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Full Length Research Paper

Cholinesterase inhibitory activity of *Morinda lucida*

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Morinda lucida **was found common in recipes given by Traditional Medical Practitioners in South Western Nigeria either as memory enhancer or anti-ageing. The oven dried leaves were extracted with 80% methanol and screened for both acetyl (AChE) and buteryl (BuChE) cholinesterase inhibitory activities. Fractionation of the crude extracts, followed by ethyl acetate bulk extraction, precipitation studies, phytochemical screening and anti-cholinesterase activity of the extracts and fractions are hereby reported. Both the precipitate and supernatant showed different coloured spots with vanillin in H_2SO_4 and were positive for terpenoids, anthraquinones and flavonoids while negative for alkaloids. The methanolic extract inhibited both AChE (40.15% ± 2.5) and BuChE (34.09% ± 1.93), and the activity resided more in the supernatant of the ethyl acetate fraction (82.35% AChE). This correlates with ethno medical claims and thus provides for the first time, the pharmacological basis for the folkloric use of *M. lucida* in the management of memory dysfunction in South Western Nigeria.**

Key words: *Morinda lucida*, cholinesterase, acetyl (AChE), buteryl (BuChE).

INTRODUCTION

In Nigeria, memory loss is considered a major problem in the traditional setting, particularly among the youth. The youth seek memory enhancing remedies to increase their ability to pass examinations. The old however, seek such remedies to alleviate suffering associated with old age; one of the key concerns of older adults is the experience of memory loss especially as it is one of the hallmark symptoms of Alzheimer's disease (AD). Recent research had identified a transitional state between the cognitive changes of normal ageing and AD, known as mild cognitive impairment (MCI). Several studies have indicated that MCI individuals are at an increased risk of developing AD, ranging from 1 to 25% per year. 24% of MCI patients progressed to AD in two years and 20% more over three years whereas, a study indicated that the progressions of MCI subjects was 55% in 4.5 years (Almkvist and Arrnaliz, 2003).

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Studies comparing the effect of ageing on episodic, semantic and short term memory finds that episodic memory is especially impaired in normal ageing (Nilson, 2003). These deficits may be related to impairment seen in the ability to refresh recently processed information (Johnson et al., 2002). In addition, even when equated in memory for a particular item of facts, older adults tend to be worse at remembering the source of their information (Johnson et al., 1993); a deficit that may be related to a decline in the ability to bind information together in memory (Mitchell et al., 2000).

Acetylcholine is critical for an adequately functioning memory and it is the subject of the majority of researches on for treatments for memory defects, like those found in Alzheimer's disease. Any mutual health issue that involves memory or lack of it directly relates to acetylcholine (Greenfield, 1983; Blockland, 1996).

Loss of this neurotransmitter plays an instrumental role in the pathogenesis of AD. Post mortem studies of AD patients consistently have demonstrated the loss of basal fore brain and cortical cholinergic neurons and depletion of choline acetyl transferase, the enzyme responsible for

acetylcholine synthesis (Blockland, 1996; Bartus et al., 1985; Andrews et al., 1994). The degree of this central cholinergic deficit is correlated with the severity of dementia which has led to the "cholinergic hypothesis" of cognitive deficits (Perry et al., 1986).

The cholinergic hypothesis of cognitive hypo function has provided the rationale for the current major therapeutic approach to cognitive dysfunction which holds that the enhancement or restoration of central cholinergic function may significantly improve the cognitive impairments present in cognitive disorders (Francis et al., 1999).

Currently, the only approved therapies for cognitive dysfunction are a group of indirect cholinomimetic which enhance function by inhibiting ACh degradation. Cholinesterase inhibitors (ChEIS), most especially of AChE, however constitute the most effective approach to treat the cognitive symptoms of AD (Schneider, 2000). Plants have been used to treat memory related disorders for centuries (Perry et al., 2000). The use of complementary medicine such as plant extracts in dementia therapy however varies according to the different cultural traditions (Perry et al., 1999). New naturally occurring anti-cholinesterase continues to be identified in a wide variety of plant species (Tang, 1994; Park et al., 1996).

MATERIALS AND METHODS

Collection

Fresh samples of *M. lucida* leaves, stem bark, and root bark were collected in August, 2005 from the Medicinal farm of Obafemi Awolowo University (OAU) Ile-Ife, Nigeria after proper identification by Mr. T. A. Oladele of the Department of Pharmacognosy OAU and authentication by Dr. H. Illoh of the Botany Department OAU. Voucher specimens (IFE 5672) were deposited at the herbarium in Botany Department OAU. The samples were oven dried at 40°C and powdered.

Preliminary (pilot) extraction

One hundred (100 g) each of the powered leaves, stem bark and root bark were macerated with 80% methanol for 72 h with constant shaking using the griffin mechanical shaker. The extract was concentrated *in vacuo* to dryness at 40°C.

Fractionation

The methanolic extract was partitioned into n-hexane, ethyl acetate and water. The various fractions were concentrated *in vacuo* at 40°C. Both the crude extract and the various fractions were tested for AChE and BuChE inhibitory activity.

Bulk extraction and precipitation studies

The leaves of *M. lucida* (1 kg) were extracted with 100% ethyl acetate. The extract was concentrated *in vacuo* to a small volume and lipid constituents were precipitated out by gradual addition of methanol. The precipitates were filtered and weighed. Both supernatant and precipitates were then tested for AChE inhibitory activity.

Anti cholinesterase assay

This was done both spectrophotometrically and using Thin layer chromatography (TLC) bioutographic assay. Acetyl cholinesterase (AChE) and butyryl cholinesterase (BuChE) inhibitions were determined spectrophotmetrically using acetyl thiocholine iodide (ATCHI) and buterylcholine chloride (BuCHI) as substrates, respectively by the modified method of Ellman and others (1961).
The reaction assay mixture consisted of 2000 ml 100 mM phosphate buffer pH 8.0, 100 ml of test sample stock solution in methanol (at a final concentration of 42.5 µg/ml), 100ml of enzyme AChE or BuChE solition at a final concentration of 0.003 and 0.001 µg/ml, respectively. 100 µl of dithionitrobanzoate (DTNB) (0.3 mM) was prepared in 100 M phosphate buffer pH 7.0 containing 120 mM sodium bicarbonate. The reaction mixture was vortexed and then pre-incubated in a water bath at 37°C for 30 min. The reaction was initiated by the addition of 100 µl of ATCI or BTCI at a final concentration of 0.5 mM. As a negative control, the inhibitor solution was replaced with methanol. The change in absorbance at λ_{max} 412 was then measured for a period of 5 min at ambient temperature. All assays were carried out in triplicate. Eserin ((-) physiostigmine) was used as positive control. The percentage inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{a-b}{a} \times 100$$

Where a = ΔA/min of control; b = ΔA/min of test sample; ΔA = change in absorbance. The crude methanolic extract, the various fractions, the ethyl acetate extract, the precipitate and the supernatants were subjected to this test.

The TLC bioatographic assay was carried out according to Rhee et al. (2001a) by spotting the various samples on precoated (G60 PF 254) TLC aluminum plate, followed by development in appropriate solvent system. The developed plates were air dried and first sprayed with 2.55×10^{-3} units/ml of the acetylcholinesterase enzyme until saturated, incubated at 37°C for at least 20 min and then sprayed with 0.5 mM of the substrate (ATCHI or BuCHI) and then DTNB.

RESULTS AND DISCUSSION

Research has correlated the severity of cognitive decline in AD alterations of the cholinergic functions (Mountjoy et al., 1984). This has led to the hypothesis that impaired learning and memory could be ameliorated by the restoration of cholinergic neurotransmission. *M. lucida* was found prominent in herbal preparations used for the traditional management of memory dysfunctions in certain parts of Nigeria. Thus the plant was investigated in this study for its ability to inhibit cholinesterase enzymes, thereby increasing the level of acetylcholine in the brain and ultimately improving cholinergic transmission.

Table 1 shows the percentage cholinesterase inhibition of the methanolic extract of the different plant parts. The leaves methanolic extracts gave 40.15 and 34.09%

Table 1. Percentage cholinesterase inhibition of the different parts of *M. lucida* (methanolic extracts).

Part	% inhibition AChE	% inhibition BuChE
Leaves	40.15±2.57	34.09±1.93
Stem bark	20.34±0.76	2.50±0.06
Root bark	13.20±0.78	0.75±0.02

Table 2. Percentage cholinesterase inhibition of the various leaf fractions.

Fraction	% inhibition AChE	% inhibition BuChE
N hexane	16.13±1.33	13.63±2.60
Ethyl acetate	63.21±0.59	69.08±1.22
Aqueous	28.75±1.87	24.66±1.69

Table 3. Results of precipitation studies.

Sample	% yield	% inhibition AChE
Precipitate	2.89	53.20±1.54
Supernatant	3.86	82.35±1.98

towards AChE and BuChE, respectively. Thus, the active constituents appear to be more concentrated in the leaves than any other parts of the plant. This will make further research on the plant very convenient without the risk of causing injury to the plant, since leaves are generally the most regenerative part of a plant.

The leaf extract was partitioned into different solvents and assayed for both AChE and BuChE inhibitory activity as reported in Table 2. It was observed that on partitioning, the active constituent was most extractible in the ethyl acetate fraction, giving a percentage inhibition of 63.21% (AChE) and 69.08% (BuChE). Further partial purification by precipitating the ethyl acetate fraction with methanol showed that the supernatant was more active (82.35% AChE) than the precipitate (53.20% AChE). This shows that the activity of the extract increases with purification, thus giving a potential hope for the isolation of the active principle.

Both precipitate and supernatant were also subjected to qualitative AChE and BuChE inhibitory activity. Figures 1 and 2 shows the TLC bioautographic AChE and BuChE inhibitory results, respectively of the precipitate and supernatant. White spots on yellow background is indicative of positive activity. The activity seems to be more pronounced in the supernatant when compared with the precipitate (Table 3, Figures 1 and 2).

Previous studies have shown that *M. lucida* possess pharmacological activities like antitumor, antibacterial and antifungi (Anke et al., 1980), antimalaria (Obih et al., 1985), molluscicidal (Adewumi and Adesogan, 1983),

Figure 1. TLC bioautographic assay of precipitate and supernatant (AChE). Where 2 = *Morinda lucida*; a = precipitate; b = supernatant. Solvent system: N-hexane - chloroform (3:7).

Figure 2. TLC bioautographic assay of precipitate and supernatant (BuChE). Where 2 = *Morinda lucida*; a = precipitate; b = supertatant. Solvent system: N-hexane - chloroform (3:7).

tranquilizing and diuretic (Ohi, 1986). The current study indicates that the leaves of *M. lucida* inhibits both acetylcholnesterase and butyrylcholinesterase, thus having the potential to enhance cholinergic function and may have a role in ameliorating cholinergic memory impairment. The study also showed that purification increases the activity of the extract and thus presents the possibility for the isolation of the active principles.

Conclusion

This study has justified the inclusion of *M. lucida* in recipes used by traditional medical practitioners in South Western Nigeria for the management of memory dysfunctions.

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