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CONTENT

Research Article

Review Articles

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Research article

Entada phaseoloides SEED KERNELS MITIGATES DEPRESSION LIKE BEHAVIOUR IN RATS: A PRECLINICAL FINDING

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Abstract

Background: *Entada phaseoloides (L.) Merr.(Fabaceae) is a traditional medicine mainly used to treat diabetes, inflammation, urolithiasis, edema, and has been proven to be effective in managing disorders related to the Central Nervous System.*

Objective: *The main objective of the current study was to investigate possible antidepressant effects of the characterized hydroalcoholic extract of E. phaseoloides seed kernel using validated rodent model(s).*

Methods: *Rats were divided into 5 groups comprising of 6 rats (150-200 g) in each group. Group I rats were administered with the vehicle, Group II rats were treated with the standard drug Impiramine (10 mg/kg/day p.o.), while Group III, IV, and V rats were treated with different dose of the extract (30, 100, and 300 mg/kg/day, p.o.).The antidepressant effect of the characterized hydroalcoholic extract of E. phaseoloides seed kernel was assessed using forced swimming test and tail suspension test in rats of both sexes. Additionally, oxidative stress parameters like superoxide dismutase, and catalase activities, malondialdehyde, reduced glutathione, and nitrite levels were assessed using hippocampus of the rats.*

Results: *The immobility time in both the behavioural tests were significantly ($P<0.05$) decreased in those rats that were treated with the extract in a dose dependent manner. Additionally, the extracts significantly ($P<0.05$) alleviated oxidative stress markers in the hippocampal region of brain of rats.*

Conclusion: *Results suggest that the hydroalcoholic extract of E. phaseoloides seed kernel possess potential antidepressant activity.*

Keywords: Depression; Hippocampus; Oxidative stress; *Entada phaseoloides*

Introduction

Depression is a prevalent, recurrent mental condition marked by enduring sorrow, diminished interest, and incapacity to do everyday tasks, disrupted sleep and eating, feelings of worthlessness, hopelessness, guilt, and low self-esteem. The World Health Organization (WHO) report indicates that over 300 million individuals globally experience depression, which is recognized as a primary contributor to disability and suicide [1]. According to the Global Burden of Diseases, Injuries, and Risk Factors Study (GBS) 2019, depression is one of the two most disabling mental disorders alongside anxiety, both ranking among the top 25 leading causes of burden worldwide [2]. Depending on the number and severity of symptoms, depression can be categorized as: mild to moderate depression, major to severe depression, and bipolar affective disorder [3]. Although the etiology behind this complex disease is still not fully understood, the occurrence of the disease is thought to be related to psychosocial,

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biological, and genetic risk factors [4].Increased level of cortisone due to impaired feedback regulation of the HPA (Hypothalamic-Pituitary-Adrenal) axis, possibly due to the glucocorticoid receptor and induced depressive disorder, neurotransmitter dysregulation such as dopamine, noradrenaline, and serotonin (5-HT) in the CNS (central nervous system) are some of the major hypotheses of depression [5].There is evidence that has received significant attention suggesting that the hippocampus may have a role in the pathophysiology of depressive disorders. The dysregulation of glucocorticoid secretion and excitatory amino acid neurotransmitters could result in both potentially reversible remodeling and irreversible cell death in the hippocampus of patients with depression[6].Moreover, extensive studies on rodent and humans have shown that a large proportion of patients with major depression showed decreased hippocampus volume and has shown that its mnemonic functions and neuroplasticity are highly sensitive to stress which is manifested by increased cortisol levels[7].Although there are various chemically synthesized drugs that acts by different mechanisms, these drugs are associated with serious side effects, low response rate, less effective specially in patients suffering from more depressive symptoms, resistance, and relatively high price [8,9,10]. In recent years, an increasing number of studies have demonstrated the use of herbal remedies and plant products as therapeutic agents for the treatment of various condition including depression [3].Plant products are known to have fewer adverse effects, significant therapeutic effects, ability to function in synergy with multiple components, abundant, low cost, and easy availability [11].In the recent years, the exploration of novel antidepressants derived from medicinal plants and the efforts to identify their active extract, or key active compounds have emerged as a prominent area of research[12,13,14].

Entada phaseoloides (L.) Merr. (Fabaceae) is a climber, evergreen, large and woody plant, which is widely distributed in many parts of tropical Asia, central and eastern Himalayas [15].Chinese medical material such as “Nanfang Caomu Zhuang” and “Bencao Gangmu” documented the use of this plant as a folk medicine for the treatment of cancer, diabetes mellitus, hyperlipidemia, and stomachache [16]. The reported pharmacological activities include anti stress [17], memory enhancing [18], antidiabetic [19,20,21,22], antitumor [16,23], antiviral [24], analgesics and anti-inflammatory [25,26], anti-arthritic [27], anti-ulcer [28], anticomplement and antimicrobial [29], and antioxidant activities [30,31,32]. Investigations on the phytoconstituents of seed have shown the presence of flavonoids, alkaloids, steroids, saponins, glycosides, tannins, terpenoids, and phenolic compounds [17, 25, 33].However, to date, the active extracts and active compound from *E. phaseoloides*has not been explored for its antidepressant properties and hence, efforts have been made to explore the antidepressant effect of the hydroalcoholic extract of *E.phaseoloides* seed kernel using validated preclinical models.

Materials and Methods

Collection, identification and preparation of plant material

The seeds of *E. phaseoloides* were collected from Mokokchung district, Nagaland, Northeast India and authenticated as mentioned previously in our study [33]. A voucher specimen number bearing BSI/ERC/Tech/2020/1063 has been recorded in the herbarium. The hydroalcoholic extract of *E. phaseoloides* seed kernels (HAEP) was prepared by cold maceration according to the method previously reported in our study [33] in which qualitative and quantitative characterization of the extract has also been provided.

In vivo pharmacological studies

Rattus norvegicus, Wistar albino rats (150-200 g, 6-12 weeks old) of both sexes were divided into five groups, each consisting of six animals (n=6). They were housed in groups (same-sex) in polycarbonate cages with paddy husk bedding kept under controlled conditions (22 ± 3 ˚C, 12 h light-dark cycle, 55 ± 55% humidity). Laboratory standard rodent chow and water were offered *ad libitum*. The extract and

standard suspensions prepared in 0.3% carboxymethyl cellulose (CMC) were given orally at a volume of 10 ml/kg b.w. while the control animals were treated with the vehicle (0.3% CMC) at a volume of 10 ml/kg b.w.. The effect of various doses of the characterized HAEP (30, 100, and 300 mg/kg b.w./day, *p.o.*) and the corresponding reference drug (Imipramine 10 mg/kg b.w./day, *p.o.*) in the behaviour of animals were studied on day 8^{th} and day 10^{th} of pre-treatment(s) using validated behavioural rodent models i.e., Forced swimming test (FST) and Tail suspension test (TST), respectively. The doses of HAEP (30, 100, and 300 mg/kg b.w./day, *p.o.*) were selected based on the previous observations made from the acute and sub-chronic toxicity studies[34], while in case of imipramine, dose was selected as per previous pharmacological reports. HAEP of different doses and imipramine were administered once a day until the experiment day while the normal control group received an equivalent amount of 0.3% CMC. All behavioural studies were executed in the naive experimental rats between 09:00 and 15:00 h in noise free experimental rooms. The experimental work received approval from the Institutional Animal Care Committee (Approval No. IAEC/DU/157, Dated. 24/11/2020), Dibrugarh University (Regd. No. 1576/GO/ERe/S/11/CPCSEA, Dated. 30/10/2018) established for the purpose of control and supervision of experiments on animals. All animal studies were performed in compliance with the "Guide for the Care and Use of Laboratory Animals" issued by the National Academy of Sciences, The National Academies Press, Washington, D.C. The overview of the study design of this present study is presented in Figure 1.

Forced swimming test

This model was used to determine the antidepressant effect of various treatments in rodents and was conducted as demonstrated by Porsolt et al. [35]. The experimental set up consisted of a cylindrical container measuring 45x20 cm which was filled with water up to a height of 38 cm maintained at 25±2 ˚C. The set up was arranged in such a way to prevent the rat from touching the bottom of the cylinder with its hind limb or tail, or from climbing over the edge of the cylinder. After 1 hr of oral administration of various treatments, animals were placed individually in the cylinder containing water and the escape-oriented behavior of the rats was examined by recording the total immobility time for 3 min. Animals were considered immobile when they did not try to escape and stayed floating without struggling, except for essential movements required to keep their head above the surface of the water.

Tail suspension test (TST)

Tail suspension test (TST) was also performed to study the antidepressant effect of various treatments as described by Steru *et al.* [36]. After 1 hr of oral application of various treatments, the animals were suspended approximately 50 cm above the floor and were held by their tail with an adhesive tape. In this test, the escape-oriented behavior of the rats was examined by recording the total immobility time for 3 min. Animals were accepted immobile when the rats did not make any struggle to get rid of the tape.

Estimation of oxidative stress parameters

Immediately after performing all the behavioural observation in TST, the animals were sacrificed by decapitation and their brains were removed immediately following the protocol described by Spijker [37]. The hippocampus was excised from the entire brain and rinsed with chilled normal saline. A 10% (w/v) homogenate was prepared with 0.1 M phosphate buffer containing 1% Triton-100X (pH 7.4) and subjected to centrifugation at 1575 x g for 15 minutes. The supernatant obtained post-centrifugation was collected and utilised for biochemical studies.

Estimation of lipid peroxidation (LPO)

Lipid peroxidation was estimated by measuring malondialdehyde (MDA) level which was determined in the form of thiobarbituric acid-reactive substances using the procedure described by Wills [38].In brief, 0.5 ml of supernatant and 0.5 ml of tris-HCl were incubated at 37 ˚C for 2 h. Subsequent to incubation, 1 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged at 1000 xg for 10 min. 1 ml of 0.67% thiobarbituric acid was combined with 1 ml of supernatant, and the tubes were subjected to boiling water for 10 min. Subsequent to cooling, 1 ml of distilled water was added, and absorbance was then measured at 532 nm. Lipid peroxidation was expressed as nanomoles of malondialdehyde per milligram of protein (nmol MDA/mg protein).

Estimation of reduced glutathione (GSH)

The estimation of GSH was conducted according to the methodology outlined by Jollowet al. [39]. In brief, 1 ml of supernatant was precipitated with 1 ml of sulfosalicylic acid (4%). The samples were kept for 1 h at 4 ˚C and thereafter subjected to centrifugation at 1200 xg at 4 ˚C for 15 min. The assay mixture contained 0.1 ml supernatant, 2.7 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml 5,5′-dithiobis-(2-nitro benzoic acid) (DTNB or Ellman's reagent, 0.1 mM in phosphate buffer, pH 8.0), resulting in a total volume of 3 ml. The yellow colour developed was measured immediately at 412 nm. Reduced glutathione was expressed as microgram of reduced glutathione per milligram of protein (µg/mg protein).

Estimation of superoxide dismutase (SOD)

SOD activity was conducted using the method described by of Kono [40]. Briefly, the assay system consisted of 0.1 mM EDTA, 50 mM sodium carbonate and 96 mM of nitro blue tetrazolium (NBT). In a cuvette, 2 ml of the above mixture was combined with 0.05 ml of supernatant and 0.05 ml of hydroxylamine hydrochloride, adjusted to pH 6.0 with NaOH. The auto-oxidation of hydroxylamine was monitored by measuring the change in optical density at 560 nm for 2 min at 30/60 s intervals. The activity of SOD was expressed as units of superoxide dismutase per milligram of protein (SOD units/mg protein).

Estimation of catalase (CAT)

CAT activity was estimated by following the procedure as demonstrated by Claiborne[41]. Briefly, 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M), and 0.05 ml of supernatant were added and the changes in absorbance was recorded at 240 nm. Catalase activity was expressed as units of hydrogen peroxide consumed per minute per milligram of protein (H_2O_2 consumed/min/mg protein).

Estimation of nitrite

Nitrite level was measured using the Greiss reagent and served as an indicator of nitric oxide production and was performed by following the methodologyoutlined by Green et al. [42]. Briefly, 500 µl of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% napthaylaminediaminedihydrochloric acid in water) was added to 100 µl of supernatant, and the absorbance was measured at 546 nm. Nitrite level was measured from a standard curve for sodium nitrite and was expressed as nanogram per milligram of protein (ng/mg protein).

Estimation of protein concentration

Protein estimation of the homogenates was performed following the method demonstrated by Lowry et al.[43]. A standard curve was established by using Bovine serum albumin (BSA). Briefly in a 96-microwell plate, 100 µl of brain homogenates or BSA was put in its respective well, followed by the addition of 200 µl of biuret reagent (2 g sodium potassium tartrate tetrahydrate, 1g copper sulphate, 10 ml of 1 N NaOH, and made up the volume to 100 ml with distilled water) and mixed thoroughly with repeated pipetting. The mixture was then allowed to incubate at room temperature for 10-15 min. After incubation, 20 µl of Folin reagent (1 volume of Folin-Ciocalteu's reagent diluted in 15 volume of distilled water) was added to each well. The colour was allowed to develop for 30 min at room temperature. All absorbance readings were made using Thermo Scientific Multiskan Go plate reader at 655 nm.

Statistical analysis

The results are expressed as mean ± Standard Error of Mean (SEM). Differences among different treatment groups were determined by one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparable test. GraphPad Prism-8.0.2 software (GraphPad Software Inc., CA, USA) was used for statistical analysis. A P-value less than 0.05 ($P<0.05$) was considered as the threshold (95% level of confidence) for statistically significant. Statistically significant changes obtained from the above-mentioned tests were designated by the superscripts.

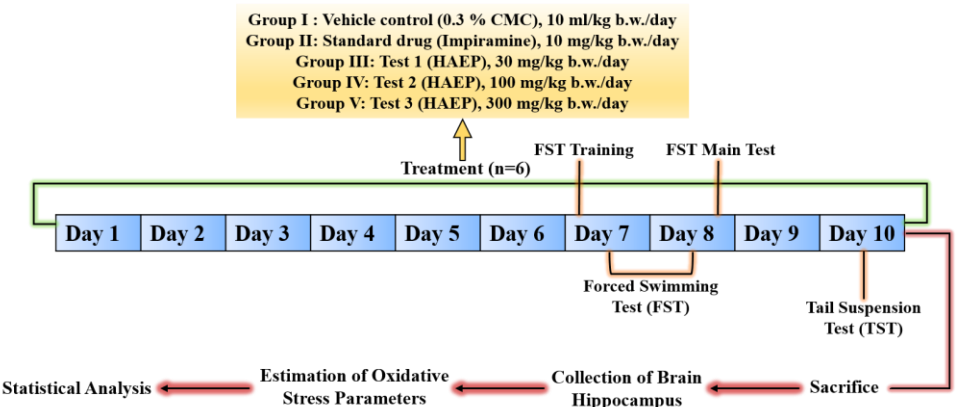

Figure 1: Overview of the study design illustrating animal groups with its corresponding doses, treatment period, behavioural experiments and biochemical assessments performed in the present study. CMC = Carboxymethyl cellulose, HAEP = characterized hydroalcoholic (70% ethanol) extract of Entadaphaseoloides.

Results

Antidepressant effect of HAEP on rats in forced swimming test (FST)

Figure 2 illustrates the effect of repeated daily administration of characterized HAEP (30, 100, and 300 mg/kg/day, *p.o.*) in immobility time (sec.) on the FST. Data analysed by one-way ANOVA showed that rats pretreated with the HAEP (100 and 300 mg/kg/day, *p.o.*) as well as imipramine (10 mg/kg/day, *p.o.*) showed significant ($P<0.01$, $P<0.0001$, and $P<0.001$, respectively) reduction in immobility time (sec.) *i.e*. more escape-oriented behaviour when compared with vehicle-treated control group.

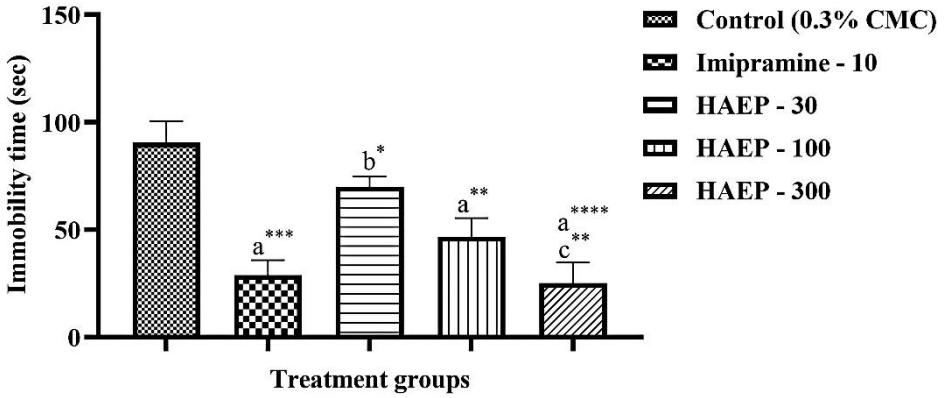

Figure 2:Antidepressant effect of various treatments on rats in forced swimming test. Values are presented as mean ± S.E.M (n=6 each group). Data were analysed by one way ANOVA followed by Tukey's Multiple Comparable test where, 'a', 'b', and 'c' indicates significant difference (*$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$) as compared to vehicle (0.3% CMC) treated group, Imipramine (10 mg/kg) treated group, and HAEP (300 mg/kg) treated group, respectively. HAEP = characterized hydroalcoholic (70% ethanol) extract of *Entadaphaseoloides*.

Antidepressant effect of HAEP on rats in tail suspension test (TST)

Figure 3 depicts the effect of repeated daily administration of characterized HAEP (30, 100, and 300 mg/kg/day, *p.o.*) in immobility time (sec.) in the TST. Data analysis by one-way ANOVA showed the animals that were pretreated with the HAEP (300 mg/kg/day, *p.o.*) and Imipramine (10 mg/kg/day, *p.o.*) showed significant ($P<0.01$, and $P<0.001$, respectively) decrease in immobility time (sec.) *i.e.* more escape-oriented behaviour when compared with vehicle-treated control group.

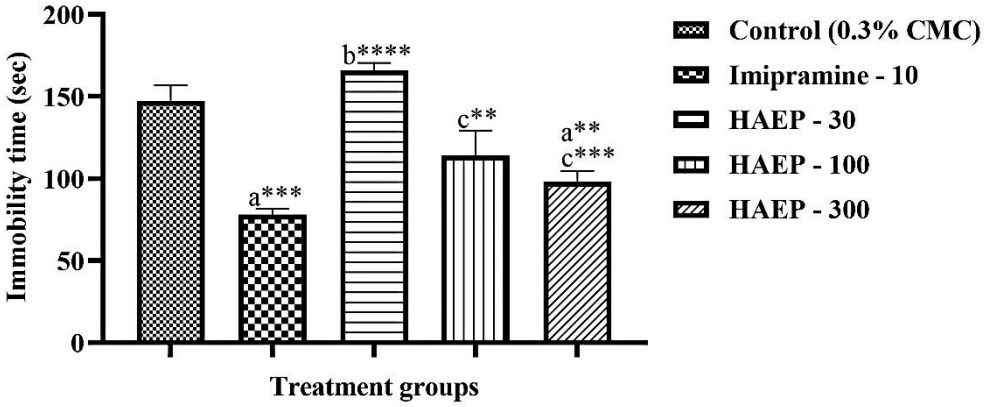

Figure 3:Antidepressant effect of various treatments on rats in tail suspension test. Values are presented as mean ± S.E.M (n=6 each group). Data were analysed by one way ANOVA followed by Tukey's Multiple Comparable test where, 'a', 'b', and 'c' indicates significant difference (***P*< 0.01, ****P*< 0.001, *****P*< 0.0001) as compared to vehicle (0.3% CMC) treated group, Imipramine (10 mg/kg) treated group, and HAEP (300 mg/kg) treated group, respectively. HAEP = characterized hydroalcoholic (70% ethanol) extract of *Entada phaseoloides*.

Effect of HAEP on the oxidative stress in rat hippocampus

Figure4 represents the result of various treatment on the different oxidative stress markers in the hippocampus of rats. HAEP treatment on stress induced rats significantly ($P < 0.05$) ameliorated the increased the level of LPO(Figure3A) and NO (Figure 3E) levels and increased the activity of antioxidant enzymes namely SOD(Figure3B)and catalase activity(Figure3C), as well as GSH(Figure3D) levels when compared with the vehicle treated group.

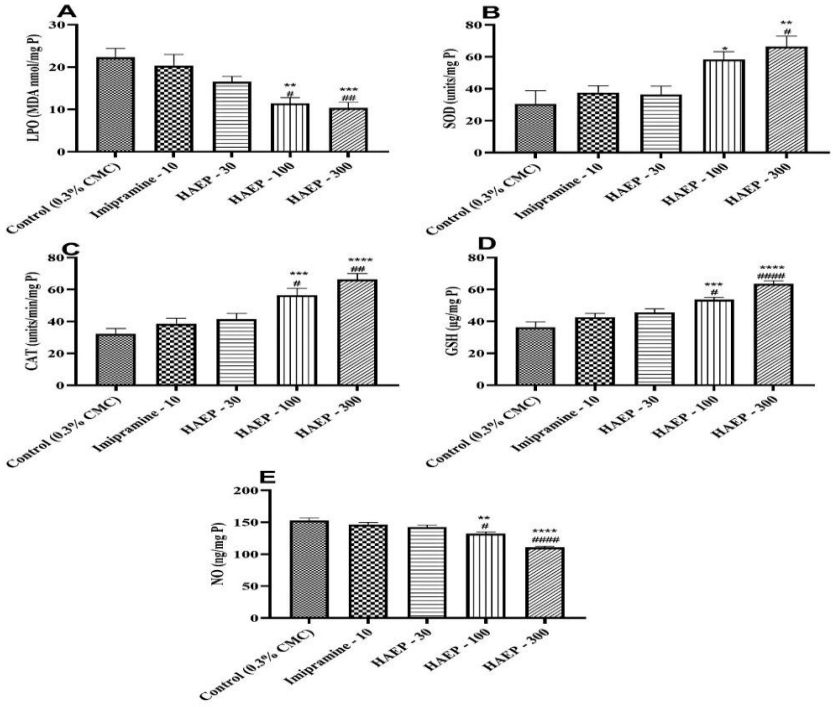

Figure 4: Effect of various treatments on the oxidative stress in hypothalamus of rats A. LPO, B. SOD, C. CAT, D. GSH, and E. NO. Values are presented as mean ± S.E.M (n=6 each group). Data were analysed by one way ANOVA followed by Tukey's Multiple Comparable tests, where '*' indicates significant difference as compared to vehicle (0.3% CMC) treated group ($p<0.05$). HAEP = characterized hydroalcoholic (70% ethanol) extract of *Entada phaseoloides*, LPO = Lipid peroxidation, SOD = Superoxide dismutase, CAT = Catalase, GSH = Glutathione, NO = Nitric oxide.

Discussions

The principal objective of the current study was to investigate potential antidepressant-like effect of the characterized hydroalcoholic extract of *Entada phaseoloides*(HAEP) seed kernel, a traditional medicinal plant mostly used in diabetes, inflammation, urolithiasis, cancer, and CNS disorders on two validated rodent models *i.e.* FST and TST. These two models are the most widely followed animal models for screening potential antidepressants because of their sensitivity to most of the classes of antidepressants like selective serotonin reuptake inhibitors, tricyclic antidepressants, and monoamine oxidase inhibitors [36, 44, 45]. These models rely on behavioural despair where the animals when exposed to some inescapable unfavourable condition lose its ability of resistance. This behavioural despair is also observed in people as a part of depressive symptoms. Rodent behavioural tests for depression are often referred to as "depressive-like behaviour" tests, since they primarily involve identifying certain behavioural traits that resemble symptoms of depression in humans [46]. The immobility time which was observed in the FST and TST is usually considered to as behavioural despair in animals as it reflects depression in humans [47]. FST was developed by Porsolt and colleagues in 1970s to study the phenomenon of learned helplessness and investigate the effects of antidepressant treatments on rats [44]. Since then, it has become popular tool in studies on behaviour resembling depression. It is a common test used for evaluation of the efficacy of anti-depressant drugs and the effects of various behavioural and neurobiological manipulations in basic and preclinical research [48]. The widespread use of this test is mostly due to its ease of use, reliability across laboratories and capability to detect a wide range of antidepressant agents [49]. In the current study, seven days of treatment with HAEP (100 and 300 mg/kg b.w.; *p.o*) significantly reduced the duration of immobility. There are studies that showed that the decrease in the duration of immobility time was linked to antidepressant property [49, 50, 51]. Similarly, TST was developed in the 1980s to study the antidepressant effects in rodents. The test examines the impact of short-term stress on rodents, which differs significantly from human depression. In humans, depression is primarily caused by long-term behavioralissues resulting from genetic vulnerability and environmental exposures[52]. It is probable that the reason for the continued use of this test to assess depression in laboratory animals is due to the absence of more reliable alternatives [53]. The present study demonstrated that treatment with HAEP (100 and 300 mg/kg b.w.; *p.o*) for ten days showed a significant decrease in immobility time which indicates potential antidepressant properties of HAEP.

Clinical evidence indicates the involvement of hippocampus in depression, where a reduction in hippocampal volume has been observed in depressed patients [54]. This reduction in hippocampal volume was observed to be mitigated by antidepressant medication, which demonstrated enhanced increased neurogenesis in the adult hippocampus in preclinical studies [54,55,56]. To supplement this point, it has been shown that patient with multiple depressive episodes have decreased hippocampal volume relative to those with patients experiencing their first depressive episode and healthy controls [57,58]. The brain-derived neurotrophic factor (BDNF) which plays a major role in neuronal growth, survival, maturation and synaptic plasticity in the brain is present in the hippocampus[59,60,61].Studies have indicated that chronic stress can both deplete and suppress the synthesis of BDNF in the hippocampus and prefrontal cortex[56,62,63]. The findings that serum BDNF levels are decreased in individuals with depression and can be restored through antidepressant treatment has significant implications for the role of BDNF as a biomarker of depression and the pursuit of effective treatment[64,65].Additionally, the postmortem studies in humans indicate that the levels of BDNF are markedly reduced in the hippocampus and prefrontal cortex of depressed individuals who died by suicide, in comparison to controls, while antidepressant medication normalises brain BDNF levels[66,67]. There is growing evidence that oxidative stress, resulting from elevated production of reactive oxygen and nitrogen species, plays a significant role in the pathogenesis of various psychiatric disorders and may influence the onset or adverse progression of psychiatric disorders through alterations in the brain structure[68,69]. Increased oxidative damage was seen in postmortem hippocampal tissue of patients with major depressive disorder [70].An elevation

in lipid peroxidation correlates with depression as it is attributed to increased levels of proinflammatory cytokines that generates free radicals and enhance monoamines metabolism. Malondialdehyde is the end product of lipid peroxidation, and elevated levels of malondialdehyde was observed in patients with depression compared to healthy control [71,72]. Also, there are also evidences that non-enzymatic antioxidant molecules like glutathione levels were also found to be disturbed during depression [73, 74]. While the activities of enzymatic antioxidants like catalase [75] and superoxide dismutase [76]were reduced in depressed patients[77]. The current study demonstrated that the animals that were treated with HAEP showed decreased lipid peroxidation and nitric oxide levels and enhances glutathione levels and antioxidant enzymes like catalase and superoxide dismutase in the hippocampus of the rats. Thus, the results of the present study suggest a potential antidepressant efficacy of *Entada phaseoloides* (L.) Merr. seed kernel.

Conclusion

The hydroalcoholic extract of *Entada phaseoloides* seed kernel exhibited a significant antidepressant-like property in the validated FST and TST test for depression in rats. The extracts alleviate the oxidative stress in the hippocampal region of the brain which further suggests that modulation of oxidative stress pathway could be one of the mechanisms involved in the antidepressant efficacy of the seed kernel making it a potential therapeutic candidate for the treatment and management of depression. Further, there is a need for mechanistic and clinical studies for the validation of its antidepressant potential.

List of Abbreviations

WHO:World Health Organisation

HAEP: Hydroalcoholic extract of *Entada phaseoloides*

LC-MS: Liquid chromatography-mass spectrometry

p.o.: Per oral

b.w.: Body weight

GBS: Global Burden of Diseases, Injuries, and Risk Factors Study

HPA: Hypothalamic-Pituitary-Adrenal

CMC: Carboxymethyl cellulose

FST: Forced swimming test

TST: Tail suspension test

LPO: Lipid peroxidation

MDA: Malondialdehyde

GSH:Glutathione

SOD: Superoxide dismutase

CAT: Catalase

BSA: Bovine serum albumin

BDNF: Brain-derived neurotrophic factor

Human and Animal Rights

Research Involving Humans

Not applicable

Research Involving Animals

The experimental work was approved by the Institutional Animal Care Committee (Approval No. IAEC/DU/157, Dated. 24/11/2020), Dibrugarh University (Regd. No. 1576/GO/ERe/S/11/CPCSEA, Dated. 30/10/2018) constituted for the purpose of control and supervision of experiments on animals. Each of the animal experiments was conducted in accordance “Guide for the Care and Use of Laboratory Animals” published by the National Academy of Sciences, The National Academies Press, Washington, D.C

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Declaration of competing interests

The authors declare no conflict of interest.

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YOGA AND ITS STREAMS: AN OVERVIEW OF *ASANAS*, *PRANAYAMA*, MEDITATION AND RELAXATION TECHNIQUES

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Abstract

Background

The impact of Yoga in everyday life is undeniable. It not only emphasizes on body but also the mind. Thus, practicing Yoga helps in improving physical and mental health. Yoga is believed to connect universe with individual shelf. It increases physical strength, mental strength and create an emotional balance and make an individual spiritually grounded. Yoga is in practice since ancient time, even before any human existence prevail. And has been in constant evolvement for the betterment and requirement of the contemporary public. It has been divided into different phases or eras like vedic, pre-classical, classical and post classical. In each era various adaptations have been notice that's objective was for the benefits of the public. Yoga is an interdisciplinary field encompassing various techniques required for healthy living such as Asanas, Pranayama, Meditation and various relaxation methods to free the body, mind and soul from worldly sufferings and attainment of Moksha.

Objective

This review paper is to get a complete idea about Yoga, its evolutionary background , its present day importance in the life of contemporary people, its developmental trends over the years, its uses and different streams of Yoga. Focusing on the attainment of mental and spiritual connection and thus leading to a healthy life avoiding diseases and fulfillment of good health. This review focus on

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different Asanas (yogic postures), different Pranayamas (breathing techniques) and different Dhyana i.e. meditation techniques.

Methods

Relevant literature was collected and analyzed from peer-reviewed journals, publications and standard reference textbooks. Information related to concept and historical evolution of yoga, ancient origins and texts related to yoga, importance of yoga, major streams of yoga, eight limbs of yoga, an overview on asanas, pranayama,meditation and relaxation technique and also technical evolution in the field of yoga.

Discussion

Yoga has also many physiological effects on the body and also cognitive and psychological benefits. There are many benefits that has been seen among the practitioners like the blood circulation increases and make the person skin glow, helps in weight management, keeps in check of Autonomic Nervous System by regulating the sympathetic and parasympathetic activities, improving cardiovascular and respiration rate, improving mental health. Due to modern trends in the field of Yoga and more people inclining to it, has lead to increase in professionalizing, thus leading more engagement of people, which inversely leads to economic growth.

Conclusion

Practitioning yoga has been seen to be impactful in the lives of the practitioners, thus, leading to a healthy mind and a healthy life. Implementing AI and computational real time monitoring has aced this practice more. Proper knowledge regarding practice of Asanas, Pranayamas and meditation techniques with the help of modern techniques can help to emphasize more on the benefits of humans.

Keywords: Yoga, *Asana*, *Pranayamas*, *Dhyana*

1.Introduction

1.1. Definition and Concept of Yoga

Yoga is mainly derived from a Sanskrit word “Yuj”, whose meaning is to unite. If more elaborated it defines the union of one’s soul to the cosmic consciousness. Two distinct units are being connected i.e. individual soul and universal self [1]. It is an integrative discipline encompassing postures referred as Asana, pace breathing referred as Pranayama and meditation referred as Dhyana to intensify physical, mental and spiritual well being [2].Yoga is a holistic practice that has been in use through generations. Practices that are encouraged in Yoga strongly revolves around one’s disciplined lifestyle referring as Yama and Niyama, detoxifying or cleansing procedures known as Kriya , concentration referring as Dharana [3]. Yoga aims to overcome worldly sufferings and reaching a state of emancipation or Moksha [4].

1.2. Historical Evolution of Yoga

Yoga has been in practice since the beginning of human culture and society. In yogic philosophy, Lord Shiva has been considered as the very first yogi or Adiyogi. It is believed that on the banks of the lake Kantisarovar at the Himalayas, Lord Shiva has transferred his yogic knowledge to the seven sages or Saptarishis. The Saptarishis took this yogic science to different places across the world encompassing Asia, Middle East, North and South America. Agastya Saptarishi in India crafted the Yogic culture [4].

Yoga’s history claims back to over 5,000 years and has led to changes from early prehistoric spiritual rituals and meditation into a global system for health and well being [5]. Traditionally this transformation is classified into four main periods: the Vedic, Pre-Classical, Classical and Post-Classical eras [6-8].

1.3. Ancient Origins and Vedic Roots

1.3.1. Pre-Vedic and Indus Valley Civilization

Archaeological traces like seals depicting figures in yogic postures dates back to the Indus Valley Civilization (circa 3000 BCE), confirms the intertwined of spiritual and physical disciplines even before the codification of religious systems [9-11].

1.3.2. The Vedic Period

At the foundational level, yoga was established during the Vedic period (circa 1500 to 500 BCE) through the formulation of the Vedas, the oldest Sanskrit texts [11, 12]. The earliest scriptures first connect yoga with ritual activities like sacrificial fire rituals and the spiritualization of the physical body [13-15].The word Yoga was found in Rig Veda, where it was define and linked with the terminologies of discipline and the art of connection [16-18].

1.4 Philosophical Codification and Classical Yoga

1.4.1. The Upanishads and Bhagavad Gita

In the Pre-Classical period (800-400 BCE), the focus turned to an inner spiritual quest as depicted in Upanishads [13, 19].These texts provide the fundamental aspect of Moksha as well as define yoga as an activity that uses two methods i.e. controlling one's breath and meditate, to join individual consciousness with that of God. The Bhagavad Gita further expanded these ideologies by highlighting different paths such as Karma which means action, Bhakti which means devotion and Jnana which means knowledge to proper understand oneself [20, 21].

1.4.2. Patanjali and the Yoga Sutras

In Classical period (circa 200 BCE to 500 CE) , sage Patanjali systematically coded the Anciient ascetic practices[22][23]. He provided around 194 to 195 aphorisms that gave a definite framework to the philosophy[24]. This "Raja Yoga" outlined mostly on moral guidelines, physical postures (asanas) , breath control (pranayama) and meditation (dhyana) [25].

1.5. Medieval Transitions and Hatha Yoga

1.5.1. Post-Classical Development

This period occurs between around 800 CE and 1700 CE, it included the teachings of impactful figures like the Acharyatrayas [26]. Yoga methods where during this time was included into the customs and culture of Hindu ascetics, such as Nath and Ramanandi orders [27].

1.5.2. The Rise of Hatha Yoga

Hatha yoga developed through the fusion of Tantrism and other traditions, emphasizing on the physical body as a medium for spiritual liberation [28, 29].

Though previous texts lacked certain key mentions of some modern postures, works like 17th century Gheranda Samhita began to document poses like Bhujangasana[30]. This period made a proper shift from purely mental discipline toward more body oriented practices favourable to modern audience [31].

1.6. The Modern Era and Global Expansion

1.6.1. Introduction to West

Yoga received international recognition in the late 19th and early 20th century, mainly due to Swami Vivekananda [32]. Due to his reconfigurement of Yoga Sutras and publication of Raja Yoga in 1896, attracted new age audience [33]. Other masters like T. Krishnamacharya played an impactful role in the 20th century by reconceptualizing postures within an active flow [34].

1.6.2. Modern Worldwide Wellness

Yoga in present day has reformed into a multi-billion dollar industry globally with an estimation of 300 billion practitioners [35]. Certain new age adaptations include various styles such as Power Yoga, Hot Yoga and Aerial Yoga [36, 37]. Yoga is now performed for its believe of therapeutic benefits and globally celebrated through the declaration of United Nations of International Yoga Day on 21st of June [38].

1.7 Importance of Yoga in Health and Wellness

Yoga serves as a powerful therapeutic and preventive tool, offering advantages in controlling stress and treating various chronic medical conditions [39].Meditation helps in providing internal physical thus ensuring health benefits [39, [40].

Yoga provides benefits such as :

Musculoskeletal and Respiratory Benefits: Practice of Hatha yoga improves strength, body flexibility and increase blood flow [41, 42]. Systematic training leads to improvement in Respiratory functions [42].

Anthropometric and Metabolic Improvements: Regular practicing of yoga showcases positive changes in body leading to decrease in body weight, body mass index and waist circumference [43]. Thus yoga is beneficial in increasing g metabolic rate and improving insulin sensitivity [44]. It shows significant decrease

in triglycerides and thus leading to decrease in heart related problems and illness such as type 2 diabetes [45].

Cardiovascular Health and Regulation: Yoga has a great impact in reducing blood pressure and lipid management. Meta-analysis of randomized controlled trials indicate that yoga influences in significant control in lipid profiles, that helps in lowering total cholesterol and low density lipoprotein (LDL) cholesterol [46].

Autonomic and Vascular function: The primary mechanism for cardiovascular improvement is the stimulation of the vagus nerve, which improves parasympathetic tone and baroreflex sensitivity [47]. Increased heart rate in yoga practitioners indicates a more healthier and more flexible autonomic nervous system [48].

Mental well-being and Stress Management: Yoga is widely used in reduction of symptoms related to stress, anxiety and depression in both clinical and healthy from populations [49]. Studies conclude that yoga practitioners can be effective as standard medication for treating certain mood disorders [50].

Endocrine Regulation and Cortisol: Yoga is largely impactful in controlling the down regulation of the hypothalamic pituitary adrenal axis (HPA) [51].This regulation includes in decrease of primary stress hormone i.e. cortisol levels. Yoga practice leads to increase release beneficial chemicals such as serotonin, melatonin and oxytocin [52].

Chronic Pain and Digestive health: Yoga provides an impactful non-pharmacological intervention for controlling chronic primary pain, including low back pain and musculoskeletal disorders [53].It has been shown to reduce pain intensity and disability while improving physical function in affected individuals[54]. It is helpful in treating youths and adults with irritable bowel syndrome, yoga also regulates the autonomic nervous system and reduce visceral sensitivity[55].

Support for severe illness: In oncology, yoga is utilized as an optional supportive intervention to manage symptoms such as fatigue and psychological distress in cancer survivors [56]. It aids in recovery from intensive by improving overall quality of life and physical endurance [57]. Yoga serves as an adjunct therapy for other serious conditions, including cardiovascular disease, rheumatoid arthritis and

respiratory illness like COPD [58]. Its multi-component nature makes it as a versatile tool for enhancing well-being across diverse medical histories [59].

2. Philosophy of Yoga

Yogic philosophy is a comprehensive spiritual system that focuses on achieving a peaceful mind and stable mental state for higher purposes [60]. It is rooted in ancient scriptures and ethical disciplines that guide a practitioner's physical and spiritual life [61].

The primary foundational texts include the Yoga Sutras, the *Bhagavad Gita* and the *Hatha Yoga Pradipika* [62]. Each text offers a unique perspective on the path of spiritual realization and mental clarity [63].

2.1. Core Ethical Principles of Yoga

Yoga teaches specific ethical restraints and positive duties that foster spiritual growth and physical well-being [64].

2.1.1 The Yamas

The Yamas are ethical restraints that define how a practitioner interacts with the world. These principles include ahimsa, which refers to non-violence and satya which represents truthfulness. Additionally, practitioners are encouraged to follow asteya (non- stealing), brahmacharya (right use of energy) and aparigraha (non-greed). These guidelines serve as the first limb of yoga, establishing a moral foundation for the practitioner [64].

2.1.2 The Niyamas

Niyama represents the second limb of yoga and involves spiritual observances and self- discipline. These are positive duties intended to facilitate healthy spiritual and physical living. Following these disciplines helps practitioners maintain the necessary internal environment for deeper meditative practices [64, 65].

2.2 Ultimate Purpose of Yoga

The primary goal of yoga is to promote an integrated, peaceful mind rather than serving as an end in itself. It acts as a means to stabilize mental activity, preparing the individuals for their highest spiritual purpose. By integrating the lessons from the Bhagavad Gita and other scriptures, practitioners aim to achieve a state of

mental equilibrium.This stabilization of the mind is central to all forms of yoga described in the foundational Hatha Yoga Pradipika and other key texts [65, 66].

3. Eight Limbs of Yoga (Ashtanga Yoga)

Ashtanga yoga is a powerful discipline that integrates dynamic physical postures with a rigorous philosophical framework to promote mental and physical well-being. The practice, which means "eight limbs" in Sanskrit, aims to help individuals achieve a deep state of conscious awareness and live in harmony with the universe [66].

It was popularized by K. Pattabhi Jois during the twentieth century, Ashtanga Vinyasa yoga is often promoted as a dynamic form of medieval practice [67]. This specific style originated with the vinyasa concepts introduced by the yoga master Krishnamacharya [68]. As an Indian yoga guru, Jois founded the Ashtanga Yoga Research Institute and played a pivotal role in making the discipline popular globally [69].

The term "Ashtanga" translates directly to "eight limbs" represents the fundamental components of the yoga practice [66]. These limbs, derived from Patanjali's system, provide a comprehensive guide for living a balanced and ethical life [70]. The eight limbs consist of the following elements:

Yama- These are moral restrictions or restraints that guide behavior [70].

Niyama- These refer to internal disciplines or observances [70].

Asana- This limb focuses on the physical postures used throughout the practice [70].

Pranayama- This involves the control of breath and the regulation of energy [70].

Pratyahara- This stage involves the withdrawal of the senses from external distractions [70].

Dharana- This refers to the practice of focused concentration [70].

Dhyana- This stage represents a state of deep meditation [70].

Samadhi- This is the final state of conscious awareness or enlightenment where one lives in harmony with the universe [70].

4. Major Streams of Yoga

Yoga is primarily expressed through several major streams or paths that cater to different human temperaments and lead to the same spiritual goal. These pathways include the four classical paths of Karma Yoga, Bhakti Yoga, Jnana Yoga, and Raja Yoga, as well as physical disciplines like Hatha Yoga [71].

4.1.The Four Classical Paths of Yoga

The Bhagavad Gita serves as a foundational text that outlines four main spiritual paths designed to help practitioners achieve self-realization. These paths are often compared to the branches of a single tree or tributaries of a river, as they all share the same source and destination [71].

4.1.1.Karma Yoga: The Path of Action

- Definition: Karma Yoga is the path of selfless service and action.
- Practice: It involves performing one's duties to the best of their ability while letting go of attachment to the results or outcomes.
- Focus: This stream focuses on work and action as a means of spiritual growth [71].

4.1.2. Bhakti Yoga: The Path of Devotion

- Definition: Bhakti Yoga is the path of unconditional love, devotion, and worship.
- Practice: Practitioners channel their emotions toward a higher power or the divine to foster inner peace and humility.
- Focus: It is particularly suited for those who are naturally more emotional or devotional in nature [71].

4.1.3. Jnana Yoga: The Path of Knowledge

- Definition: Jnana Yoga, also spelled Gyana or Jyana, is the path of wisdom, philosophy, and intellectual understanding.
- Practice: It involves the study of scriptures and the use of the intellect to discern reality from illusion.
- Focus: This path is centered on achieving spiritual insight through knowledge and deep understanding [71].

4.1.4. Raja Yoga: The Royal Path

- Definition: Raja Yoga is frequently referred to as the royal path or the path of control.
- Practice: It emphasizes meditation, concentration, and the systematic control of the mind.
- Variations: In some traditions, it is associated with Patanjali Yoga (Ashtanga Yoga) or Dhyana Yoga [71].

4.2. Additional Major Streams and Disciplines

While the four classical paths are central to yogic philosophy, other streams focus on the physical and energetic aspects of the human experience.

Hatha Yoga: This is the yoga of physical discipline, focusing on postures and harmonizing the body's energies.

Tantra Yoga: Some traditions include Tantra as a major branch, focusing on the expansion of consciousness.

Kundalini Yoga: This school focuses on awakening the latent spiritual energy located at the base of the spine [71].

5. *Asanas* (Yogic Postures)

Asana is a Sanskrit term for a body posture, originally used to describe a sitting meditation pose. Over time, the term has been extended within practices such as Hatha Yoga and modern yoga to include a wide variety of physical positions [72].

5.1. Definition and Historical Roots

The earliest source of the term, which refers to a posture or seat, is found in scriptures that are over two thousand years old [73]. While it is now associated with various physical exercises, it was historically a general term for a sitting meditation pose [72].

5.2. Historical Evolution

In classical yoga texts, specifically the Yogasūtra of Patañjali from approximately the 4th or 5th century CE, the term referred exclusively to a posture for practicing seated meditation [73]. The transition from strictly seated meditation to the diverse

range of postures seen today occurred later through the development of different yoga traditions [72].

5.3. Classification of Postures

Modern practices involve a diverse array of movements that can be categorized by the body's orientation and the type of movement performed [74]. These postures are generally classified into the following groups:

- Basic Orientations: Postures may be standing, seated, or reclining in either prone or supine positions.
- Functional Movements: This category includes twists, forward bends, and backbends.
- Advanced Forms: These involve more complex physical engagements such as arm balances and inversions [74].

5.4. Health Benefits and Examples

Practicing these body postures is often motivated by the desire to enhance overall health and physical well-being. Specific movements are frequently utilized to address mobility problems and improve functional strength [75].

One of the most popular examples is Tadasana, commonly known as Mountain Pose, which is used to improve posture, strengthen the legs, and promote a sense of grounding. Other well-known poses, such as Adho Mukha Svanasana, provide various physiological benefits ranging from increased flexibility to improved circulation [75].

6. *Pranayama* (Breathing Techniques)

Pranayama is an ancient breathing technique originating from India that involves the deliberate control and modification of breath to enhance physiological and psychological well-being. By regulating the flow of vital energy, it serves as a natural method to calm the mind, reduce stress, and improve cardio respiratory health [76].

6.1. Origin, Historical and Spiritual Context

The term is derived from two Sanskrit words: "prana," which signifies the breath of life or vital energy, and "ayama," which means regulation, control, or expansion.

Consequently, the practice is defined as a set of techniques designed to control the life force within the human body through various breathing patterns.

The practice has origins in ancient India, with records dating back thousands of years. It is described in foundational Hindu texts, such as the Upanishads, and remains a core component of yogic traditions. The primary spiritual and mental goal is to reduce mental chatter while enhancing concentration and clarity [77].

6.2. Classification of Breathing Techniques

There are at least 14 distinct types of breathing exercises, each offering unique health benefits. These techniques are generally categorized by their pace and specific methods of execution [78].

Slow-paced Techniques: These practices, such as Nadi-Shodhana (alternate nostril breathing), primarily focus on enhancing parasympathetic activity and promoting relaxation.

Rapid-breathing Techniques: Practices like Bhastrika (bellows breath) involving rapid diaphragmatic movements can have heterogeneous effects on the nervous system.

Specialized Methods: Techniques like Ujjayi (ocean breath) and Sitali (cooling breath) are used to reduce anxiety or remove excess heat from the body.

Unilateral Nostril Breathing: This involves breathing through a single nostril to influence brain dominance and autonomic functions.

6.3. Physiological Effects on the Body

Autonomic Nervous System Regulation- Scientific assessments indicate that yogic breathing influences the autonomic nervous system by altering the balance between sympathetic and parasympathetic activities [79].

Cardiovascular and Respiratory Health- The practice has demonstrated significant therapeutic effects on heart rate and blood pressure. In clinical settings, it has been shown to improve the quality of life for patients with specific conditions like Bronchial Asthma, Hypertension, Chronic Obstructive Pulmonary Disease [79].

6.4. Cognitive and Psychological Benefits

Neuromodulation and Brain Function- The practice is considered a self-directed form of neuromodulation, similar in effect to contemporary approaches like vagus nerve stimulation or transcranial magnetic stimulation [79].

Mental Health and Stress Management- Breathing exercises are highly effective for managing clinical stress and anxiety. By promoting emotional regulation and physiological resilience, they serve as a low-risk, home-based adjunctive care method for mental health [79].

7. Meditation Techniques

Yoga meditation encompasses a diverse range of practices designed to enhance mental clarity, physical well-being, and emotional stability through techniques such as focused attention and conscious relaxation. These methods, which include yoga nidra, trataka, and dhyana, provide systematic approaches to achieving inner transformation and stress management [80].

7.1. *Yoga Nidra*: The Practice of Yogic Sleep

7.1.1. Definition and Method

Yoga Nidra, often translated as "yogic sleep," is a guided relaxation technique that facilitates a profound state of conscious awareness between wakefulness and sleep. Unlike traditional yoga which emphasizes physical poses, this practice is typically performed in shavasana (corpse pose) and relies on mental imagery and body scanning. While its roots are ancient, the modern systematized version was introduced in the 1960s by Swami Satyananda Saraswati to make it accessible to everyone from beginners to advanced practitioners [81].

7.1.2. Psychological and Physiological Impact

Routine practice of Yoga Nidra has been shown to reduce symptoms of mild depression, anxiety, and perceived stress. It works directly with the autonomic nervous system, shifting balance toward parasympathetic activity to elicit deep physiological rest. Research indicates that it can also improve sleep quality by reducing sleep onset latency and increasing total sleep time in individuals with insomnia [82].

7.2.*Trataka*: Focused Gazing Meditation

7.2.1. Definition and Method

Trataka is a powerful yogic purification and meditation technique that involves gazing steadily at a single point or object without blinking. A common application of this practice involves focusing on a candle flame, which serves as a physical anchor for the mind. The goal is to still the eyes, as steady eyes are believed to help still the mind and deepen overall concentration [83].

7.2.2. Benefits for Focus and Vision

By fixing the gaze, practitioners can enhance their mental focus and internal awareness [83]. Ancient texts suggest that Trataka can eradicate diseases associated with vision and improve optical health. It is often used as a preparatory stage for deeper meditation by clearing the mind of external distractions [84].

7.3. Classical Stages: Dharana and Dhyana

7.3.1. Sequential Mental States

Traditional yoga texts, including Patanjali's Yoga Sutras, describe meditation as a progression through specific mental states. The first stage, Dharana, involves focused attention or meditative focusing on a single object. This leads to dhyana, a state of effortless meditation or mental expansiveness where the practitioner maintains heightened awareness without active effort [85].

7.3.2. Neurophysiological Correlates

Scientific investigations show that Dharana improves performance in attention-based tasks. In contrast, Dhyana is associated with reduced sympathetic activity and increased vagal tone, indicating a deeper state of relaxation [85].

7.4. Clinical Evidence and Therapeutic Benefits

Yoga meditation has been found to positively influence biological markers of stress, specifically by reducing total cortisol levels and steepening diurnal cortisol slopes. Controlled trials have also demonstrated its effectiveness in lowering resting blood pressure and improving heart rate variability [86].

A synthesis of research across various populations highlights the broad utility of these techniques [86].

Mental Health: Significant reductions in anxiety, depression, and stress levels have been recorded in both healthy and clinical cohorts.

Cognitive Function: Regular practice has been shown to improve cognition and memory, particularly in individuals with cognitive impairment.

Physical Health: Benefits extend to managing non-communicable diseases like hypertension and diabetes through better hormonal and metabolic regulation.

Accessibility: Techniques like mantra meditation and Yoga Nidra are cost-effective and non-invasive, making them practical for digital delivery and integration into daily life [86].

8. Challenges and Future Perspectives

Yoga faces significant methodological hurdles in clinical research and a shift toward commercialization, but its future is being shaped by integration with advanced technology and a focus on global sustainability [87].

8.1. Research and Methodological Hurdles

8.1.1. Limitations in Clinical Evidence

Most clinical trials involving yoga are relatively small in size and frequently fail to explore common medical conditions systematically. A primary challenge in conducting a Randomized Controlled Trial for yoga is the difficulty of implementing double-blind designs, as the nature of the intervention makes blinding both participants and instructors nearly impossible [87].

8.1.2. Cultural and Philosophical Divergence

There is a growing tension between the traditional spiritual roots of yoga and its modern Western adaptation, which often focuses solely on physical postures or "asanas". In the West, yoga has become virtually synonymous with movement-based practice, even though there is little historical evidence that such movement was ever the primary focus of ancient traditions [88].

8.2. Technological Integration and Innovation

8.*2.1. Artificial Intelligence and Machine Learning*

Artificial Intelligence is revolutionizing yoga therapy by providing enhanced personalization and real-time monitoring. Machine learning algorithms can now

analyze patient data, such as medical history and diagnostic results, to tailor interventions for specific conditions like chronic pain or cardiovascular disease. AI-driven virtual assistants use natural language processing to guide users through sessions, offering adaptive guidance and modifications based on the practitioner's performance [89].

8.2.2. Internet of Things (IoT) and Wearables

The integration of the Internet of Things into yoga practice involves using smart mats and wearable sensors to provide independent guidance without a tutor. Wearable devices can capture body movements, heart rate, and breathing patterns to ensure optimal alignment and safety during practice. These non-intrusive sensors provide real-time feedback on posture and movement quality, helping practitioners avoid injuries such as ligament ruptures or strokes caused by incorrect form. However, collecting high-quality training data and ensuring the privacy of practitioners remain significant challenges in this field [89].

8.3. Clinical and Market Future Outlook

8.3.1. Market Projections and Economic Growth

The global yoga market is experiencing a massive growth trajectory, valued at approximately USD 113.7 billion in 2024 and projected to reach USD 167.5 billion by 2030. Other forecasts suggested the industry could reach up to USD 215 billion by 2025 as wellness trends continue to rise globally. North America currently dominates this market, holding a 33.27% share as of 2025. This economic expansion is fueled by an increasing global interest in mind-body medicine and a proactive approach to self-care among the aging population [90].

8.3.2. Professionalization of Yoga Therapy

To fully integrate yoga into modern healthcare, there is a push to establish yoga therapy as a formal medical department with recognized MD degrees. Standardized clinical protocols are being developed to provide evidence-based "yoga prescriptions" that can be tailored to individual needs. These efforts aim to overcome barriers such as a lack of specialized training for teachers and the high cost of traditional wellness facilities. Moving forward, increasing the participation

of prestigious medical institutions in yoga research will be essential for validating its restorative and preventive abilities [90].

9. Conclusion

From this review we can get a proper idea about Yoga and its different streams. Importance of Asanas, Pranayama, meditation and different relaxation techniques. This review give us insights about Historical and ancient roots of Yoga and yogic culture, evolutionary development over different phases by different yogic practitioners and the impact towards its modern culture. Regularly practicing yoga has lead to healthy lifestyle of individual as well as preventing and even curing certain diseases, leading to a peaceful mind. Yoga has created an important benchmark over time in the economic growth and the people associated with it has seen an increase in trends over the years. Inclusion of Artificial intelligence in the field of yoga includes real time monitoring to guide users while providing adaptive guidance. Wearable devices have also been a great helpful to capture body movements and postures while preventing injuries.

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COMPARATIVE REGULATORY FRAMEWORK OF HERBAL COSMETICS IN INDIA: ROLE OF CDSCO, AYUSH, FSSAI, BIS AND DGFT

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Abstract

Background: *India's herbal cosmetics sector is expanding, driven by preference for plant-derived and Ayurvedic products. Regulatory landscape is involving authorities—Central Drugs Standard Control Organization (CDSCO), Ministry of AYUSH, Food Safety and Standards Authority of India (FSSAI), Bureau of Indian Standards (BIS), and Directorate General of Foreign Trade (DGFT)—posing challenges for compliance, market access, and consumer safety.*

Objective: *To review and compare Indian frameworks governing herbal cosmetics, elucidating roles, recent amendments, and interfaces of CDSCO, AYUSH, FSSAI, BIS, and DGFT, and analyzing impact on product classification, manufacturing, safety, labeling, trade, and enforcement.*

Methods: *A comprehensive literature review was conducted using peer-reviewed articles, official notifications, regulatory guidelines, and market reports published between 2021 and 2026. Key regulatory documents, amendments, and case studies were analyzed. Comparative tables and figures were constructed to illustrate regulatory overlaps and distinctions.*

Conclusion: *India's regulatory framework for herbal cosmetics is robust but fragmented. Greater inter-agency coordination, harmonization with international*

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standards, and clarity in product classification are essential for industry growth, consumer safety, and global competitiveness.

Keywords: CDSCO, AYUSH, FSSAI, BIS, DGFT, export policy

1.Introduction

India's herbal cosmetics sector is at the confluence of tradition and modernity, reflecting the country's rich *Ayurvedic* heritage and the global shift towards natural, sustainable beauty products [1]. The market for herbal cosmetics in India reached USD 1.06 billion in 2025 and is projected to grow at a CAGR of over 10% through 2034, driven by consumer demand for *plant-based, chemical-free* products, and the influence of *Ayurveda* and traditional knowledge systems [2]. This growth is further fueled by rising disposable incomes, increased awareness of skin health, and the proliferation of e-commerce platforms.

Despite this momentum, the regulatory landscape for herbal cosmetics in India remains complex and multi-layered. The Central Drugs Standard Control Organization (CDSCO) oversees cosmetics regulation under the Drugs and Cosmetics Act, 1940, and the Cosmetics Rules, 2020, with significant amendments in 2025. The Ministry of AYUSH regulates *Ayurvedic*, *Siddha,* and *Unani* products, emphasizing Good Manufacturing Practices (GMP) and traditional knowledge protection. The Bureau of Indian Standards (BIS) prescribes mandatory standards for cosmetic ingredients and finished products, while the Food Safety and Standards Authority of India (FSSAI) governs ingestible products and food-cosmetic borderline items. The Directorate General of Foreign Trade (DGFT) manages import/export policy, HS codes, and export facilitation for herbal cosmetics.

This intricate regulatory framework, while designed to ensure product safety, quality, and consumer protection, often results in overlapping jurisdictions, ambiguous product classifications, and compliance challenges for manufacturers and exporters. The emergence of *phytopharmaceuticals*, *nutraceuticals*, and *borderline* products further complicates the regulatory environment, necessitating a

nuanced understanding of statutory definitions, licensing pathways, and enforcement mechanisms.

The present review critically examines the comparative regulatory framework for herbal cosmetics in India, focusing on the roles and recent reforms of CDSCO, AYUSH, FSSAI, BIS, and DGFT. It analyzes key areas such as product classification, GMP, labeling, safety testing, claims, pharmacovigilance, trade policy, and international harmonization, providing evidence-based insights and recommendations for stakeholders.

2. CDSCO: Statutory Role, Cosmetic Rules 2020 and Amendments 2025, SUGAM Portal

The Central Drugs Standard Control Organization (CDSCO) is the apex regulatory authority for cosmetics in India, operating under the Ministry of Health and Family Welfare. The CDSCO's mandate includes licensing, import registration, enforcement, and post-market surveillance of cosmetics, including herbal variants.

The regulatory authority structure is bifurcated:

- Central Licensing Authority (CDSCO/DCGI): Responsible for import registration (COS-2), new cosmetic approvals, and central enforcement.
- State Licensing Authorities (SLAs): Responsible for domestic manufacturing licensing (COS-8), facility inspections, and local enforcement [3,4].

2.2 Cosmetics Rules 2020 and 2025 Amendments

The Cosmetics Rules, 2020, marked a comprehensive restructuring of India's cosmetics regulation, separating cosmetics from drugs and introducing digitized, category-specific pathways [5]. Key features include:

- Electronic Licensing via SUGAM Portal: All applications for import, manufacturing, and new cosmetic approvals are processed online, enhancing transparency and efficiency.
- Product Classification: 14 import categories under the Fourth Schedule, with a dedicated "New Cosmetic" category requiring reference safety data and toxicology studies.

- Ingredient Standards: Mandatory compliance with BIS IS 4707:2025 for ingredients, including lists of Generally Recognized as Safe (GRAS) and Generally Not Recognized as Safe (GNRAS) substances.
- GMP Requirements: Manufacturers must comply with GMP as per the Seventh Schedule, with batch-wise record retention and periodic inspections.
- Labeling and Claims: Strict labeling requirements under Rule 34, with prohibitions on false or misleading claims (Rule 36) and mandatory ingredient disclosure.
- Animal Testing Ban: Absolute statutory ban on animal testing for cosmetics since 2014.
- Post-Market Surveillance: Mandatory reporting of foreign regulatory actions, state inspections, and complaint mechanisms via SUGAM.

The Cosmetics (Amendment) Rules, 2025 (G.S.R. 513(E)), introduced targeted reforms for greater regulatory clarity and enforcement [5,6]:

- Clarification of "use before" and "expiry date" definitions.
- Electronic or hardcopy record retention for three years or six months post-expiry.
- Central Cosmetics Laboratory designated for appellate testing.
- Enhanced powers for license suspension/cancellation by SLAs.
- Export labeling flexibility to accommodate importing country requirements.

2.3 SUGAM Portal

The SUGAM portal is the centralized digital platform for all CDSCO cosmetic applications, including import registration (COS-1/COS-2), manufacturing licensing (COS-5/COS-8), and new cosmetic approvals (COS-12). The portal streamlines documentation, fee payments, and regulatory correspondence, reducing processing times and enhancing compliance monitoring.

2.4 Product Classification and Borderline Products

CDSCO's 2025 guidelines provide explicit criteria for classifying products as cosmetics or drugs, particularly for borderline products such as sunscreens, anti-dandruff shampoos, and skin-lightening [7]. Key decision criteria include:

- Function: Beautification/cleansing (cosmetic) vs. diagnosis/treatment (drug).
- Claims: Aesthetic claims (cosmetic) vs. therapeutic/functional claims (drug).
- Ingredients: Presence of scheduled drugs (e.g., clindamycin, hydroquinone, minoxidil) triggers drug classification.
- SPF Claims: SPF >30 or extended protection claims require drug licensing.

This classification is critical, as misclassification can result in regulatory violations, recalls, and penalties.

2.5 Licensing and Dossier Requirements

- Import Registration (COS-1/COS-2): Requires appointment of an Indian representative, product classification, submission of mandatory documents (Free Sale Certificate, Certificate of Analysis, IS 4011:2018 test report, ingredient list, no-animal-test declaration), and CDSCO scrutiny (180 working days).
- Manufacturing License (COS-5/COS-8): Application via SUGAM, facility inspection, GMP self-declaration (COS-7), and indefinite validity with five-year retention fees.
- Loan License (COS-9): For brand owners using third-party manufacturers, both licenses must remain valid.

2.6 Enforcement, Recalls, and Penalties

CDSCO and SLAs conduct routine and risk-based inspections, with powers to seize, recall, or destroy non-compliant or spurious cosmetics. Penalties include license suspension/cancellation, product seizure, and criminal prosecution under the Drugs and Cosmetics Act [8].

3. Ministry of AYUSH: Regulation of Herbal Products, Schedule T, AYUSH Certification Schemes

3.1 Statutory Role and Regulatory Framework

The Ministry of AYUSH (Ayurveda, Yoga & Naturopathy, Unani, Siddha, and Homeopathy) is responsible for the regulation, promotion, and development of traditional medicine systems in India. Herbal products classified as *Ayurvedic*, *Siddha*, or *Unani* (ASU) medicines are regulated under the Drugs and Cosmetics Act, 1940, and the Drugs and Cosmetics Rules, 1945, with specific provisions for GMP (Schedule T), licensing, and pharmacovigilance [9,10].

3.2 Schedule T: Good Manufacturing Practices (GMP)

Schedule T prescribes detailed GMP requirements for ASU medicines, ensuring authenticity, quality, and safety of raw materials and finished products [10].

Table 1: Key GMP Requirements under Schedule T

Area	Requirement
Factory Premises	Adequate space for raw material storage, manufacturing, QC, finished goods, rejected goods
General Hygiene	Clean, ventilated, pest-free environment; fire safety; proper drainage
Raw Materials	Authentication, traceability, segregation, FIFO procedure, testing by in-house or approved labs
Packaging	Clean, dry containers; proper storage of packaging materials
Finished Goods	Quarantine until QC approval; distribution records for recall
Health & Hygiene	Medical examination, uniforms, sanitation for workers
Machinery	Suitable equipment for each dosage form; SOPs for cleaning and maintenance
Batch Records	Detailed records of raw materials, tests, manufacturing steps, QC, packaging
Quality Control	In-house or government-approved lab; reference books, controlled

	samples, shelf-life records
Market Complaints	Register for complaints, investigations, corrective actions, adverse reactions

These standards are mandatory for all ASU manufacturers and are enforced through periodic inspections and audits by State Licensing Authorities.

3.3 Licensing and Certification

- Manufacturing License: Issued by State AYUSH Licensing Authorities, based on compliance with Schedule T and technical staff qualifications.
- Loan/Third-Party Manufacturing License: For brand owners using external manufacturers, with documented quality agreements.
- AYUSH Certification Schemes:
    - *AYUSH Standard Mark:* For domestic regulatory compliance.
    - *AYUSH Premium Mark:* For export-quality certification, based on WHO-GMP or importing country's standards.

3.4 Pharmacovigilance and Adverse Event Reporting

The Ministry of AYUSH operates the National Pharmacovigilance Program for ASU & H Drugs, collecting and analyzing adverse event data to enhance patient safety [11]. Manufacturers are required to maintain complaint registers, investigate adverse reactions, and report serious events to regulatory authorities.

3.5 Labeling, Claims, and Advertising

AYUSH products must comply with labeling requirements, including product name, dosage form, ingredient list, batch number, manufacturing/expiry dates, license number, directions for use, and caution statements. Claims must be truthful, evidence-based, and non-misleading, with oversight by the Advertising Standards Council of India and the Central Consumer Protection Authority.

3.6 Evidence-Based Research and Phytopharmaceuticals

The rise of *phytopharmaceuticals*—purified, standardized plant fractions with defined bioactive compounds—has led to a new regulatory pathway under CDSCO, requiring scientific evidence, clinical trials, and Schedule M GMP compliance [12].

3.7 Export Facilitation

The Ayush Export Promotion Council (AYUSHEXCIL) supports exporters with certifications, trade delegations, and capacity-building events, enhancing global market access for AYUSH products [13].

4. FSSAI: Interface with Herbal Cosmetics, Food-Cosmetic Borderline and Indigestible Products

4.1 Statutory Role

The Food Safety and Standards Authority of India (FSSAI) regulates food products, including health supplements, nutraceuticals, and ingestible items that may overlap with cosmetics (e.g., edible lip balms, beauty supplements) [14].

4.2 Food-Cosmetic Borderline

Products intended for ingestion (e.g., nutricosmetics, beauty drinks) are regulated as foods under the Food Safety and Standards Act, 2006, and the Food Safety and Standards (Health Supplements, Nutraceuticals, Food for Special Dietary Use, Food for Special Medical Purpose, Functional Food and Novel Food) Regulations, 2016. Such products require FSSAI licensing, safety assessments, and compliance with labeling and advertising standards.

4.3 Labeling and Claims

FSSAI mandates clear labeling of ingredients, nutritional information, batch number, manufacturing/expiry dates, and FSSAI license number. Claims must be substantiated and non-misleading, with strict prohibitions on therapeutic or disease-related claims for food products.

4.4 Testing and Enforcement

FSSAI-notified laboratories conduct safety and quality testing for food products, including those at the food-cosmetic interface. Enforcement actions include product recalls, penalties, and prosecution for non-compliance.

4.5 Challenges and Overlaps

The overlap between FSSAI and CDSCO arises for products that are both ingestible and have cosmetic claims. Clear classification and regulatory guidance are essential to avoid dual compliance burdens and enforcement ambiguities.

5. BIS: Indian Standards for Cosmetics, Mandatory Standards and IS Codes (Including IS 4707:2025)

5.1 Statutory Role

The Bureau of Indian Standards (BIS) is the national standards body, responsible for developing, publishing, and enforcing Indian Standards for cosmetics, ingredients, and testing methods [15,16].

5.2 IS 4707:2025 and Related Standards

- IS 4707 (Part 2):2025: List of GNRAS and restricted ingredients, specifying banned substances, concentration limits, and safety requirements. Mandatory from February 9, 2026.
- IS 4707 (Part 3):2025: List of preservatives allowed in cosmetics with restrictions.
- Other IS Codes: Cover specific product categories (e.g., IS 3959 for skin powders, IS 6608 for skin creams, IS 9875 for lipsticks, IS 7123 for hair oils).

Table 2: BIS Heavy Metal Limits in Cosmetics

Parameter	Limit
Lead	≤20 ppm
Arsenic	≤2 ppm
Mercury (eye products)	≤70 ppm
Mercury (other products)	≤1 ppm
Hexachlorophene	Prohibited (except soap ≤1%)

5.3 Mandatory Certification and QCOs

BIS certification is mandatory for certain cosmetic categories, with Quality Control Orders (QCOs) expanding coverage. Manufacturers must obtain BIS licenses, undergo facility inspections, and comply with IS standards for product safety, quality, and labeling.

5.4 Testing Infrastructure

BIS operates a network of recognized laboratories for conformity assessment, heavy metal testing, preservative efficacy, and microbiological safety. NABL accreditation ensures reliability and international acceptance of test results [17,18].

5.5 Labeling and Packaging

BIS standards prescribe labeling requirements, including product name, manufacturer details, batch number, net content, ingredient list, and warnings. Packaging must ensure product integrity, tamper-evidence, and compliance with Legal Metrology rules.

6. DGFT and Trade: Import/Export Policy, HS Codes, Restrictions, Export Facilitation for Herbal Cosmetics

6.1 Statutory Role

The Directorate General of Foreign Trade (DGFT), under the Ministry of Commerce and Industry, formulates and implements India's import/export policy, including for herbal cosmetics [19].

6.2 Import/Export Policy and HS Codes

- Import Policy: Import of cosmetics requires a valid Import Export Code (IEC), CDSCO import license (COS-1), and compliance with BIS standards. HS Code 33041000 applies to most cosmetic products, with a basic customs duty of 20% and additional GST.
- Export Policy: Exporters must comply with destination country regulations, obtain Free Sale Certificates, and ensure labeling meets importing country requirements. DGFT facilitates exports through trade agreements, market access initiatives, and export promotion councils (e.g., AYUSHEXCIL).

6.3 Restrictions and Prohibitions

- Import of cosmetics tested on animals after November 12, 2014, is prohibited.
- Products containing banned substances (e.g., hexachlorophene, lead, arsenic) are not permitted.

- Export of certain herbal ingredients may be restricted under the Biodiversity Act and CITES.

6.4 Export Facilitation

DGFT supports exporters with trade delegations, buyer-seller meets, and capacity-building events. The Ayush Export Promotion Council (AYUSHEXCIL) provides certifications, market intelligence, and advocacy for herbal cosmetics exporters [20].

7. Product Classification and Borderline Products: Cosmetic vs Drug vs Ayurvedic Medicine vs Nutraceutical

7.1 Classification Criteria

Product classification is pivotal, as it determines the applicable regulatory pathway, licensing, and compliance requirements. Key criteria include:

- Intended Use: Beautification/cleansing (cosmetic), diagnosis/treatment (drug), traditional use (Ayurvedic medicine), nutritional benefit (nutraceutical).
- Claims: Aesthetic (cosmetic), therapeutic (drug), preventive/curative (Ayurvedic), health supplement (nutraceutical).
- Ingredients: Presence of scheduled drugs, active pharmaceutical ingredients, or traditional herbs.
- Dosage Form: Topical (cosmetic), oral (nutraceutical/food), both (borderline).

7.2 Regulatory Pathways

- Cosmetics: Regulated by CDSCO under Cosmetics Rules 2020/2025, BIS standards, and Legal Metrology rules.
- Drugs: Regulated by CDSCO under Drugs and Cosmetics Act, Schedule Y, and pharmacopoeial standards.
- Ayurvedic Medicines: Regulated by Ministry of AYUSH under Schedule T, with GMP and traditional knowledge protection.
- Nutraceuticals: Regulated by FSSAI under Health Supplements and Nutraceuticals Regulations.

7.3 Challenges and Case Studies

Borderline products (e.g., anti-dandruff shampoos, SPF creams, ingestible beauty supplements) often face regulatory ambiguity, leading to compliance challenges, enforcement actions, and market delays [21].

8. Good Manufacturing Practices (GMP): Schedule T, Schedule M-II, WHO-GMP and Manufacturing Licensing

8.1 GMP for Cosmetics

- Schedule M-II (Drugs and Cosmetics Rules 1945): Previously applied to cosmetics, now replaced by the Seventh Schedule of Cosmetics Rules 2020, prescribing GMP for cosmetic manufacturing.
- Seventh Schedule: Requires hygienic premises, category-specific equipment, quality control systems, and qualified personnel. Manufacturers must submit GMP self-declaration (COS-7) and undergo periodic inspections.

8.2 GMP for ASU Medicines

- Schedule T: Prescribes GMP for *Ayurvedic*, *Siddha*, and *Unani* medicines, covering premises, raw material authentication, process controls, quality assurance, and documentation [9,10,23].
- WHO-GMP: Internationally recognized standards for herbal medicines, increasingly required for exports.

8.3 Manufacturing Licensing

- COS-8 License: Issued by State Licensing Authorities for cosmetic manufacturing, valid in perpetuity with five-year retention fees.
- AYUSH Manufacturing License: Issued by State AYUSH Licensing Authorities, based on Schedule T compliance.

9. Labeling, Claims, Advertising and Legal Metrology Compliance

9.1 Labeling Requirements

Labeling is governed by Rule 34 of the Cosmetics Rules 2020, BIS standards, and the Legal Metrology (Packaged Commodities) Rules 2011 [24].

Mandatory Declarations:

- Product name, manufacturer name/address, batch number, manufacturing/expiry dates, net content, ingredient list (INCI nomenclature), warnings/directions, import registration number (COS-2), license number, MRP, country of origin (for imports), importer details.

Export Labeling: Labels for export must comply with importing country laws, with flexibility for manufacturer identification via code numbers.

9.2 Claims and Advertising

- Rule 36 (Cosmetics Rules 2020): Prohibits false or misleading claims. Claims must not imply therapeutic or medicinal effects, which would trigger drug classification.
- Advertising Standards: Oversight by Advertising Standards Council of India and Central Consumer Protection Authority. Digital promotions are increasingly monitored.

9.3 Legal Metrology Compliance

Cosmetics are regulated as packaged commodities, requiring compliance with declarations on net content, MRP, batch number, and manufacturer/importer details.

10. Safety Testing, Contaminants and Analytical Methods (Heavy Metals, Pesticides, PFAS, Stability)

10.1 Safety Testing

Manufacturers must conduct safety testing for heavy metals, microbial contamination, preservatives, and stability, as per BIS standards (e.g., IS 4707, IS 4011) and CDSCO requirements.

Key Parameters:

- Lead, arsenic, mercury, cadmium, chromium, nickel, antimony.
- Microbial limits, preservative efficacy, stability under Indian climatic conditions.
- PFAS and other emerging contaminants.

10.2 Analytical Methods

Testing is conducted in NABL-accredited, BIS-recognized, or FSSAI-notified laboratories, using validated methods (e.g., HPLC, GC-MS, TLC, DNA barcoding).

10.3 Stability Studies

Manufacturers must conduct accelerated and real-time stability studies to validate shelf life, especially under Indian climatic conditions.

11. Pharmacovigilance and Adverse Event Reporting for Herbal Products and Cosmetics

11.1 Cosmetics

CDSCO mandates reporting of adverse events, product recalls, and foreign regulatory actions. The SUGAM portal facilitates complaint submission and tracking.

11.2 AYUSH Products

The Ministry of AYUSH operates the National Pharmacovigilance Program for ASU & H Drugs, with adverse event reporting via the Ayush Suraksha portal [25]. Manufacturers must maintain complaint registers and investigate adverse reactions.

11.3 International Harmonization

India is aligning pharmacovigilance practices with international standards (e.g., ICH-GCP, EMA guidelines) to enhance consumer safety and global acceptance [8,11].

12. Registration, Licensing and Dossier Requirements for Domestic Manufacture and Import

12.1 Cosmetics

Import Registration (COS-1/COS-2): Requires submission of technical dossiers, safety data, ingredient lists, and compliance certificates via SUGAM.

Manufacturing License (COS-5/COS-8): Requires GMP self-declaration, facility inspection, and technical staff qualifications.

12.2 AYUSH Products

Manufacturing License: Requires compliance with Schedule T, technical staff qualifications, and product approval for proprietary formulations.

Export Certifications: Free Sale Certificate, Certificate of Analysis, WHO-GMP documentation.

12.3 Phytopharmaceuticals

CDSCO Approval: Requires CTD-based dossier, preclinical and clinical data, GMP compliance (Schedule M), and post-marketing surveillance plan [11,12].

13. Standards and Testing Infrastructure: NABL, BIS Labs, FSSAI Notified Labs and Recognized Testing Facilities

India has a robust network of testing laboratories, including:

NABL-Accredited Labs: For heavy metal, microbial, and stability testing.

BIS-Recognized Labs: For conformity assessment and IS standard compliance.

FSSAI-Notified Labs: For food-cosmetic borderline products.

Central Cosmetics Laboratory: Appellate authority for sample analysis.

14. Enforcement, Recalls, Penalties and Recent Case Studies

Regulatory authorities conduct routine and risk-based inspections, with powers to seize, recall, or destroy non-compliant products. Penalties include license suspension/cancellation, product seizure, and criminal prosecution. Recent case studies highlight enforcement actions against non-compliant imports, misbranded products, and false claims [5,26].

15. International Harmonization and Comparative Frameworks (EU THMPD, FDA MoCRA, ASEAN)

India is increasingly harmonizing its regulatory framework with international standards:

EU THMPD: Traditional Herbal Medicinal Products Directive requires 30 years of traditional use, pre-market approval, and pharmacovigilance [27].

FDA MoCRA: Modernization of Cosmetics Regulation Act introduces mandatory registration, safety substantiation, and PFAS bans.

ASEAN Cosmetic Directive: India is aligning ingredient lists, labeling, and GMP with ASEAN guidelines to facilitate exports.

16. Phytopharmaceuticals Pathway and Scientific Evidence Requirements under CDSCO

Phytopharmaceuticals are regulated as new drugs under CDSCO, requiring:

- Purified, standardized plant fractions with defined bioactive markers.
- Preclinical and clinical data for safety and efficacy.
- GMP compliance (Schedule M).
- Post-marketing surveillance and periodic safety updates [7,11].

17. Industry Stakeholders, Trade Associations and Certification Bodies

Key stakeholders include:

Indian Beauty & Hygiene Association (IBHA): Apex body for the beauty and personal care industry, advocating for regulatory reforms and industry standards [28].

Ayush Export Promotion Council (AYUSH EXCIL): Supports exporters with certifications and market access.

Certification Bodies: Provide AYUSH Standard/Premium Marks, WHO-GMP, and organic certifications.

18. Market Trends, Size, Consumer Perceptions and Export Data for Herbal C osmetics

The Indian herbal cosmetics market reached USD 1.06 billion in 2025, projected to grow to USD 2.61 billion by 2034 (CAGR 10.18%) [2]. Key trends include:

- Rising demand for Ayurvedic and plant-based ingredients.
- Growth of sustainable, eco-friendly, and organic products.
- Increasing e-commerce and digital marketplace penetration.
- Export growth to EU, US, Middle East, and ASEAN markets.

Consumer perceptions are shaped by gender, marital status, beauty appeal, advertising, and peer recommendations. Gender and marital status significantly influence preference for herbal cosmetics, while age and frequency of use have less impact [29].

19. Intellectual Property, Traditional Knowledge Protection, TKDL and Biodiversity Act Implications

India protects traditional knowledge and biodiversity through:

Traditional Knowledge Digital Library (TKDL): Prevents misappropriation of Ayurvedic, Unani, and other traditional formulations in international patent offices [30].

Biological Diversity Act, 2002 (Amended 2023): Regulates access to biological resources, ensures fair benefit sharing, and supports community-led conservationstatic.pib.gov.in.

People's Biodiversity Registers: Document local species, ecosystems, and traditional knowledge for conservation and benefit sharing.

20. E-Commerce and Online Marketplace Regulation for Herbal Cosmetics

E-commerce sellers of cosmetics are fully regulated under the Drugs and Cosmetics Act, 1940, and Cosmetics Rules, 2020. Key requirements include:

- Valid import/manufacturing licenses (COS-1, COS-8).
- Labeling compliance (Rule 34), including ingredient lists, batch numbers, MRP, and importer details.
- Prohibition of false or therapeutic claims.
- Marketplace platforms (Amazon, Flipkart, Nykaa) require license documentation and compliance verification.
- Enforcement actions include delisting, product seizure, and legal notices [31].

22. Conclusion

India's regulatory framework for herbal cosmetics is comprehensive but fragmented, involving multiple authorities—CDSCO, AYUSH, FSSAI, BIS, and DGFT—each with distinct mandates, standards, and enforcement mechanisms. While this multi-agency approach ensures robust consumer protection, quality assurance, and traditional knowledge preservation, it also creates challenges in product classification, compliance, and market access, especially for borderline and e-commerce products.

Recent reforms, including the Cosmetics Rules 2020 and 2025 Amendments, digitization via the SUGAM portal, expansion of BIS standards, and harmonization with international frameworks, have enhanced regulatory clarity, transparency, and enforcement. However, further efforts are needed to:

- Strengthen inter-agency coordination and regulatory harmonization.
- Clarify product classification criteria for borderline products.
- Enhance GMP, safety testing, and pharmacovigilance systems.
- Support industry stakeholders with capacity building, export facilitation, and certification.
- Protect traditional knowledge and biodiversity through robust legal frameworks.

By addressing these challenges, India can foster innovation, ensure consumer safety, and position itself as a global leader in herbal cosmetics.

Conflict of interest None to declare

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TRADITIONAL KNOWLEDGE DIGITAL LIBRARY (TKDL) AND REGULATORY FRAMEWORKS IN AYUSH: ROLE OF GOVERNMENT INITIATIVES AND RESEARCH COUNCILS

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Abstract

Background: *Traditional medicinal knowledge represents an important component of the cultural heritage and healthcare practices of Indigenous communities in India. Passed down orally through generations, this knowledge contributes significantly to community well-being and biodiversity conservation. However, the lack of systematic documentation and the limitations of existing intellectual property frameworks create challenges in recognizing and protecting its medicinal value. Although India has made considerable progress in safeguarding traditional knowledge, the diversity of Indigenous medical systems necessitates more comprehensive and culturally sensitive approaches.*

Objective: *The present study aimed to examine the status of traditional medicinal knowledge among Indigenous communities in India and to explore mechanisms for its recognition and protection within the country's intellectual property rights (IPR) framework. It also sought to identify appropriate approaches for ensuring that Indigenous communities are able to exercise and benefit from their rights over such knowledge.*

Methods: *The study was based on secondary sources of information. A focused literature review was conducted using PubMed, Google Scholar, and other relevant*

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online and offline resources between March 2021 and September 2023. Keywords used in the search included traditional knowledge, intellectual property rights, tribes, medicinal plants, ethnomedicine, folklore, medical anthropology, World Health Organization (WHO), World Intellectual Property Organization (WIPO), Traditional Knowledge Digital Library (TKDL), Patent Act, and India. Relevant scientific articles, reports, and policy documents were critically reviewed.

Discussion: *The findings indicate that traditional medicinal knowledge in India remains fragmented and largely undocumented, limiting its effective legal recognition. A uniform approach is unlikely to address the diverse cultural and regional contexts of Indigenous communities. A multi-tiered documentation strategy, combined with a human rights-based approach emphasizing cultural rights, may provide a more suitable framework for recognizing Indigenous communities as the legitimate custodians of their knowledge.*

Conclusion: *The development of appropriate guidelines and inclusive legal frameworks is essential for integrating Indigenous medicinal knowledge into India's IPR regime. Such measures would facilitate the preservation, recognition, and equitable utilization of traditional knowledge while safeguarding the rights and cultural heritage of Indigenous communities.*

Keywords: TKDL, IPR, WIPO

1.Introduction

1.1 Traditional knowledge and medicinal heritage of India

India is widely known for its rich cultural diversity, ancient traditions, and deep-rooted heritage of extensive skills and knowledge systems. Due to remarkable biodiversity and long history of utilizing medicinal plants, the country is often described as a "herbal emporium". For centuries, people in India have relied on herbs not only for treatment of illnesses but also as part of everyday health practices. Although various many medicinal plants and even certain animal-derived

resources possess therapeutic value, many peopleremain unaware of their formal recognition and classification within traditional medical systems.

Traditional medical knowledge is primarily represented through systems such as Ayurveda and Siddha, which are often regarded as indigenous systems of medicine that originate and evolved from within Indian culture itself and continue to reflect its philosophical and holistic approach to health and well-being [1]. Additionally, it also emphasizes prevention and the maintenance of a balanced and healthy lifestyle.

India's traditional medical heritage is also shared with neighbouring countries such as Bangladesh, Pakistan, Nepal, and Sri Lanka, where similar practices and influences can be observed [1]. Among these systems, Siddha medicine occupies a significant place, particularly in the Tamil-speaking regions of southern India. It represents one of the oldest codified systems of healing and continues to be practiced in various parts of the country to play an important role in healthcare and traditional healing practices.

Another significant component of India's traditional healthcare landscape is the Unani system of medicine Ayurveda and Siddha; the Unani system is also widely practiced across India. Introduced by Arabian scholars and physicians around 1350 AD, Unani medicine became integrated into the Indian healthcare landscape over time and is now an important component of the country's traditional medical framework.Other systems such as Yoga and Naturopathy have also evolved into distinct disciplines, although they share foundational principles with Ayurveda, particularly in their emphasis on balance, discipline, and holistic wellness[2]. In addition, Sowa Rigpa, meaning "which translate knowledge of healing" in the Bhoti language, represents the traditional Tibetan system of medicine practiced in the Himalayan regions of India. It is especially followed in states and regions such as Sikkim, Himachal Pradesh, Arunachal Pradesh, Darjeeling in West Bengal, and the Union Territory of Ladakh [1].

Together, these diverse systems reflect India's pluralistic and integrative approach to health, where traditional knowledge coexist and continues to play a vital role in both cultural identity and healthcare practices.

The Government of India has established the Department of Indian Systems of Medicine and Homeopathy in 1995 in accordance with the methods of WHO for integration of complementary and alternative medicine in the public health system. In 2003, the Department was renamed as AYUSH (Ayurveda, Yoga, Unani, Siddha and Homeopathy Medical Systems). Subsequently, on 9th November 2014, the Ministry of Ayush was created with the objective to develop and promote the Ayush systems of healthcare through the public healthcare framework and revive the invaluable traditional knowledge of Indian medical systems [2].

Despite their immense value, traditional knowledge systems have faced threats of misappropriation and unauthorized patent claims to prevent deterioration and safeguards India's rich medicinal heritage, the Indian government has launched the Traditional Knowledge Digital Library (TKDL) in collaboration with the Council of Scientific and Industrial Research (CSIR), Ministry of Science and Technology and Department of Ayurveda, Yoga, Unani, Siddha and Homeopathy (AYUSH) to prevent Traditional Knowledge from misuse and unauthorized patent claims [3].

1.2 Traditional Knowledge Digital Library (TKDL): An overview

The Traditional Knowledge Digital Library (TKDL) is a huge database that preservedand protect digitized texts of various indigenous medical practices and medicinal herbs including both codified and non-codified. According to the Council of Scientific and Industrial Research (CSIR), a considerable 70% of Indians earn their living through traditional medical practices including the use of plant-based remedies like neem, turmeric, ArogyaPacha [4].Global patent, U.S. patent application related to the wound-healing properties of turmeric drew global attention to the issue of biopiracy, where elements of India's traditional healing knowledge were being claimed and commercially exploited without proper acknowledgement or benefit-sharing. Similar instances demonstrated the vulnerability of indigenous knowledge systems, in which traditional practices from Indian systems of medicine had been accessed by international entities over several decades without adequate legal protection [4].

In response to these challenges, the Government of India established the Traditional Knowledge Digital Library (TKDL) as a defensive mechanism to prevent the misappropriation of traditional knowledge by documenting and digitizing ancient medical systems in a structured and searchable format. Through the use of modern information technology tools and a systematic classification system known as the Traditional Knowledge Resource Classification (TKRC), the library translates and categorized traditional formulations and practices into multiple international languages and formats that are compatible with patent examination procedures.[4].

So far, more than 4.54 lakh formulations and practices derived from codified Indian traditional medical systems under AYUSH have been documented in the TKDL. This extensive large-scale effort has made to strengthens this knowledge by enabling to patent offices worldwide, helping to recognized prior traditional knowledge and prevent the granting of patents on knowledge that already exist. Furthermore, the Traditional Knowledge Resource Classification (TKRC) system facilitates the translation of ancient Indian texts available in various regional languages such as Hindi, Arabic, Urdu, Tamil, Sanskrit, and others into widely used international languages including English, French, Spanish, German, and Japanese, the Traditional Knowledge Resource Classification (TKRC) system plays a crucial role in overcoming language barriers. This systematic translation process enhances the identification of prior art and ensures the accessibility of traditional Indian medical knowledge to patent examiners and researchers across the world [4].

1.3 Rationale and Significance of the Study

In the contemporary era of rapid technological expansion and artificial intelligence, there is a growing concern that indigenous knowledge systems related to traditional healing practices may gradually weaken or become lost in transmission to future generations as many of which have been transmitted orally across generations which make it vulnerable to disappear if they are not adequately documented and preserved [5]. In this context, digital innovation emerges not only as a technological advancement but also as a powerful means to provide opportunities for preserving, organizing, and disseminating valuable traditional know andit helps document and

safeguard the rich diversity of traditional healing practices in India, thereby contributing to broader humanitarian and scientific development [5].

The Traditional Knowledge Digital Library (TKDL) acts as an important bridge between fading oral traditions and modern digital knowledge systems [4,5]. Consequently, It helps preserve, organize, and disseminate traditional medicinal knowledge in a structured format, ensuring its protection from misappropriation while promoting global recognition of India's indigenous heritage [4].

Therefore, this study aims to highlight:

1. The role of the Traditional Knowledge Digital Library (TKDL) in the preservation and protection of indigenous knowledge systems [5].
2. It also seeks to emphasize the importance of integrating tribal and community-based healing practices into the TKDL framework to achieve more comprehensive documentation of traditional knowledge [5].

1.4 Methodology

This study adopts a qualitative research approach with a particularemphasis on the Traditional Knowledge Digital Library (TKDL) as a model to evaluate effective digitization strategies for traditional knowledge preservation. The analysis is based on content analysis of secondary sources through a comprehensive review of published literature and relevant documents collected from scholarly databases and platforms such as Google Scholar, PubMed, Springer Nature, and ResearchGate [5]

2. Role of TKDL in Intellectual Property Rights (IPR)

The Traditional Knowledge Digital Library (TKDL) initiative has been developed to address several challenges associated with the protection indigenous knowledge systems, particularly in the context of Intellectual Property Rights (IPR) [6]. The interaction between traditional knowledge with modern patent frameworks has frequently resultedin concerns such as misappropriation, biopiracy, and the unintended exploitation of indigenous communities. In many cases, the commercialization of traditional knowledge has been perceived as leading to inequitable benefit distribution, have contributed to mistrust among local and tribal

communities toward formal systems of commercialization and intellectual property protection [6].

Within this context, TKDL plays a crucial role as a defensive mechanism by documenting, classifying, and translating traditional knowledge into format that are readily accessible to patent examiners globally. Although, the library has achieved considerable success in preventing wrongful, several studies also highlight certain limitations, which are increasingly viewed as opportunities for further improvement. Current research continues to explore ways to strengthen TKDL and enhance its inclusivity, thereby makingthe repository more inclusive and representatives. [6]

2.1 TKDL and Patent Law in India

The Indian Patent Act, 1970 contains several provisions that are directly relevant to the protection of traditional knowledge and the prevention of its misappropriation. Section 3 of the Act defines categories of inventions that are not patentable. In particular, Section 3(p) excludes the duplication of known traditional or indigenous knowledge from patentability, as such knowledge do not satisfy the requirement of novelty. Similarly, Section 3(e) states that substances obtained through simple admixture not exhibiting synergistic effects are not patentable. Other important provisions relevant to traditional knowledge include Sections 3(b), 3(c), 3(d), 3(f), 3(h), and 3(j), further establish all of the boundaries of patent eligibility [8].

In addition, under Section 25(1)(k) allows any person may file for pre-grant opposition to a patent application if the claimed invention is anticipated by prior traditional or indigenous knowledge, whether documented or orally transmitted, in India or elsewhere. Such opposition can be filed by any person through Form 7(A) after the publication of the patent application but prior the grant of the patent. Although no strict time limit is prescribed, it is generally advisable to file such opposition within six months of publication [8].

Similarly, Section 25(2)(k) provides for post-grant opposition, allowing any interested party to challenge a granted patent if evidence shows that the claimed invention had already existed in the form of traditional or indigenous knowledge.

This opposition can be filed using Form 7 within one year from the date of publication of the grant [8].

Furthermore, the Indian government has issued guidelines for patent examiners and controllers to strengthen proper identification and examination of applications involving traditional knowledge. These guidelines establish a systemic approach for identifying, screening, classification, and review of TK-related patent applications [9]. The key provisions include:

1. All patent applications potentially related to traditional knowledge must be properly identified, screened, and classified. The designated authority is responsible for ensuring correct classification and routing of such applications under the appropriate Patent Cooperation Treaty (PCT) categories.
2. In exceptional cases, misclassified applications may be reviewed or reclassified by the technical head.
3. A designated group leader or controller oversees TK-identified applications, ensuring proper verification and assignment to qualified examiners for detailed assessment.
4. Examiners are required to conduct comprehensive prior-art searches, including searches within the TKDL database and other relevant sources, to determine novelty and inventive step.
5. Patentability requirements, particularly novelty and inventive step, must be strictly assessed in accordance with the Patents Act, 1970.
6. In cases involving biological resources sourced from India, applicants must obtain prior approval from the National Biodiversity Authority, and examiners are responsible for ensuring compliance with this requirement.
7. Under Section 11A of the Patents Act, the Controller General of Patents, Designs and Trademarks is required to maintain and publish a dedicated list of patent applications related to traditional knowledge. This list should be regularly updated and made publicly accessible on the official website, including details such as application number, filing date, title of invention, and applicant name. Additionally, granted patents based on traditional knowledge (since July 1, 2012) must also be

published with relevant details, including patent number and application information.

2.2 Structure, Coverage, and Global Impact of TKDL

The Traditional Knowledge Digital Library (TKDL) was developed through a collaborative initiative of the Council of Scientific and Industrial Research (CSIR) and the Ministry of AYUSH with the objective to systematically preserve and protect the traditional knowledge held by India's indigenous and local communities. Over the years, it has evolvedthe database into more than 4,24,000 formulations derived from classical Indian medical systems such as Ayurveda, Unani, and Siddha, making it one of the most comprehensive repositories of traditional medicinal knowledge worldwide [3].

The TKDL has been widely recognized for its defensive protection mechanism, which has effectively prevented the misappropriation and wrongful patenting of Indian traditional knowledge. As of March 25, 2022, the database incorporates information from 281 classical texts from traditional Indian medical systems. It is made to enhance accessibility in five international languages—English, German, French, Japanese, and Spanish—thereby significantly enhancing its global usability and accessibility [3].

The system is built on a unique classification framework known as the Traditional Knowledge Resource Classification (TKRC) which has been aligned with the International Patent Classification (IPC) and containsmore than 5,000 subgroups related to medicinal plants and traditional therapeutic practices and organizes knowledge systematically into sections, classes, subclasses, groups, and subgroups, enabling efficient retrieval and examination of prior art [3].

Access to TKDL is governed through TKDL Access (Non-Disclosure) Agreements, and has been provided under with approximately fifteen major patent offices across the world. This controlled access allows both protection of sensitive traditional knowledge and its use as prior art in patent examination processes.The impact of TKDL has been significant at the global level. More than 271 patent applications have been either rejected, withdrawn, or amended based on prior art evidence retrieved from the TKDL database demonstrating the efficiency of the database as a

defensive intellectual property tool. These outcomes were achieved within a relatively short time frame and without substantial legal or financial costs, highlighting the efficiency of TKDL as a defensive intellectual property tool. Consequently, TKDL is widely acknowledged as an innovative model and a strong deterrent against biopiracy. At present, it is reported to contain approximately 34 million pages covering around 270,000 pharmaceutical formulations presented in a patent compatible format, reinforcing its role as a global standard for traditional knowledge protection, particularly for knowledge-rich developing countries.One of the most significant contributions of TKDL is ensuring that patent examiners across jurisdictions have direct and reliable access to prior art related to traditional knowledge. This access plays a critical role in preventing the granting of erroneous patents based on pre-existing indigenous knowledge [3].

2.3 Regulatory Framework for Traditional Knowledge

Indigenous traditional knowledge faces significant legal challenges in despite its immense, cultural, medicinal and economic significance achieving formal recognition within the existing Intellectual Property Rights (IPR) system, primarily due to its oral, practice-based, and collectively held nature, which lacks identifiable individual ownership. The IPR framework, by contrast, is designed to protect individual innovation and clearly defined authorship, with the primary objectives of safeguarding creative works, assigning ownership rights, and ensuring economic benefits are attributed to identifiable creators. As such, IPR operates within a market-based system where rights are linked to identifiable individuals and commercial value.Since traditional knowledge (TK) and other forms of informal knowledge often fall outside this framework and key barriers to their legal protection include difficulties in identifying the original knowledge holders and establishing clear ownership rights. Since TK is usually transmitted orally and embedded within community practices, it is often perceived as part of the public domain, despite being collectively owned and culturally significant. This perception leads to the assumption that such knowledge is freely accessible to all, thereby undermining the rights of indigenous communities [10].

The current IPR system does not adequately recognize collective ownership, largely because the authorship of traditional knowledge is difficult to determine and is often undocumented. This lack of documentation creates legal uncertainty and limits the ability of communities to claim formal protection. Consequently, although traditional knowledge may hold substantial commercial value—particularly in sectors such as medicine—its benefits are rarely equitably shared with its original custodians.In the late 90s in response to these limitations, the World Intellectual Property Organization (WIPO), in collaboration with UNESCO, has been developing model provisions for a sui generis system aimed at protecting expressions of folklore. Furthermore, in 2000, WIPO established the Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge, and Folklore (IGC-GRTKF) to examine the relationship between intellectual property, biodiversity, and traditional knowledge and explore appropriate mechanisms for their protection [10,11]

Over the years, the committee has examined various approaches, including temporary IP protections for traditional knowledge holders and the development of long-term legal frameworks to recognize collective rights. It has also explored the possibility of creating community-based ownership systems which would enable indigenous groups to hold and benefit from intellectual property rights collectively.One proposed approach suggests that once individual contributors can be identified, their rights could be transferred to the community, thereby preserving collective ownership without altering the existing IPR structure. It is widely acknowledged that different categories of traditional knowledge may require different degreesand forms of protection, indicating the need for a flexible, rather than uniform, legal framework.Neverthless, these approaches face several challenges, particularly due to inconsistent national laws regarding the legal recognition of communities as collective entities. Moreover, the lack of comprehensive documentation further complicates the issue, as undocumented knowledge cannot easily be subjected to formal ownership rights without creating legal ambiguity.Despite extensive discussions within WIPO, no universally accepted solution has yet emerged. While some scholars advocate for the creation of

sui generis system specifically designed for traditional knowledge protection, others supportreforming existing IPR frameworks to include flexible, community-oriented provisions. However, there remains limited consensus regarding how to address the fundamental challenges of identifying ownership, ensuring documentation, and establishing enforceable rights over traditional knowledge [11]

2.4 Role of CBD, TRIPS, and Cultural Aspects in TK Protection

The protection of Traditional Knowledge (TK) can be effectively understood through a human rights perspective, particularly when viewed in relation to moral rights and cultural identity. Within this approach, TK is not only treated as a form of knowledge but also as an expression of cultural heritage and community identity. This perspective establishes a connection between the cultural foundations of TK and the collective rights of indigenous communities, offering a more inclusive and practical protection mechanism.From this perspectives, human rights instruments recognize TK largely through the lens of moral and cultural rights. In contrast, the Trade-Related Aspects of Intellectual Property Rights (TRIPS) Agreement does not explicitly provide protection for TK, as it primarily focuses on trade-related intellectual property such as patents, copyrights, and trademarks, which are based on identifiable individual ownership and commercial innovation. Consequently, as a result, TK often remains outside the direct scope of TRIPS protection [12].

However, indigenous and local communities are explicitly protected under key international human rights instruments, including the International Covenant on Civil and Political Rights (ICCPR) and the International Covenant on Economic, Social and Cultural Rights (ICESCR). These international covenants recognize the right of individuals and communities to enjoy their culture, both individually and collectively, and to participate in cultural life “in community with others” (Article 27, ICCPR; Article 15(1), ICESCR). This provision is particularly significant for indigenous peoples, as it acknowledges cultural participation as a collective right rather than purely an individual entitlement.The Human Rights Committee (HRC) and the Committee on Economic, Social and Cultural Rights, which are treaty-monitoring bodies under these covenants, have further clarified that indigenous intellectual innovations, traditional practices, and cultural expressions fall within the

scope of protected cultural rights. Through interpretative instruments such as General Comment 23 (1994) on Article 27 of the ICCPR and General Comment 21 (2009) on Article 15(1) of the ICESCR, these bodies affirm that indigenous knowledge systems and cultural expressions must be safeguarded as part of the right to culture. States that are party to these treaties are therefore legally obligated to respect and protect such rights.In addition to human rights frameworks, the Convention on Biological Diversity (CBD) plays a central role in linking biodiversity conservation with the protection of traditional knowledge. Article 8(j) of the CBD specifically calls upon member states to respect, preserve, and maintain the knowledge, innovations, and practices of indigenous and local communities relevant to the conservation and sustainable use of biological diversity. It also encourages the equitable sharing of benefits arising from the utilization of such knowledge. The implementation of Article 8(j), along with the work of its dedicated working groups, has strengthened the connection between environmental governance and cultural protection [12].

Within this evolving legal framework, indigenous traditional knowledge is increasingly understood through the concept of "biocultural rights," which integrates cultural rights, biodiversity conservation, and community-based knowledge systems. Although not yet comprehensively codified in a single legal instrument, biocultural rights emerge from the interaction between the CBD provisions and international human rights standards applicable to indigenous peoples.Together, the CBD framework and international human rights law provide a normative foundation for the protection of traditional knowledge. While the TRIPS Agreement establishes a global intellectual property regime, it does not adequately address the collective and cultural dimensions of TK. Therefore, effective protection requires an integrated approach that combines international legal frameworks with national implementation strategies [12].

Ultimately, while international instruments such as the CBD and human rights treaties offer strong normative guidance, the actual safeguarding of indigenous traditional knowledge depends on how effectively states translate these obligations into domestic laws and policies.

3. Government Initiatives for AYUSH and Traditional Knowledge

It is now widely recognized that traditional medical systems play a significant role in achieving Sustainable Development Goal 3 (SDG-3), which aims to ensure healthy lives and promoting well-being for all. In this regard, AYUSH systems of Indian origin are increasingly gaining recognition as legitimate and complementary healthcare options not only within India but also globally.In recent years, the Government of India (GoI) has undertaken several important initiatives to strengthen the global visibility, acceptance, and scientific validation of AYUSH systems. One of the most significant developments is the establishment of the World Health Organization's first Global Centre for Traditional Medicine (GCTM) in India, which seeks to integrate traditional medical knowledge with modern scientific approaches. Additionally, the celebration of the International Day of Yoga has played a major role in promoting India's traditional health practices on a global platform. The creation of AYUSH Knowledge Cells in various countries has further facilitated to the dissemination of authentic traditional medical knowledge worldwide.The relevance of AYUSH systems was particularly highlighted during the COVID-19 pandemic, during which traditional interventions were widely explored and subjected to scientific evaluation, including clinical trials in India and several other countries. This period marked a renewed global interest in evidence-based traditional medicine and strengthened the demand for integrative healthcare approaches.A key element of India's governance approach in this sector has been transparency, accountability, and citizen-centric development, as emphasized by the Government of India. Public outreach such as Mann Ki Baat played an important role to communicate public health awareness and promote traditional medicine systems. In several episodes, the Prime Minister has highlighted the importance of Yoga and Ayurveda in preventive healthcare and holistic well-being. He has also emphasized the need for scientific validation and evidence-based research to enhance global acceptance of traditional medical systems[13].

In this context, the Government's vision of integrating traditional and modern medicine is further reflected in the establishment of the Global Centre for Traditional Medicine (GCTM), which aims to utilize traditional medical knowledge through modern science and technology for the benefit of global health and environmental sustainability.The Ministry of AYUSH (MoA) continues to collaborate actively with national and international stakeholders to promote research, education, training, awareness, and commercialization of validated traditional knowledge systems. In this regard, the Council of Scientific and Industrial Research (CSIR),contributed to research in medicinal plants, phytopharmaceuticals, herbal formulations, and integrative approaches such as Ayur genomics.One of the most notable collaborative initiatives between CSIR and the Ministry of AYUSH is the Traditional Knowledge Digital Library (TKDL), originally developed in 2001 to prevent the misappropriation of India's traditional knowledge through erroneous patenting. The TKDL serves as a defensive intellectual property tool and a global prior art database. It currently contains approximately 4.4 lakh formulations from classical systems such as Ayurveda, Unani, Siddha, Sowa Rigpa, and yoga practices [13,3].

In a significant policy decision, the Government of India approved broader access to the TKDL database in August 2022, extending its use beyond patent offices. This decision is expected to enhance research, innovation, and global engagement with traditional knowledge systems.Integration of CSIR and Ministry of AYUSH have also strengthened their collaboration through research and clinical validation of traditional medicines. For example, joint clinical studies on AYUSH interventions during COVID-19 have contributed to emerging scientific evidence supporting formulations such as Ashwagandha for immune support and AYUSH-64 for therapeutic use. These efforts highlight the increasing integration of traditional knowledge with evidence-based modern science.Further strengthening this ecosystem, the Government has promoted initiatives such as SVASTIK (Scientifically Validated Societal Traditional Knowledge), led by CSIR-NIScPR. This national communication campaign aims to disseminate scientifically validated traditional knowledge through digital platforms, social media, webinars,

publications, and multilingual outreach. So far, more than 28 traditional knowledge practices across fields such as Ayurveda, metallurgy, water management, agriculture, and mathematics have been scientifically validated and disseminated under this initiative.In addition to CSIR, the Ministry of AYUSH collaborates with several national institutions, including the Ministries of Tribal Affairs and Women & Child Development, the Department of Biotechnology, the Department of Science and Technology, IITs, AIIMS, and various universities. These multi-sectoral collaborations reflect India's integrated approach to strengthening traditional knowledge systems through scientific validation, policy support, and global engagement.Overall, these coordinated efforts position India as a global leader in traditional medicine, with the aim of ensuring that AYUSH systems contribute meaningfully to global health, sustainable development, and the preservation of indigenous knowledge systems [13].

4. Challenges in Preserving Traditional Knowledge in the Digital Era

The preservation of Traditional Knowledge (TK) in the digital age faces multiple interconnected challenges, ranging from linguistic erosion to technological, legal, and cultural barriers. One of the most critical issues is the rapid decline of Indigenous languages, which serve as the primary medium for transmitting oral traditions. According to UNESCO, nearly half of the world's scripts—many associated with minority and Indigenous languages—remain unsupported by digital platforms. This lack of digital compatibility accelerates language loss and disrupts intergenerational knowledge transfer.For example, the Tuvan language of Siberia, spoken by nomadic herders, is not fully supported by Unicode systems, limiting its usability in digital environments and posing a serious threat to its long-term survival. In addition, existing intellectual property (IP) frameworks often fail to protect TK effectively, as they prioritize individual ownership rather than collective rights. This structural limitation enables the unauthorized use and commercialization of traditional practices. Nwauche (2023) highlights this concern, noting that approximately 67% of 79 patents related to medicinal plants such as neem and turmeric—both integral to South Asian traditional knowledge systems—

were granted without proper consent or benefit-sharing arrangements.These challenges are further intensified by technological inequalities. Many Indigenous and rural communities lack access to reliable internet connectivity, digital infrastructure, and modern recording tools, resulting in a persistent "digital divide." For instance, around 80% of Indigenous settlements in the Amazon Basin reportedly lack stable electricity, making systematic digital archiving extremely difficult.

Cultural sensitivity also remains a major concern. The digitization of sacred rituals, healing practices, or culturally significant knowledge without proper community consent can lead to misinterpretation, misrepresentation, or commodification. A 2024 study on metaverse-based heritage preservation warns that poorly managed digital representation may distort cultural meaning and weaken the spiritual integrity of traditional knowledge systems [4,14]

4.1 Strategies for Effective Preservation

Despite these challenges, digitization—when implemented ethically and inclusively—can serve as a powerful tool for safeguarding TK. The Traditional Knowledge Digital Library (TKDL) of India stands as a leading example, successfully encoding approximately 290,000 medicinal formulations into a multilingual digital database. By translating classical Sanskrit and other traditional texts into five United Nations languages, TKDL has played a significant role in preventing biopiracy and has successfully blocked over 300 wrongful patent applications since its inception.Similarly, UNESCO's "Missing Scripts" initiative focuses on preserving endangered writing systems such as N'Ko and Vai by promoting digital standardization and linguistic inclusion. However, long-term preservation efforts require participatory and community-led approaches. As demonstrated in KwaZulu-Natal, South Africa, digital literacy programs that train Indigenous communities in technologies such as blockchain documentation and 3D modeling significantly enhance local ownership and improve preservation outcomes [3,4]

Legal and policy reforms are equally essential. The World Intellectual Property Organization (WIPO) has advocated for sui generis frameworks that include mechanisms such as prior informed consent (PIC) and equitable benefit-sharing to prevent the exploitation of Indigenous knowledge. Community-driven initiatives such as India's Honey Bee Network further illustrate effective models of ethical knowledge documentation by ensuring that Indigenous innovators retain control over their knowledge while benefiting from its dissemination.Education also plays a vital role in TK preservation. Programs involving youth participation in documenting oral histories, traditional ecological knowledge, and cultural practices—such as those implemented among Māori and Native American communities—have proven effective in sustaining cultural continuity across generations [3,4].

Emerging technologies offer additional opportunities. Blockchain-based systems used by First Nations communities in Canada enable secure, timestamped documentation of traditional knowledge, allowing controlled access through smart contracts while preventing unauthorized reproduction. Similarly, Australia's "Living Archive of Aboriginal Languages" integrates artificial intelligence with elder supervision to document endangered languages while maintaining cultural accuracy.Collaborative efforts between governments, NGOs, and technology companies further strengthen preservation initiatives. Microsoft's AI for Cultural Heritage project works with the Tembé community in Brazil to develop speech recognition tools for endangered languages. Likewise, the Sami Council's "Sëastallamat" project in the Nordic region uses virtual reality and digital storytelling to revitalize oral traditions among Indigenous youth [4].

5. Case Studies and Best Practices

Several global case studies demonstrate the effectiveness of hybrid, community-centered approaches to TK preservation. The Indian Honey Bee Network exemplifies a successful model by crowdsourcing over 10,000 traditional knowledge entries through local field researchers, ensuring that communities maintain ownership of their knowledge while enabling ethical dissemination. An

impact assessment (2022) reported a 40% increase in rural innovation recognition, enabling communities to engage in fair commercialization practices [3].

In South Africa, the IsiZulu language digitization initiative uses AI-based transcription tools to document oral histories while preserving linguistic nuance and cultural meaning. At the policy level, WIPO's Intergovernmental Committee (IGC) has developed frameworks that integrate intellectual property protection with cultural sovereignty, reinforcing the importance of balancing legal systems with Indigenous rights.National education policies also contribute significantly. For example, Bhutan's integration of traditional knowledge into school curricula aligns with UNESCO's Education for Sustainable Development goals and supports ecological knowledge transmission. Similarly, Canada's Indigenous-led blockchain projects provide secure, decentralized platforms for protecting TK from misuse.However, not all initiatives have been successful, highlighting the importance of Indigenous participation in all stages of knowledge preservation. A failed 2015 attempt to digitize Maasai pastoral knowledge in Kenya demonstrated the consequences of excluding elders from the research process. Likewise, a 2023 Māori-led evaluation found that nearly 60% of digital archives misrepresented taonga (cultural treasures), emphasizing the risks of inadequate cultural oversight.Successful scalable models such as the Arctic Council's EALLU project demonstrate how TK preservation can also support sustainable livelihoods. By training Sami and Nenets youth in ethnographic filmmaking, the program has documented reindeer herding knowledge across eight countries, bridging generational gaps while creating opportunities for cultural and economic sustainability [4].

6. Conclusion

Integrated healthcare is increasingly being viewed as the future of healthcare delivery, and India has taken significant steps toward building a strong foundation for this approach by combining the strengths and expertise of traditional systems with those of modern medicine. Traditional medicine represents only one aspect of the vast body of traditional knowledge that exists worldwide. In recent years,

growing concerns regarding climate change and sustainable development have renewed interest in traditional ecological knowledge (TEK) and its potential contributions to environmental conservation and resilience. India's initiatives aimed at revitalizing and promoting AYUSH systems demonstrate a commitment not only to strengthening healthcare but also to contributing to global discussions on the preservation and utilization of traditional knowledge. Nevertheless, achieving equitable recognition between traditional and contemporary knowledge systems remains a continuing challenge. Governments and international organizations have an important responsibility to foster knowledge equity and create policies that ensure the fair integration, protection, and utilization of diverse knowledge systems for the benefit of present and future generations.

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GLOBAL AND REGIONAL CHALLENGES IN HERBAL MEDICINE SAFETY: A FOCUS ON REGULATION AND QUALITY CONTROL

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Abstract

Background: *Herbal medicines are widely used across the world for primary healthcare, disease prevention, and complementary therapeutic applications. However, the increasing global demand for herbal products has raised significant concerns regarding their safety, quality, efficacy, and regulatory oversight. Variations in cultivation practices, manufacturing processes, and regulatory requirements contribute to inconsistencies in product quality and consumer protection.*

Objective: *This review aims to examine the major global and regional challenges associated with herbal medicine safety, with particular emphasis on regulatory frameworks, quality control measures, and strategies for ensuring safe and effective use of herbal products.*

Methods*: A comprehensive review of published literature, regulatory guidelines, and policy documents was conducted using scientific databases and authoritative reports. Relevant studies addressing herbal medicine regulation, quality assurance, safety monitoring, contamination, adulteration, standardization, and pharmacovigilance were selected and critically analyzed. Data were extracted and synthesized to identify common challenges and emerging trends across different geographical regions.*

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Discussion: *Significant disparities exist in regulatory requirements, quality standards, and safety monitoring systems among countries. Challenges such as adulteration, contamination with heavy metals and pesticides, microbial contamination, misidentification of plant materials, and insufficient post-marketing surveillance continue to affect herbal medicine safety. Furthermore, limitations in standardization and harmonization hinder international trade and consumer confidence.*

Conclusion: *Strengthening regulatory frameworks, implementing robust quality control systems, enhancing pharmacovigilance, and promoting international harmonization are essential to ensure the safety, quality, and global acceptance of herbal medicines. Collaborative efforts among regulators, researchers, manufacturers, and healthcare professionals are critical for addressing existing challenges and safeguarding public health.*

Keywords: Herbal Medicine, Safety Challanges, Pharmacovigilance, Consumer Protection

1. Introduction

Herbal medicine refers to the therapeutic use of plants, their parts, and products such as extracts, essential oils, gums, resins, and exudates obtained from plants [1].These plant-based preparations are used to support normal physiological functions and, in conventional medicine, to help treat, prevent, or manage diseases in humans and animals [2].

However, only 5–15% of higher plant species have been scientifically studied, leaving many potential bioactive compounds undiscovered. Herbal medicine continues to play an important role in healthcare, especially in rural areas [3]. India is one of the world's 12 mega biodiversity regions, enriched by diverse agro-climatic and vegetative zones. It hosts vast biological wealth, including over 45,000 plant species and thousands of fungi, algae, lichens, and microorganisms. Around 7,500 species of higher plants are known for their medicinal value [4].

Standardization and identification of herbs are essential for quality control in herbal medicines.Techniques such as HPLC, GC, spectroscopy, chemical assays, and reference standards are used for analysis. Authenticity testing methods, including macroscopic and microscopic examination, chromatography, DNA barcoding, and chemical profiling, help verify the correct plant species and prevent adulteration[5].

Regulatory authorities play an important role in ensuring the safety, quality, and efficacy of herbal medicines. Effective regulations and international collaboration help ensure the safe, rational, and standardized use of herbal medicines for public health benefits [5,6].

The increasing use of herbal medicines worldwide has highlighted the need for proper safety monitoring and regulation. Adverse effects may occur due to wrong plant identification, contamination, adulteration, overdose, misuse, or interactions with other medicines [1, 7].

Assessing the safety of herbal products is complex because factors such as plant origin, processing methods, and lack of proper botanical identification can affect their quality and effects. Therefore, accurate scientific naming, proper documentation, and collaboration among botanists, pharmacologists, and healthcare professionals are essential to ensure the safe use of herbal medicines [1, 8].

In the modern era, globalization have expanded people's access to different medical systems. Patients can now choose among various forms of healthcare and consult practitioners trained in different traditions .In many countries, traditional medical systems coexist with modern biomedicine [1, 9].Today, globalization has created a situation where multiple medical systems coexist, leading to ongoing debates about their integration into modern healthcare systems and how they should be regulated and valued [1, 2].

Traditional and complementary medicine, including traditional and faith healing, is widely used in mental health care [1].Proper toxicity testing and active safety monitoring are needed to protect users [2].

- Plants contain many bioactive compounds, especially secondary metabolites like phenols and tannins, many of which have antioxidant properties.

- Around 25% of all modern drugs are derived from plants, with about 121 active compounds in use.
- About 11% of WHO essential medicines are plant-derived [3].

2. Overview of Herbal Medicines

2.1 World Health Organization Perspective

According to WHO estimates, traditional and alternative medicine use is very high in developing countries:

The global medicinal plant market is valued at about US $60 billion annually and is growing at around 7% per year.

A major share of pharmaceuticals is derived from natural sources:

About 42% of best-selling drugs in 1997 were biologicals, natural products, or derived from them

These were worth approximately US $17.5 billion [4].

2.2 Ayurveda

Ayurveda, meaning the "science of life," is an ancient Indian system of medicine developed between 2500 and 500 BCE. It is a holistic approach that focuses on maintaining balance among five basic elements, three functional energies (Vata, Pitta, Kapha), body tissues, and waste products. According to Ayurveda, disease occurs when this balance is disturbed, and treatment aims to restore it through diet, lifestyle, and natural remedies. Important classical texts include the *Charaka Samhita*, *Sushruta Samhita*, *Ashtanga Hridaya*, and *Bhava Prakasha*, which describe hundreds of medicinal plants along with animal, mineral, and metal-based preparations [5].

Ayurveda are made of five elements and governed by three doshas—Vata, Pitta, and Kapha. Health depends on the balance of these doshas along with proper functioning of body tissues (Dhatus), waste removal (Malas), and digestive fire (Agni). When this balance is disturbed, disease occurs. Treatment in Ayurveda focuses on restoring balance through diet, lifestyle, and therapies such as Panchakarma, which detoxifies and rejuvenates the body [6].

2.3 Traditional Chinese Medicine

Traditional Chinese Medicine (TCM) is an ancient, fully institutionalized healthcare system that includes herbal medicine, acupuncture, moxibustion, massage, dietary therapy, and physical exercises [7].

Traditional Chinese Medicine (TCM) has strong potential in preventing and treating complex diseases such as autoimmune disorders, cardiovascular diseases, and cancer, and it is also an important source for modern drug discovery [8]. TCM mainly uses herbal medicines, with over 11,000 recorded plant species and about 700 commonly used herbs, often combined into thousands of formulas that act on multiple biological targets [9].

2.4 Unani

Unani medicine is a traditional system of medicine, mainly based on herbs, practiced in the Indian subcontinent. It originated from the teachings of Hippocrates [20].

It is based on the concept that each individual has a unique temperament influenced by four humors and factors such as age, mental state, climate, and environment. Treatment is tailored to the patient's temperament using diet therapy, pharmacotherapy, and regimental therapies [10, 11].

Unani medicine focuses on both disease treatment and health promotion.

It has shown effectiveness in clinical studies with minimal side effects, and recent efforts have improved the standardization, quality control, and safety of its herbal formulations [11].

2.5 Indigenous medicine systems

India has an ancient heritage of traditional medicine. The materiamedica of India provides a great deal of information on the folklore practices and traditional aspects of therapeutically important natural products [12, 13].

With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems, the evaluation of the rich heritage of traditional medicine is essential [13].

The government and the private sector are exploring all of the possibilities for the perfect evaluation of these systems in order to effectively adopt the therapeutic approaches available in original systems of medicine as well as to help in generating data to put these products on the national health program [14].

2.6 Forms of Herbal Products

2.6.1 Powders

These are powdered herbal preparations made from plant materials that can be used directly or added to foods, drinks, insufflations, or applied to wounds. They are usually finely ground parts of plants intended for specific therapeutic effects [15, 16].

Their stability depends on the type of herb used and the moisture content in the packaging. For example, powdered extracts of the root of *Nauclea latifolia*, an African antimalarial plant, have been shown to remain stable for over a year when stored in sealed glass containers at tropical room temperature [15].

2.6.2 Extracts

Plant extracts and herbal by-products contain bioactive compounds such as phenols, alkaloids, terpenoids, and essential oils that can serve as alternatives to conventional chemotherapies and vaccines [16].

These plants also provide important nutrients like proteins, carbohydrates, vitamins, fats, and minerals that support animal growth and physiological functions [16].

2.6.3 Capsules

Capsules are solid dosage forms in which drugs and suitable fillers are enclosed in gelatin shells[17]. Herbal capsules commonly contain finely powdered plant materials or herbal extracts mixed with excipients [18].

Stability studies have shown that many herbal capsule formulations remain effective and stable for long periods under normal and accelerated storage conditions [19].

2.6.4 Decoctions

Decoctions are traditional herbal preparations made by boiling herbs in water to extract soluble constituents. They are commonly prepared from mixtures of several herbs and are especially suitable for hard plant materials such as roots and barks [20].

Studies on Sijunzi decoction demonstrated variations in active components compared to individual herb extracts, while Cassia fistula decoction extracts showed good chemical stability during storage, though with lower antifungal activity [21].

2.6.5 Essential oils

Herbal oils are suspensions or solutions of herbal materials in an oily vehicle and are often referred to as macerated oils. They differ from essential oils, which are obtained through distillation of plant materials [22, 23]. Herbal oils commonly contain plant materials such as leaves rich in essential oils [22].

The stability and shelf life of herbal oils largely depend on the type of oil used during extraction, as different essential oils possess varying levels of stability [23].

3. Global Challenges in Herbal Medicine Safety

3.1 Variability in phytochemical composition

Main Causes of Phytochemical Variation

- Genetic composition and developmental stage
- Environmental and geographical factors
- Harvesting, processing, and storage
- Microbial contamination and insect infestation
- Pesticides and environmental pollutants
- Adulteration and substitution

➢ *Curcuma longa* (Turmeric)

Reported curcumin content ranged from:

- 1.06–5.65% in commercial turmeric products
- 3.07–9.58% in Thai samples

➢ *Ocimum basilicum* (Basil)

Tanzanian basil had major components different from Kenyan basil

➢ *Plantago major* [24].

3.2 Harvesting and storage issues

Dried plant materials should be stored in clean, dry, and well-ventilated buildings with controlled temperature and humidity, protected from pests, rodents, and

animals [40].Regular inspections and quality-control measures should be carried out before and during packaging to remove contaminants and inferior materials [25].

Fresh medicinal plants should be refrigerated at 2–8°C, and frozen products should be stored below –20°C [26].

3.3 Contamination and Adulteration

Herbal medicinal products (HMPs) are commonly adulterated or contaminated with harmful substances such as dust, pollen, insects, rodents, parasites, toxic heavy metals [27]. These impurities can cause serious health problems, including liver damage, metabolic disorders, brain haemorrhage, coma, and even death [28].

3.4 Heavy Metals

Researchers analyzed the levels of copper (Cu), cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg). The study also measured how much of the heavy metals dissolved into herbal decoctions. Zinc and copper had the highest dissolution rates, while mercury, arsenic, and lead dissolved in smaller amounts [28].

3.5 Pesticides

Researchers tested for 168 different pesticides and found that 89.2% of the samples contained one or more pesticide residues. However, most samples had relatively low residue levels, with 60.5% containing less than 0.02 mg/kg [29].

3.6 Microbial contamination

The study examined bacterial and fungal contamination levels and tested for harmful pathogens such as *Escherichia coli*, Salmonella spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [30].

Water samples were also tested for coliform bacteria and E. coli using the Colilert method and standard microbiological procedures [31].

3.7 Herb – Drug Interactions

Pharmacovigilance systems are essential for detecting and assessing these risks [32].National pharmacovigilance centres help identify harmful combinations through surveillance and increase awareness among health professionals, consumers, and regulators [33].

3.8 Misidentification of Medicinal Plants

Medicinal plants are widely used in traditional medicine but their identification is often difficult due to complex manual methods, lack of expertise, and similarities between species, which can lead to misidentification and unsafe use [34]. To reduce errors, a Convolutional Neural Network (CNN) based system using EfficientNet-B0 was developed to distinguish similar-looking plants [35].

3.9 Self- Medication and Irrational Use

Self-medication refers to the use of medicines by individuals to treat self-recognized symptoms or conditions[36]. It can be beneficial by improving access to treatment. however, it also carries risks, especially when done irresponsibly, including incorrect diagnosis, delayed medical care, adverse drug reactions [37].

4.Regional Challenges in Herbal Medicine Regulation

4.1India

In India, herbal medicines are regulated under the Drugs and Cosmetics Act, 1940 and Rules, 1945, with specific provisions for Ayurveda, Unani, Siddha, and Homoeopathy systems under the Ministry of AYUSH. Manufacturers must obtain a license before producing or marketing herbal drugs [38, 39]. The Act also regulates aspects such as formulation, quality, labeling, packaging, and export. Good Manufacturing Practices (GMP) under Schedule “T” ensure quality standards, and official pharmacopoeias provide reference standards [39].

The Ministry of AYUSH has introduced revised regulations for ASU (Ayurveda, Siddha, Unani) medicines, including Good Clinical Practice (GCP) guidelines published in March 2013 for clinical trials [40].

4.2 China

China has identified ten priority areas for developing regulatory science in Traditional Chinese Medicine (TCM). These include modernizing the regulatory system, improving quality control and manufacturing processes, and enhancing clinical evaluation using real-world data [41]. Other priorities focus on re-evaluating TCM injections, setting standards for classical formulas, and strengthening pharmacovigilance through diverse data sources. The framework also

emphasizes evaluating integrative medicine, promoting innovation, and improving regulatory capacity, along with encouraging international collaboration in TCM regulatory science [42].

4.3 Southeast Asia

Most existing studies on TCAM have been conducted in high-income countries, while data from lower- and middle-income regions—such as ASEAN countries and the Lower Mekong region—remain limited, even though usage is known to be high [55].ASEAN are geographically and strategically important, particularly in regional development initiatives such as China's Belt and Road Initiative. Because China and ASEAN countries share similar ecological conditions, medicinal biodiversity, and cultural traditions related to medicine, they have strong potential for cooperation in traditional medicine research and development [43].

4.4 Europe

Directive 2004/24/EC established a common EU definition for traditional herbal medicinal products (THMPs) and introduced a simplified registration system. It also created the Herbal Medicinal Products Committee within the European Medicines Agency to develop herbal monographs and support product registration. The Directive helped harmonize the regulation of herbal medicines across the EU, with registrations increasing over time and possible future expansion to other traditional non-herbal products [44].

4.5 United States

The European Union generally classifies herbal remedies as medicines, although differences among member states have led to inconsistent market categories. Efforts by the European Agency for the Evaluation of Medicinal Products aim to harmonize regulations.The United States classifies herbal products as dietary supplements, resulting in less strict regulation, though proposals have been made to create a separate regulatory framework for certain botanical products [45].

4.6 Canada

In Canada, more than 70,000 natural health products (NHPs) have been authorized for sale, and over 2,000 sites have been licensed to manufacture them. Health

Canada uses active and collaborative measures to address issues such as contamination, adulteration, and misleading advertising [46].

4.7 Africa

In South Africa, legislation to regulate Traditional Health Practitioners (THPs) was approved in 2004, and efforts have continued to develop regulations for African Traditional Medicines (ATMs) [47].Research and development are supported by the Council on Scientific and Industrial Research, universities, and the Medical Research Council. Key challenges include developing regulations, strengthening collaboration between researchers and traditional healers, and improving resources for pre-clinical and clinical studies [48].

5.Quality Control of Herbal Medicines

5.1 Authentication of Raw Materials

Ensuring the authenticity of herbal raw materials is a major challenge because high-demand or scarce plant species are often adulterated or substituted, leading to poor-quality products and potential safety risks [49].

DNA analysis is often combined with chromatographic, metabolomic, proteomic, and genomic approaches to improve accuracy. Despite advances in authentication technologies, the herbal industry continues to seek methods that are simple, rapid, and cost-effective [50].

5.2 Physicochemical Evaluation

Medicinal plants are valuable sources of therapeutically active compounds, making the authentication and quality assessment of herbal products essential for ensuring their safety, efficacy, and consistency. Quality herbal medicines must meet established standards for identity, purity, composition, and physical, chemical, and biological characteristics, as well as comply with regulatory manufacturing requirements [51].

5.3 Phytochemical Evaluation

This highlights the urgent need to develop systematic approaches and well-designed methodologies for the standardization of herbal raw materials and formulations. This discusses various phytochemical standardization techniques, including

preliminary phytochemical screening, fingerprint profiling, and quantification of marker compounds in herbal raw materials and polyherbal formulations [52].

5.4 Analytical Techniques

A variety of analytical techniques have been developed to generate fingerprint profiles of herbal medicines, proving to be valuable tools for ensuring consistent composition by establishing uniformity criteria.

Advances in analytical methodologies are ushering in a new era of herbal drug development by providing rapid and reliable tools for quality assessment, enabling manufacturers to establish standardized specifications and obtain regulatory approval for marketing [53].

5.5 Good Manufacturing Practices(GMP)

Good Manufacturing Practice (GMP) for herbal drugs refers to a set of quality assurance guidelines that ensure medicinal products derived from natural sources are consistently produced and controlled according to quality standards [54].

In countries like India, where herbal medicine plays a significant role in healthcare due to rich biodiversity and traditional use, implementing GMP is essential to ensure reliable production and to meet global demand for safe and effective herbal products [55].

5.6 Stability Testing

Stability testing is a crucial part of the development and quality assurance of herbal drugs and products (HDPs). It evaluates how environmental factors such as temperature, humidity, and storage conditions affect the chemical composition, safety, efficacy, and shelf life of herbal medicines [56].

Major challenges in stability testing of herbal products include their complex chemical composition, variability in raw materials, selection of appropriate markers, and the influence of enzymes on product stability [57].

6.Pharmacovigilance and Safety Monitoring

6.1 Herbal Pharmacovigilance

Pharmacovigilance for herbal medicines involves the detection, assessment, understanding, and prevention of adverse effects associated with the use of herbal products [58].

However, applying conventional pharmacovigilance methods to herbal medicines presents challenges due to issues such as variations in naming, sourcing, composition, and patterns of use. In the UK, the main system for monitoring adverse drug reactions (ADRs) is the Yellow Card Scheme, but reporting rates for herbal medicines remain low because many users self-medicate and do not report side effects [59].

Future improvements may include enhanced reporting mechanisms, patient participation, compliance with regulatory requirements, and the application of pharmacogenetics and pharmacogenomics to better understand individual responses to herbal medicines [60].

6.2 Adverse Drug Reaction Reporting

Spontaneous reporting systems remain the primary method for detecting ADRs, but under-reporting is common since many users self-medicate and do not seek professional advice. To address this issue, some countries have introduced reporting schemes specifically for herbal medicine practitioners and linked them to national pharmacovigilance systems [61].

New regulations in regions such as the European Union, Australia, and Canada require manufacturers of traditional herbal medicines to participate in pharmacovigilance programs. Future improvements may include enhanced reporting systems, patient involvement, modified monitoring methods, and the use of pharmacogenetics to improve the safety and effectiveness of complementary medicines [62].

6.3 Role of Healthcare Professionals

Herbal medicines are among the oldest forms of treatment used by humans and have played a significant role in the development of healthcare throughout history [63].

Today, herbal products occupy a unique position between medicines and dietary supplements, often resulting in unclear regulatory oversight. This lack of clear regulation can raise concerns regarding product quality, safety, and efficacy. Therefore, understanding the history, role, and regulation of herbal medicines is important for integrating them safely and effectively into modern healthcare systems while meeting the needs of consumers seeking alternative treatment options [64].

6.4 International Monitoring Systems

Herbal medicines are widely used worldwide and continue to play an important role in healthcare. However, concerns remain regarding their composition, effectiveness, and safety due to variations in quality and lack of standardization [65].

Therefore, effective pharmacovigilance and safety monitoring are essential. Improved reporting of adverse reactions, along with accurate identification of herbal products using international Latin binomial nomenclature and standardized classification systems such as the Herbal Anatomical Therapeutic Chemical (ATC) classification, can help ensure better assessment of their safety and support global monitoring efforts [66].

6.5 Challenges in Reporting Herbal Toxicity

Although herbs have long been valued for their therapeutic benefits, their use can sometimes result in toxicity, adverse reactions, and herb–drug interactions, which may lead to serious health complications [67].

Many consumers believe that herbal products are completely safe because they are natural, often leading to underreporting of adverse effects. This problem is especially significant in developing countries, where limited scientific data, inadequate toxicity studies, poor documentation of adverse reactions, and lack of information on herbal composition create challenges for safety assessment [68].

Therefore, stronger regulations, improved pharmacovigilance systems, and standardized protocols for safety and toxicity testing are essential to ensure the safe use of herbal medicinal products [69].

7. Role of International Organization and Regulatory Bodies

7.1 World Health Organization

WHO's core functions include:

- Setting international health norms and standards.
- Monitoring health trends and disease outbreaks.
- Supporting and coordinating international responses.
- Providing technical guidance and leadership [70].

7.2 U.S. Food and Drug Administration

Federal Food, Drug, and Cosmetic Act (FFDCA)**,** so the FDA **c**annot regulate them as a separate category. Instead, a product's regulatory status depends on its intended use, which is determined mainly by its labeling, health claims, and marketing information.

Manufacturers must first determine whether their product is classified as a food or a drug, as each category has different safety, labeling, and regulatory requirements [71].

8.Emerging Technologies and Future Perspectives

8.1 AI in herbal authentication

AI technologies such as machine learning and deep learning are being used to identify medicinal herbs, standardize product quality, optimize formulations, predict herb–drug interactions, and support personalized treatments.
These applications help address challenges such as adulteration, inconsistent quality, and limited scientific validation. However, the use of AI faces obstacles, including insufficient high-quality data, the complexity of herbal compounds, high computational demands, ethical concerns, and the lack of transparency in some AI models [72].

8.2 Omics technologies

Metabolomics is a rapidly advancing technology that enables the comprehensive identification and profiling of metabolites in medicinal plants, supporting herbal drug discovery, quality control, and a better understanding of biological systems [73].

Key analytical platforms used in plant metabolomics include GC-MS, LC-MS, and LC-SPE-NMR, with GC-MS and LC-MS being the most widely used due to their high sensitivity and ability to analyse numerous metabolites simultaneously.Additionally, it highlights the role of metabolomics in ensuring the quality of medicinal plants, addressing research challenges, and advancing herbal medicine through the integration of biotechnology and modern analytical tools [74].

8.3 Nanotechnology in herbal formulations

It emphasizes the importance of developing novel drug delivery systems (NDDS) for herbal medicines to enhance their therapeutic effectiveness.

NDDS,particularly nanotechnology-based delivery systems, can improve the sustained release of herbal compounds, increase patient compliance, reduce toxicity, and enhance bioavailability. The integration of nanocarriers with traditional herbal medicine offers promising opportunities for the treatment of chronic diseases such as asthma, diabetes, and cancer, making herbal therapies safer and more effective [75, 76].

9. Conclusion

Herbal medicines play a vital role in global healthcare, but their safety is compromised by issues related to quality, regulation, and standardization. Addressing these challenges requires coordinated efforts from regulatory authorities, researchers, and healthcare professionals. Strengthening regulatory frameworks, improving quality control systems, and enhancing pharmacovigilance are essential to ensure the safe use of herbal medicines worldwide

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CHALLENGES OF HEAVY METAL AND PESTICIDE COMPLIANCE TESTING IN HERBAL COSMETICS

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Abstract

Background: *The global demand for herbal cosmetics has surged due to the perception that natural ingredients are inherently safer than synthetic alternatives. However, botanical raw materials are highly vulnerable to environmental contamination. Toxic heavy metals (such as lead, cadmium, arsenic, and mercury) and synthetic pesticide residues accumulate in plants via contaminated soil, irrigation water, and agricultural applications. Ensuring consumer safety through rigorous compliance testing is imperative, yet technically challenging.*

Objective: *This review critically evaluates the technical, analytical, and regulatory hurdles associated with the detection, quantification, and compliance verification of heavy metals and pesticide residues in complex herbal cosmetic formulations.*

Methods: *A comprehensive literature search was conducted across major scientific databases and regulatory standards (WHO, US FDA, EU Cosmetics Regulation) were analyzed, focusing on matrix interference, sample preparation techniques, chromatographic and spectroscopic detection methodologies, and global limit variations. Herbal cosmetics contain complex, heterogeneous mixtures of lipids, essential oils, waxes, and secondary plant metabolites. These compounds exert significant matrix effects—causing severe ion suppression/enhancement in LC-MS/MS, peak overlaps in GC-MS/MS, and spectral interferences or incomplete digestion in atomic spectroscopy (ICP-MS, AAS). Furthermore, lack of unified*

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global limits specifically established for cosmetic formulations (as opposed to oral pharmaceuticals or foods) complicates international regulatory compliance.

Conclusion: *Heavy metal and pesticide compliance testing in herbal cosmetics is hindered by complex matrix interferences, sample preparation limitations, high testing costs, and fragmented international regulations. Overcoming these challenges necessitates the development of validated, matrix-specific sample clean-up techniques, high-throughput multi-analyte methods, and global harmonization of regulatory safety thresholds to protect consumer health and streamline international trade.*

Keywords: Herbal Cosmetics, Heavy Metal Analysis, Pesticide Residues, Compliance Testing, Matrix Effects, Regulatory Harmonization, ICP-MS, LC-MS/MS.

1.Introduction

The global cosmetic industry has undergone a substantial paradigm shift toward "clean beauty," natural products, and formulations perceived as environmentally responsible. This transition has been driven by increasing consumer interest in botanical ingredients, sustainability, transparency of ingredient sourcing, and the perception that naturally derived substances are inherently safer than synthetic chemicals. Herbal cosmetics may broadly be described as personal-care products containing botanical extracts, essential oils, herbal preparations, or other plant-derived active ingredients. Such products are frequently marketed as gentle, biocompatible, and safer alternatives to conventional synthetic formulations. However, the natural origin of an ingredient does not, by itself, guarantee its purity or toxicological safety. The safety profile of a herbal cosmetic depends not only on the intrinsic properties of its botanical constituents but also on agricultural practices, environmental exposure, processing, storage, manufacturing, and the possibility of contamination with undesirable substances.

Botanical raw materials present a particularly complex safety challenge because plants can accumulate contaminants from their surrounding environment.

Medicinal and aromatic plants may absorb and accumulate potentially hazardous elements from contaminated soil, irrigation water, atmospheric deposition, fertilizers, and other environmental sources. The extent of accumulation varies considerably according to plant species, plant part, geographical origin, soil characteristics, pH, climatic conditions, and the chemical form and bioavailability of the contaminant. Heavy metals and other potentially toxic elements, including lead, arsenic, cadmium, mercury, nickel, and chromium, are therefore important quality-control concerns for botanical materials. The World Health Organization (WHO) specifically recognizes contaminants and residues as important components of quality assessment for herbal materials, while regulatory guidance for herbal products emphasizes the establishment of appropriate specifications and testing procedures [1,2].

Agricultural practices represent an additional and potentially significant source of contamination. Modern cultivation of medicinal and cosmetic plants may involve the use of insecticides, herbicides, fungicides, acaricides, and other plant-protection products. Although these substances are intended to control pests and improve agricultural productivity, inappropriate application, excessive use, environmental persistence, or inadequate pre-harvest intervals can result in measurable residues remaining on or within harvested plant material. Some persistent compounds may also remain in soil and water for prolonged periods, creating opportunities for continued environmental exposure. The WHO has emphasized that pesticides are intrinsically toxic substances and that their use requires appropriate regulation, monitoring, and risk assessment [3,4].

The potential health significance of such contaminants is particularly relevant when botanical ingredients are incorporated into products intended for repeated topical application. Cosmetic exposure can occur through direct contact with the skin, and the extent of exposure may depend on the concentration of the contaminant, duration and frequency of application, formulation characteristics, site of application, and physicochemical properties of the contaminant. Certain metals, for example, may cause sensitization or systemic toxicity under sufficient exposure conditions, whereas pesticide residues encompass a chemically diverse

group of substances with potentially different toxicological profiles. Reported toxicological endpoints associated with particular pesticides or toxic elements can include irritation and sensitization, effects on neurological or reproductive systems, organ toxicity, genotoxicity, and carcinogenicity. Consequently, the presence of a contaminant should not be interpreted solely on the basis of its detection; appropriate risk assessment requires consideration of its concentration, exposure pathway, toxicological characteristics, and relevant regulatory limits [3-5]

For these reasons, quality and safety assessment of botanical materials requires systematic control of contaminants and residues throughout the supply chain. International organizations and regulatory authorities have established frameworks addressing the quality of herbal materials, contaminants, pesticide residues, and related analytical requirements. The WHO has published specific guidelines for assessing the quality of herbal medicines with reference to contaminants and residues, covering important considerations for the control of undesirable substances in herbal materials [1]. The European Medicines Agency (EMA), although primarily concerned with medicinal products rather than cosmetics, similarly requires appropriate specifications, test procedures, and acceptance criteria for herbal substances and preparations used in medicinal products. These principles illustrate the broader regulatory expectation that botanical materials should be adequately characterized and controlled rather than assumed to be safe solely because of their natural origin [2].

In the United States, the regulatory framework for cosmetics differs from that applied to medicinal products. Cosmetic products and ingredients generally do not undergo pre-market approval by the US Food and Drug Administration (FDA), with the important exception of color additives; nevertheless, cosmetics must be safe when used according to their labeling or customary conditions of use. The FDA can take regulatory action against cosmetics that are adulterated or otherwise unsafe. Importantly, FDA surveillance activities specifically include the assessment of potentially hazardous contaminants such as arsenic, cadmium, chromium, cobalt, lead, mercury, and nickel. However, it has to be noted that the Modernization of Cosmetics Regulation Act of 2022 (MoCRA), has significantly

updated the US FDA's oversight over cosmetic safety, facility registration, adverse event reporting, and mandatory recall authority [5,6].

Despite the existence of regulatory expectations, compliance testing of herbal cosmetics remains analytically demanding. A major difficulty arises from the inherent chemical complexity of botanical materials. Unlike a formulation containing a relatively small number of well-characterized synthetic ingredients, a plant extract may contain hundreds of chemically diverse constituents, including polyphenols, flavonoids, tannins, terpenoids, alkaloids, pigments, sugars, lipids, proteins, organic acids, and volatile compounds. Their concentrations can also vary according to plant species, cultivar, geographical origin, season of collection, cultivation conditions, plant part, harvesting stage, extraction technique, and processing conditions. Consequently, two batches of nominally identical botanical ingredients may display substantially different chemical profiles.

This chemical complexity creates significant matrix effects during instrumental analysis. Co-extractives may interfere with chromatographic separation, suppress or enhance detector responses, contaminate analytical instruments, or produce signals that overlap with those of target analytes. Highly lipophilic constituents can complicate extraction and chromatographic analysis of pesticide residues, whereas polyphenolic and pigment-rich components may interfere with spectroscopic or chromatographic detection. In addition, analytes may occur at trace or ultra-trace concentrations against a background containing abundant naturally occurring compounds. The analytical method must therefore achieve adequate selectivity, sensitivity, accuracy, precision, recovery, and robustness while minimizing matrix-induced interference.

Sample preparation is consequently one of the most critical stages of compliance testing. An appropriate procedure must efficiently extract the contaminants of interest while removing as many interfering constituents as possible. For heavy-metal analysis, digestion or other matrix-decomposition procedures may be required before elemental determination. For pesticide-residue analysis, extraction and clean-up procedures must account for pesticides spanning a wide range of polarities, volatilities, and chemical stabilities. Method selection may therefore

involve chromatographic and spectrometric techniques, such as liquid chromatography coupled with mass spectrometry or gas chromatography coupled with mass spectrometry, depending on the chemical characteristics of the target residues. Elemental contaminants may require highly sensitive atomic or mass-spectrometric approaches. The complexity of the botanical matrix makes method validation particularly important because a method demonstrating acceptable performance in a simple solvent system may not necessarily perform equivalently in a complex herbal formulation.

Another challenge is the diversity of botanical formulations available on the market. Herbal cosmetics may take the form of creams, lotions, gels, shampoos, soaps, oils, serums, masks, powders, and other dosage-like presentations. These matrices differ substantially in their proportions of water, oils, waxes, surfactants, emulsifiers, polymers, fragrances, pigments, and botanical extracts. Consequently, a single analytical procedure may not be universally suitable for every product category. Matrix-specific extraction, dilution, clean-up, and validation strategies may be necessary to ensure reliable quantification.

Regulatory harmonization also remains an important consideration. Requirements applicable to herbal medicinal products cannot automatically be transferred to cosmetics because the legal classification, intended use, exposure assumptions, and regulatory frameworks may differ between jurisdictions. Nevertheless, international regulatory approaches share a common underlying principle: botanical ingredients and finished products must be appropriately controlled for contaminants that could compromise consumer safety. The EMA's current guidance on herbal products explicitly addresses specifications, testing, contaminants, manufacturing, and other quality attributes, while WHO guidance provides a dedicated framework for contaminants and residues in herbal materials [1,2,7].

Therefore, compliance testing of herbal cosmetics should be regarded as a multidisciplinary analytical problem rather than a simple screening exercise. Reliable assessment requires control of raw-material quality, traceability of botanical sources, appropriate sampling strategies, validated analytical methods,

suitable reference materials, rigorous quality-assurance procedures, and scientifically justified acceptance criteria. Particular attention must be given to method selectivity and matrix effects because false-positive, false-negative, or inaccurate quantitative results can arise when complex botanical matrices are analyzed without adequate preparation and validation.

Against this background, the present review critically examines the analytical, methodological, and regulatory challenges associated with compliance testing of herbal cosmetics. Particular emphasis is placed on the occurrence and toxicological relevance of heavy metals and pesticide residues, challenges associated with complex botanical matrices, sample-preparation strategies, instrumental analytical techniques, method validation, quality-control requirements, and differences among major international regulatory frameworks. A systematic understanding of these factors is essential for developing reliable testing strategies capable of ensuring the safety, quality, authenticity, and regulatory compliance of herbal cosmetic products.

2. Heavy Metal Contamination and Testing Challenges

2.1 Sources and Health Hazards of Heavy Metals

Heavy-metal contamination represents one of the most important chemical-safety concerns associated with botanical raw materials used in herbal cosmetics. Unlike contaminants that are introduced exclusively during manufacturing, heavy metals may enter the botanical supply chain at several stages, beginning with cultivation and continuing through harvesting, processing, storage, and formulation. Plants can absorb potentially toxic elements from contaminated soil, irrigation water, atmospheric deposition, industrial emissions, mining activities, and agricultural inputs. Their subsequent concentration within roots, leaves, stems, flowers, fruits, or seeds depends on plant species, soil characteristics, environmental conditions, and the chemical form and bioavailability of the metal. Consequently, geographical origin and cultivation practices are important determinants of the contaminant profile of herbal raw materials [1,9].

Agricultural practices can further contribute to the introduction or mobilization of heavy metals. Certain phosphate fertilizers, for example, may contain trace amounts of cadmium, while contaminated irrigation water and sewage-derived amendments can introduce additional metallic contaminants into agricultural soils. Industrial effluents and atmospheric emissions represent particularly important sources in regions located close to mining, metal-processing, manufacturing, or other industrial activities. Once deposited in agricultural soils, heavy metals are generally persistent and cannot be readily degraded through conventional biological processes. They may therefore accumulate over time and become available for uptake by plants [1,10].

Processing equipment can constitute another potential source of contamination. Contact between botanical materials and inappropriate metallic surfaces, containers, pipelines, grinding equipment, or extraction apparatus may introduce trace elements into the final material. The possibility of contamination is particularly relevant during repeated processing operations, where corrosion, abrasion, or inadequate equipment maintenance can contribute to metal transfer. Therefore, effective control of heavy metals requires a comprehensive approach extending beyond analysis of the finished cosmetic product to include qualification of raw materials, environmental monitoring, appropriate equipment selection, and good manufacturing practices [1,9].

The toxicological significance of heavy metals varies according to the specific element, chemical species, concentration, duration of exposure, and route of administration. The principal elements of concern in botanical and cosmetic products include lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg).

Lead (Pb): Lead is a non-essential and toxic element that can accumulate in biological systems following repeated exposure. It is of particular concern because there is no recognized physiological requirement for lead and because even relatively low exposure can have adverse effects. Chronic lead exposure is associated primarily with neurological toxicity, with children being especially vulnerable to developmental and cognitive effects. Lead can also adversely affect the hematopoietic, renal, cardiovascular, and reproductive systems. In cosmetic

products, the presence of lead may result from contaminated mineral or botanical raw materials, environmental exposure, pigments, or manufacturing-related contamination. Because herbal ingredients may be repeatedly applied to the skin, controlling lead contamination is an important component of raw-material and finished-product quality assessment [11,12].

Cadmium (Cd): Cadmium is another non-essential toxic element that can enter plants through contaminated soil, fertilizers, irrigation water, and atmospheric deposition. The element is efficiently taken up by certain plant species and may consequently accumulate in botanical materials. Chronic cadmium exposure is particularly associated with renal toxicity, including damage to renal tubular function, and may also adversely affect the skeletal system. The International Agency for Research on Cancer (IARC) classifies cadmium and cadmium compounds as carcinogenic to humans (Group 1)[13]. Because cadmium is persistent in the environment and can accumulate in biological tissues, monitoring its concentration in botanical raw materials is particularly important.

Arsenic (As): Arsenic is a naturally occurring element that can also be introduced into agricultural environments through industrial activities, contaminated groundwater, mining, and certain agricultural practices. Its toxicity is strongly dependent on chemical form, with inorganic arsenic generally presenting greater toxicological concern than many organic arsenic compounds. Chronic exposure to inorganic arsenic has been associated with characteristic skin manifestations, including hyperpigmentation and hyperkeratosis, as well as cardiovascular, neurological, and developmental effects. Long-term exposure is also associated with an increased risk of several cancers, including skin, lung, and bladder cancer [14]. These toxicological properties make arsenic contamination a significant consideration when assessing the safety of botanical materials.

Mercury (Hg): Mercury is a highly toxic element that exists in several chemical forms with differing absorption and toxicity profiles. Exposure to inorganic mercury compounds can affect the nervous system and kidneys, while certain organic mercury species have particularly significant neurological toxicity. Cosmetic products, especially products marketed for skin lightening, have

historically been associated with mercury contamination or intentional mercury-containing ingredients. Topical exposure can produce local dermatological effects as well as systemic exposure under certain circumstances. For this reason, mercury requires particular attention during the quality assessment of cosmetic and botanical products [15, 16].

The pathway from environmental contamination to the finished herbal cosmetic can be conceptualized as a continuum. Heavy metals present in soil, irrigation water, industrial emissions, or other environmental sources may first become available for plant uptake. Following cultivation and harvesting, contaminants can persist through drying, grinding, extraction, and other processing operations. Depending on their physicochemical properties and the processing procedure employed, they may subsequently become distributed within botanical extracts or concentrated in particular fractions. The resulting contaminant burden may then be incorporated into the final cosmetic formulation.

This pathway is further complicated by the chemical characteristics of the finished formulation. Herbal cosmetics may contain substantial quantities of lipids, waxes, oils, resins, pigments, proteins, and polyphenolic compounds. These constituents can create substantial matrix effects during analytical determination. Consequently, the detection and quantification of trace metals in finished products cannot always be achieved through direct analysis without appropriate sample preparation. Acid digestion or other matrix-decomposition procedures may be required to convert the complex formulation into a suitable analytical solution. However, digestion conditions must be carefully optimized to ensure efficient decomposition while minimizing contamination and analyte losses [1,17].

Volatility and chemical instability present additional analytical challenges for certain elements. Mercury, and some arsenic species under particular analytical conditions, may be susceptible to losses during inappropriate digestion, heating, storage, or sample handling. Such losses can result in an underestimation of the actual contaminant concentration. Conversely, contamination introduced through reagents, laboratory equipment, vessels, or the laboratory environment can produce falsely elevated results. Therefore, trace-element analysis requires stringent

procedural blanks, appropriate reference materials, validated digestion procedures, and suitable calibration and quality-control strategies.

Inductively coupled plasma mass spectrometry (ICP-MS) is among the highly sensitive techniques available for multi-element determination and can provide detection limits suitable for trace and ultra-trace analysis. Nevertheless, complex cosmetic matrices can generate spectral and non-spectral interferences that influence analytical accuracy. Polyatomic ions, matrix-derived species, and variations in ionization efficiency may interfere with the measurement of selected isotopes. Appropriate sample preparation, internal standards, interference-reduction strategies, and method validation are therefore essential for reliable ICP-MS determination [17].

The analytical pathway for heavy metals can consequently be summarized as in Figure 1.

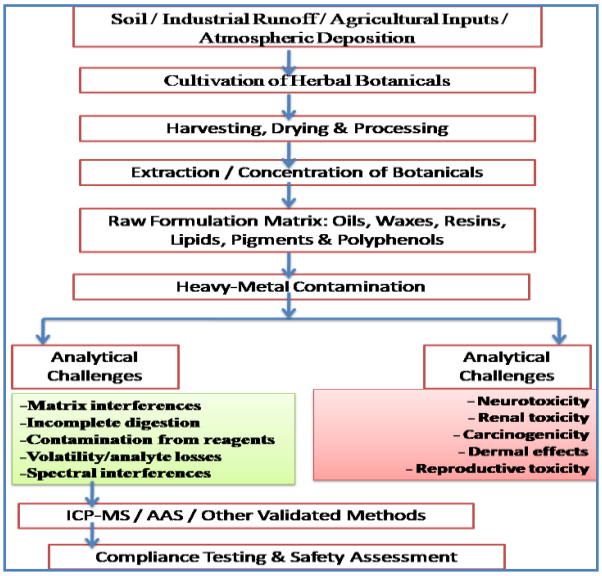

Figure 1. Analytical pathway for heavy metals

Thus, heavy-metal control in herbal cosmetics should not be viewed simply as an end-product testing requirement. It represents a “farm-to-formulation” quality-control problem, in which environmental conditions, agricultural practices, processing procedures, formulation composition, analytical methodology, and regulatory limits are interconnected. Effective compliance therefore requires

appropriate supplier qualification, control of botanical sourcing, good agricultural and collection practices, suitable manufacturing controls, validated analytical methods, and scientifically justified acceptance criteria [1, 8].

2.2 Analytical Techniques for Heavy Metal Detection

Accurate determination of heavy metals in herbal cosmetics requires highly sensitive and selective analytical techniques capable of measuring trace and, in some cases, ultra-trace concentrations in chemically complex matrices. The analytical procedure generally involves several sequential stages, including representative sampling, homogenization, digestion or extraction of the cosmetic matrix, instrumental determination, calibration, and appropriate quality-control procedures. The choice of analytical technique depends on the target elements, expected concentration range, formulation matrix, required detection limits, sample throughput, and available instrumentation. Among the principal techniques used for elemental analysis are Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [17-19].

2.2.1 Atomic Absorption Spectroscopy (AAS)

Atomic Absorption Spectroscopy is a well-established technique for the quantitative determination of metallic elements. The fundamental principle of AAS is based on the absorption of electromagnetic radiation by free ground-state atoms. During analysis, the sample is converted into free atoms, generally through a flame or an electrothermal atomizer. A light source emitting radiation characteristic of the target element is directed through the atomized sample, and the decrease in radiation intensity resulting from atomic absorption is measured. Under appropriate conditions, the measured absorbance is proportional to the concentration of the element in the sample [17,20].

Two commonly used configurations are Flame Atomic Absorption Spectroscopy (FAAS) and Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)**.**

Flame AAS (FAAS) uses a flame to atomize the sample. It is relatively straightforward, robust, and suitable for the determination of elements present at low-to-moderate concentrations. FAAS is commonly applied to elements such as

lead, cadmium, copper, iron, zinc, and nickel. However, its sensitivity is generally lower than that of graphite furnace AAS or ICP-MS, and individual elements are normally measured sequentially rather than simultaneously. Consequently, analysis of numerous elements can require considerable instrument time [17, 20].

Graphite Furnace AAS (GFAAS) employs an electrically heated graphite tube for atomization. The sample is introduced into the graphite furnace and subjected to programmed drying, pyrolysis, and atomization steps. Because the sample is confined within a small atomization volume and atomization can be carefully controlled, GFAAS provides substantially greater sensitivity than FAAS and is particularly useful for trace-level determination of elements such as lead and cadmium. The technique is therefore valuable when the concentration of a target element is too low for reliable determination by conventional flame AAS [15, 20].

A major advantage of AAS is its relatively mature and well-established methodology. Instruments are widely available; analytical procedures are comparatively well understood, and operating costs may be lower than those associated with high-end multi-element mass spectrometric systems. Nevertheless, conventional AAS is limited in its ability to perform rapid multi-element analysis. Matrix effects can also influence analytical accuracy, particularly when complex cosmetic formulations contain substantial quantities of oils, waxes, pigments, surfactants, or botanical co-extractives. Appropriate sample digestion and matrix matching are therefore essential.

2.2.2 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

ICP-OES, also referred to as Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), is a multi-element analytical technique based on the emission of characteristic electromagnetic radiation by atoms and ions excited within a high-temperature plasma. In a typical instrument, the liquid sample is converted into an aerosol and transported into an argon plasma generated by radio-frequency energy. The extremely high plasma temperature causes desolvation, atomization, and excitation of the constituent elements. As excited atoms and ions return to lower-energy states, they emit radiation at element-specific wavelengths.

The intensity of these emission lines is used to determine the concentration of the corresponding elements [17, 18].

One of the principal advantages of ICP-OES is **its** multi-element capability. A single sample introduction can allow numerous elements to be measured within the same analytical run, making the technique considerably more efficient than sequential single-element AAS when a broad elemental profile is required. ICP-OES also provides a comparatively wide linear dynamic range and good analytical precision, making it suitable for routine determination of elements occurring at low concentrations in botanical and cosmetic materials [17, 18].

ICP-OES is particularly useful when heavy metals are present at concentrations above its practical detection range. It can be applied to the simultaneous determination of elements such as Pb, Cd, As, Hg, Cr, Ni, Cu, and Zn following appropriate sample preparation. However, its sensitivity for some elements is generally inferior to that achievable by ICP-MS. Spectral overlap between emission lines can also occur, particularly in complex matrices, requiring careful wavelength selection and appropriate background correction [17, 18].

For herbal cosmetics, ICP-OES provides an attractive compromise between sensitivity, multi-element capability, analytical throughput, and operational cost. It is particularly appropriate for routine quality-control laboratories that require simultaneous monitoring of several elemental contaminants but do not necessarily require the ultra-trace sensitivity provided by mass spectrometry.

2.2.3 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Inductively Coupled Plasma Mass Spectrometry is one of the most powerful instrumental techniques available for trace and ultra-trace elemental analysis. It combines an inductively coupled plasma as the ionization source with a mass spectrometer for separation and detection of ions according to their mass-to-charge ratio (m/z) [19, 21].

During analysis, the sample is introduced into an argon plasma where the solvent is removed and the constituent elements are atomized and ionized. The resulting ions are extracted from the plasma into the mass spectrometer, separated according to their mass-to-charge ratios, and detected. Because the technique measures ions

rather than optical emission signals, extremely low concentrations of many elements can be determined [19, 21].

The principal advantages of ICP-MS include very high sensitivity, low detection limits, broad elemental coverage, and rapid multi-element analysis**.** Depending on the element, instrument configuration, sample preparation, and analytical conditions, detection limits can extend into the parts-per-trillion (ppt) range. This capability is particularly valuable when regulatory specifications require determination of contaminants at very low concentrations. ICP-MS can simultaneously determine a broad range of elements, allowing Pb, Cd, As, Hg, Cr, Ni, and other potentially toxic elements to be assessed within a single analytical sequence [19, 21].

Another important advantage is the ability to obtain isotopic information. Because ICP-MS separates ions according to mass-to-charge ratio, different isotopes of an element can potentially be distinguished. This feature can provide additional analytical information and can assist with interference correction or specialized source-identification studies.

However, the high sensitivity of ICP-MS does not eliminate analytical challenges. In fact, its sensitivity makes rigorous contamination control particularly important. Trace quantities of metals originating from laboratory vessels, reagents, tubing, sample-preparation equipment, or the surrounding laboratory environment can become analytically significant. Matrix components can additionally produce non-spectral effects such as signal suppression or enhancement. Spectral interferences, including polyatomic-ion formation, may also affect selected analytes. These problems can be controlled through appropriate digestion procedures, reagent blanks, internal standards, collision/reaction-cell technologies where appropriate, interference correction, and rigorous method validation [19, 21].

2.2.4 Comparative Evaluation of the Major Techniques

The three principal techniques differ considerably in sensitivity, elemental coverage, throughput, and analytical complexity.

Table 1: Comparative depiction of major techniques for determination of heavy metals

Parameter	FAAS	GFAAS	ICP-OES	ICP-MS
Primary principle	Atomic absorption using flame atomization	Atomic absorption using electrothermal atomization	Optical emission from plasma-excited atoms/ions	Mass-to-charge separation of plasma-generated ions
Sensitivity	Low-to-moderate	High	Moderate-to-high	Very high
Multi-element analysis	Limited/sequential	Limited/sequential	Yes	Yes
Trace analysis	Suitable	Highly suitable	Suitable	Excellent
Ultra-trace analysis	Limited	Better	Limited for some elements	Excellent
Typical strengths	Simplicity and routine analysis	High sensitivity for selected elements	Rapid multi-element analysis	Ultra-trace, multi-element determination
Major limitations	Sequential analysis and lower sensitivity	Slower, element-specific analysis	Spectral interferences	High cost and susceptibility to contamination/matrix effects
Suitability for	Good after digestion	Good for selected trace	Very good after	Excellent, provided matrix

complex herbal matrices	metals	appropriate digestion	and interference control are adequate

Overall, AAS remains a practical and established technique for routine determination of selected heavy metals**,** particularly where the number of target elements is limited. GFAAS is advantageous when higher sensitivity is required for individual elements**.** ICP-OES offers efficient multi-element analysis with good sensitivity and throughput**,** making it useful for routine elemental profiling. ICP-MS generally provides the highest analytical sensitivity and the broadest multi-element capability, making it particularly suitable for regulatory compliance testing where heavy metals must be detected at trace or ultra-trace concentrations (Table 1) [17-19, 21].

It is important, however, to avoid treating ICP-MS as automatically superior under every analytical circumstance. The reliability of any elemental-analysis method ultimately depends on the complete analytical workflow, including sampling, digestion, calibration, blank correction, interference management, reference materials, recovery studies, and method validation. In complex herbal cosmetic matrices, inadequate sample preparation can compromise even the most sensitive instrumental technique. Therefore, the selection of an analytical method should be based on the required analytical performance and characteristics of the formulation rather than instrumentation alone.

2.3 Challenges in Heavy Metal Analysis

Although modern elemental-analysis techniques such as AAS, ICP-OES, and ICP-MS provide excellent sensitivity and precision, reliable heavy-metal determination in herbal cosmetics remains challenging because of the complex composition of cosmetic matrices. Creams, ointments, lipsticks, balms, oils, and other semi-solid preparations may contain substantial proportions of fats, waxes, pigments, polymers, surfactants, resins, and botanical extracts. These constituents can interfere with sample preparation, analyte recovery, aerosol generation, plasma stability, and instrumental detection. Consequently, obtaining a representative and

homogeneous analytical solution is often as important as the choice of the detector itself [15, 18, 20].

2.3.1 Limitations of Sample Digestion

Sample preparation is a critical step in trace-metal analysis because the organic matrix must be sufficiently decomposed before instrumental determination. Herbal cosmetics containing high concentrations of lipids and waxes, such as cold creams, lipsticks, ointments, and oil-based preparations, can be particularly difficult to digest. Inadequate decomposition may leave residual carbonaceous material or incompletely solubilized organic components, resulting in poor analyte recovery and inconsistent measurements [15, 18].

Microwave-assisted digestion is widely used to improve the decomposition of complex organic matrices. Strong oxidizing acids, particularly nitric acid (HNO_3), are commonly employed, sometimes in combination with hydrogen peroxide (H_2O_2). Hydrochloric acid (HCl) may also be incorporated when justified by the target analytes and analytical method. Closed-vessel microwave systems offer several advantages over conventional open-vessel heating because they provide controlled temperature and pressure conditions and can improve digestion efficiency while reducing contamination and analyte losses [15, 22].

Nevertheless, highly lipophilic cosmetic matrices may remain difficult to decompose completely. Excessive organic loading can result in residual carbon or other incompletely oxidized material entering the analytical system. During ICP-based analysis, residual carbon and other matrix components may contribute to deposits on the sample-introduction system, injector, torch, interface cones, and related components. Such deposits can progressively impair aerosol transport and plasma conditions, potentially producing signal drift, memory effects, or reduced analytical sensitivity [18, 22].

Incomplete digestion can also result in heterogeneous analyte distribution. If particulate or waxy residues remain after digestion, metals associated with these fractions may not be transferred quantitatively into the final analytical solution. This produces negative bias in the measured concentration. Conversely, contamination from digestion vessels, acids, laboratory equipment, or other

reagents can cause artificially elevated results. Therefore, digestion procedures must be optimized and validated for each major class of cosmetic matrix rather than assuming that a single digestion protocol will provide equivalent performance across all formulations [15, 22].

Appropriate quality-control measures should include reagent blanks, laboratory control samples, certified reference materials where available, duplicate preparations, and matrix-spike recovery experiments. Recovery studies are particularly useful for determining whether the digestion procedure quantitatively transfers the target elements from the complex cosmetic matrix into the analytical solution.

2.3.2 Volatilization and Analyte Losses

Volatilization represents another important source of analytical error, particularly for mercury and certain volatile or chemically labile species of arsenic. During conventional open-vessel digestion or prolonged heating, volatile forms of an analyte may be released from the sample and lost to the atmosphere. Such losses can result in significant underestimation of the actual contaminant concentration [16, 20].

Mercury (Hg) requires particular attention because of its volatility and complex chemical behavior. Open-vessel heating can promote the conversion of mercury-containing species into volatile forms, which may escape before instrumental determination. Closed-vessel microwave digestion can reduce this risk by maintaining the analyte within a controlled system during decomposition. However, the digestion conditions must still be carefully selected because excessive temperature, unsuitable reagents, or prolonged treatment may affect analyte recovery [16, 23].

Arsenic presents an additional challenge because its analytical behavior depends strongly on chemical speciation. Different inorganic and organic arsenic species have different volatilities, chemical stabilities, and responses in analytical systems. Consequently, total arsenic determination and arsenic-speciation analysis represent distinct analytical objectives and may require different sample-preparation and instrumental approaches.

Specialized techniques can be used when particularly high sensitivity is required for volatile elements. Cold Vapor Atomic Absorption Spectroscopy (CVAAS) is especially suited to mercury determination because mercury can be chemically reduced to elemental mercury vapor and subsequently transported to the detector without conventional flame atomization. This provides highly sensitive determination of mercury and minimizes some of the matrix problems associated with direct analysis [16].

Similarly, Hydride Generation Atomic Absorption Spectroscopy (HGAAS) can be used for elements capable of forming volatile hydrides, particularly arsenic and selenium. In hydride-generation procedures, the target element is converted chemically into a volatile hydride, which is separated from much of the original sample matrix before detection. This separation can substantially improve sensitivity and reduce matrix-related interference [20, 24].

Therefore, the analytical strategy for volatile elements should consider not only the sensitivity of the detector but also the chemical form of the analyte throughout sample preparation. Closed-vessel digestion, controlled temperature programs, appropriate stabilizing reagents, and validated sample-transfer procedures can all contribute to improved analyte recovery.

2.3.3 Polyatomic and Isobaric Interferences in ICP-MS

ICP-MS provides extremely low detection limits, but its high sensitivity is accompanied by potential spectral interferences. These arise when ions generated within the plasma or sample matrix possess the same nominal mass-to-charge ratio as the isotope being measured. Such interfering ions can produce a signal that is incorrectly attributed to the target analyte, resulting in a positive analytical bias [18, 26].

Polyatomic interferences are particularly important in ICP-MS. They arise when atoms or ions originating from the plasma gas, acids, solvents, or sample matrix combine to form molecular ions. For example, chlorine-containing matrices can produce argon-chloride species that occur at the same nominal mass as the principal arsenic isotope, $^{75}As^{+}$. This is especially relevant when hydrochloric acid

or chloride-rich matrices are present. The resulting interference can make accurate determination of trace arsenic considerably more difficult [25, 26].

Other polyatomic species may arise from combinations involving argon, oxygen, nitrogen, carbon, hydrogen, and matrix-derived elements. Their formation depends on plasma conditions, sample composition, acid concentration, and instrumental configuration. Because herbal cosmetics may contain substantial amounts of organic material and various mineral or inorganic components, the magnitude of these interferences can vary significantly between formulations.

Isobaric interferences represent a different phenomenon. They occur when isotopes of different elements have identical or extremely similar nominal masses and therefore cannot be distinguished by conventional unit-resolution mass spectrometry alone. Appropriate isotope selection, mathematical correction, higher-resolution instrumentation, or alternative analytical approaches may therefore be required depending on the target element and matrix.

One of the principal approaches for controlling polyatomic interferences is the use of a collision/reaction cell (CRC)**.** In collision-cell operation, an inert gas such as helium is introduced into the ion path. Polyatomic ions generally possess larger collision cross-sections than atomic analyte ions and undergo more collisions with the cell gas. Under helium kinetic energy discrimination (KED) conditions, these interfering polyatomic species lose more kinetic energy and can subsequently be discriminated against, whereas analyte ions are transmitted more efficiently [15,24].

Reaction-cell technology provides another approach in which a reactive gas is introduced to promote chemical reactions involving the interfering species or analyte. The choice between collision and reaction modes depends on the specific interference and analytical objective. Modern ICP-MS instruments may therefore employ collision/reaction cells to improve selectivity and enable reliable measurement of elements that would otherwise suffer substantial spectral interference.

2.3.4 Matrix Effects and Instrument Contamination

In addition to spectral interferences, complex herbal cosmetic matrices can generate non-spectral matrix effects**.** High concentrations of dissolved solids or residual organic material can modify aerosol formation, transport efficiency, plasma characteristics, and ionization behavior. These effects may cause signal suppression or enhancement and consequently compromise quantitative accuracy [18, 24].

Organic matrices can also increase carbon loading in the plasma. Excessive carbon can alter plasma conditions and contribute to deposits on interface cones and other components of the ICP-MS sample-introduction system. Progressive accumulation may affect sensitivity, increase background signals, and reduce instrument stability. Dilution, improved digestion, appropriate sample-introduction systems, and optimized plasma conditions can help minimize these effects [19, 24].

Internal standards are routinely used in ICP-MS to compensate, to some extent, for short-term variations in instrumental response and sample-introduction efficiency. However, internal-standard correction cannot compensate for every type of matrix effect. Consequently, calibration strategy, matrix matching, standard addition, dilution, and appropriate certified reference materials may be required depending on the analytical method.

2.3.5 Strategies for Overcoming Analytical Challenges

The major challenges associated with heavy-metal analysis of herbal cosmetics and their corresponding control strategies can be summarized as follows:

Table 2: Challenges and control strategy for heavy-metal analysis of herbal cosmetics

Analytical challenge	Primary consequence	Recommended control strategy
High lipid and wax content	Incomplete digestion and poor analyte recovery	Optimized closed-vessel microwave digestion
Residual organic carbon	Cone/tubing deposits and signal instability	Improved oxidation, dilution, and matrix control

Volatile Hg species	Underestimation due to analyte loss	Closed-vessel digestion and validated Hg-specific methods
Volatile arsenic species	Potential analyte loss/speciation changes	Controlled digestion and appropriate stabilization
Polyatomic ions	False-positive or biased results	CRC, helium KED, or reaction-cell methods
Isobaric overlap	Incorrect isotope assignment	Alternative isotopes, correction equations, or high-resolution MS
High dissolved-solids content	Signal suppression and instrument contamination	Dilution and optimized sample introduction
Reagent/laboratory contamination	False-positive results	High-purity reagents, procedural blanks, and contamination control
Matrix-dependent recovery	Inaccurate quantification	Matrix spikes, standard addition, and certified reference materials

Overall, heavy-metal analysis of herbal cosmetics is not simply an instrumental detection problem. It is an integrated sample-preparation, chemical-interference, and measurement-validation problem**.** The analytical method must demonstrate that the target elements are quantitatively recovered from the specific cosmetic matrix, remain stable throughout preparation, and can be distinguished from potentially interfering species. For this reason, method validation should include parameters such as selectivity, linearity, accuracy, precision, recovery, detection and quantification limits, robustness, and assessment of matrix effects (Table 2)[16, 22, 26].

Among the available strategies, closed-vessel microwave digestion provides improved control of complex organic matrices and reduces the risk of

contamination and volatilization compared with conventional open-vessel procedures. For ICP-MS, collision/reaction-cell technology, particularly helium KED, provides an effective means of reducing important polyatomic interferences. However, these technologies should be regarded as components of a validated analytical workflow rather than standalone solutions. Proper sampling, digestion, calibration, quality control, and interference assessment remain essential for generating defensible compliance results.

3. Pesticide Residue Contamination and Testing Challenges

Pesticide-residue contamination represents a major quality and safety concern in herbal cosmetics because botanical raw materials may retain residues of agricultural chemicals used during cultivation, harvesting, storage, and post-harvest processing. Unlike a single synthetic contaminant, pesticide residues encompass a chemically diverse group of compounds with markedly different physicochemical properties, including differences in polarity, volatility, thermal stability, molecular weight, persistence, and affinity for plant tissues. This diversity makes comprehensive screening considerably more demanding than the determination of a limited number of predefined contaminants [1, 27, 28].

The occurrence and concentration of pesticide residues in botanical materials depend on several factors, including the pesticide selected, application rate and frequency, physicochemical characteristics of the compound, environmental conditions, crop characteristics, harvesting interval, and post-harvest handling. Lipophilic pesticides may preferentially associate with plant waxes and oils, whereas more polar compounds may remain associated with aqueous or cellular fractions. Consequently, extraction procedures designed for one chemical class may provide poor recovery for another. A suitable analytical strategy must therefore accommodate a broad range of pesticide chemistries while minimizing interference from the complex botanical matrix [1, 27].

3.1 Diversity of Agrochemicals

A major obstacle in comprehensive agrochemical screening is the extensive diversity of pesticide classes employed in agricultural production. Pesticides used

on medicinal and aromatic plants can include insecticides, herbicides, fungicides, acaricides, and other plant-protection products. Their chemical behavior differs substantially, creating challenges in extraction, chromatographic separation, ionization, detection, and quantification. The principal groups of concern include organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs), synthetic pyrethroids, and carbamates, although numerous additional pesticide classes may also be encountered [1, 27, 28].

3.1.1 Organochlorine Pesticides (OCPs)

Organochlorine pesticides constitute an important group of historically significant agricultural chemicals. Representative compounds include DDT, lindane, and endosulfan. Many OCPs are characterized by high lipophilicity and environmental persistence, although their physicochemical and toxicological properties vary among individual compounds. Their persistence and affinity for organic matter can facilitate their retention in soil and plant-associated lipid fractions [29, 30].

In botanical raw materials, lipophilic OCP residues may become associated with surface waxes, cuticular materials, oils, and other non-polar fractions. This characteristic can complicate analytical extraction because the same extraction conditions that efficiently recover lipophilic pesticides may simultaneously extract substantial quantities of naturally occurring waxes, terpenoids, pigments, and other non-polar constituents. These co-extractives can subsequently interfere with chromatographic separation and detection.

The historical persistence of several OCPs is particularly relevant to medicinal and cosmetic plants because residues may sometimes reflect not only recent pesticide application but also contamination originating from previously treated soils or environmental deposition. Certain OCPs have been restricted or prohibited in many jurisdictions because of their persistence and toxicological or environmental concerns. Nevertheless, their monitoring can remain important because persistent residues may continue to be detected in environmental and agricultural matrices [29, 30].

3.1.2 Organophosphorus Pesticides (OPPs)

Organophosphorus pesticides represent another major group of agricultural chemicals and include compounds such as chlorpyrifos and malathion. Compared with many highly persistent organochlorines, organophosphorus compounds generally exhibit different degradation patterns and physicochemical properties. However, individual OPPs vary considerably in polarity, volatility, stability, and environmental persistence, meaning that the group cannot be treated as chemically homogeneous [1, 28].

Many organophosphorus compounds can be effectively analyzed using chromatographic techniques, particularly gas chromatography coupled with mass spectrometric detection (GC-MS/MS) or, for compounds with appropriate physicochemical characteristics, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Their analytical behavior can be influenced by thermal stability and matrix composition. Thermally labile compounds may undergo degradation during inappropriate injection or chromatographic conditions, potentially resulting in reduced recovery or the formation of degradation products [27, 31].

In herbal cosmetic analysis, OPPs can be particularly challenging because botanical extracts contain naturally occurring compounds that may co-elute with target pesticides. Matrix-derived components can also affect detector response, particularly in mass-spectrometric methods, resulting in signal suppression or enhancement. Consequently, matrix-matched calibration, isotopically labelled internal standards where available, and appropriate sample clean-up can be important for accurate quantification [27, 31].

3.1.3 Synthetic Pyrethroids

Synthetic pyrethroids are widely used insecticidal compounds derived structurally from the pyrethrins found in chrysanthemum flowers. Examples include compounds such as permethrin, cypermethrin, deltamethrin, and related substances. Many pyrethroids are relatively hydrophobic and tend to associate with organic matter and lipid-rich fractions [1,28].

Their lipophilicity presents an analytical challenge in herbal cosmetics because non-polar extraction procedures may simultaneously recover large quantities of plant waxes, essential oils, pigments, and other hydrophobic constituents. These matrix components can complicate chromatographic separation and contribute to matrix effects during mass-spectrometric detection. Some pyrethroids also possess stereoisomeric or positional-isomeric forms, creating additional considerations for chromatographic resolution and accurate quantification when individual isomers are of regulatory interest.

3.1.4 Carbamate Pesticides

Carbamates constitute another important group of pesticides used for insect and pest control. Their physicochemical characteristics vary considerably, but many carbamate pesticides can be analyzed effectively by liquid chromatographic techniques because of their polarity and thermal behavior. LC-MS/MS is therefore frequently useful for multi-residue determination of compounds that are unsuitable for conventional gas chromatographic analysis [27, 31].

A particular analytical challenge arises from the broad chemical diversity within the carbamate group. Extraction conditions optimized for one compound may not provide equivalent recovery for another. Furthermore, some carbamates can undergo degradation or transformation under inappropriate pH, temperature, or storage conditions. Sample handling and extraction conditions must therefore be carefully controlled to maintain analyte integrity.

3.1.5 Implications of Chemical Diversity for Multi-Residue Analysis

The contrasting characteristics of these pesticide classes demonstrate why comprehensive pesticide-residue analysis cannot rely on a single simplistic extraction or detection procedure. A method optimized for highly lipophilic OCPs may not provide adequate recovery for more polar compounds, while conditions suitable for thermally stable compounds may be unsuitable for thermolabile analytes. Similarly, a highly non-polar extraction can introduce substantial quantities of botanical lipids and waxes, whereas an overly selective extraction may fail to recover important pesticide classes.

Table 3:Primary analytical challanges of major pesticide class

Pesticide group	Representative compounds	Important characteristics	Major analytical challenge
Organochlorines (OCPs)	DDT, lindane, endosulfan	Persistent, generally lipophilic	Co-extraction of waxes and lipids; persistent environmental residues
Organophosphorus compounds (OPPs)	Chlorpyrifos, malathion	Diverse polarity and stability	Matrix effects; thermal or chemical instability of some compounds
Synthetic pyrethroids	Permethrin, cypermethrin, deltamethrin	Generally hydrophobic; some stereochemical complexity	Lipid/wax co-extraction and chromatographic resolution
Carbamates	Carbaryl, carbofuran	Variable polarity; many amenable to LC analysis	Variable extraction recovery and analyte stability

The diversity of pesticide residues also necessitates the use of complementary analytical platforms. GC-MS/MS is particularly valuable for sufficiently volatile and thermally stable pesticides, whereas LC-MS/MS is advantageous for more polar, thermally labile, or non-volatile compounds. The use of tandem mass spectrometry provides enhanced selectivity because precursor and product ions can be monitored for individual analytes (Table 3)[27, 31].

Modern multi-residue workflows frequently employ a QuEChERS-type extraction and clean-up approach**,** with modifications according to the botanical matrix and pesticide panel. The original QuEChERS concept was developed for efficient multi-residue pesticide analysis and combines extraction, partitioning, and

dispersive clean-up in a comparatively rapid workflow. However, highly complex herbal matrices often require additional optimization because essential oils, pigments, waxes, polyphenols, and other co-extractives can produce substantial matrix effects [32, 33].

Consequently, the analytical challenge extends beyond simply detecting whether a pesticide is present. A defensible compliance result requires reliable identification and quantitative determination at concentrations relevant to applicable regulatory limits. This requires validated extraction procedures, adequate recovery, selectivity, sensitivity, precision, appropriate calibration strategies, and systematic monitoring of matrix effects [27, 31].

Overall, the chemical diversity of agrochemicals is a fundamental reason why pesticide-residue testing of herbal cosmetics is analytically complex. The wide variation in polarity, lipophilicity, volatility, thermal stability, and chemical structure among pesticide classes necessitates carefully designed multi-residue methods rather than a universal analytical procedure. This complexity becomes even greater when the pesticides are present within botanical matrices containing naturally occurring lipids, waxes, essential oils, pigments, and secondary metabolites. Therefore, effective compliance testing generally requires optimized sample preparation coupled with complementary chromatographic and mass-spectrometric techniques.

3.2 Advanced Chromatographic Extraction and Detection

Modern pesticide-residue compliance testing increasingly relies on hyphenated chromatographic techniques coupled with tandem mass spectrometry because of their high sensitivity, selectivity, and ability to detect multiple residues in complex botanical matrices. Gas chromatography-tandem mass spectrometry (GC-MS/MS) is particularly suitable for volatile, relatively non-polar, and thermally stable pesticides, including many organochlorine pesticides and synthetic pyrethroids. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is preferred for non-volatile, polar, or thermolabile compounds, including many carbamates, organophosphates, and newer systemic pesticides [22, 25].

For sample preparation, QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) is widely used for multi-residue pesticide extraction because it combines efficient extraction with relatively simple clean-up and can accommodate a broad range of pesticide chemistries. However, complex herbal matrices may require modification of the conventional QuEChERS procedure to minimize co-extraction of lipids, pigments, essential oils, and polyphenols that can cause matrix effects during mass-spectrometric detection [16, 26].

3.3 Challenges in Pesticide Residue Testing

Pesticide-residue analysis in herbal cosmetics is complicated by the highly heterogeneous nature of cosmetic matrices and the large number of compounds that may need to be monitored simultaneously. Major challenges include matrix-induced ion suppression or enhancement, contamination and fouling of chromatographic systems, analyte degradation, and the difficulty of developing a single extraction and clean-up procedure that provides acceptable recovery across a chemically diverse pesticide panel [22, 26].

3.3.1Matrix Effects: Ion Suppression and Enhancement

Herbal cosmetic matrices may contain substantial amounts of chlorophylls, carotenoids, essential oils, fatty acids, waxes, and other naturally occurring constituents. During extraction, these compounds may be co-extracted with pesticide residues and subsequently co-elute with target analytes. In electrospray ionization (ESI) LC-MS/MS, co-eluting substances can compete with analyte molecules for available charge during droplet formation, producing ion suppression and consequently causing underestimation of pesticide concentrations. Conversely, certain matrix components may increase detector response, resulting in ion enhancement and potentially inaccurate quantitative results. Matrix-matched calibration, isotopically labelled internal standards, and additional sample clean-up can help minimize these effects [22,26].

3.3.2 Instrument Fouling and Analyte Degradation

The high lipid, wax, pigment, and resin content of herbal cosmetics can introduce substantial non-volatile material into chromatographic systems. In GC-MS/MS, these co-extractives may accumulate in the injection liner, inlet, and column,

resulting in increased maintenance requirements, reduced chromatographic performance, and changes in analyte response. Thermally sensitive pesticides may also undergo degradation within the heated injector when unsuitable injection conditions or excessive matrix loading are present. Appropriate extract dilution, matrix clean-up, inlet maintenance, and optimized injection conditions are therefore essential for maintaining system performance [28, 34].

3.3.3 Target Multiplicity and Broad Chemical Diversity

Modern compliance programs may require simultaneous screening of hundreds of pesticide residues covering a wide range of polarity, volatility, molecular weight, and chemical stability. This makes development of a universal extraction and clean-up procedure particularly challenging. Dispersive solid-phase extraction (d-SPE) sorbents such as primary secondary amine (PSA), C18, and graphitized carbon black (GCB) remove different classes of matrix components. However, excessive clean-up can also remove or retain certain target pesticides, reducing recovery. PSA is useful for removing organic acids and other polar matrix components, C18 helps reduce lipids, while GCB can remove pigments such as chlorophyll. GCB, however, may strongly retain certain planar pesticide molecules, making its concentration and application highly dependent on the target analyte panel [16, 26].

Thus, pesticide-residue testing of herbal cosmetics requires a balance between effective matrix removal and maximum analyte recovery. Because no single clean-up strategy is optimal for every pesticide and cosmetic matrix, laboratories may need matrix-specific modifications, complementary GC-MS/MS and LC-MS/MS methods, matrix-matched calibration, and rigorous recovery and matrix-effect studies to ensure reliable compliance results.

4. Method Validation and Regulatory Compliance Hurdles

Reliable compliance testing of herbal cosmetics requires analytical methods that are not only sensitive but also demonstrably accurate, precise, selective, robust, and fit for purpose. The principal difficulty arises from the fundamentally different chemical behavior of elemental contaminants and pesticide residues. Heavy metals

are inorganic elements that must generally be liberated from the formulation matrix before instrumental measurement, whereas pesticide residues represent hundreds of structurally diverse organic compounds requiring efficient extraction, separation, and selective detection. Consequently, validation strategies must be adapted to the contaminant class and the complexity of the cosmetic matrix [16, 22, 26].

4.1 Comparative Analysis of Contaminant Testing Challenges

The major analytical differences between heavy-metal and pesticide-residue testing are summarized in Table 4.

Table 4: Comparative Analysis between heavy-metal and pesticide-residue testing

Analytical parameter	**Heavy metals (ICP-MS/AAS)**	**Pesticide residues (LC-MS/MS & GC-MS/MS)**
Primary contaminants	Pb, Cd, As, Hg, Ni, Cr	OCPs, OPPs, carbamates, pyrethroids
Sample preparation	Closed-vessel microwave digestion, commonly using HNO_3/H_2O_2	QuEChERS, SPE, or liquid-liquid extraction
Major matrix effects	Residual carbon, incomplete digestion, polyatomic spectral interference	Ion suppression/enhancement, co-elution, non-volatile matrix contamination
Typical clean-up components	Oxidizing acids and controlled digestion conditions	PSA, C18, GCB, $MgSO_4$ and related sorbents
Major instrumental challenges	Volatilization of Hg, incomplete decomposition of wax-rich matrices, spectral interference	Simultaneous determination of numerous compounds with widely different physicochemical properties
Representative ICP-MS interference	$^{40}Ar^{35}Cl^{+}$ may interfere with $^{75}As^{+}$	Not applicable
Calibration considerations	Matrix matching, internal standards, standard reference materials	Matrix-matched calibration, internal standards, preferably isotopically labelled standards
Principal validation concerns	Digestion efficiency, recovery, spectral selectivity,	Extraction recovery, matrix effects, selectivity, chromatographic separation, analyte stability

contamination control

For heavy-metal analysis, sample preparation is primarily concerned with achieving complete or sufficiently quantitative decomposition of the organic formulation. Closed-vessel microwave digestion using nitric acid, with hydrogen peroxide where appropriate, can facilitate oxidation of complex cosmetic matrices. However, waxes, oils, pigments, and resins may remain problematic and can contribute to carbon loading or incomplete digestion. ICP-MS additionally requires assessment and control of spectral and non-spectral interferences. For example, the formation of $^{40}Ar^{35}Cl^{+}$ can interfere with measurement of $^{75}As^{+}$ in chloride-containing matrices. Collision/reaction-cell technology, appropriate isotope selection, and optimized sample preparation can reduce such effects [16, 19].

Pesticide-residue analysis presents a different challenge. The objective is generally to extract and detect a large number of chemically diverse compounds simultaneously. QuEChERS-based extraction, solid-phase extraction, and liquid-liquid extraction can provide effective approaches, but the optimal conditions depend on analyte polarity, volatility, stability, and affinity for the matrix. Excessive clean-up can reduce recovery of certain pesticides, whereas insufficient clean-up can produce substantial matrix effects and contamination of chromatographic systems [22, 26].

The choice of clean-up reagents must therefore be carefully balanced. Primary secondary amine (PSA) can remove organic acids and other polar matrix constituents, C18 is useful for reducing lipid-associated components, and graphitized carbon black (GCB) can remove pigments such as chlorophyll. However, GCB may also retain certain planar pesticide molecules, making indiscriminate use potentially detrimental to analyte recovery. Magnesium sulfate ($MgSO_4$) is commonly used in QuEChERS procedures to facilitate water removal and promote partitioning [26].

Regulatory interpretation introduces another layer of complexity. Heavy-metal specifications may be derived from toxicological risk assessments and regulatory frameworks applicable to cosmetics, foods, medicines, or botanical materials,

depending on the jurisdiction and product classification. Similarly, pesticide-residue limits are often established for specific agricultural commodities and individual pesticide-commodity combinations. Such limits cannot automatically be transferred to finished herbal cosmetics without considering the applicable legal framework, intended use, route of exposure, and relevant risk assessment. Therefore, laboratories must establish which regulatory limits and standards are legally applicable to the particular product and market rather than relying on generalized contaminant thresholds [1, 6, 35].

4.2 Matrix-Matched Calibration versus Isotopic Labeling

Matrix effects represent one of the most important sources of quantitative uncertainty in pesticide-residue analysis. Co-extracted botanical and cosmetic constituents can alter ionization efficiency in LC-MS/MS or influence detector response in other chromatographic systems. Consequently, calibration performed exclusively in a neat solvent may produce inaccurate results because the response of the pesticide in the actual cosmetic matrix can differ substantially from its response in the calibration solution [24, 25].

One commonly used strategy is matrix-matched calibration, in which pesticide standards are prepared in an extract or formulation matrix that is as similar as possible to the samples but free from the target analytes. The resulting calibration curve incorporates the matrix-dependent response and can therefore provide more accurate quantification than solvent-based calibration when significant matrix effects are present [21, 22].

However, obtaining a genuinely contaminant-free blank herbal cosmetic matrix can be difficult. Botanical ingredients may naturally contain pesticide residues originating from cultivation or environmental exposure, while complex commercial formulations may contain numerous undocumented ingredients or contaminants. Even when a blank matrix is available, demonstrating that it is free from every compound in a multi-residue analytical panel can be impractical. Differences between botanical species, extraction procedures, oils, waxes, pigments, and other formulation components can also mean that a single matrix-matched calibration does not accurately represent every sample type.

An alternative strategy is the use of isotopically labelled internal standards (ILIS)**.** These compounds are chemically or isotopically analogous to the target analytes but contain stable isotopes such as ^{13}C or ^{15}N. Because the labelled standard generally behaves similarly to its corresponding analyte during extraction, chromatography, and ionization, it can compensate for variations in sample preparation and matrix-dependent instrumental response [22, 34].

The principal limitation of comprehensive ILIS-based approaches is economic and practical. Using a separate isotopically labelled standard for every pesticide in a multi-residue panel can be expensive, particularly when hundreds of analytes must be monitored. In addition, not every pesticide has a commercially available labelled analogue. Laboratories may therefore employ a combination of labelled internal standards, structurally similar surrogates, matrix-matched calibration, and periodic matrix-effect assessments rather than relying exclusively on one approach. A practical strategy for routine laboratories is therefore to combine matrix-matched calibration with selected isotopically labelled internal standards, prioritizing ILIS for compounds known to exhibit substantial matrix effects or variable analytical recovery. This approach can provide a reasonable compromise between analytical reliability and operational cost.

4.3 Key Method-Validation Requirements

For both heavy-metal and pesticide-residue testing, method validation is necessary to demonstrate that the analytical procedure is suitable for its intended purpose. Important validation characteristics include:

Selectivity/specificity: ability to distinguish target contaminants from matrix components and other substances.

Linearity and working range: demonstration that detector response is appropriately related to analyte concentration over the intended measurement range.

Accuracy and recovery: assessment of how closely measured concentrations correspond to accepted or fortified concentrations.

Precision: evaluation of repeatability and intermediate precision.

Limit of detection (LOD): lowest concentration that can be reliably distinguished from background under defined conditions.

Limit of quantification (LOQ): lowest concentration that can be quantified with acceptable precision and accuracy.

Robustness: ability of the method to remain reliable following small, deliberate variations in analytical conditions.

Matrix effects: evaluation of signal suppression or enhancement caused by the sample matrix.

Stability: demonstration that analytes remain sufficiently stable during extraction, storage, preparation, and analysis [22, 36].

For heavy metals, additional emphasis should be placed on digestion efficiency, reagent blanks, contamination control, recovery from certified reference materials or fortified samples, and correction of spectral interferences. For pesticide residues, extraction recovery, matrix effects, chromatographic selectivity, analyte stability, and confirmation of detected residues are particularly important.

Ultimately, method validation provides the evidentiary foundation for regulatory compliance. A result should not be considered reliable merely because an instrument produces a numerical concentration. The laboratory must be able to demonstrate that the reported value is supported by a validated procedure capable of controlling the specific sources of uncertainty associated with the complex herbal cosmetic matrix [16, 22, 26, 36].

4.4 Regulatory Ambiguity and Lack of Harmonization

A major challenge in the compliance testing of herbal cosmetics is the absence of a single, globally harmonized framework specifying maximum permissible levels of heavy metals and pesticide residues specifically for cosmetic products. Regulatory requirements differ substantially among jurisdictions and may also depend on whether a botanical product is legally classified as a cosmetic, traditional medicine, herbal medicinal product, or another category. Consequently, laboratories and manufacturers may face difficulties when establishing universally applicable acceptance criteria for contaminants in herbal cosmetic products.

4.4.1 Extrapolation from Food and Pharmaceutical Standards

In the absence of cosmetic-specific limits for certain contaminants, manufacturers and laboratories may refer to limits or guidance developed for other product categories, including foods, dietary supplements, herbal medicines, or pharmaceutical substances. Such extrapolation must be approached cautiously because the exposure scenarios are not identical. Oral products involve gastrointestinal absorption and systemic exposure following ingestion, whereas cosmetics are predominantly associated with dermal exposure, although incidental ingestion, inhalation, or exposure through damaged skin may also be relevant depending on the product. Furthermore, cosmetic products can be applied repeatedly over prolonged periods and to relatively large areas of the body. Therefore, contaminant limits established for an oral product cannot automatically be assumed to provide an equivalent level of protection for a topical cosmetic. Risk assessment should consider the route of exposure, frequency and duration of application, amount of product used, body surface area exposed, dermal absorption, and systemic toxicity of the contaminant [36, 37].

4.4.2 Regional Regulatory Discrepancies

Regulatory approaches also differ considerably between jurisdictions. In the European Union, Regulation (EC) No. 1223/2009 establishes a stringent framework for cosmetic-product safety. Substances listed as prohibited or restricted in the relevant annexes cannot be intentionally incorporated into cosmetic products, while the regulation also requires manufacturers to demonstrate that products are safe under normal or reasonably foreseeable conditions of use. For technically unavoidable traces of prohibited substances, their presence may be considered only where they are technically unavoidable under good manufacturing practice and the product remains safe [37].

The United States follows a different regulatory model. The US Food and Drug Administration (FDA) generally does not pre-approve cosmetic products or their ingredients before marketing, with certain exceptions such as color additives. However, cosmetics must be safe for consumers under customary conditions of use, and adulterated or otherwise non-compliant products may be subject to

regulatory action. The FDA has also conducted testing and surveillance for potentially hazardous elemental contaminants, including lead, arsenic, cadmium, mercury, chromium, cobalt, and nickel [35, 38].

Consequently, a concentration that may be treated as a technically unavoidable impurity under one regulatory framework may require additional assessment or corrective action under another. This creates particular difficulties for manufacturers distributing the same herbal cosmetic formulation across multiple markets. Rather than relying on a single universal contaminant threshold, companies may need to identify the most stringent applicable requirements and demonstrate compliance through appropriate risk assessment and analytical testing.

The problem is further complicated by differences in regulatory terminology and analytical expectations. Terms such as *impurity*, *contaminant*, *trace element*, *residue*, *technically unavoidable trace*, and *maximum permitted level* may have different regulatory implications depending on the jurisdiction and product category. Similarly, analytical methods, reporting limits, sampling requirements, and expectations for demonstrating compliance may differ between regulatory systems [35, 36].

For herbal cosmetics, this lack of harmonization is particularly significant because botanical raw materials naturally exhibit variability in chemical composition and may be exposed to environmental contaminants during cultivation. A single global specification may therefore be difficult to establish without considering botanical source, manufacturing process, product type, exposure conditions, and the toxicological characteristics of individual contaminants.

A more scientifically consistent regulatory approach would involve product-specific risk assessment supported by validated analytical testing**,** rather than indiscriminate transfer of limits established for unrelated product categories. International harmonization of terminology, contaminant definitions, analytical methods, reporting requirements, and risk-assessment principles would substantially reduce uncertainty for manufacturers and testing laboratories while improving consumer protection [35, 39].

5. Conclusion and Future Directions

The assessment of heavy metals and pesticide residues in herbal cosmetics is an essential component of product safety, quality assurance, and consumer protection. Although botanical ingredients are widely perceived as natural and inherently safe, their cultivation and processing can introduce environmental contaminants, agrochemical residues, and other undesirable substances. Reliable compliance testing is therefore necessary to ensure that the natural origin of a cosmetic ingredient does not become a substitute for systematic safety assessment.

However, analytical testing of herbal cosmetics presents considerable challenges. The complex composition of creams, oils, waxes, resins, pigments, essential oils, and botanical extracts can interfere with extraction, digestion, chromatographic separation, and instrumental detection. Heavy-metal analysis may be affected by incomplete matrix decomposition, analyte volatilization, contamination, and spectral interferences, whereas pesticide-residue analysis is complicated by matrix-induced ion suppression or enhancement, instrument fouling, analyte degradation, and the large number of chemically diverse residues that may need to be monitored simultaneously. In addition, differences among national regulatory frameworks create uncertainty regarding permissible contaminant levels, analytical requirements, and interpretation of compliance results.

Future research should therefore focus on developing analytical approaches that are more selective, automated, sensitive, and specifically adapted to complex herbal cosmetic matrices. Advanced sample clean-up technologies represent one important area of development. Novel sorbents, including magnetic nanoparticles, molecularly imprinted polymers, and other selective extraction materials, could potentially improve removal of lipids, waxes, pigments, and resins while retaining target contaminants. Automation of extraction and clean-up procedures could additionally improve reproducibility, reduce analyst-dependent variability, and increase laboratory throughput.

The increasing availability of high-resolution mass spectrometry (HRMS) provides another promising direction. Techniques such as liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) and gas

chromatography-quadrupole time-of-flight mass spectrometry (GC-QTOF-MS) can provide accurate-mass measurements and facilitate both targeted and non-targeted analysis. In addition to routine monitoring of known pesticide residues, HRMS can assist in the detection and characterization of unexpected or previously unidentified contaminants, degradation products, and transformation products. Such capabilities could be particularly valuable for complex botanical formulations in which the complete contaminant profile may not be known in advance.

Finally, greater international regulatory harmonization is needed. Development of internationally accepted contaminant limits, standardized terminology, validated analytical procedures, and matrix-specific standard operating procedures for topical herbal cosmetics would reduce discrepancies between jurisdictions. Regulatory frameworks should consider the distinctive exposure characteristics of topical products rather than relying solely on limits developed for foods, oral medicines, or dietary supplements. Harmonized requirements would also facilitate international trade, simplify compliance testing, and provide laboratories with clearer criteria for method development and validation.

Overall, the future of herbal-cosmetic safety testing will depend on integrating advanced sample preparation, high-resolution instrumental analysis, robust validation, digitalized laboratory workflows, and scientifically based regulatory frameworks**.** Such developments would enable more comprehensive detection of contaminants while reducing analytical uncertainty. Ultimately, a harmonized and technologically advanced testing framework would strengthen consumer protection while supporting the responsible growth of the global herbal cosmetics sector.

Future Research Priorities

Priorities that needs to be focused more significantly in the future should emphasize on the following aspects:

1. **Optimized sample clean-up:** Development of selective, automated sorbents such as magnetic nanoparticles and molecularly imprinted polymers specifically designed for oily, wax-rich, and resinous herbal cosmetic matrices.

2. **High-resolution mass spectrometry:** Greater adoption of LC-QTOF-MS and GC-QTOF-MS for targeted, suspect, and non-target screening, including identification of unknown contaminants and transformation products.
3. **Harmonized cosmetic regulations:** Establishment of internationally consistent permissible contaminant levels, analytical requirements, and validated matrix-specific standard operating procedures designed specifically for topical herbal cosmetics.
4. **Integrated risk-based testing:** Development of testing strategies that combine contaminant occurrence, analytical concentration, dermal exposure, toxicological data, and product-specific usage patterns to provide a more scientifically defensible assessment of consumer risk.
5. **Reference materials and proficiency testing:** Expansion of certified reference materials and interlaboratory proficiency-testing programs specifically covering complex herbal cosmetic matrices to improve comparability and reliability between testing laboratories.

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RECENT PROGRESS IN MARINE NATURAL PRODUCTS (MNPs) : EMERGING TRENDS AND FUTURE PROSPECTS IN DRUG DISCOVERY

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Abstract

Background:

Marine ecosystems are an exceptional source of structurally diverse natural products with immense pharmaceutical, agricultural, and biotechnological potential. Several marine natural products (MNPs) have been approved for clinical use, while many others are under investigation. Recent advances in high-throughput cultivation, metagenomics, genome mining, synthetic biology, and artificial intelligence (AI) have significantly accelerated the discovery of novel marine bioactive compounds.

Objective:

This review highlights recent developments in marine natural product discovery and explores the potential of metabolic engineering and microbial cell factories as sustainable platforms for the production of marine-derived bioactive compounds.

Methods:

Relevant literature on marine natural product discovery, AI-assisted biosynthetic gene cluster prediction, multi-omics approaches, synthetic biology, and metabolic engineering was critically reviewed to evaluate current progress, challenges, and future prospects in sustainable MNP production.

Results and Discussion:

Advanced omics technologies integrated with AI have enhanced the identification of cryptic biosynthetic gene clusters and previously inaccessible marine metabolites.

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Despite these advances, commercialization of MNPs remains constrained by low natural abundance, structural complexity, high production costs, and environmental concerns associated with marine harvesting and chemical synthesis. Metabolic engineering and synthetic biology have emerged as promising alternatives by enabling the development of engineered microbial cell factories capable of producing valuable marine metabolites sustainably and at scale.

Conclusion:

The integration of AI, multi-omics technologies, synthetic biology, and metabolic engineering is transforming marine natural product research. Engineered microbial platforms offer a sustainable and economically viable strategy for large-scale MNP production, facilitating the translation of marine bioactive compounds into therapeutics and industrial products while minimizing environmental impacts.

Keywords: Marine natural products (MNPs), genome mining, metabolomics, synthetic biology, artificial intelligence (AI), sustainable production

1.Introduction

Natural products (NPs) have historically been an incomparable source of pharmaceutical agents, contributing to more than 60 % of approved drugs worldwide. Within this vast domain, products which are derived from marine environment have gained notable attention owing to their structural novelty and diverse biological activities [1]. The marine environment spans more than 70 % of the Earth's surface and hosts unique ecological niches characterized by extreme temperature, salinity, and pressure gradients and these conditions lead to drive the evolution of distinct metabolic pathways and chemical defence mechanisms among marine organisms [2].Marine creatures such fungi and bacteria offer a multiplicity of useful natural products that reveal several uses across manyfields, including medicine and cosmetics. Among these, marine natural products (MNPs) that could have pharmaceutical value are especially important for modern day medicine.The marine ecosystem is a rich source of chemically diverse, biologically potent natural products, and serves as an invaluable resource in the

ongoing research in the field of drug discovery.The discovery of new MNPs which are reported annually continues to increase, with over 1,000 new compounds described in 2023 alone [3,4].Since the isolation of Spong thymidine and Spong uridine from the Caribbean sponge *Tethya crypta* in the 1950s, which inspired the development of cytarabine and vidarabine, MNP research has evolved from classical isolation approaches to genomics-driven discovery [5]. Strong antifungal capabilities against dangerous yeasts, which pose health concerns because of their virulence and drug resistance, are demonstrated, for instance, by turbinmicin taken from a marine microbe.

However, while many marine compounds exhibit potent bioactivity, their development into viable therapeutics remains constrained by challenges in sustainable sourcing, structural complexity, and scalable production. Advances in "omics" technologies, bioinformatics, and synthetic biology are now bridging these gaps, enabling the transition from random discovery to targeted and predictive exploration. This review discusses the key technological advances, emerging concepts, and foreseeable directions that shape the future of MNP discovery and application [6,7].

The market for MNPs is huge; estimations project that marine-derived pharmaceuticals could reach USD 10 billion in market value in 2021 and increase yearly by more than 8% by 2026. Nevertheless, the expenses related with manufacturing these drugs are considerable. Treatments like eribulin for breast cancer may cost up to USD 10,000 per month; brentuximab for lymphoma runs over USD 30,000 [8].

Most pharmaceutical MNPs arise from the secondary metabolism of marine creatures, hence increasing their structural complexity and impeding total chemical synthesis. Therefore, getting these chemicals usually calls on extraction methods based on harvesting or culturing marine creatures. Since the most promising MNPs in these species often occur in minute quantities, culturing bacteria, fungus, or microalgae can be more easily controlled than harvesting invertebrates [9,10].

Although these concentrations may be enough for preliminary research, they fall short of the bigger volumes required in clinical trials and possible

commercialization. Therefore, only relying on wild collecting is not a sensible choice. The restrictions observed in sourcing MNPs reflect those already experienced with plant-derived medications, therefore driving the investigation of substitute supply methods utilizing metabolic engineering and synthetic biology techniques coming into early 2000s [11-13].

By creating medications from biosynthetic genes derived from one organism in another bacterial or yeast, this approach uses genes from one organism in another. Modified microorganisms are now regarded as helpful for producing plant-based medicines after artemisinin production became successful. This debate emphasizes how contemporary advances in biotechnological techniques for making these medicines using engineered microorganisms might provide fresh and creative production choices in the future [14,15].

2. Marine Natural Products: Sources And Chemical Diversity

2.1 Marine Microorganisms

The seas cover a great portion of the planet and habitat for numerous live species able of producing natural molecules because they have developed a wide range of substances with distinctive qualities and evolved in the sea.Marine organisms are sometimes better sources of bioactive chemicals than those seen on land andabout 28,500 marine natural products—such polysaccharides, peptides, and alkaloids—had been discovered by year's end 2016 [16]. These compounds exhibit several biological activities such as anticancer, antibacterial, antifungal, and antiviral effects; therefore, they are very promising leads for the discovery of new medicinal product.

Due to unique structures and features of marine organisms still researchon marine microorganisms not enough or comparatively limited therefore restricting our knowledge of their possible use. Marine organisms are capable of tolerating extreme environmental condition—high pressure, low temperatures, high acidity, and great salinity—and some are specially have adapted to thrive in them and support genetic and metabolic variety. As a result, marine microbes can create distinctive protective chemicals with diverse biological activities from rare

metabolites. These substances could be helpful in the healthcare, cosmetic, and pharmaceutical industries [17,18].

Marine microorganisms contain biomolecules which are less harmful and more effective medicinally than conventional enzymes, seawater resembles human blood plasma. This has promoted more study on marine bacteria as sources of biologically active chemicals andemphasizing their capacity to generate antibiotics, anticancer agents, enzymes, and polysaccharides, about 400 reviews on "marine microorganisms" have been published over the last five years. Most of these evaluations focus on the chemical makeup and structure of metabolites, biological activities and marine microbial diversity [1,2].

Recent discoveries reported that many compounds originally attributed to macrofauna which are synthesized by symbiotic or associated microorganisms [8,9]. Marine bacteria, actinomycetes, cyanobacteria, and fungi produce unique broad spectrum of secondary metabolites, including polyketides, non-ribosomal peptides, and alkaloids [10,11]. The Salinispora genus, for instance, produces salinosporamide A (marizomib), a potent proteasome inhibitor in advanced clinical trials for glioblastoma.

Marine fungi, such as Aspergillus, Penicillium, and Emericella species, have also emerged as prolific producers of polyketides and alkaloids with cytotoxic and antimicrobial activities [19]. These microbial sources offer advantages in cultivation and genetic manipulation compared with macro-organisms.

2.1.1 Marine Bacteria: an unexplored source of novel natural products

The surface of marine organisms, such as corals, fish, sponges and algae serves as a habitat for marine bacteria since these microorganisms commonly have symbiotic or mutualistic relationships with higher organisms.Though they are abundant, only a small fraction (0.01%) of marine microbes has been researched and defined. Studies reported that a great number of bacteria and archaea groups still to be uncovered. Finding novel bioactive substances is greatly promised in marine bacteria, but just 179 such compounds were discovered in 2016. One instance is the strain *Micromonospora harpali*, which creates fresh antibacterial substances against some microorganisms.Including MRSA, Gram-positive bacteria are strongly antibacterial

against Actinomycete Streptomyces sp. MBTI36. Thiocoraline, made by Micromonospora sp., inhibits DNA polymerase-α and some of which under clinical trials for myeloma and other cancers. Salinispora tropica produces Salinosporamide A, under clinical testing for numerous malignancies [20,21].

Two cyclodepsipeptides, P11A and P11B, generated by Streptomyces sp. P11-238 slow glioma cell growth and combined with liposomal doxorubicin, (-)-Ecteinascidin 743, found in *Ecteinascidia turbinate* and produced by a related bacterium, is utilized to treat soft tissue sarcoma and ovarian cancer; P11A can stop the cell cycle at the G0/G1 phase and lead to death of the cancer cell while reducing specific tumour enzyme activities [22].

From 2017 to 2021, several novel bioactive metabolites from marine bacteria were discovered Pyrroloformamides C and D from Streptomyces sp. CB02980 demonstrated moderate antibacterial activity against MRSA and other bacteria; Streptoseomycin from *Streptomyces seoulensis* A01 had outstanding antibacterial action. Anthranilate derivatives from Streptomyces sp. CMN-62 showed antiviral activity against the influenza A virus; alkaloids bulbimidazoles A, B, and C from Microbulbifer sp. DC3-6 revealed antibacterial effects against specific bacterial strains [23].

Still, only a few papers on novel bioactive metabolites from marine bacteria were published in prestigious chemistry publications during this time. The knowledge of the secondary metabolism of marine bacteria is still constrained. Still, marine bacterial metabolites provide great promise towards treating viral, bacterial, and cancers [24].

2.1.2 Marine Fungi: Prominent Source for Bioactive Molecules

Marine fungus generallyclassified into two categories: obligate and facultative, however their variety and ecological function are still under discussion. Since they can grow in several marine habitats including hydrothermal zones, saltmarshes, and the deep ocean, the definition of marine fungi is not quite precise. Found in almost every marine environment—including sediments, the saltwater column, and in conjunction with other marine life like sponges, corals, and algae. An essential

source of advantageous compounds including antimicrobials, antioxidants, and anticancer agents [25,26].

Many marine fungi have shown captivating biological actions. Forexample, a marine Aspergillus fungus generates dehydroxychlorofusarielin B which is effective against drug-resistant *Staphylococcus aureus*. Linear peptides like simplicilliumtides A, E, G, and H, generated by another fungus *Simplicillium obclavatum*, showed low cytotoxicity against specific leukemia cell types. Strong antimalarial action is provided by Chaetoxanthone B found in Chaetomium sp. *Phomopsis lithocarpus* produced novel benzophenone derivatives; one chemical, tenellone H, demonstrated cytotoxic effects on particular cancer cell lines. Malformin C, created by *Aspergillus niger*, is distinguished for its strong anti-HIV-1 activity; Stachybotrys gave stachyflin which give moderate activity against influenza A virus. Because a sizable part of microbial bioactive metabolites come from fungi, these results highlight the possibility of marine fungal research to uncover novel medicine candidates [27,28].

Similar secondary metabolites have been discovered from the Neosartorya, a group linked to Aspergillus. Researchers observed that metabolites differ even inside the same species depending on their environment. Indole alkaloids, terpenoids, and polyketides are among the metabolites discovered; Neosartorya compounds have demonstrated antibacterial and anticancer properties, emphasizing the necessity of more study into their pharmacological and biological characteristics [25].

2.2 Marine Macroorganism

Marine macro-organisms such as sponges, tunicates, mollusks, bryozoans, and macroalgae have historically served as the significant sources of MNPs [5]. Sponges (Porifera) alone account for nearly 30 % of all reported compounds, including alkaloids, terpenoids, peptides, and polyketides with diverse biological activities [6].These microorganisms can account for a large percentage of the organism's weight and variety. Symbiosis is this intimate connection between many different forms of marine plants and animals that include both interior and external associates. Among the typical symbionts are bacteria, archaea, and single-celled eukaryotes.

Tunicates yield metabolites such as ecteinascidins and didemnins—potent cytotoxins that led to clinically approved drugs like trabectedin (Yondelis) for soft tissue sarcoma [7].

Macroalgae produce sulfated polysaccharides, phlorotannins, and halogenated phenols with antioxidant and antiviral properties. Marine mollusks and bryozoans also contribute a wide array of bioactive compounds, often derived from their associated microorganisms.

These macroorganisms can account for a large percentage of the organism's weight and variety. Symbiosis is this intimate connection between many different forms of marine plants and animals that include both interior and external associates. Among the typical symbionts are bacteria, archaea, and single-celled eukaryotes [9,10]

Frequently more active than free-living ones are the microbes found on marine flora and animals. By creating secondary metabolites that enable the host to live in challenging settings, they have significant functions. Studies have found, for instance, that a microbe from Caribbean seagrass created a bioactive metabolite. Because of their therapeutic and industrial potential, these metabolites can play significant biological roles, including in drug development [11,12].

Either encouraging or inhibiting larval settlement, microbes connected with marine organisms can influence it. Those who stop settlement could cause antifouling chemicals to emerge. Studies indicate that, in contrast to those floating freely in the water, the germs on marine invertebrate surfaces display more potent antibacterial and antifouling capabilities. Some investigations shown that the bacteria are the source of certain metabolites believed to be produced by the host organism. Studies on sponges have found previously unknown metabolites created by their symbiotic bacteria, so demonstrating the promise of these microbial relationships as a source of fresh biologically active substances. This has driven more scientific research of metabolites created by bacteria related to marine macroorganisms [1,2].

2.3 Chemical Classes And Structural Features

Marine metabolites commonly feature halogenation, high oxygenation, and uncommon ring systems, reflecting adaptation to marine ecological pressures [12]. The major structural classes include:

2.3.1 Marine Derived Alkaloids

Marine organisms, including sponges, tunicates, mollusks, algae, and microorganisms, synthesize a range of alkaloids that serve as crucial components of their defence mechanisms. These alkaloids exhibit specific structural variations and possess prominent biological activities. Notably, sponges are rich in bioactive alkaloids that demonstrate efficacy against both bacterial and fungal pathogens. These organisms have a variety of marine environments, spanning from shallow reefs to the depths of the sea, and have developed complex chemical defences tailored to their ecological niches. The structural diversity of the alkaloids derived from sponges make them as promising candidates for the development of novel antimicrobial agents, potentially expanding thetreatment modalities available for managing microbial infections. Figure 2 provides a detailed illustration of the chemical structures of these alkaloids, further emphasizing their complexity and potential utility in biomedical applications [28,29].

2.3.2 Marine Derived Amino Acids

Obtained from several marine creatures including bacteria, fungus and algae, marine-derived amino acid provides a bright area for the creation ofnew antibacterial medicines. Significantlyagainst drug-resistant microorganisms, these compounds have shown strong antibacterial and antifungal activities. Among them Halicylindramides, notably halicylindramides A, show prominent antifungal capabilities against M. ramanniana, therefore emphasizing the relevance of their constitution for efficacy. Low minimum inhibitory concentration (MIC) values characterize rhodopeptins from Rhodococcus sp. Another important group demonstrates efficacy against a variety of fungi such as C.albicans and C. neoformans. Moreover, marine derived amino acids have significant antibacterial capabilities particularly cyclic heptapeptides obtained from marine streptomyces culture that exhibit activity against number of bacteria including S. aureus and MRSA [30,31].

2.3.3 Marine Derived Peptides

Peptides which are obtained from marine sources are acquiring more recognition for their use against bacterial and fungal infections,especially with the increased

emergence ofantimicrobial resistance (AMR). Ilamycins, significantly ilamycin B1, are cyclic oligopeptides produced by marine bacteria, for example,*Streptomyces atratus*, that are effective against drug-resistant pathogens such as *Mycobacterium tuberculosis* (M. tuberculosis) and other cyclic oligopeptide examples include kahalalide F, effective against *Candida albicans*, and surfactin which is known to be active against (MRSA). Cyclic peptides are peptides that range from 2–20 amino acids present in a cyclic conformation and are generally produced by non-ribosomal peptide synthetases. The ilamycin family includes ilamycin B1–F, ilamycins G–R, and serves as sufficient antimicrobial activity by the mechanism of the disruption of bacterial cell walls, including drug-resistant strains of M. tuberculosis. Recently,structure and function relationship of cyclic oligopeptides revealed to be receptive and a function of increases to the amino acid side chains which enhances the antimicrobial activity. Cyclic depsipeptides are other marine derived peptides that are defined by the presence of amide and ester bonds, which provides them with a unique structure and advantages against antimicrobial properties. Didemnin B is a cyclic depsipeptide that has showed antifungal activity [32].

2.3.4 Marine-Derived Polyketide

In drug discovery marine derived polyketides serves as a potential candidate for for new antimicrobial agents because of their unique chemical properties and modes of action. Polyketides from marine organisms, including bacteria, fungi, and algae, display potent biological activity, mainly against human pathogens. Marine-derived polyketides are valuable to researchers because many display unique chemical structures that reduces the risk for cross-resistance with other existing antibiotics compared to terrestrial-derived polyketides. Marine-derived polyketides shown to exhibit wide range of biological activities such as multiple antimicrobial modes of action, including inhibition of cell wall synthesis, impairment of membrane integrity, interference with protein synthesis and nucleic acid synthesis, and inhibition of several key metabolic pathways. The combination of the various antimicrobial mechanisms of polyketides allows for improving the potency of the compound and limit the ability to develop resistance. A specific group of compounds are depicted as dicitrinones, and dicitrinone E has been derived from the

starfish-associated fungi Penicillium sp. GGF 16-1-2. Dicitrinone E was shown to exhibit very robust antifungal activity against *Colletotrichum gloeosporioides* in a similar dosage range (LD50 0.00958 mg/mL to 0.01614 mg/mL). There was also evidence for the isolation of five novel polyketides, including two chromones, two phenyl derivatives, all of which showed some level of antifungal activity [31].

2.3.5 Marine-Derived Naphthoquinones

Naphthoquinones derived from marine sources serve a unique naphthalene ring structure with two ketone groups. They are generally obtained from different organisms, including sponges, algae, and bacteria, and they exhibit a wide range of biological activities. Particularly Marine-derived naphthoquinones are well-known for their antimicrobial activity. Because compounds can work is by disrupting the electron transport chain in microbial cells, which reduces ATP production and leads to cell death. Some of the naphthoquinones can also introduces into the DNA of microorganisms and prevent replication and transcription, inhibiting reproduction and consequent cellular functioning. Moreover, marine-derived naphthoquinones can also induce reactive oxygen species (ROS) formation in microbial cells, leading to oxidative damage to proteins, lipids, and DNA, in turn leading to dysfunction and possibly cell death [32,33].

2.3.6 Marine-Derived Terpenoids

Terpenoids derived from marine sources possess numerous structurally complex compounds that are often composed of multiple isoprene units and these structures are further modified through unique biosynthetic pathways that exist in marine environments. These modifications, including but these are not limited to halogenation, glycosylation, and modifications producing distinct ring structures, can significantly impact the biological activity of marine-derived terpenoids. Recent highlighted observed that some marine-derived terpenoids exhibit various forms of activity against fungi and bacteria, makes them excellent molecular scaffolds for the development of novel classes of antimicrobial agents. Unique bioactive character and actions of marine-derived terpenoids, including disruption of microbial (including bacterial and fungal) cell membranes, inhibition of cell wall synthesis, and inhibition of protein and nucleic acid synthesis, suggest that they could have

significant effect in the treatment of resistant bacterial strains and other infections associated with biofilms. Other marine derived terpenoids have been found to alter the immune response in addition to their bioactivities against microorganisms,enhancing the host response to infection. For example, data has reported the activity of squalene and derivatives, which disrupt microbial membranes leading to the lysis and death of the pathogen. Differences of the target in the mechanism makes marine-derived terpenoids promising molecular scaffolds leading to the discovery and engineering of agents to develop new therapeutic approaches to infectious diseases [34,35].

2.3.7 Marine-Derived Polysaccharides

polysaccharides derived from marine sources which are complex carbohydrates comprised of long chains of monosaccharide units, with some unique structural characteristics including sulfation, branching, and acetylation that give different biological activities, both antimicrobial and antifungal effects; as a result marine derived polysaccharides have been investigated for industrial applications (e.g., pharmaceutical and food packaging applications) because of the ability of inhibiting microbial adhesion, biofilm formation disrupt cell membranes and modulate immune responses; various polysaccharides which are derived from marine sources such as chitin, chitosan, polysaccharide, fucoidan and alginate sustaining known abilities to enhance the activity of various classes of antibiotics, thus reducing antibiotic dose and potential side effects. Marine natural products (MNPs) and their sources (e.g., sea urchins, sea squirts (ascidians), sea cucumbers, sea snakes, sponges, soft corals, marine algae,microalgae) are introduced as key biomedical properties because they consist bioactive molecules with potential therapeutic effects, including antioxidant, anti-infection, anti-inflammatory, anticoagulant, anti-diabetic effects, cancer treatments, and enhancement of human immunity. This review focuses more on the potential benefits of MNPs - combating infections from the coronavirus - SARS-CoV-2 and related variants (alpha, delta, omicron) [36,32].

3. Recent Progress In Bioactive Marine Natural Products For Drug Discovery

3.1 Anticancer Agents

Marine cyanobacteria are established as abundantly rich sources of potent bioactive compounds, especially with modified peptides and peptide–polyketide hybrids. Structurally relevant frame work of possible clinical applicability include dolastatin 10, dolastatin 15, and cryptophycin-52, which have been demonstrated to target tubulin and disturb microtubule dynamics, causing picomolar cytotoxicity. Synthetic derived analogues such as monomethyl auristatin E (MMAE) and MMAF are included as cytotoxic payloads in multiple antibody-drug conjugates (ADCs) including brentuximab vedotin, polatuzumab vedotin, and enfortumab vedotin, as ADC technology has facilitated the clinical efficacy of cyanobacterial pluton. In addition to tubulin-targeting compounds, the report discusses new mechanism of action that have been discovered recently, such as the inhibition of the Sec61 protein-translocation channel (apratoxins and coibamides), inhibition of histone deacetylases (HDAC) (largazole and santacruzamate A), and inhibition of the proteasome (carmaphycins). These discoveries are an extension of therapeutic options but have identified first-in-class leads in the targeted treatment of cancer. By evaluating structure activity relationship progresses made in the total synthesis, semi-synthetic modification, and rigorous biosynthetic studies has also facilitated the availability of compounds [37].

3.2 Antimicrobial/Antifungal Agents

The emerging threat of antimicrobial resistance (AMR) has generated interest in natural products which are derived from marine ecosystem, detailed overview of the findings of marine natural product research with respect to antibiofilm and antibacterial activity against pathogenic microorganisms that exhibit resistance. All compounds which are classified in a series of chemical classes, e.g.,alkaloids, aminoacids/peptides, polyketides, naphthoquinones, terpenoids, and polysaccharides that belong to a range of marine resources, for example, microbes, algae, sponges, tunicates, and mollusks, with antibacterial and antifungal activity against drug resistant organisms [37].

3.3 Other Therapeutic Areas (Anti-Inflammatory, Neuroprotective, Antiaging)

Marine natural products (MNPs) emphasize significant potential in the field of drug discovery, particularly for anticancer, antimicrobial, and neuroprotective applications. The marine environment comprised of unique chemical diversity, offering novel molecular scaffolds sourced from bacteria, fungi, algae, and invertebrates that have different structure from natural products found on land. This uniqueness posseses new opportunities for developing innovative therapies in the field of drug discovery.Drugs that are clinically approved generally derived from marine sources, such as Ziconotide, Trabectedin, and Cytarabine, stressing them as successful examples that demonstrate the translational potential of MNPs as viable therapeutic agents [36,37].

4. Recent Advances in Discovery Technologies

4.1 Metagenomics And Genome Mining

Marine ecosystems posseses a vast and largely untapped microbial diversity, many of which remain undiscovered through traditional laboratory methods. These uncovered microbial communities represent an immense reservoir of bioactive secondary metabolites. Introduction of metagenomics and genome mining has revolutionized the field of exploration of marine natural products by enabling access to the biosynthetic potential of both cultured and uncultured microorganismsRecent studies discuss the role of marine derived natural products (MNPs), extracted from numerous marine organisms, including sponges, soft corals, sea urchins, ascidians, sea cucumbers, sea snakes, and macro- and micro-algae, as possible sources of immunomodulatory, antiviral, and cardio-protective agents. A 2024 comprehensive review by Hu et al., entitled “Marine Natural Products and Human Immunity: New Biomedical Resources for Anti-Infection of SARS-CoV-2 and Related Cardiovascular Disease”, highlights multiple mechanism of action of MNPs that include limiting the entry and replication of the virus, decreasing anti-inflammatory signalling, antioxidant and immune enhancing effects, and favourably modulating cardiovascular risk pathways. The study demonstrated MNPs regarding their hypothesized use in SARS-CoV-2 and variants (i.e., Delta and Omicron). It

provides a summary of marine-derived compounds that inhibited various viral targets, such as 3C-like protease, viral spike protein-ACE2 receptor interface, entry into host cells and cofactors, based on the studies in silico and in vitro studies. Metagenomics and genome mining have transformed the discovery of marine derived products from unanticipated screening to genome informed exploration by unlocking the biosynthetic potential of the uncovered microbial majority [38,39].

4.2 LC–MS/MS and NMR-Based Metabolomics

Metabolomics is the comprehensive analysis of metabolites or small molecules within a biological system- has become a backbone in the research of marine derived products.Among various hyphenated techniques, liquid chromatography mass spectrometry (LC-MS/MS) and nuclear magnetic resonance (NMR) spectroscopy have serve as the most powerful techniques that combine one or more separation and detection methods so that it can provide comprehensive and accurate results. These are complimentary techniques for identification, characterization, and quantification of marine derived metabolites. Their introduction in the products which are derived from marine ecosystem has accelerated the discoveries of novel compounds from complex marine matrices including sponges, algae, cyanobacteria and deep-sea microorganisms which are remain uncultured. LC-MS/MS based molecular networking has facilited dereplication and identification of structurally related compounds and NMR spectrometry helps in the structural elucidation of novel macrolides, depsipeptides and sterols from marine microorganisms. Integration with metagenomics and genome mining data to validate and predicted biosynthetic products of uncovered marine bioactive by using advanced approaches such as metagenome-guided genome mining, metabolomics- genomic correlation and CRISPER- based activation of silent biosynthetic gene clustures (BGCs) [39].

4.3 Synthetic Biology And Heterologous Expression

Many BGCs remain silent or poorly expressed under conventional laboratory condition which limiting their access to their chemical diversity. The emergence of synthetic biology and heterologous expression systems has revolutionized the field to express various marine biosynthetic pathways for sustainable production of novel natural products. This integrates the principles of molecular biology, bioinformatics,

and construct new biological systems or reprogram existing ones for desired biosynthetic functions. Heterologous expression involves transferring and expressing marine biosynthetic genes in a well characterized, genetically tractable host organisms such as *Streptomyces coelicolor, Escherichia coli, Saccharomyces cerevisiae*, and *Aspergillus nidulans*biosynthetic gene clusters (BGCs) into more manageable host organisms, including Streptomyces coelicolor, Escherichia coli, Saccharomyces cerevisiae, and *Aspergillus nidulans*. This innovative approach reduces the challenges of cultivating slow growing or uncultivated marine microbes and enables the scalable production of valuable metabolites that might otherwise be difficult to obtain from natural sources [38,39,40].

4.3.1 Heterologous Expression And Pathway Refactoring

Pathway refactoring posseses systematic redesign and reconstruction of biosynthetic gene clustures to improve the expression, yield or development of novel compounds. Refactoring emphasizes on rational reengineering of regulatory elements, gene organization, and expression control to achieve predictable and enhanced biosynthesis novel analogues.Advances in DNA synthesis and modular cloning allow for quick reconstruction of large biosynthetic gene clusters (BGCs).Examples include efficient production of marizomib in Streptomyces and polyketide synthesis in engineered *E. coli* [39,40].

4.3.2 Combinatorial Biosynthesis And Precursor Engineering

Synthetic biology enables combinatorial assembly of enzymes from multiple pathways that involves the rational recombination of genes, modules, or domains from different biosynthetic pathways to yield new chemical structuresand Swapping tailoring enzymes (e.g., halogenases, glycosyltransferases) generates analogues with altered pharmacological profiles [28]. Precursor-directed biosynthesis further expands chemical diversity by applying modified substrates that are incorporated into natural scaffolds [40].

4.3.3 Automated Design And Genome Editing Tools

The use of CRISPR–Cas9, CRISPR–Cpf1, and recombineering systems promotes precise editing of MNP gene clusters in the native host or engineered heterologous host organism. It promotes transcriptional activation in many bacteria and

cyanobacteria. Many marine BGCs are divergent from terrestrial counterparts. Deep learning (DeepBGC) and clustering (BIG-SPACE) helps in the identification of remote homologs and group novel families and assists in pathway design and similarity analysis, respectively [39,38].

4.3.4 Cell-Free Biosynthesis And Microbial Consortia

Cell free biosynthesis and microbial consortia are two rapidly emerging strategies in the discovery and production of marine natural products and complementing automated genome mining by enabling the expression of silent or cryptic biosynthetic gene clusters (BGCs) from marine microorganisms. Cell-free systems provide rapid prototyping of enzyme modules without the constraints of cellular metabolism. In parallel, synthetic co-culture systems recreate natural symbioses (e.g., sponge–bacteria consortia) to induce secondary metabolism [30].

Overall, synthetic biology links the gap between discovery and supply, allowing sustainable access to marine metabolites by reducing ecological impact.

5. Artificial Intelligence And Machine-Learning Applications

By introducing artificial intelligence and machine learning application in the field of marine natural products accelerates every stage from genome mining and structure prediction to biosynthetic pathway design, metabolite identification, and bioactivity prediction. The integration of artificial intelligence (AI) into MNP discovery represents a major paradigm shift. Machine-learning (ML) models assist in predicting chemical structures, bioactivity, and biosynthetic potential from genomic and spectral data [36,39].

5.1 AI-Driven Genome Mining

It comprises artificial intelligence (AI), machine learning (ML), and bioinformatics to predict, classify, and prioritize biosynthetic gene clusters (BGCs) hidden in the genome of marine microorganisms, many of which are uncovered and poorly characterized. Deep-learning algorithms trained on BGC databases (e.g., MIBiG, antiSMASH outputs) can predict new biosynthetic architectures and chemical classes with high accuracy rely on rule-based algorithms that detect conserved domains or motifs [31]. Tools like DeepBGC and RiPP utilizes a bidirectional

recurrent neural network for identifying novel or divergent clusters with minimal sequence similarity to known compounds.several networks to classify BGCs. NeuRiPP and RiPPMiner (for Ribosomally Synthesized and Post Translationally modified Peptides) utilizes deep learning to identify RiPP precursor peptides and modification of enzymes [39].

5.2 Metabolomics And Spectral Prediction

AI models such as Spec2Vec and MS2DeepScore represent the new generation and metabolomic informatics in the discovery of natural products derived from marine environment (MNPs). This tool reduces the limitation of traditional cosine based spectral matching by utilizing deep learning and machine learning to capture chemical relationships and structural similarities between metabolites, facilitating dereplication and annotation [32]. Integration of these tools with GNPS molecular networks provides a more comprehensive chemical-space view.

5.3 Bioactivity And Property Prediction

This is an emerging interdisciplinary area that combines marine bioresources, nanotechnology, and AI based prediction models to design and evaluate biogenic gold nanoparticles with specific pharmacological or biotechnological functions. Quantitative structure–activity relationship (QSAR) models, random-forest classifiers, and deep-learning architectures (e.g., graph neural networks) used for prediction of antimicrobial, cytotoxic, or antiviral potential directly from structural descriptors [39].

5.4 AI-Assisted Design And Optimization

AI assisted design in MNPs can be visualized as a closed loop system, where computational models and Generative models, such as variational autoencoders and transformer-based molecular generators, have been used to design analogues inspired by marine scaffolds to improve performance [34]. Reinforcement-learning frameworks can optimize synthetic routes and identify potential derivatives with improved ADMET profiles.

These developments indicate that AI-assisted “data driven discovery” will increasingly complement classical chemistry and biology in the coming decade.

6. Emerging Trends in Marine Natural-Product Research

6.1 Host–Microbe Interactions And Symbiosis

Symbiotic association often define the chemical phenotype of the host, influencing the synthesis, modification, and ecological function of marine natural products. A large proportion of secondary metabolites of marine ecosystem originate from symbiotic bacteria or fungi residing within sponges, tunicates, and corals, algae and marine invertebrates that serves as aprimary source of bioactive secondary metabolites. Genomic evidence demonstrates that the majority of sponge-associated metabolites are bacterial in origin. Understanding these associations through metagenomics and in-situ imaging has reported that microbial consortia contribute significantly to the host's chemical defence and marine symbiosis exist across a spectrum of relationship, from transient associations to stable mutualistic partnership [41,42].

The "holobiont" concept—considering host and microbiome as a functional unit—has thus become essential to MNP research.

6.2 Chemical Ecology And Metabolite Function

By understanding the ecological function of secondary metabolites which act as a chemical signals, defence agents, and ecological mediators, influencing competition, predation, symbiosis, and microbial community structure not only provides insight into evolutionary adaptation but also guides the biotechnological exploration or MNPs for pharmaceutical and industrial use. For example, antifouling peptides from marine bacteria have inspired environmentally friendly coating technologies [37,43]. Chemical-ecology–guided approaches are now used to predict bioactivity classes from ecological roles, streamlining discovery pipelines.

6.3 Blue Biotechnology And Sustainable Bioprospecting

With growing conservation concerns, researchers focus on sustainable exploitation of marine resources. Cultivation of producing organisms in aquaculture systems, microbial fermentation, or synthetic-biology platforms reduces harvesting pressure on fragile ecosystems. "Blue biotechnology" integrates genomics, robotics, and green chemistry to ensure ethical and sustainable use of marine biodiversity and prioritizing culture- independent and genome-based approaches by developing

invitro and heterologous expression system to reduce to reduce wild harvesting and employing AI assisted predictive tools for virtual screening of bioactive potential [44-46].

6.4 Integration Of Multi-Omics Approaches

Traditional single omics approaches, including genomics or metabolomics offer valuable but fragmented insights, their integration with other omics techniques such as transcriptomics, proteomics, and metabolomics provides a system level perspective linking genes, enzymes, metabolites, and ecological function. Multi-omics integration allows correlation between gene expression and metabolite production, providing a systems-level understanding of biosynthesis regulation. Bioinformatic pipelines such as OmicsNet and MetaboAnalyst now support multi-layer data visualization and hypothesis generation. This holistic framework enables the identification of biosynthetic gene clustures, prediction of metabolite function, elucidation of regulatory mechanisms underlying natural product biosynthesis [44,45].

7. Challenges and Bottlenecks in Marine-Natural-Product Research

Despite rapid progress, several persistent challenges obstruct the full translation of marine metabolites into industrial and pharmaceutical applications.

7.1 Rediscovery And Dereplication

Traditional culture-based screening often leads to frequent re-isolation of well-known metabolites due to limited strain diversity in culture collection, overlapping biosynthetic pathways among related taxa and conventional bioassay guided fractionation, which tends to Favor previously characterized bioactivities. Most of the critical issues in marine bioprospecting generally originated due to re-isolation of known compounds. While LC–MS molecular networking and databases such as Marin Lit and the discovery of Marine derived Natural Products (DMNP) have improved dereplication, spectral overlap and limited database coverage still cause redundancy The incorporation of in-silico fragmentation tools and AI-based annotation is gradually reducing these bottlenecks but not eliminating them completely [39,42].

7.2 Activation Of Silent Biosynthetic Gene Clusters

Comparative genomics indicates that less than 10 % of BGCs in marine microorganisms are expressed under laboratory conditions and majority remain "silent" or "cryptic", meaning that they are transcriptionally inactive or expressed at undetectable levels due to absence of natural ecological triggers (e.g., symbiotic signals, competition, or stress conditions), transcriptional repression by regulatory proteins, epigenic modification, insufficient precursor supply and low cellular energy or unfavourable culture conditions. Unlocking these "silent" clusters requires manipulation of regulatory circuits through promoter exchange, CRISPR-based activation, or co-culture stimulation. Epigenetic modifiers (e.g., histone-deacetylase inhibitors) have also been used to trigger cryptic metabolite production in marine fungi [46,44].

7.3 Supply And Scalability

Supply issue arises primarily from the low yield of bioactive compounds in marine organisms, restricted access to natural habitats, and difficulties in large scale production. Many promising metabolites occur in minute quantities or in organisms that cannot be sustainably collected. Heterologous expression and total synthesis address supply constraints but remain complex for large polyketides and depsipeptides. Bioprocess engineering, precursor optimization, and cell-free biosynthetic systems are being developed to enhance production yields [39,44,47,48].

7.4 Structural Complexity And Synthetic Challenges

Marine natural products often possess high stereochemical density, and unique functional motifs contribute to exceptional biological activities, often unmatched by terrestrial analogs. Total synthesis routes, though successful for halichondrins and bryostatins, require multistep sequences that limit economic viability. Semi-synthetic analogues, inspired by marine scaffolds, offer a more practical approach for lead development [44,49].

7.5 Regulatory, Ethical, And Conservation Concerns

Bioprospecting in marine ecosystems must adhere to the Nagoya Protocol and national access-and-benefit-sharing regulations. Deep-sea sampling and coral-reef

collection raise ethical and environmental considerations due to overexploitation, climate change, and pollution responsible bioprospecting practices. Sustainable aquaculture, microbial fermentation, and in-silico exploration minimize ecological disturbance while ensuring compliance with biodiversity treaties [42,43, 44].

7.6 Data Integration And Standardization

Among heterogenicity, fragmentation, lack of standardization, data base posseses significant barriers to effective data sharing, reproducibility, and large-scale analysis. Integration of genomic, metabolomic, and ecological data into interoperable repositories will be essential for improving reproducibility and large-scale comparative analyse [50].

8. Future Perspectives and Directions

MNP research is entering into a transformative era driven by breakthroughs in synthetic biology, omics integration, automation, and artificial intelligence which focusses on sustainability and translational of MNPs [51].

8.1 AI-integrated Multi-Omics Platforms

Combining metagenomics, transcriptomics, proteomics, and metabolomics within AI-driven frameworks will enable predictive discovery pipelines and pathway prediction. Deep-learning models can rank BGC novelty, predict metabolite classes, and simulate biosynthetic logic prior to empirical work [39, 44,45].

8.2 High-Throughput Cultivation And Miniaturized Screening

Microfluidic cultivation, diffusion chambers, and single-cell isolation will accelerate the culturability of marine microbes. Coupling these with miniaturized LC–MS analytics and automated bioassays allow rapid evaluation of metabolic potential at scale [52].

8.3 Synthetic-Biology Chassis Optimization

Development of “marine-mimetic” chassis organisms with enhanced halogenation capacity, redox flexibility, and membrane tolerance will facilitate efficient heterologous expression. Modular biosynthetic toolkits—comparable to Golden Gate or Bio Brick standards—will make pathway engineering routine [42,38].

8.4 Computational Chemistry And Cheminformatics Synergy

Advances in quantum-mechanics/molecular-mechanics (QM/MM) simulation and molecular-docking workflows can predict binding affinities of marine scaffolds to disease targets, accelerating hit-to-lead transitions. Virtual-screening of MNP libraries via cloud platforms will become integral to preclinical trials [48,49].

8.5 Eco-Innovation And Blue Circular Economy

Future marine bioprospecting must align with blue-economy principles, ensuring ecological integrity and local community benefits. Integration of MNP discovery with carbon-neutral aquaculture, waste-valorisations, and biomaterial innovation will extend impact beyond pharmaceuticals [53].

8.6 Translational Networks And Open Data

Establishing open-access repositories for BGCs, spectral data, and ecological metadata—alongside FAIR (Findable, Accessible, Interoperable, Reusable) data standards—will enhance collaboration across institutions. Public–private partnerships can bridge academic discovery and industrial development [54-57].

9. Conclusion

Marine natural products continue to represent one of the most chemically diverse and pharmacologically rich sources of new molecules on Earth. The transition from traditional isolation to genomics- and AI-driven exploration has transformed discovery efficiency and sustainability and the field now moves toward data-driven, sustainable, and interdisciplinary discovery ecosystems, capable of converting the vast, untapped marine chemical space into tangible benefits for medicine, industry, and society. However, persistent challenges—especially rediscovery, supply limitations, and regulatory complexity, cultivation difficulties—require coordinated solutions combining computational prediction, synthetic biology, and ethical bioprospecting. Through international collaboration and responsible innovation, the future of marine natural products promises both scientific advancement and stewardship of ocean biodiversity and the future direction of MNP research lies in combining advanced analytical technologies with computational prediction, and synthetic biology to achieve scalable production of high value compounds.

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