

Comparative impact of solvent extracts of *Spondias mombin* leaves on *in vitro* antioxidant and acetylcholinesterase inhibitory activities

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 with the aid of a mechanical grinder. Following maceration of the powdered plant materials in each of the three extraction solvents (methanol, n-hexane, and ethyl acetate), the resulting solutions were then 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, AChEhasbeen 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 (Hogplum) 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, *ìjìkàrà*, *Uyuru* 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]. Moreover, 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 *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°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°C in a water bath. On cooling, the mixture's absorbance was measured at 532nm and 600nm, respectively. The following equation provided the MDA equivalents per 1 cm² of tissue:

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

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 500µL 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 \pm 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, at 0.05 mg/L and 0.2 mg/L, the ethyl acetate leaf extract was higher than that of the n-hexane extract. The opposite effect was observed for the two solvents at 0.1 mg/L and 0.2 mg/L.

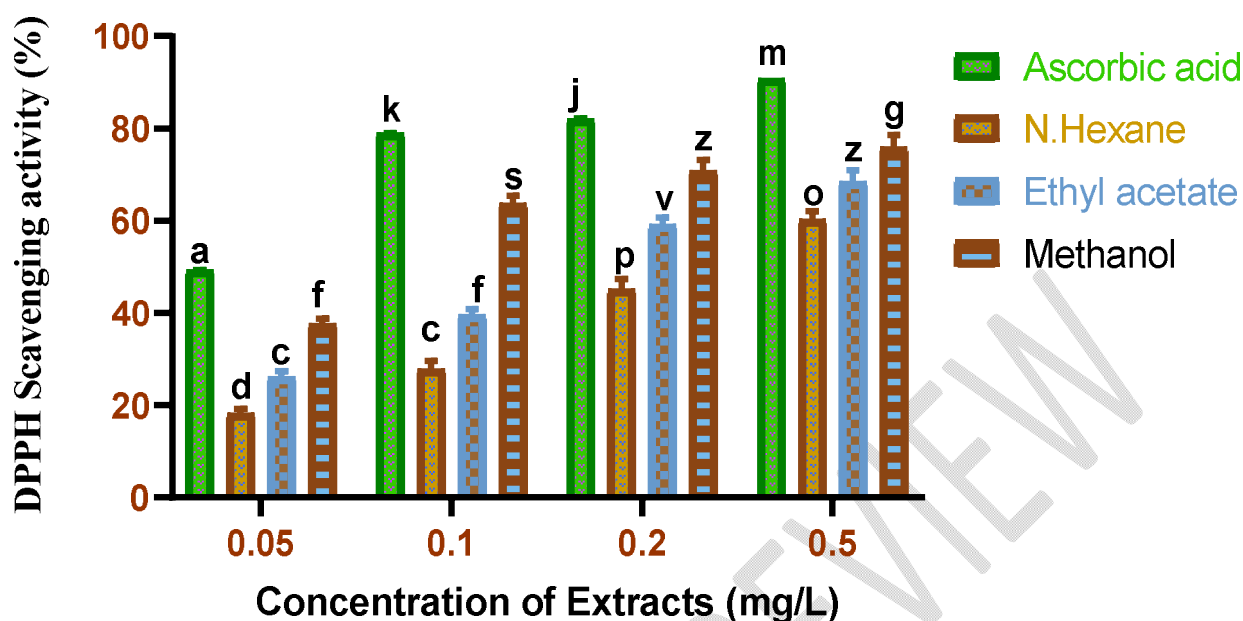


Fig. 1: Influence of various solvent extracts of *S. mombin* leaves on 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. The bars are expressed as the Mean \pm SD (standard deviation) of three replications (n=3). Bars marked with different alphabets differ significantly at $p < 0.05$.

3.2. Effects of various solvent extracts of *S. mombin* leaves on lipid peroxidation

Figure 2 below 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.

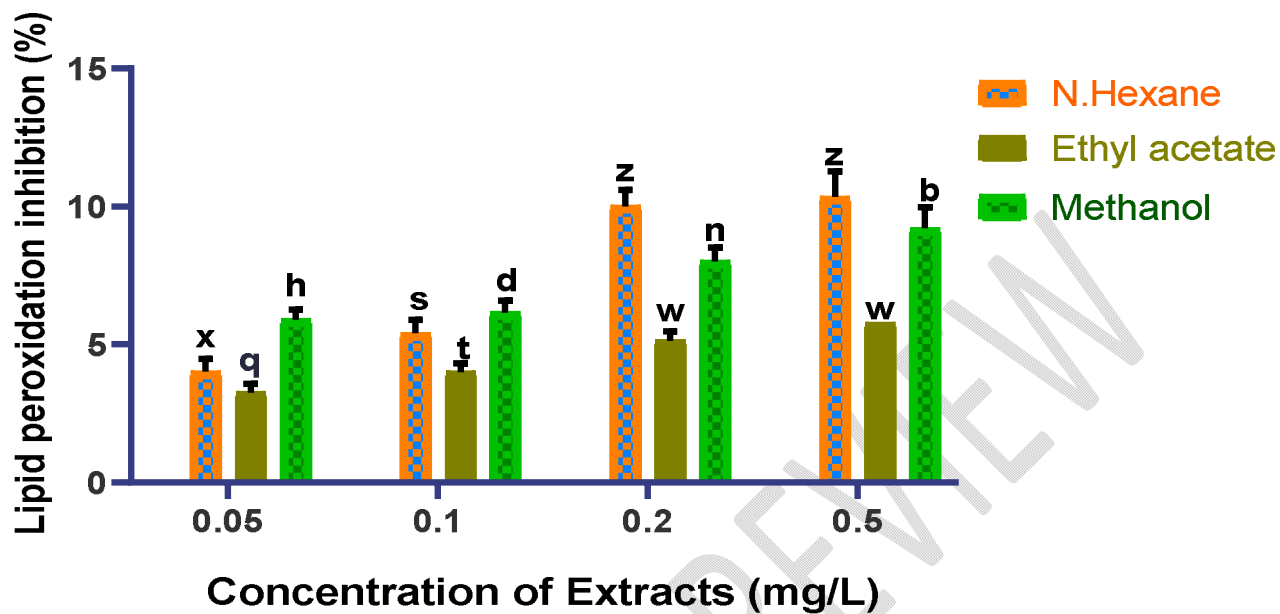


Fig. 2: Influence of various solvent extracts of *S. mombin* leaves on lipid peroxidation. The bars are expressed as the Mean \pm SD (standard deviation) of three replications (n=3). Bars marked with different alphabets differ significantly at $p < 0.05$.

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 below. The order of inhibitory ability of the various solvent extracts is as follows:

methanol > ethyl acetate > n-hexane.

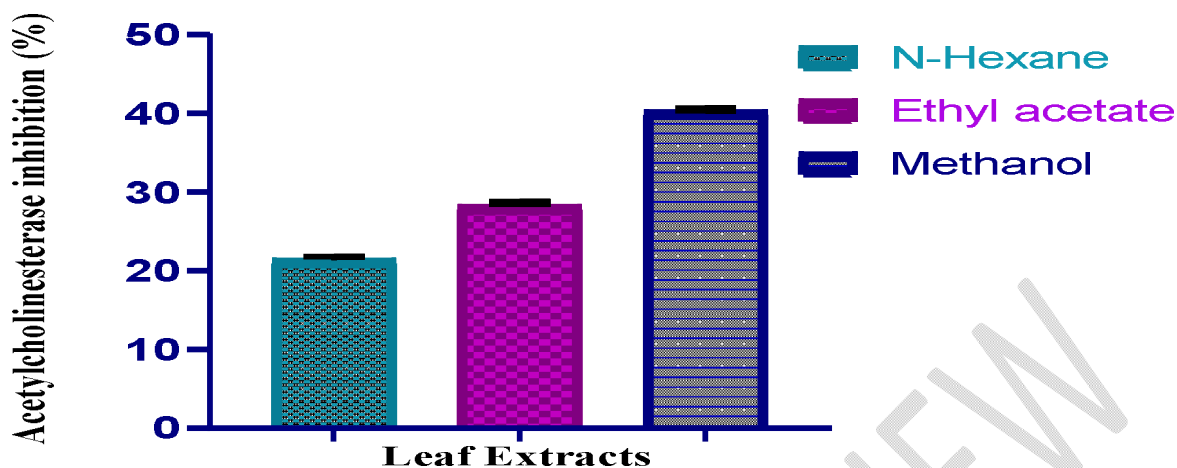


Fig. 3: Influence of various solvent extracts of *S. mombin* leaves on acetylcholinesterase inhibitory activity

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 and require high concentrations to have a measurable impact. 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]. 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 gallic 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 shows 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 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.

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