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Comparative Impact of Solvent Extracts of *Spondias mombin* Leaves on *In-vitro* Antioxidant and Acetylcholinesterase Inhibitory Activities

Uduenevwo Francis Evuen [a*] and Enyohwo Dennis Kpomah [b]

[a] Department of Biochemistry, College of Natural and Applied Sciences, Western Delta University, P.M.B. 10, Oghara, Delta State, Nigeria.
[b] Department of Biochemistry, Federal University, Otuoke, Bayelsa State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The underlying cause of a number of neurological disorders is oxidative stress. Given the dearth of medications now available to treat such disorders and their accompanying detrimental impacts, an urgent need exists for the global identification of brand-new antioxidants and acetylcholinesterase (AChE) inhibitors. This study evaluated the comparative impacts of the antioxidant and acetylcholinesterase activities of n-hexane, ethyl acetate, and methanol extracts of *S. mombin* leaves. The dried leaf samples of the plant were triturated. Following maceration of the powdered

**Corresponding author: E-mail: evuen.udu@wdu.edu.ng, francdei@yahoo.com;*

plant materials in each of the three extraction solvents (methanol, n-hexane, and ethyl acetate), the resulting solutions were separately subjected to lyophilization. The *in-vitro* antioxidant analysis was determined by employing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and lipid peroxidation (LPO) assays. Acetylcholinesterase (AChE) inhibitory abilities of the various extracts were also evaluated using a standard protocol. The methanol extract showed the greatest DPPH scavenging (75.91%) and AChE inhibitory (40.17%) properties in the DPPH and AChE inhibitory assays, respectively. The DPPH and lipid peroxidation assays also demonstrated that all extracts had dose-dependent antioxidant properties. In addition, evaluations of each extract's ability to inhibit lipid peroxidation revealed that, at initial concentrations of 0.05 mg/L and 0.1 mg/L, the methanol extract displayed stronger LPO inhibitory effects (5.90%, 6.15%) than the n-hexane (4.00%, 5.4%) and ethyl acetate (3.26%, 3.99%) extracts. Nonetheless, the n-hexane extract showed a greater LPO inhibitory effect (10.00%, 10.34%) at higher dosages of 0.2 mg/L and 0.5 mg/L respectively. The results of this study have shown that methanol is the best solvent for exploiting the pharmacological benefits of *S. mombin* leaves thereby reasserting the numerous applications of the plant in traditional medicine. It has also paved the way for the development of novel therapeutic alternatives for a range of neurodegenerative disorders and other health concerns.

Keywords: Acetylcholinesterase inhibitor; natural antioxidant; lipid peroxidation; solvent extracts; neurodegenerative disorders.

1. INTRODUCTION

An increasingly older population is a recipe for the global predominance of age-linked neurological illnesses such as Parkinsonism, Huntington's, and Alzheimer's diseases in this era. Among the neurodegenerative diseases, Alzheimer's disease characterized by disorientation, memory loss, altered cognitive and emotional function, inflammation, amyloid plaque deposition, and activity changes in neurotransmitters, is one of the most prevalent [1]. The concentration of the neurotransmitter, acetylcholine in the synaptic cleft is increased by acetylcholinesterase (AChE) inhibition, improving cholinergic neurotransmission [2]. Consequently, AChE has been the most alluring therapeutic target for the alleviation of the symptoms associated with Alzheimer's disease despite the uncovering of several pathophysiological routes. Furthermore, emerging shreds of evidence indicate that oxidative stress contributes to the development of Alzheimer's disease [3,4].

Conventionally, a coordinated and balanced network of antioxidant systems in the body counteracts the production of excessive free radicals during its regular metabolic activities [5]. However, these defense mechanisms can occasionally be knocked off by different disease conditions [6]. Additionally, Alzheimer's disease has been associated with enhanced levels of DNA and protein oxidation as well as lipid peroxidation in the brain of patients suffering from the disease [7]. In such oxidative stress-induced conditions, supplementation with an exogenous source of antioxidants will be required to boost the body's internal antioxidant system. Accordingly, antioxidants have been demonstrated to mitigate the harmful effects resulting from oxidative stress during the pathogenesis of Alzheimer's disease [8]. As a consequence, a growing understanding exists that a therapeutic component that can modify a pathophysiological process may offer a superior clinical effect. In this regard, plants are established producers of a wide variety of bioactive chemicals that influences both physiologic and biochemical functions in the body [9].

It's interesting to note that a significant portion of the world's population today maintains the practice of traditional medicine that supports the application of plants for the treatment of a variety of maladies [10]. Moreover, to preserve good health and stave off diseases caused by oxidative stress, antioxidants are frequently employed as components in dietary supplements [11]. A wide range of chemical components that scavenge free radicals are found in plants, including endogenous metabolites, carotenoids, flavonoids, anthocyanins, vitamins, etc. Phenolic compounds' ability to quench oxygen-derived free radicals by proton or electron donation coupled with their health-promoting prospects has caused them to gain prominence among the antioxidants present in plants [12,13]. In the hunt for new, safe, and potent remedies for oxidative stress-induced illnesses such as neurodegenerative disorders, natural antioxidants derived from plants such as

Spondias mombin may provide a viable treatment option.

Spondias mombin Linn (Hog plum) belongs to the family, Anacardiaceae. The tree is small and it thrives well in tropical coastal regions in South America, Africa, and Asia. In Africa, it can be found in the Republic of Congo, the Central African Republic, and Nigeria among others. The pinnate leaves bear 5-8 leaflets arranged in opposite pairs, along with one terminal leaflet [14,15]. When crushed, the leaves smell like turpentine [16]. Local names used for the plant in Nigeria include *óghìghèn* in Urhobo, *akikan* in Itsekiri, *óghèéghè, Okighan* in Edo, *Tsáádàr Másàr* in Hausa, *íjíkàrà*, U*vuru* in Igbo, *Iyeye, Ekikan, Olosan* in Yoruba, and *Nsukakara* in Ibibio [17,18].

Spondias mombin has been used as a traditional remedy for a number of ailments. In Nigeria, the leaves have been useful in combating bacterial infections, halting the propagation of viral infections, managing Candida infections, and eliminating parasitic worms. They have been additionally employed to ease nervousness and seizures, treat eye ailments and stomach pain, soothe coughs, enhance digestion, and stimulate the uterus [19]. Some pharmacological properties reported for the leaves of the plant include antioxidant, antimicrobial, antidiarrheal, muscle relaxant, abortifacient, anti-malarial, sedative, antifertility, antipsychotic, and anti-inflammatory [20, 21]. Similarly, Asuquo et al. [22] reported that the administration of aqueous leaf extract of *S. mombin* greatly enhanced the learning and cognitive abilities of Wistar rats. In addition, traditional healers in Nigeria have reportedly utilized preparations of the plant's leaf extracts to improve memory and cure some patients with mental illnesses [23,24]. Furthermore, the cytotoxic attribute of the plant has also been reported [25].

Several kinds of secondary metabolites, such as phenolics, essential oils, alkaloids, saponins, sterols, polysaccharides, triterpenes, amino acids, macro and micro elements are abundant in *Spondias mombin* [14,26]. Moreover, high-performance liquid chromatography-diode array detector (HPLC-DAD) analysis of the leaf extract of *S. mombin* revealed the presence of nine phenolic compounds including Rutin, Chlorogenic acid, Quercetin, Catechin, Epicatechin, Kaempferol, Caffeic acid, Isoquercetin and Ellagic acid [27]. Additionally, it has been observed that various food compositions and categories require different extraction solvents [28]. Thus, finding the most suitable extraction solvent for a given sample type is a necessity. Given the aforementioned, it is vital to reassert the antioxidant and acetylcholinesterase inhibitory properties of various solvent extracts of *S. mombin,* in pursuit of the most promising therapeutic options for neurodegenerative and oxidative stress-induced disorders hence, the present evaluation.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Samples

The leaves of *S. mombin* were harvested in Otuoke town, Ogbia Local Government Area of Bayelsa State, Nigeria. The plant was certified as authentic by the Niger Delta University's herbarium, located on Wilberforce Island in Bayelsa State. A voucher number, NDU P008, was assigned for proper documentation and future reference.

2.2 Sample Preparation and Extraction

After being thoroughly cleaned of earthy materials, the leaves were kept for two weeks at room temperature to dry. Thereafter, they were triturated with the aid of a mechanical grinder. Following maceration of 750 g of the powdered plant material in 2 Litres of each of the three extraction solvents (methanol, n-hexane, and ethyl acetate) for 72 hours, the resulting solutions were then separately subjected to lyophilization to obtain the crude methanol (CME), crude n-hexane extract (CNH), and crude ethyl acetate (CEA) powdered extracts.

2.3 Assessment of Antioxidant Properties and Acetylcholinesterase Inhibitory Activity of *S. mombin* Leaf Extracts

2.3.1 DPPH radical scavenging activity

With a few minor adjustments, the method published by Sabir & Rocha [29] was used to assess the DPPH radical scavenging activity of *S. mombin* leaf extracts. To a 0.5 mL (0.25 mM in methanol) solution of DPPH, various extract concentrations (0.05, 0.1, 0.2, and 0.5 mg/L) were added. The mixture was agitated and allowed to remain at room temperature for 30 min before the absorbance of the mixture was assessed at 517 nm employing the DPPH solution as a blank. The standard, ascorbic acid,

was used in a similar manner at the same progressive concentrations. In the preparation of the control, neither the test sample nor the standard was used, and the absorbance was measured at the specified wavelength. Subsequently, the free radical scavenging activities of the extracts were calculated as follows:

$$\frac{s-t}{s} \times 100$$

Where, *s* = absorbance of control
t = absorbance of test

2.3.2 Lipid peroxidation assay

Using the method of Heath & Packer [30], lipid peroxidation (LPO) inhibition by the various leaf extracts of the plant was assessed. Leaf tissue extract weighing 0.5 g was homogenized in 0.1% (w/v) TCA. The homogenate then underwent a 10-minute centrifugation (15000 g, 40 ^{0}C). Then, the supernatant was recovered and a 0.5% TBA in 20% TCA was added to it. For 25 minutes, the mixture was heated at 95 ^{0}C in a water bath. On cooling, the mixture's absorbance was measured at 532 nm and 600 nm, respectively. The following equation provided the MDA equivalents per 1 cm^2 of tissue:

MDA equivalents (nmol.cm^{-1}) = 1000[(Abs $_{523nm}$ - Abs $_{600nm}$)/155]

2.3.3 Acetylcholinesterase inhibitory assay

According to the modified procedure developed by Rocha et al. [31], the AChE activity of the plant was evaluated. Fifty microlitres (50 µL) of the leaf extract, 100 µL of the 5,5'-dithiol-bis-2-nitrobenzoic acid solution, 200µL of acetylcholinesterase solution, and 500 µL of phosphate buffer (pH 7.0) were included in the reaction mixture. An addition of 100 µL of 0.05 M acetylcholine iodide solution to the reaction mixture initiated the reaction. The mixture was then left to sit at room temperature for 20 minutes. At a wavelength of 412 nm, the UV-Visible spectrophotometer was used to measure the absorbance values of the extracts. Without the test sample or standard, the control was prepared, and absorbance was measured at the specified wavelength. The percentage inhibitory activities of the various extracts were computed as follows:

$$\frac{m-n}{m} \times 100$$

Where, *m* = absorbance of control
n = absorbance of test

2.4 Data Analysis

The Statistical significance was deemed to exist at 95% ($p < 0.05$). Data analysis was conducted with the aid of the Statistical Package for the Social Sciences (SPSS) version 21. The one-way analysis of variance (ANOVA) followed by the Tukey post hoc test was used to analyze the data. Results were expressed as mean ± standard error of the mean.

3. RESULTS

3.1 Effects of Various Solvent Extracts of *S. mombin* Leaves on DPPH Scavenging Activity

The influence of various solvent extracts of *S. mombin* leaves (n-hexane, ethyl acetate, and methanol) on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity are shown in Fig. 1. All extracts showed dose-dependent increments in their inhibitory activities at all concentrations. However, the methanol extract of the leaves showed the greatest inhibition of DPPH at all doses. Concomitantly, the n-hexane extract exhibited the lowest DPPH scavenging activity among the three solvent extracts of the leaf examined.

3.2 Effects of Various Solvent Extracts of *S. mombin* Leaves on Lipid Peroxidation

Fig. 2 reveals the impact of various solvent extracts of *S. mombin* leaves on lipid peroxidation. The methanol extract exhibited stronger LPO inhibitory effects than the n-hexane and ethyl acetate extracts at initial concentrations of 0.05 mg/L and 0.1 mg/L. Contrarily, the n-hexane extract showed a greater LPO inhibitory effect at higher dosages of 0.2 mg/L and 0.5 mg/L. Concurrently, the ethyl acetate extract exhibited the lowest inhibitory effect on LPO at all concentrations of the plant extract.

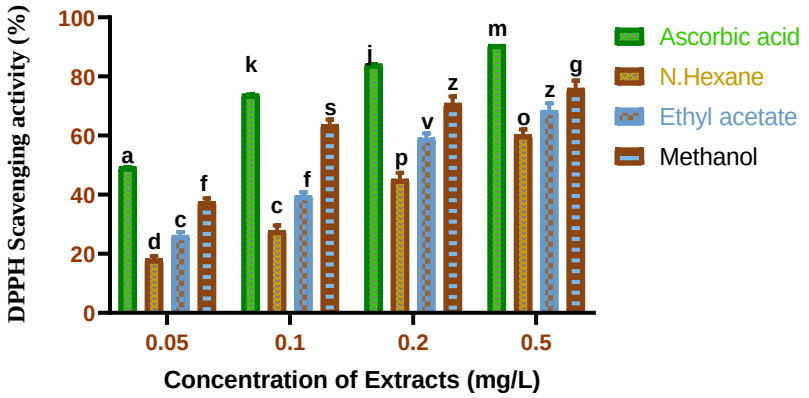

Fig. 1. Influence of various solvent extracts of *S. mombin* leaves on 2.2 diphenyl-1-pycrylhydrazyl (DPPH) scavenging activity. The bars are expressed as the Mean ± SD (standard deviation) of three replications (n=3). Bars marked with different alphabets differ significantly at *p*< 0.05

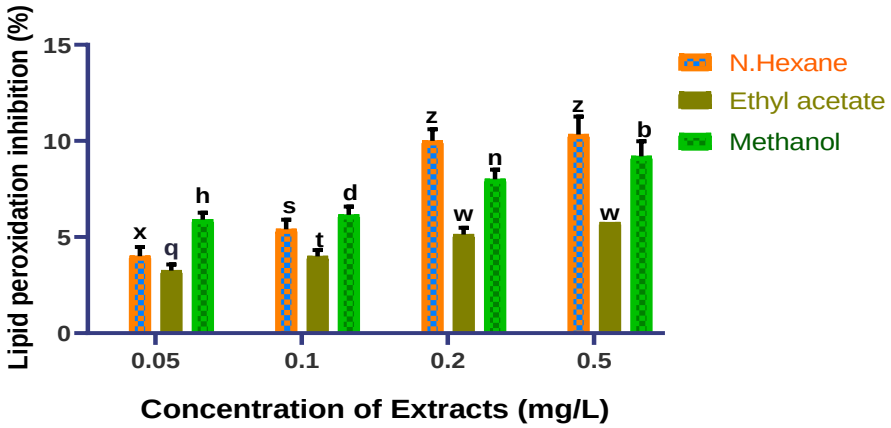

Fig. 2. Influence of various solvent extracts of *S. mombin* leaves on lipid peroxidation. The bars are expressed as the Mean ± SD (standard deviation) of three replications (n=3). Bars marked with different alphabets differ significantly at *p*< 0.05

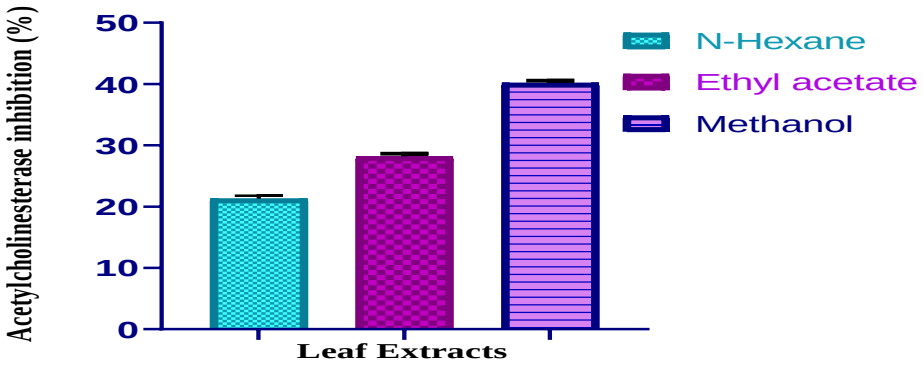

Fig. 3. Influence of various solvent extracts of *S. mombin* leaves on acetylcholinesterase inhibitory activity

3.3 Effects of Solvent Extracts of *S. mombin Leaves* on acetylcholinesterase Inhibitory Activity

The inhibitory capacities of the various crude extracts of *S. mombin* leaves are revealed in Fig. 3. The order of inhibitory ability of the various solvent extracts is as follows:

Methanol > ethyl acetate > n-hexane.

4. DISCUSSION

4.1 Effects of Various Solvent Extracts of *S. mombin* Leaves on DPPH Scavenging Activity

The most crucial phase in the assessment of the antioxidant potency of a plant's components is extraction. Moreover, the quality and quantity of secondary metabolites harnessed in a plant sample are influenced by the type of solvent employed to extract them [32]. Studies on natural antioxidants now frequently use the DPPH test. The fact that this procedure is straightforward and extremely sensitive serves as its justification. A hydrogen donor's role as an antioxidant is the foundation of this test. In addition, DPPH is among the few chemically stable nitrogen radicals that are readily available in commerce [33].

In the DPPH assay, the hydrogen-accepting free radical, DPPH is decreased by an antioxidant. Accordingly, the present investigation revealed that all extracts showed dose-dependent increments in their inhibitory activities at all concentrations. This is an indication of their free-radical scavenging potency. However, the exhibition of the greatest DPPH-scavenging ability by the crude methanol extract shows that the antioxidants present in this extract are stronger than those of its counterparts. This is a piece of corroboratory evidence that the antioxidant capacities of the components in a crude extract may differ depending on the types of reactive species [34]. Furthermore, it has been noted that no one chemical compound has been shown to be capable of reacting with every type of radical [35].

The observed lower antioxidant attribute of the n-hexane extract at 0.05 mg/L and 0.2 mg/L compared with that of the ethyl acetate extract, implies that the antioxidants in the n-hexane extract of the leaf are weak radical-scavengers at the said concentrations. Furthermore, it is interesting to note that extracts generated using polar (methanol) and nonpolar (n-hexane) solvents exhibited a differential ability to react to the DPPH radical. This might be connected to how diverse the extracts from various solvents' secondary chemical profiles are characterized [36]. Accordingly, similar investigations conducted in the past [37,38] have demonstrated that extracting *S. mombin* with polar solvents improved its antioxidant capabilities when compared with reference antioxidant compounds.

The resultant DPPH-scavenging activity observed for the various leaf extracts could possibly be attributed to their polyphenolics constituent. Moreover, previous studies [39,40] have shown that the antioxidant capacity of the extract and/or fractions is directly proportional to their phenolic concentration. Thus, the highest free-radical scavenging activity as seen in the crude methanol leaf extract of the plant may be a function of a maximum concentration of its phenolic constituents.

4.2 Effects of Solvent Extracts of *S. mombin* Leaves on Lipid Peroxidation

Lipid peroxidation and some of its well-known by-products have been connected to a number of diseases. Consequently, minimizing the harmful effects of reactive oxygen species in various clinical circumstances may be achieved by reducing the development of lipid peroxidation products or by scavenging them with antioxidants derived from plants. In addition, contemporary research has uncovered a number of prospective therapeutic agents that have the potential to deter LPO-mediated cell signaling pathways [41,42].

The present study reveals that all leaf extracts exhibited a dose-dependent increment in their lipid peroxidation inhibitory capacities. Moreover, the ability of the methanol and n-hexane extracts to exhibit stronger and weaker LPO inhibitory effects at lower and higher concentrations respectively could also be traced to the increased solubility of the leaf's active components in n-hexane than in methanol at lower concentrations and vice versa. Furthermore, Shetty et al. [43], have reported that plants from the genus Spondias have been found to have high antioxidant activity, which has primarily been linked to their flavonoids and phenol content. Accordingly, Ajaegbu et al. [36]

confirmed that the presence of several established phenolic antioxidant compounds such as gallocatechin, ellagic acid, the derivatives of quercetin, gallic acid, and kaempferol, detected in the leaf extract of *S. mombin* leaves during their investigation, is responsible for its reported antioxidant activity. In the same vein, other notable antioxidant compounds such as Chlorogenic acid, Rutin, Quercetin, Catechin, and Epicatechin have also been discovered in the leaf extract of the plant [27].

It is worth stating that, a large proportion of the polyphenolic compounds detected in the leaf samples of *S.mombin* from the studies mentioned above are flavonoids. Due to their phenolic hydroxyl groups, several flavonoids are discovered to be potent antioxidants that can efficiently scavenge reactive oxygen species. Nevertheless, Fidrianny et al. [44] have reported that a negative correlation (indirect proportionality) existed between the phenolic content of a plant and its antioxidant ability. Though this study did not ascertain the total phenolic and total flavonoid contents of the leaf extracts, the values obtained for the percentage DPPH-Scavenging abilities of the plant's extracts are generally higher than those of their corresponding lipid peroxidation inhibitory capacities. This trend in results is similar to findings from previous studies [45,46].

4.3 Effects of Various Solvent Extracts of *S. mombin* Leaves on Acetylcholinesterase Inhibitory Activity

The cardinal enzyme responsible for the decomposition of acetylcholine, acetylcholinesterase (AChE), is regarded as a viable therapeutic target for the management of neurological illnesses such as Alzheimer's disease, senile dementia, and Parkinson's disease [47]. Moreover, the plethora of plants in the natural world is undoubtedly a potential source of AChE inhibitors. Indeed, several botanicals have historically been employed to improve cognition, and are regarded as vital ingredients for the development of medications for various neurodegenerative disorders today [48]. Thus, the need to assess the capacity of *S. mombin* leaf extracts to impede the usual breakdown of acetylcholine; arresting neurodegeneration cannot be overemphasized.

The current investigation revealed that the n-hexane, ethyl acetate, and methanol crude extracts exhibited varying degrees of acetylcholine inhibitions with the methanol extract displaying the best inhibitory activity. This finding indicates that the extract's impact on an impaired cholinergic system could improve how neural signals are transmitted. This may be achieved by deactivating the constraints placed on the cholinergic system [49]. It could also be inferred that a neuroprotective mechanism was displayed by the said extracts on the cholinergic system. This is an affirmation of the neuroprotective effects reported for the plant [22,27]. Moreover, the acetylcholinesterase inhibitory attribute displayed could be linked to the well-known anticholinesterase compounds, Campesterol, Betulin, and Phytol, isolated from the plant's leaf extract [47].

The report of Darvesh et al. [50] has also shown that flavonoids are part contributors to the neuroprotective effects of plants. This further affirms the identification of several flavonoid compounds in the leaf extracts of this plant by previous authors [27,37,51-53]. Plausibly, the methanol extract of the leaf may have contained a higher proportion of the phenolic compounds reported in the aforesaid studies hence, its highest level of acetylcholinesterase inhibition. The results of this study are in agreement with those of previous authors [23,53,54] who had also reported the anticholinesterase activity of the leaf extract of *S. mombin.*

5. CONCLUSION

This study offers pertinent data that may be useful in the hunt for effective cholinesterase inhibitors and natural antioxidants from plant materials that can be used to treat neurodegenerative and other illnesses occasioned by oxidative stress. The methanol extract demonstrated high levels of cholinesterase inhibitory and free radical scavenging capacities thereby mitigating the detrimental effects caused by free radicals during the pathogenesis of neurodegeneration. This outcome supports the plant's ethnomedicinal usages including improvement of cognition and treatment for psychosis. Consequently, *S.mombin* leaf extracts particularly that of the crude methanol represent a promising pharmacotherapeutic agent that might be further explored for the treatment of Alzheimer's disease and other neurodegenerative conditions that calls for agents capable of fixing cholinergic

impairments. However, further studies on the *in vivo* antioxidant activity, phytotoxicity, and cytotoxicity assessments of the leaf extracts of *S.mombin* are advisable for a diversified usage of the plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Guo T, Zhang D, Zeng Y, Huang TY, Xu H, Zhao Y. Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease. Molecular Neurodegeneration. 2020;15(1):40.
2. Ahammed S, Afrin R, Uddin N, Al-Amin Y, Hasan K, Haque U, Monirul Islam KM, Alam H, Tanaka T, Sadik G.. Acetylcholinesterase Inhibitory and Antioxidant Activity of the Compounds Isolated from *Vanda roxburghii.* Advances in Pharmacological and Pharmaceutical Sciences. 2021;5569054. Available:https://doi.org/10.1155/2021/5569054
3. Butterfield DA, Drake J., Pocernich C, Castegna A. Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid β-peptide.Trends Molecular Medicine. 2001;7:548-554.
4. Tonnies E, Trushina E. Oxidative stress, synaptic dysfunction, and Alzheimer's disease. Journal of Alzheimer's Disease. 2017; 57(4):1105–1121.
5. He L, He T, Farrar S, Linbao J, Liu T, Ma XI.. Antioxidants and Cellular Homeostasis by Elimination of Reactive Oxygen Species. Cell Physiology and Biochemistry. 2017;44:532-553.
6. Hazra B, Biswas S, Mandal N.. Antioxidant and free radical scavenging activity of *Spondias pinnata*. BMC Complementary and Alternative Medicine. 2008;8:63.
7. Lyras L, Cairns NJ, Jenner A, Jenner P, Halliwell B.. An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease. Journal of Neurochemistry. 2002; 68(5):2061–2069.
8. Karim N, Khan H, Khan I, Guo O, Sobarzo-Sánchez E, Rastrelli L, Kamal MA. An Increasing Role of Polyphenols as Novel Therapeutics for Alzheimer's: A Review. Medicinal Chemistry. 2020;16(8): 1007-1021.
9. Evuen UF, Okolie NP, Apiamu A. Evaluation of the mineral composition, phytochemical and proximate constituents of three culinary spices in Nigeria: a comparative study. Scientific Reports. 2022;12:20705.
10. Hlila MB, Omri A, Jannet HB, Lamari A, Aouni M, Selmi B. Phenolic composition, antioxidant and antiacetylcholinesterase activities of the Tunisian *Scabiosa arenaria*, Pharmaceutical Biology. 2013; 51(5):525-532.
11. Evuen UF, Apiamu A, Owuzo PO. Comparative Assessment of the Nutritional and Antioxidant Status of *Euphorbia heterophylla (Euphorbiaceae)* and *Morinda lucida (Rubiaceae)* Plants. Nigerian Journal of Pharmaceutical and Applied Science Research. 2016;5(2):49–57.
12. Evuen UF, Okolie NP. Antioxidant Prospects of Three African Ethnomedicinal Spices. Western Delta University Journal of Natural and Applied Sciences. 2022; 2(1):49-64.
13. Pandey KB, Rizvi SI . Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cell Longevity. 2009;2:270-278.
14. Mondal A, Bhar R, Sinha SN. Ethnomedicinal value and Biological Activities of *Spondias mombin* L- A Concise Review. Asian Research Journal of Current Science. 2021; 3(1): 87-94.
15. Njoku PC, Akumefula MI. Phytochemical and nutrient evaluation of Spondais mombin leaves. Pakistan Journal of Nutrition. 2007;6(6):613–615.
16. Eze EA, Danga SP, Okoye FB. Larvicidal activity of the leaf extracts of Spondias mombin Linn.(Anacardiaceae) from various solvents against malarial, dengue and filarial vector mosquitoes (Diptera: Culicidae). Journal of Vector Borne Diseases. 2014;51(4):300.
17. Aigbokhan EI. Annotated checklist of vascular plants of southern Nigeria, a quick reference guide to the vascular plants of southern Nigeria: a systematic approach. Benin: Uniben Press; 2014.
18. Burkill HM. The useful plants of west tropical Africa, 1985; 1
19. Nusrat AH. Hepatoprotective toxicological assessment of *Spondias mombin* L. (Anacardiaceae) in rodents [dissertation].

Kwame Nkrumah University of Science and Technology. 2010;22-26.

20. Ayoka AO, Akomolafe RO, Iwalewa EO, Akanmu MA, Ukponmwan OE. Sedative, antiepileptic and antipsychotic effects of *Spondias mombin* L. (Anacardiaceae) in mice and rats. Journal of Ethno Pharmacology. 2006;103:166-175.
21. Odoh UE, Nwadimkpa AL. Evaluation of oxytocic and haematological effects of methanol extract of the root bark of *Spondais mombin* Linn (Anacardiaceae). Journal of Pharmacognosy and Phytochemistry. 2020;9(3):41-47.
22. Asuquo OR, Udonwa UN, Eluwa MA, Ekanem TB. Effects of *Spondias mombin* leaf extract on the cytoarchitecture of the cerebral cortex and on learning and memory in Wistar Rats. International Journal of Science and Research. 2013;2(9):5–8.
23. Elufioye TO, Oladele AT, Cyril-Olutayo CM, Agbedahunsi JM, Adesanya SA. Ethnomedicinal study and screening of plants used for memory enhancement and antiaging in Sagamu, Nigeria. European Journal of Medicinal Plants. 2012;2(3): 262–275.
24. Ayoka AO, Akomolafe R, Iwalewa E, Ukponmwan OE. Studies on the anxiolytic effect of *Spondias mombin* L (Anacardiaceae) extracts. African Journal of Traditional, Complementary and Alternative Medicines. 2005;2:153–165.
25. Ibikunle GF, Okwute SK, Ogbadoyi, EO. Cytotoxic Agents from Nigerian Plants: A Case Study of *Spondias Mombin* Linn (Anacardiaceae) Leaves. FUW Trends in Science & Technology Journal. 2017; 2(1b):510 – 513.
26. Kpomah ED, Odokwo EO. Comparative Phytochemical, Proximate, and Some Mineral Composition of the Leaves and Stem Bark of *Spondia mombin* (L. anacardiaceae). Annual Research and Review in Biology. 2020;35(6):90-98.
27. Akinmoladun AC, Adelabu AA, Saliu IO, Adetuyi AR, Olaleye MT. Protective properties of *Spondias mombin* Linn leaves on redox status, cholinergic dysfunction and electrolyte disturbance in cyanide intoxicated rats. 2021;104(2): 1–17.
28. 28. Rababah TM, Banat F, Rababah A, Ereifej K. Yang W. Optimization of extraction conditions of total phenolics, antioxidant activities, and anthocyanin of oregano, thyme, terebinth, and pomegranate. Journal of Food Science. 2010;75:626–632.
29. Sabir SM, Rocha JBT. Water-extractable phytochemicals from *Phyllamthus niruri* exhibit distinct *in-vitro* antioxidant and *in vivo* hepatoprotective activity against paracetamol-induced liver damage in mice. Food Chemistry. 2008;111:845-851.
30. Heath RL, Packer L. Photoperoxidation in Isolated Chloroplasts: I. Kinetics and Stoichiometry of Fatty Acid Peroxidation. Archives of Biochemistry and Biophysics. 1968;125:189-198.
31. Rocha JB, Emanuelli T, Pereira ME. Effects of early undernutrition on kinetic parameters of brain acetylcholinesterase from adult rats. Acta Neurobiologiae Experimentalis. 1993;53:431.
32. Venkatesan T, Choi Y, Kim Y. Impact of Different Extraction Solvents on Phenolic Content and Antioxidant Potential of _*Pinus densiflora*_ Bark Extract. Bio Med Research International. 2019; 14. DOI: 10.1155/2019/3520675.3520675
33. Moon JK, Shibamoto T. Antioxidant Assays for Plant and Food Components. Journal of Agricultural and Food Chemistry. 2009;57:1655-1666.
34. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agriculture and Food Chemistry. 2005; 53(10):4290–4302.
35. Kondo K, Kurihara M, Fukuhara K, Tanaka T, Suzuki T, Miyata N, Toyoda M. Conversion of procyanidin B-type (catechin dimer) to A-type: evidence for abstraction of C-2 hydrogen in catechin during radical oxidation. Tetrahedron Letters. 2000; 41:485–488.
36. Temesgen, S., Sasikumar, JM, Egigu MC. Effect of Extraction Solvents on Total Polyphenolic Content and Antioxidant Capacity of *Syzygium Aromaticum* L. Flower Bud from Ethiopia. Biomedical Research International. 2022;4568944.
37. Ajaegbu EE, Uzochukwu IC, Okoye FBC. Antioxidant and Anti-inflammatory Activities of Extract and Fractions of *Spondias mombin* Leaf and Isolation of its Active Principles. Tropical Journal of Natural Product Research. 2022;6(1): 80-86.
38. Bhandarkar AP, Bhat RA, Vinodraj K, Shetty MS, Shenoy GK. *In-vitro* evaluation

and efficient antioxidant. International Research Journal of Pharmacy. 2015; 6(2):164-168.

39. Sasikumar JM, Erba O, Egigu, MC. *In vitro* antioxidant activity and polyphenolic content of commonly used spices from Ethiopia. *Heliyon,* 2020;6(9):e05027.
40. Soulef S, Seddik, K, Nozha, M, Smain A, Saliha D, Hosni K.. Phytochemical screening and *in vivo* and *in vitro* evaluation antioxidant capacity of *Fargaria ananassa*, *Prunus armeniaca* and *Prunus persica* fruits growing in Algeria. Progress in Nutrition. 2020;22:236–252.
41. Shabalala SC, Johnson R, Basson AK, Ziqubu K, Hlengwa N, Mthembu SXH, Mabhida SE, Mazibuko-Mbeje SE, Hanser S, Cirilli I Tiano L, Dludla PV. Detrimental Effects of Lipid Peroxidation in Type 2 Diabetes: Exploring the Neutralizing Influence of Antioxidants. Antioxidants (Basel). 2022;11(10):2071.
42. Ramana KV, Srivastava S, Singhal SS. Lipid Peroxidation Products in Human Health and Disease. Oxidative Medicine and Cellular Longevity. 2013;583438.
43. Shetty B, Rao G, Abhineetha N, Preethika B, Reddy S. Study of protective action of *spondias pinnata* bark extract on rat liver and kidney against etoposide-induced chemical stress. Pharmacognosy Journal. 2016;8(1):24–27.
44. Fidrianny I, Suhendy H, Insanu M. Correlation of phytochemical content with antioxidant potential of various sweet potato (*Ipomoea batatas*) in West Java, Indonesia. Asian Pacific Journal of Tropical Biomedicine. 2018; 8(1): 25-30.
45. Anokwuru CP, Anyasor GN, Ajibaye O, Fakoya O, Okebugwu P. Effect of extraction solvents on phenolic, flavonoid and antioxidant activities of three Nigerian medicinal plants. Natural Science. 2011;9:53–61.
46. Upadhyay R, Chaurasia JK, Tiwari KN, Singh K. Antioxidant Property of Aerial Parts and Root of *Phyllanthus fraternus* Webster, an Important Medicinal Plant. The Scientific World Journal. 2014;692392.
47. Mukherjee PK, Kumar V, Mal M, Houghton PJ. Acetylcholinesterase inhibitors from plants. Phytomedicine. 2007;14(4):289-300.
48. Elufioye TO, Obuotor EM, Agbedahunsi JM, Adesanya SA. Anticholinesterase constituents from the leaves of *Spondias mombin* L. (Anacardiaceae). Biologics: Targets and Therapy. 2017; 11:107–114.
49. Grantham C, Geerts H. The rationale behind cholinergic drug treatment for dementia related to cerebrospinal disease. Journal of Neuronal Science. 2002; 203:131-136.
50. Darvesh AS, Carroll RT, Bishayee A, Geldenhuys WJ, Van der Schyf CJ. Oxidative stress and Alzheimer's disease: Dietary polyphenols as potential therapeutic agents. Expert Review of Neurotherapeutics. 2010;10:729–745.
51. Akanji OC. Determination of Bioactive constituents of Spondia mombin Leaves by GC-MS Analysis. World Journal of Advanced Research and Reviews. 2020;6(3):149–165.
52. Boadu A, Karpoormath R, Nlooto M. Ethnomedicinal, Phytochemistry and Pharmacological Actions of Leaf Extracts of *Spondias mombin*: A Narrative Review. African Journal of Biomedical Research. 2022;25:1-11
53. Sinan KI, Zengin G, Zheleva-Dimitrova D, Gevrenova R, Picot-Allain MCN, Dall'Acqua S, et al. Exploring the Chemical Profiles and Biological Values of Two Spondias Species (*S. dulcis* and *S. mombin*): Valuable Sources of Bioactive Natural Products. Antioxidants. 2021; 10:1771.
54. Ojo OA, Afon AA, Ojo AB, Ajiboye BO, Okesola MA, Arueleba RT, Adekita TA. Oyinloye BE. *Spondias mombim* L. (Anacardiaceae): Chemical fingerprints, inhibitory activities, and molecular docking on key enzymes relevant to erectile dysfunction and Alzheimer's diseases. Journal of Food Biochemistry. 2019; 43:e12772.

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