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EVALUATION OF THE ANTI-AMNESIC POTENTIAL AND PHYTOCHEMICAL PROFILE OF SPHAERANTHUS INDICUS AND ABRUS PRECATORIUS LEAF EXTRACTS IN RODENT MODELS

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ABSTRACT

Memory impairment and amnesia are important manifestations of several neurological disorders and may arise from oxidative stress, cholinergic dysfunction, neuroinflammation, and disturbances in neuronal signaling. Medicinal plants containing flavonoids, phenolics, alkaloids, terpenoids, and other bioactive constituents have attracted considerable attention as potential sources of cognitive-enhancing agents. The present research proposes a comparative evaluation of the anti-amnesic potential and phytochemical profiles of leaf extracts of Sphaeranthus indicus and Abrus precatorius using validated rodent models of learning and memory. The study will involve authentication, collection, drying, pulverization, and extraction of leaves using suitable solvents. Preliminary phytochemical screening will be conducted to identify major classes of secondary metabolites, followed, where feasible, by chromatographic or spectrometric profiling. The anti-amnesic activity of standardized extracts will be assessed using behavioral paradigms such as the Morris water maze, elevated plus maze transfer latency, and passive avoidance test following pharmacologically induced amnesia. Biochemical investigations may include assessment of acetylcholinesterase activity, oxidative stress markers, antioxidant enzymes, and selected inflammatory or neuronal biomarkers. Sphaeranthus indicus has documented antioxidant, neuroleptic, anxiolytic and anti-amnesic properties, including evidence that its extracts can improve memory deficits in scopolamine-treated mice. Abrus precatorius contains numerous phytochemical classes and

has reported antioxidant and neuroprotective potential, although its safety profile requires particular attention because the plant contains the highly toxic protein abrin and leaf preparations have also demonstrated toxicity in animal studies. The proposed comparative investigation may help establish relationships between phytochemical composition and cognitive effects and identify promising candidates for further neuropharmacological research.

Keywords: - Anti-amnesic activity, Sphaeranthus indicus, Abrus precatorius, phytochemical screening, memory impairment, rodents, oxidative stress, acetylcholinesterase, neuroprotection.

INTRODUCTION

Memory is a fundamental cognitive function that enables organisms to acquire, retain, consolidate, and retrieve information. Normal learning and memory depend upon the coordinated functioning of neuronal networks, particularly those involving the hippocampus, cerebral cortex, and cholinergic pathways. Disturbances in these systems can result in impaired learning, reduced memory retention, and amnesia. Cognitive impairment may be associated with aging, neurodegenerative disorders, oxidative stress, inflammation, disruption of neurotransmitter systems, and other pathological conditions. Consequently, the search for effective and safe agents capable of preventing or reducing memory dysfunction remains an important area of pharmacological and neurobiological research.

The cholinergic system has a particularly important role in learning and memory. Acetylcholine facilitates neuronal communication involved in attention, learning, and memory formation. Excessive activity of acetylcholinesterase (AChE), the enzyme responsible for hydrolyzing acetylcholine, can reduce cholinergic neurotransmission. Experimental induction of amnesia using scopolamine is therefore widely employed to investigate potential cognitive-enhancing and anti-amnesic agents. Other behavioral models, including passive avoidance and the Morris water maze, provide complementary measures of learning, spatial memory, retention, and retrieval.

Medicinal plants represent an important source of structurally diverse compounds with potential neurological activity. Flavonoids, phenolic compounds, terpenoids, alkaloids, glycosides, and other secondary metabolites may exert antioxidant, anti-inflammatory, cholinesterase-inhibitory, and neuroprotective effects. Plant-derived antioxidants may be particularly relevant because excessive generation of reactive oxygen species can damage neuronal membranes, proteins, enzymes, and nucleic acids.

Among medicinal plants of interest, Sphaeranthus indicus Linn. of the family Asteraceae has a long history of use in Indian traditional medicine. Reviews of the plant have documented traditional applications involving nervous-system-related conditions as well as pharmacological activities including anxiolytic, neuroleptic, antioxidant, anticonvulsant, and other activities. Its phytochemistry includes sesquiterpene lactones, eudesmanolides, flavonoids, sterols, alkaloids, glycosides, and volatile constituents.

Of particular relevance to the present investigation, experimental research has reported anti-amnesic activity of S. indicus. A petroleum ether fraction of its flowers was reported to improve scopolamine-induced cognitive impairment in mice in passive avoidance and water-maze tests, with findings suggesting cholinesterase-related and synergistic effects of multiple constituents. More recently, a 2026 study reported that aqueous and hydroalcoholic preparations of S. indicus improved cognitive performance in a scopolamine-induced amnesia model in Swiss albino mice. Although these findings strengthen the rationale for studying S. indicus, investigations specifically focused on standardized leaf extracts remain comparatively important.

Abrus precatorius Linn., commonly known as rosary pea or gunja, belongs to the Fabaceae family. Different parts of this plant have been used traditionally for various medicinal purposes. Phytochemical investigations have identified diverse compounds belonging to flavonoids, phenolics, terpenoids, steroids, alkaloids, organic acids, esters, proteins, and polysaccharides. A comprehensive review reported more than 166 identified or isolated compounds and documented several pharmacological activities, including antioxidant and neuroprotective effects.

Research on A. precatorius leaves has demonstrated the presence of polyphenols, flavonoids, tannins, alkaloids, saponins, β-carotene, glutathione, α-tocopherol, and vitamin C, together with measurable antioxidant activity. These properties provide a scientific basis for investigating whether leaf extracts could influence memory-related processes through antioxidant or other neuroprotective mechanisms.

However, A. precatorius presents an important toxicological consideration. The plant contains abrin, a highly toxic ribosome-inactivating protein, with the seeds being particularly dangerous. Importantly, an experimental study also reported toxic effects from aqueous leaf extract in rats at tested doses, emphasizing that leaf extracts cannot automatically be considered safe simply because the seeds are the most notorious toxic part of the plant. Therefore, any experimental investigation involving A. precatorius should incorporate appropriate toxicity evaluation, ethical approval, dose justification, and careful extract characterization.

The comparative investigation of S. indicus and A. precatorius leaf extracts is therefore scientifically relevant because the two plants possess chemically diverse constituents and reported antioxidant or neuropharmacological properties, while their anti-amnesic effects as standardized leaf preparations require further systematic evaluation.

I. PHYTOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF SPHAERANTHUS INDICUS

Sphaeranthus indicus Linn. is an important medicinal plant belonging to the family Asteraceae and is widely distributed throughout India and other tropical regions. It is commonly known as Gorakhmundi or Mundi and has been extensively used in traditional systems of medicine, particularly Ayurveda. Different parts of the plant, including leaves, flowers, roots, stems, and aerial portions, have traditionally been used for the management of various disorders. The plant has been traditionally associated with the treatment of nervous disorders, epilepsy, headache, fever, digestive problems, skin diseases, inflammatory conditions, respiratory disorders, jaundice, and other health problems. The extensive traditional use of S. indicus has stimulated considerable scientific interest in its phytochemical composition and pharmacological properties. Investigations have demonstrated that the plant contains a diverse range of biologically active constituents that may contribute individually or synergistically to its therapeutic effects.

The phytochemical composition of S. indicus is complex and may vary depending on the plant part, geographical location, environmental conditions, stage of maturity, harvesting period, and extraction method. Phytochemical investigations have identified several groups of secondary metabolites, including sesquiterpene lactones, sesquiterpenoids, eudesmanolides, flavonoids, flavonoid glycosides, phenolic compounds, sterols, alkaloids, glycosides, amino acids, sugars, and volatile constituents. Among these, sesquiterpene lactones and eudesmanolide-type compounds are considered important characteristic constituents of the plant. Several sesquiterpenoids have been isolated and structurally characterized from different parts of S. indicus, and these compounds are believed to contribute to its diverse biological activities.

Sesquiterpene lactones constitute one of the most important groups of phytochemicals identified in S. indicus. Several eudesmanolide-type compounds and related sesquiterpenoids have been reported from the plant. Compounds such as 7-hydroxyeudesm-4-en-6,12-olide, 2-hydroxycostic acid, β-eudesmol, ilicic acid, cryptomeridiol, and 4-epicryptomeridiol have been reported in phytochemical investigations. These compounds possess considerable pharmacological importance because sesquiterpenoids and sesquiterpene lactones have been associated with antioxidant, anti-inflammatory, antimicrobial, and neuropharmacological effects. Their presence may contribute significantly to the biological activities observed in different extracts and fractions of S. indicus.

Flavonoids are another significant group of phytoconstituents found in S. indicus. Different investigations have reported the presence of flavonoids, flavone glycosides, flavonoid C-glycosides, and related compounds. Flavonoids are well known for their antioxidant and free-radical-scavenging properties. They can protect biological tissues from oxidative damage by neutralizing reactive oxygen species and reducing oxidative stress. This property is particularly important in neurological research because excessive oxidative stress can result in lipid peroxidation, mitochondrial dysfunction, protein oxidation, neuronal injury, and impairment of cognitive functions. Flavonoids may also influence inflammatory pathways, neurotransmitter systems, and neuronal signaling, thereby contributing to the neuroprotective and cognitive-enhancing properties of S. indicus.

Phenolic compounds are also important components of S. indicus. Phenolic substances possess significant antioxidant activity and contribute to the overall free-radical-scavenging capacity of plant extracts. Various phenolic and flavonoid constituents, including gallic acid, chlorogenic acid, rutin, epicatechin, and naringenin, have been reported in investigated extracts of the plant. These compounds may contribute to antioxidant and anti-inflammatory effects. However, the concentration and composition of phenolic constituents can differ according to the plant material and extraction procedure. Therefore, standardization of the plant material and extract is important when evaluating its pharmacological potential.

Sterols and other lipid-soluble constituents have also been identified in S. indicus. Phytochemical investigations have reported sterols and sterol glycosides along with alkaloids, glycosides, amino acids, and sugars. Essential oils are another important chemical component of the plant and contain different monoterpenes, sesquiterpenes, oxygenated monoterpenes, and oxygenated sesquiterpenes. The chemical composition of the essential oil may vary according to the plant part and geographical origin. These volatile compounds may contribute to antimicrobial, antioxidant, insecticidal, and other biological activities. The presence of numerous classes of phytochemicals indicates that the pharmacological effects of S. indicus are likely to result from the combined actions of several constituents rather than from a single compound.

The pharmacological properties of S. indicus are extensive and have been investigated using different experimental models. Studies have reported antioxidant, anti-inflammatory, anxiolytic, neuroleptic, anticonvulsant, antidiabetic, hepatoprotective, antimicrobial, analgesic, antipyretic, bronchodilatory, immunomodulatory, nephroprotective, antihyperlipidemic, wound-healing, and neuroprotective activities. The broad pharmacological profile of the plant is consistent with its complex phytochemical composition. However, the level of experimental evidence differs among these activities, and many findings remain primarily preclinical.

The antioxidant activity of S. indicus is particularly important because oxidative stress is involved in the development of numerous pathological conditions. Extracts and fractions of the plant have demonstrated free-radical-scavenging and reducing activities in experimental antioxidant assays. Flavonoids, phenolic compounds, sesquiterpenoids, and other secondary metabolites may contribute to these effects. The brain is particularly vulnerable to oxidative stress because of its high oxygen consumption, intense metabolic activity, and high concentration of polyunsaturated fatty acids. Therefore, the antioxidant activity of S. indicus may play an important role in protecting neuronal cells from oxidative damage and may contribute to its reported neuroprotective effects.

The anti-inflammatory activity of S. indicus is another important pharmacological property. Persistent inflammation can contribute to tissue damage and is increasingly recognized as an important factor in neurological disorders. Various constituents of S. indicus, particularly flavonoids and sesquiterpenoids, may influence inflammatory processes and reduce inflammatory responses. The relationship between oxidative stress and inflammation is particularly relevant because excessive production of reactive oxygen species can activate inflammatory pathways, while inflammation can further increase oxidative damage. Consequently, the combined antioxidant and anti-inflammatory properties of S. indicus may provide an important basis for its protective effects on nervous tissue.

The central nervous system-related activities of S. indicus are especially relevant to research on cognitive impairment. Traditional medicinal applications of the plant include the management of nervous disorders, epilepsy, and mental disturbances. Experimental studies have reported anxiolytic, neuroleptic, anticonvulsant, central nervous system depressant, and neuroprotective activities. These findings suggest that the plant may influence neuronal functions through multiple mechanisms. The presence of sesquiterpene lactones, flavonoids, phenolic compounds, and other neuroactive constituents may contribute to these effects.

Of particular importance is the anti-amnesic and cognitive-enhancing potential of S. indicus. Experimental investigations have evaluated extracts and fractions of the plant in animal models of memory impairment. Studies involving scopolamine-induced amnesia have reported improvements in learning and memory following treatment with certain S. indicus preparations. Improvements have been observed in behavioral paradigms such as passive avoidance and water-maze tests. Some findings have suggested a possible relationship between the anti-amnesic effects and cholinergic mechanisms. The presence of several active constituents may also result in synergistic pharmacological effects, which is particularly relevant when investigating crude plant extracts.

The possible influence of S. indicus on the cholinergic system is highly relevant to memory research. Acetylcholine plays an important role in attention, learning, memory formation, and cognitive processing. Acetylcholinesterase is the enzyme responsible for hydrolyzing acetylcholine at cholinergic synapses. Excessive acetylcholinesterase activity can reduce cholinergic neurotransmission and consequently impair cognitive performance. Therefore, inhibition of acetylcholinesterase may help maintain acetylcholine availability and improve cholinergic neurotransmission. Some investigations of S. indicus have suggested possible cholinesterase-related mechanisms in its anti-amnesic effects, although the exact molecular mechanisms and contribution of individual phytoconstituents require further investigation.

The neuroprotective activity of S. indicus may also be closely associated with its antioxidant properties. Excessive production of reactive oxygen species can damage neuronal membranes, proteins, mitochondria, and nucleic acids. Such damage can adversely affect neuronal communication and cognitive function. Antioxidant phytoconstituents present in S. indicus may help neutralize reactive species and support endogenous antioxidant defense mechanisms. Flavonoids and phenolic compounds are particularly relevant because of their established antioxidant potential. The combined antioxidant and anti-inflammatory actions may therefore contribute to the overall neuroprotective effects of the plant.

Anticonvulsant activity has also been reported for S. indicus in experimental investigations. This property is consistent with its traditional application in nervous disorders and epilepsy. The anticonvulsant effects may involve modulation of neuronal excitability, neurotransmitter systems, antioxidant mechanisms, or other neurochemical pathways. Although the exact mechanisms remain to be fully established, this activity further supports the potential importance of S. indicus in neuropharmacological research.

Apart from its neurological effects, S. indicus has demonstrated several metabolic and organ-protective activities. Experimental studies have reported antidiabetic and antihyperglycemic effects, suggesting that the plant may influence glucose metabolism. Hepatoprotective and nephroprotective effects have also been reported in experimental models. These activities may be associated with the antioxidant and anti-inflammatory properties of its phytoconstituents. The ability to reduce oxidative injury and inflammatory responses may help explain the protective effects observed in different tissues.

Antimicrobial activity is another important pharmacological property of S. indicus. Extracts and isolated constituents have been investigated against different microorganisms, and studies have reported antibacterial, antifungal, antiviral, antiparasitic, larvicidal, and related activities. Terpenoids, phenolic compounds, flavonoids, and volatile constituents may contribute to these effects. The antimicrobial activity of the plant demonstrates the broad biological potential of its chemical constituents and supports several of its traditional applications.

Other pharmacological activities reported for S. indicus include analgesic, antipyretic, bronchodilatory, wound-healing, immunomodulatory, antihyperlipidemic, anti-arthritic, mast-cell-stabilizing, and antiulcer activities. Although these effects are not directly related to memory impairment, they demonstrate the broad pharmacological spectrum of the plant. The simultaneous occurrence of antioxidant, anti-inflammatory, metabolic, antimicrobial, and neuroactive properties suggests that S. indicus may exert multiple biological effects through different but interconnected mechanisms.

The phytochemical and pharmacological characteristics of S. indicus make it a promising candidate for investigation in relation to memory impairment and neurodegenerative disorders. The combined presence of flavonoids, phenolic compounds, sesquiterpene lactones, eudesmanolides, sesquiterpenoids, sterols, alkaloids, glycosides, and volatile constituents may contribute to its biological effects through antioxidant, anti-inflammatory, cholinergic, and neuroprotective mechanisms. However, it is important to recognize that evidence obtained from flowers, roots, or whole-plant preparations cannot automatically be generalized to leaf extracts. Specific investigation of authenticated and standardized S. indicus leaves is therefore necessary for establishing their phytochemical profile and anti-amnesic potential.

II. ANTI-AMNESIC POTENTIAL OF SPHAERANTHUS INDICUS

Sphaeranthus indicus Linn. has attracted considerable scientific interest because of its traditional association with nervous-system disorders and its experimentally demonstrated neuropharmacological activities. The plant, commonly known as Gorakhmundi or Mundi, has been used in traditional medicine for conditions associated with mental disturbances, epilepsy, anxiety, and other neurological complaints. In recent pharmacological investigations, extracts and fractions of S. indicus have demonstrated promising effects on learning, memory, anxiety, and cognitive performance in experimental animals. These findings suggest that the plant may possess anti-amnesic properties and could serve as a potential source of natural compounds for the development of agents against cognitive impairment.

The anti-amnesic potential of S. indicus is particularly associated with its rich phytochemical composition. The plant contains sesquiterpene lactones, eudesmanolides, sesquiterpenoids, flavonoids, phenolic compounds, sterols, alkaloids, glycosides, and other biologically active constituents. Among these, sesquiterpene lactones and flavonoid compounds are considered particularly relevant to its neuropharmacological effects. These constituents may exert protective effects on neuronal cells through antioxidant, anti-inflammatory, cholinergic, and other mechanisms. The combined action of several phytoconstituents may be responsible for the activity of the crude extract, rather than a single chemical component.

Oxidative stress is an important factor associated with neuronal dysfunction and memory impairment. The brain has a high metabolic requirement for oxygen and contains a large proportion of polyunsaturated fatty acids, making neuronal tissue particularly vulnerable to oxidative damage. Excessive production of reactive oxygen species can cause lipid peroxidation, mitochondrial dysfunction, protein oxidation, and neuronal injury. The antioxidant activity reported for S. indicus may therefore contribute to its anti-amnesic potential. Flavonoids and phenolic compounds present in the plant can scavenge reactive oxygen species and may support endogenous antioxidant defense mechanisms. By reducing oxidative damage, these constituents may help preserve neuronal integrity and normal cognitive function.

The anti-inflammatory properties of S. indicus may provide another mechanism underlying its neuroprotective and anti-amnesic effects. Neuroinflammation can adversely affect neuronal communication and cognitive processes when inflammatory responses become excessive or persistent. Several phytoconstituents of S. indicus, particularly flavonoids and sesquiterpenoids, have demonstrated anti-inflammatory potential. Reduction of oxidative stress and inflammatory responses may work together to protect neuronal structures and maintain the physiological processes involved in learning and memory. Thus, the antioxidant and anti-inflammatory properties of the plant may complement one another in producing beneficial effects against experimentally induced cognitive impairment.

The cholinergic system is another important target in the study of anti-amnesic activity. Acetylcholine plays a central role in learning, attention, memory formation, and cognitive processing. Acetylcholinesterase is responsible for the breakdown of acetylcholine in cholinergic synapses. Increased degradation of acetylcholine can reduce cholinergic neurotransmission and contribute to memory deficits. Research on S. indicus has suggested that some extracts and fractions may influence cholinesterase-related pathways. Inhibition or modulation of acetylcholinesterase could increase the functional availability of acetylcholine and consequently improve cholinergic neurotransmission. This provides a plausible biochemical mechanism through which S. indicus may exert anti-amnesic effects.

Experimental evidence has provided further support for the cognitive-enhancing potential of S. indicus. Investigations using scopolamine-induced amnesia models have reported improvements in learning and memory following administration of certain S. indicus preparations. Scopolamine is commonly used experimentally to produce temporary cognitive impairment through disruption of cholinergic neurotransmission. Improvements in behavioral performance following treatment with S. indicus extracts suggest that the plant may counteract some of the functional changes associated with experimentally induced amnesia. Passive avoidance and water-maze-based behavioral paradigms have been used to assess these effects, with treated animals demonstrating improved performance compared with untreated amnesic animals in reported studies.

The water-maze paradigm is particularly useful for evaluating spatial learning and memory because successful performance requires animals to learn and remember the location of a hidden escape platform. Improvement in acquisition and retention following treatment with a plant extract can therefore provide evidence of cognitive-enhancing activity. Studies involving S. indicus have reported beneficial effects in water-maze-based assessments, supporting the possibility that its constituents can influence spatial learning and memory. However, behavioral improvements should ideally be interpreted together with biochemical and histological findings to establish whether the observed effects are associated with specific neuroprotective mechanisms.

Passive avoidance testing provides another important approach for assessing memory. In this model, animals learn to avoid an environment associated with an aversive stimulus, and the retention of this learned response is subsequently evaluated. Improved retention following treatment with S. indicus preparations has been reported in experimental investigations of anti-amnesic activity. Such findings suggest that the plant may influence memory acquisition, consolidation, or retrieval. The use of more than one behavioral model is important because it allows researchers to determine whether the cognitive effects are reproducible across different types of memory assessment.

A particularly interesting finding from studies of S. indicus is that the anti-amnesic effect may involve synergistic interactions among several phytoconstituents. Some investigations of flower-derived fractions have shown significant cognitive benefits even when individual isolated constituents did not reproduce all of the effects observed with the fraction. This suggests that the pharmacological activity of the whole extract may depend on interactions between multiple chemical constituents. Such synergy is common in medicinal plants and highlights the importance of phytochemical standardization and investigation of both extracts and isolated compounds.

The neuroprotective potential of S. indicus may also involve preservation of endogenous antioxidant enzymes. Important antioxidant defenses include superoxide dismutase, catalase, glutathione peroxidase, and reduced glutathione. During oxidative stress, these systems may become depleted or impaired, leading to accumulation of reactive oxygen species and cellular damage. A plant extract capable of reducing lipid peroxidation while supporting endogenous antioxidant defenses could potentially protect neuronal tissue from oxidative injury. Therefore, assessment of parameters such as reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, and malondialdehyde can provide useful biochemical evidence when evaluating the anti-amnesic potential of S. indicus.

The reported anxiolytic and central nervous system activities of S. indicus may also have relevance to cognitive performance. Anxiety and stress can interfere with learning, attention, and memory, and therefore reduction of excessive anxiety may indirectly support cognitive function. Experimental studies have reported anxiolytic activity for S. indicus, suggesting that its effects on behavior may extend beyond direct memory enhancement. However, anxiolytic activity should be distinguished from genuine anti-amnesic activity, and appropriate behavioral controls are necessary to ensure that improvements in cognitive tests are not simply the consequence of changes in locomotor activity, sedation, anxiety, or motivation.

The phytochemical composition of the extract is likely to influence its anti-amnesic activity substantially. Different solvents extract different groups of compounds, resulting in variation in biological activity between aqueous, hydroalcoholic, methanolic, petroleum ether, and other preparations. Furthermore, the plant part used can significantly influence the chemical profile. Evidence obtained from flower extracts or whole-plant preparations should therefore not automatically be interpreted as evidence for equivalent activity in leaf extracts. For research specifically focused on S. indicus leaves, authentication of the plant material, standardized extraction, phytochemical screening, quantitative estimation of important constituents, and appropriate pharmacological evaluation are essential.

Overall, the available experimental evidence indicates that Sphaeranthus indicus possesses promising anti-amnesic potential. Its activity may result from a combination of antioxidant, anti-inflammatory, cholinergic, neuroprotective, and possibly anxiolytic mechanisms. Sesquiterpene lactones, flavonoids, phenolic compounds, and other phytoconstituents may collectively contribute to the observed effects. Nevertheless, much of the existing evidence remains preclinical, and further investigations are necessary to establish the precise molecular mechanisms responsible for its cognitive effects.

Therefore, S. indicus represents a scientifically interesting medicinal plant for further investigation in the field of memory impairment and neuroprotection. Studies involving standardized leaf extracts and validated animal models could help clarify its anti-amnesic efficacy and establish relationships between phytochemical composition and pharmacological activity. Combining behavioral tests such as the Morris water maze and passive avoidance with biochemical assessment of acetylcholinesterase, oxidative stress markers, and endogenous antioxidant enzymes may provide a comprehensive understanding of its mechanism of action. Further isolation and characterization of active constituents may ultimately help identify novel natural compounds with potential therapeutic relevance for cognitive dysfunction.

III. EXPERIMENTAL EVALUATION OF ANTI-AMNESIC ACTIVITY

The anti-amnesic activity of Sphaeranthus indicus can be experimentally evaluated using validated behavioral models of learning and memory in laboratory rodents. Such investigations are designed to determine whether the plant extract can prevent or reverse experimentally induced memory impairment. The assessment generally involves the use of appropriate control, amnesic, and treatment groups, followed by behavioral evaluation of learning and memory. Scopolamine-induced amnesia is commonly employed as a pharmacological model because disruption of cholinergic neurotransmission produces measurable deficits in learning and memory. The anti-amnesic potential of S. indicus can therefore be assessed by comparing the behavioral performance of extract-treated animals with that of untreated amnesic animals and appropriate controls.

Before pharmacological evaluation, the plant material should be properly authenticated and processed under standardized conditions. Leaves of S. indicus may be subjected to suitable extraction procedures, followed by preliminary phytochemical screening to determine the major classes of bioactive constituents. The extract should be evaluated for relevant quality characteristics and, where possible, standardized using appropriate analytical techniques. Particular attention should be given to flavonoids, phenolic compounds, sesquiterpene lactones, and other constituents that may contribute to neuroprotective and cognitive effects. The experimental protocol should be reviewed and approved by the appropriate institutional animal ethics committee, and all procedures should follow applicable animal-welfare regulations.

Experimental animals should be randomly allocated into suitable groups, including a normal control group, an amnesia control group, and one or more extract-treatment groups. A suitable positive-control treatment may also be included for comparison. Group allocation, blinding of behavioral assessment where feasible, and predefined inclusion and exclusion criteria can improve the reliability of the findings. The dose selection of the plant extract should be based on appropriate literature evidence and prior safety or toxicity assessment rather than being selected arbitrarily. Particular care should be taken to monitor general health, body weight, locomotor behavior, food and water intake, and other signs of toxicity throughout the experimental period.

The Morris water maze is an important behavioral model for evaluating spatial learning and memory. In this paradigm, rodents learn to locate a hidden escape platform using spatial cues surrounding the experimental environment. During the acquisition phase, learning performance can be evaluated by parameters such as escape latency and swimming performance. During the retention or probe phase, memory can be assessed by examining the animal's ability to remember the previous location of the platform. An improvement in spatial-learning and memory performance in animals receiving S. indicus extract compared with the amnesia control group would provide evidence supporting its potential anti-amnesic effect. Appropriate interpretation is essential because changes in swimming ability, motivation, anxiety, or locomotor activity can influence performance independently of memory.

The passive avoidance test can provide complementary evidence of memory formation and retention. In this model, animals learn to avoid entering a compartment associated with an unpleasant stimulus. Memory is subsequently evaluated by measuring the animal's retention of the learned avoidance response. A greater retention of the avoidance response in extract-treated animals compared with amnesic controls may indicate improvement in memory. Using passive avoidance together with the Morris water maze is advantageous because the two paradigms assess cognitive performance through different behavioral procedures and can provide complementary evidence of anti-amnesic activity.

The elevated plus maze can also be used to investigate learning and memory in rodents, although it is important to distinguish its cognitive component from its primary use as a model of anxiety-like behavior. In memory experiments, transfer latency and changes in performance between training and retention sessions may be evaluated. If treatment with S. indicus improves retention performance in amnesic animals, the result may support a memory-enhancing effect. However, because the elevated plus maze is sensitive to anxiety and locomotor changes, these factors should be considered when interpreting the results.

Biochemical investigations can provide additional evidence regarding the mechanisms underlying the observed behavioral effects. Acetylcholinesterase activity is particularly relevant because the cholinergic system plays an important role in learning and memory. If S. indicus extract produces behavioral improvement together with a reduction in excessive acetylcholinesterase activity, the findings may suggest involvement of cholinergic neurotransmission. However, behavioral improvement should not be attributed exclusively to acetylcholinesterase inhibition without supporting biochemical and pharmacological evidence.

Oxidative stress-related parameters can also be examined to determine whether antioxidant mechanisms contribute to the anti-amnesic effect. Parameters such as reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, and malondialdehyde may be evaluated in relevant brain tissue. Memory impairment associated with oxidative stress may involve increased lipid peroxidation and weakening of endogenous antioxidant defenses. Therefore, a treatment-associated reduction in oxidative damage accompanied by restoration or improvement of antioxidant defense parameters would support a neuroprotective contribution to the anti-amnesic activity of S. indicus.

Histopathological examination may provide further information regarding the protective effects of the extract on brain tissue. Relevant brain regions involved in learning and memory can be examined for structural alterations where scientifically justified. Comparison between normal control, amnesia control, and treatment groups may help determine whether the extract is associated with preservation of neuronal architecture. Histological findings should be interpreted alongside behavioral and biochemical results rather than being considered independently.

The experimental findings should be subjected to appropriate statistical analysis. Behavioral and biochemical data can be summarized using suitable descriptive statistics, followed by an appropriate inferential statistical method based on the experimental design and distribution of the data. Comparisons among treatment groups should be planned before analysis, and statistical significance should be interpreted together with effect sizes and biological relevance. The results should be presented clearly through tables and figures showing behavioral performance, biochemical parameters, and other relevant observations.

IV. CONCLUSION

The proposed study provides a comprehensive framework for evaluating the anti-amnesic potential and phytochemical profile of Sphaeranthus indicus and Abrus precatorius leaf extracts in rodent models. Both plants possess considerable ethnomedicinal importance and contain chemically diverse secondary metabolites. Sphaeranthus indicus has particularly strong preliminary support for cognitive-enhancing and anti-amnesic activity, including experimental evidence involving scopolamine-induced memory impairment. Abrus precatorius leaves contain polyphenols, flavonoids, and other antioxidant-related constituents and have a broader literature supporting neuroprotective potential.

However, the two plants should not be considered equivalent in terms of evidence or safety. The documented toxicity associated with A. precatorius, including evidence from aqueous leaf-extract studies, makes systematic safety evaluation essential. an integrated assessment combining phytochemical profiling, behavioral testing, cholinergic measurements, oxidative-stress markers, and toxicity evaluation can provide meaningful evidence concerning the therapeutic potential of these plants. The findings may serve as a foundation for identifying standardized botanical preparations or individual phytoconstituents for subsequent neuropharmacological investigation.

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