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Phytochemical Profiling and Antioxidant Potential of Different Solvent Fractions of Madhuca Longifolia for Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is a neurological related disease and it is most common cause for dementia. Also, AD causes many disability and important socio-economic burden, but till to date no cure available. The analysis focused on determining the total phenolic content, total flavonoid content, and antioxidant activities using DPPH radical scavenging assay, hydroxyl radical scavenging assay, and total antioxidant capacity. The results revealed significant variations among the extracts. The ethyl acetate fraction exhibited the highest total phenolic content (97.30 ± 0.47 mg GAE/g) and strong DPPH radical scavenging activity (IC_{50} = 4.81 ± 0.02 µg/mL), comparable to the standard antioxidant catechin. The aqueous fraction recorded the highest flavonoid content (175.82 ± 0.65 mg CE/g) and demonstrated superior hydroxyl radical scavenging activity (IC_{50} = 9.66 ± 0.27 µg/mL), along with the highest total antioxidant capacity (0.555 ± 0.009 absorbance at 80 µg/mL). In contrast, the petroleum ether fraction showed minimal phytochemical and antioxidant potential.

Keywords: *Antioxidant Activity, Phenolic, Sodium, Flavonoid, Alzheimer's Disease.*

I. INTRODUCTION

Alzheimer's disease is one of the most debilitating and progressive neurodegenerative conditions impacting millions of people globally. AD has become a significant global health issue among the aging population, marked by increasing cognitive decline, memory loss, behavioral disorders, and diminished functional performance. The pathological characteristics of AD encompass the extracellular accumulation of amyloid-β (Aβ) plaques, the intracellular aggregation of neurofibrillary tangles formed by hyperphosphorylated tau protein, oxidative stress, mitochondrial impairment, and the degeneration of cholinergic neurons in the cerebral cortex and hippocampus. Even while we know a lot more about how AD works on a cellular level, there are still not many good treatments that can stop or slow down the illness's growth. Traditional pharmacological interventions, including

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acetylcholinesterase inhibitors (donepezil, rivastigmine, galantamine) and NMDA receptor antagonists (memantine), offer merely symptomatic relief and frequently entail negative effects with prolonged usage. As a result, scientists are becoming more and more interested in studying natural phytochemicals from medicinal plants that protect nerve cells, fight free radicals, reduce inflammation, and stop the breakdown of acetylcholine. These compounds could be useful in treating AD in more than one way.

The Mahua tree, or Madhuca longifolia (Koenig) J.F. Macbr., is a member of the Sapotaceae family and is native to the Indian subcontinent. Ayurvedic, Siddha, and Unani traditional medicine have all praised it for its many health benefits. The plant is a big tree that loses its leaves in the winter and is found all over tropical and subtropical parts of India. Traditionally, numerous components of Madhuca longifolia, like the flowers, seeds, bark, and leaves, have been used to cure a wide range of illnesses, such as inflammation, rheumatism, diabetes, epilepsy, skin problems, ulcers, and infections caused by microbes. Recent pharmacological studies have shown that Madhuca longifolia possesses a diverse array of bioactive substances, such as triterpenoids, saponins, flavonoids, steroids, tannins, and phenolic compounds, which predominantly contribute to its extensive range of biological activity.

Recent studies have highlighted the neuropharmacological potential of Madhuca longifolia, especially its efficacy in addressing neurodegenerative disorders such as AD. Neurodegeneration is a multifactorial process triggered by oxidative stress, neuroinflammation, mitochondrial malfunction, and death of neuronal cells. The antioxidant and free radical scavenging characteristics of Madhuca longifolia are particularly pertinent in this context, given that oxidative stress is recognized as one of the initial and most crucial factors in the progression of AD.

Madhuca longifolia possesses antioxidant and anti-inflammatory effects and shows promise in modifying lipid metabolism and decreasing amyloidogenesis. Dysregulation of cholesterol metabolism has been associated with the processing of amyloid precursor protein (APP) and the subsequent production of Aβ peptides. The bioactive sterols and triterpenes in the plant may affect the balance of lipids in neuronal membranes, which could help stop amyloid plaques from building up. Moreover, the neuroprotective properties of Madhuca longifolia encompass its capacity to alleviate mitochondrial malfunction and apoptotic cell death, which are critical aspects of Alzheimer's disease pathophysiology. Mitochondria are the main source of energy for neurons and a common target for oxidative damage. Research indicates that natural antioxidants derived from Madhuca longifolia safeguard mitochondrial integrity by sustaining membrane potential, preventing cytochrome-c release, and attenuating caspase activation.

II. REVIEW OF LITERATURE

Khare, Pragati & Khare, Noopur. (2023) This study aimed to investigate the biochemical alterations in the brains of Swiss albino mice and determine if the ethanolic leaf extract flavonoid fraction of Madhuca longifolia prevented colchicine-induced cognitive impairment and oxidative damage. Additionally, total phenols, total flavonoids, and HPTLC were evaluated in the research. Over the course of 28 days, the study was conducted on a model that had been provoked by colchicine. While

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physiological indicators like glutathione and nitric oxide were measured, behavioral tests like the Morris water maze and passive avoidance paradigm were used. Each of the eight groups of forty-eight Swiss albino mice consisted of six animals. The following procedures were used to analyze the data: one-way ANOVA, Dunnett's test, and finally, evaluation. In the Morris water maze test, mice given an extract from Madhuca longifolia leaves showed a substantial reduction in escape latency. In the passive avoidance situation, the transfer latency of mice was significantly increased. The GSH intensity increased significantly ($P<0.001$) while the levels of total protein, NO, and AChE decreased distinctly ($P<0.001$) in the extract of Madhuca longifolia leaves. When it comes to protecting neurons from the damage that colchicine does to memory, Madhuca longifolia is the way to go.

Dhoubhadel, Kusum et al., (2023) There is a severe lack of research in the scientific literature on the phytochemical analysis of bark from M. longifolia. The methods used in this work include solvent polarity-based extraction, chemical screening of extracts, antioxidant and antibacterial activity assessment, GC-MS profiling of the hexane extract, and quantification of sugars, gallotannins, condensed tannins, flavonoids, and phenolics in different extracts. Extracts in ethyl acetate, methanol, and 50% aqueous methanol contained the most phytochemicals, according to our findings. These phytochemicals comprised terpenoids, phenolics, flavonoids, tannins, and glycosides. In the DPPH experiment, the radical scavenging effect of the methanol extract was noticeable, as evidenced by an IC50 value of 18.86 ± 1.07 µg/mL. In the antibacterial test, inhibiting zones of 15–22 mm were seen for ethyl acetate, methanol, and 50% aqueous methanol extracts against S. aureus and E. coli. Squalene, β-amyrin, β-amyrin acetate, lupeol, lupeol acetate, and cis-3,14-Clerodadien-13-ol were among the nine molecules detected in the hexane extract using GC-MS analysis. These compounds were tentatively identified by comparing their mass fragmentation patterns with those in the standard NIST database. Supporting its long-standing uses, this study shows that extracts from M. longifolia bark include antioxidants, antibacterial, and anti-inflammatory properties.

Peter Simon, Jerine et al., (2020) Traditional Ayurvedic medicine places great value on the whole Madhuca longifolia plant, including its wood, blossoms, fruits, leaves, and seeds. The goal of our research is to examine the medicinal qualities of M. longifolia leaves by in vitro, pharmacological, and computational investigations. Various in vitro tests were carried out on progressively diluted ethanolic, methanolic, and water-based leaf extracts of Moringa longifolia. These included Total Phenolic Content, DPPH assay, catalase activity, and peroxidase activity. Female Wistar albino rats were tested for pharmacological effects utilizing the hot-plate, analgesic, antipyretic, and ulcerogenic methods. The nuclear receptor and active compounds of M. longifolia leaf were the subjects of the in silico study that was carried out utilizing the PatchDock web server. Molecular ligands were obtained from the Corina Molecular Network, while three-dimensional structures of nuclear receptors were obtained from the RCSB Protein Data Bank. All of the leaf extracts demonstrated antioxidant activity in the in vitro test, with the aqueous extract showing promise at 1:2 serial dilutions. Compared to other M. longifolia extracts, the beneficial pharmacological effects of the aqueous leaf extract seen in rats at an oral dose of 500 mg/kg body weight are more pronounced. So, M. longifolia leaf has antioxidant, pharmacological, and binding affinity properties, according to our research.

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Sinha, Jyoti et al., (2017) Mahua, or Madhuca longifolia, is a plant species of the Sapotaceae family. Due to its high monetary worth, this tree is extensively cultivated in India. In addition to being a nutritious food source, the Mahua tree may have medicinal properties that might help with a variety of health issues. Reviewing prior studies on mahua fruit, seed, and flowers, this study zeroes down on the value-added applications of mahua flowers. Phytochemical studies on mahua have uncovered a wealth of nutrients, including sugar, vitamins, proteins, alkaloids, phenolic compounds, and more. An abundance of medicinal research has focused on mahua's ethnomedical properties, including its antibacterial, anticancer, hepatoprotective, antiulcer, antihyperglycemic, and analgesic effects, among many others. In addition to being a liquor ingredient, mahua flower has many other potential uses in the kitchen, including but not limited to: biscuits, cakes, laddus, candies, bars, jam, jelly, sauces, and many more sweets and treats. Specifically, this research aims to provide light on the potential for economic growth and employment prospects in the food and pharmaceutical sectors as a result of commercializing mahua fruit, seed, and flowers.

Annalakshmi, R. et al., (2012) To identify the phytochemical components and assess the physico-chemical constants of Madhuca longifolia, we set out to do a preliminary phytochemical screening in this work. The measured physicochemical constants are good enough to be considered acceptable by phytochemical standards. Plant phytochemical research uncovered a number of potential therapeutic phytocompounds.

III. MATERIAL AND METHODS

Chemicals used

Donepezil, galantamine, acetylthiocholine iodide, S-butyrylthiocholine iodide, DPPH (2,2′-diphenyl-1-picrylhydrazyl), ammonium molybdate, Folin–Ciocalteu reagent, thiobarbituric acid (TBA), trichloroacetic acid (TCA), 2-deoxy-D-ribose, 5,5′-dithio-bis-(2-nitro) benzoic acid (DTNB), Triton X-100, aluminum chloride, potassium ferricyanide, and Tris-HCl were obtained from Sigma-Aldrich, Bengaluru, India. Catechin, ascorbic acid, and gallic acid were acquired from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Methanol, ethyl acetate, chloroform, and petroleum ether were procured from Merck Life Science Private Limited, Mumbai, India.

All other chemicals and reagents utilized in this investigation were of analytical grade were obtained from reputable Indian sources.

Homogenization of Animal's Brain

Research using brain tissue was the only justification for the use of mice. The experimental room was furnished by the Animal House with 5- or 6-week-old Swiss Albino mice. A constant supply of water and food was given to the mice. Researchers followed all relevant international ethical guidelines when dealing with animals in the lab.

Brain tissue was collected from mice by cervical dislocation after sodium pentobarbital sedation. The brain tissues were quickly washed with ice-cold saline.

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Plant Collection, Extraction and Fractionation

The Madhuca longifolia plant was collected in April 2020 from Hyderabad, Telangana after obtaining the owner's consent. The use of any plant-based material in this research does not violate any federal, state, or municipal laws.

After being rinsed with distilled water, cut into tiny pieces, and shade dried for a few days, the plant material was ground into a coarse powder using a grinding machine. Using the hot extraction process in a Soxhlet system, 500 g of powder was passed through a cotton bed and Whatman filter paper number 1 to obtain the crude methanol extract (CME). Through the use of a rotary evaporator, the filtrate was vacuo concentrated to produce a semisolid mass weighing 18.5 g. We started by mixing 10 g of CME with 200 ml of 10% methanol. Then, we added 3.2 g of petroleum ether, 2.5 g of chloroform, 1.4 g of ethylacetate, and 2.9 g of water to separate the components. Each fraction was kept in the refrigerator at 4 °C until they were ready to be used again.

Phytochemical Analysis

Phytochemical Screening of The Plant Extract

Steroids, tannins, alkaloids, saponins, and flavonoids were among the phytochemical groups identified by qualitative testing of the different fractions using the methods previously detailed.

Quantitation of TPC

Utilizing the Folin-Ciocalteu method, we ascertained the total phenolic concentration of the Madhuca longifolia extractives. A 10% Folin-Ciocalteu reagent and a 7.5% sodium carbonate solution were combined with half a milliliter of the sample in 2.5 milliliters. Next, the mixture was left to settle for 20 minutes in the dark at a temperature of 25 degrees Celsius. The reaction mixture's absorbance at 760 nm was measured using a spectrophotometer. We determined the phenolic content by pulling the gallic acid standard curve out to its maximum.

Quantitation of Total Flavonoid Content (TFC)

Utilizing a colorimetric method involving aluminum chloride, the flavonoids contained in the Madhuca longifolia extract were quantified. A combination of 5.6 ml of distilled water, 1 ml of plant extract, 10% AlCl3, 1 M potassium acetate, methanol, and waited for 30 minutes at room temperature before adding the following components. The reaction mixture's absorbance at 420 nm was measured using a spectrophotometer. The flavonoid content was determined by extending the standard curve for catechin.

Antioxidant Activity

Total Antioxidant Capacity Assay

The solution was treated with 5-80 µg/ml of plant extract and then cooked in a water bath at 95 °C for 90 minutes. The solution already included 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. After the combination cooled to room temperature, its absorbance was measured at 695 nm in comparison to a blank. To make comparisons, the compound catechin was used as a reference.

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DPPH Radical Scavenging Assay

The DPPH radical scavenging capacities of Madhuca longifolia extracts were assessed using a modified version of Choi et al.'s (2000) technique. For the sake of comparison, the component catechin was used. For 30 minutes, a mixture of 0.135 mM methanolic DPPH and a methanol solution of a plant extract or reference chemical was let to sit in the dark. Solutions with concentrations ranging from 6.25 to 100 µg/ml were shown. At 517 nm, the absorbance of the reaction mixture was recorded.

Determination of Hydroxyl Radical Scavenging Activity

The ability of Madhuca longifolia extracts to scavenge hydroxyl radicals was assessed using a modified version of the technique developed by Elizabeth et al. (1990). For the sake of comparison, the component catechin was used. Included in the 1 ml reaction mixture were the following substances: 2.8 mM 2-deoxy-2-ribose, 20 mM phosphate buffer (pH 7.4), 100 µM FeCl3, 100 µM EDTA, 1 mM H2O2, and 100 µM ascorbic acid. After that, the combination was left to incubate at 37 °C for another half an hour. From 6.25 to 100 µg/ml, the plant extract or reference chemical concentration varied. Half a milliliter of the reaction mixture was mixed with one milliliter of trichloroacetic acid (2.8%) and one milliliter of triethanolamine (1%). The mixture was then heated in a water bath at 90 °C for 15 minutes. I used a spectrophotometer to measure the combination at 532 nm in comparison to an appropriate blank solution after it cooled to room temperature.

Isolation and Characterization of An Active Compound from The Bioactive Extract

The 5.6 g Ethylacetate Fraction of Madhuca longifolia was eluted using column chromatography after a gradient of n-hexane, dichloromethane, and methanol was run. Silica gel 60 was used as the stationary phase.

Statistical Analysis

Every experiment was carried out three times. The data were provided as the mean ± the standard deviation. We used Excel 2010 for all of our quantitative and qualitative analysis. A T-test was used to determine the statistical significance between the average values (P-value < 0.05).

IV. RESULTS AND DISCUSSION

Phytochemical Analysis

The presence of tannins, phenolics, flavonoids, alkaloids, phytosterols, and saponins was revealed in a preliminary phytochemical analysis of the CME. Based on the qualitative examination of all four fractions, it was found that flavonoids and phenolics were present, with the ethyl acetate and water fractions having the highest quantities.

The ethyl acetate fraction had the highest phenolic concentration (97.30 ± 0.47 mg GAE/g dried extract), followed by CME (79.97 ± 0.63 mg GAE/g dried extract), the aqueous fraction (61.07 ± 0.19 mg GAE/g dried extract), the chloroform fraction, and the Petroleum Ether Fraction, according to analyses of total phenolic and flavonoid content in the extractives (Table 1). The maximum flavonoid content was found in the aqueous fraction (175.82 ± 0.65 mg CE/g dried extract), followed by the ethylacetate fraction (144.36 ± 0.53 mg CE/g dried extract), chloroform fraction, petroleum ether fraction, and CME (174.10 ± 1.03 mg CE/g dry extract).

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Table 1: Phytochemical Analysis and Antioxidant Activity of The Extract and Fractions of Madhuca Longifolia

Sample	Total phenolic content (mg GAE/g dried extract)	Total flavonoid content (mg CE/g dried extract)	DPPH IC50 (µg/ mL)	Hydroxyl radical scavenging IC50 (µg/mL)	Total antioxidant capacity (absorbance at 80 µg/mL)
CME	79.97 ± 0.63b	174.10 ± 1.03b	10.11 ± 0.43d	42.61 ± 0.52d	0.458 ± 0.007d
Petroleum Ether Fraction	7.36 ± 0.15e	2.67 ± 0.21e	27.48 ± 0.51f	160.36 ± 2.37f	0.349 ± 0.006f
Chloroform fraction	33.51 ± 0.18d	33.47 ± 0.32d	21.54 ± 0.47e	105.49 ± 2.03e	0.362 ± 0.006e
Ethylacetate fraction	97.30 ± 0.47a	144.36 ± 0.53c	4.81 ± 0.02a	28.18 ± 0.41c	0.531 ± 0.008b
Aqueous fraction	61.07 ± 0.19c	175.82 ± 0.65a	8.59 ± 0.05c	9.66 ± 0.27a	0.555 ± 0.009a
Catechin	–	–	5.12 ± 0.10b	14.91 ± 0.24b	0.487 ± 0.003c

Means in each column with different subscript letters (a b, c, d, e, f) differ significantly ($P < 0.05$)

Antioxidant Activity

The antioxidant efficacy of Madhuca longifolia extractives was evaluated utilizing various in vitro models, including DPPH and hydroxyl free radical scavenging, as well as total antioxidant activity.

Table 1 shows that the ethyl acetate fraction exhibited the most potent free radical scavenging capacity in the DPPH and hydroxyl radical scavenging assays, evidenced by the lowest IC_{50} value for DPPH (4.81 ± 0.02 µg/mL), surpassing the standard antioxidant catechin (5.12 ± 0.10 µg/mL). It demonstrated significant hydroxyl radical scavenging capability (IC_{50} = 28.18 ± 0.41 µg/mL). The aqueous fraction exhibited remarkable hydroxyl radical scavenging activity (IC_{50} = 9.66 ± 0.27 µg/mL), surpassing all other materials, including catechin (IC_{50} = 14.91 ± 0.24 µg/mL). The petroleum ether fraction had the lowest antioxidant activity in both experiments, as evidenced by its highest IC_{50} values.

The trend identified in the total antioxidant capacity assay corroborates these findings, with the aqueous fraction exhibiting the maximum absorbance (0.555 ± 0.009), indicating greater overall antioxidant capability, succeeded by the ethyl acetate fraction (0.531 ± 0.008). In contrast, the petroleum ether fraction had the lowest antioxidant capacity (0.349 ± 0.006).

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V. CONCLUSION

The results showed that the ethyl acetate and aqueous fractions had the most phenolic and flavonoid chemicals, which were substantially linked to their higher antioxidant activity in all tests. These fractions exhibited exceptional free radical scavenging efficacy, exceeding the conventional catechin in several parameters, hence underscoring its considerable neuroprotective potential. Oxidative stress is a crucial factor in the development and progression of Alzheimer's disease. Madhuca longifolia has strong antioxidant qualities, which makes it a viable natural source of bioactive chemicals that can help protect brain cells from oxidative damage. This work provides a scientific foundation for the traditional utilization of Madhuca longifolia and advocates for its continued investigation in the development of plant-derived medicinal compounds for the management of Alzheimer's disease.

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