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Evaluation of Antioxidant and Acteylcholinesterase-inhibitory Properties of Methanol Extracts of *Nauclealatifolia*, *Cymbopogon citratus* and *Cocos nucifera*: An *In vitro* Study

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Authors' contributions

This work was carried out in collaboration between all authors. Author OAA designed the study, supervised and write-up the manuscript. Author FAA carried-out the experimentation and analysis of samples.

Original Research Article

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ABSTRACT

Aims: To evaluate the *in vitro* antioxidant and acetylcholinesterase (AChE)-inhibitory potentials of methanol extracts of *Nauclea latifolia* (NL), *Cymbopogon citratus* (CC) and *Cocos nucifera* (CN).
Study Design/Methodologies: The antioxidant and AChE- inhibition activities were evaluated using standard *in vitro* methods viz; DPPH (2,2-diphenyl-1-picrylhydrazine), nitric oxide ($NO^{\cdot}$), hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) radical scavenging assays as well as reducing power, Fe^{2+}/ascorbate-induced lipid peroxidation (LPO) and AChE inhibition assays.
Place and Duration of the Study: The study and analyses were carried out at the Department of Biochemistry, University of Ibadan between March and June 2012.
Results: Extract of NL has the highesttotal phenol and flavonoids contents. The antioxidant activities of the extracts followed the order; CN> NL> CC. Based on DPPH radical scavenging, extract of CN was the most effective. The DPPH scavenging potential of CN, NL and CC were 88%, 82% and 76%, respectively relative to catechin

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(standard) (91%). The IC_{50} for the scavenging of hydroxyl radicals by CN, CC and NL were 145.3, 148.8 and 162.3 µg/mL, respectively while catechin is 178.6µg/mL. The reducing powers of CN and NL were statistically similar to catechin. At100 µg/mL, extracts of CN, CC and NL inhibited hepatic LPO by 41%, 22% and 29% respectively. Importantly, extract of CN significantly ($p<0.05$) inhibited brain LPO and promotes $NO^{\cdot}$ scavenging by 48% and 23%, respectively. Also, CN at 250 and 500 µg/mL significantly ($p<0.05$) inhibited AChE activities by 33% and 75%, respectively.

Conclusion: CC, NL and CN exhibited strong antioxidant activities but only CN has significant AChE-inhibitory potential.

Keywords: Acteylcholinesterase; antioxidant; free radicals; lipid peroxidation.

1. INTRODUCTION

Oxidative stress is the imbalance between cellular production of reactive oxygen species (ROS) and ability of cells to scavenge them. ROS encompasses a variety of partially reduced metabolites of oxygen which can be classified into oxygen-derived free radicals and oxygen-centered non-radicals [1]. A free radical is any chemical species (not necessarily derived from oxygen), capable of independent existence and which contains one or more unpaired electrons. This unpaired electron(s) usually gives a considerabledegree of reactivity to the species. Radicalsderived from oxygen represent the most important class of radical species generated in living systems [2]. ROS cause damage to cellular components including lipids, proteins and nucleic acids, leading to subsequent cellular death [3]. Oxidative stress disorders caused by the actions of ROS are linked with many diseases such as neurodegenerative conditions like Alzheimer's disease [4]. Alzheimer's disease (AD) is an age-related neurological disorder characterized by progressive memory loss which may lead to dementia. The main treatment strategy of AD is to maintain adequate levels of acetylcholine (ACh) at neurotransmission sites [5]. Thus, the inhibition of acetylcholinesterase (AChE), an enzyme that breakdown acetylcholine, prevents the hydrolysis of ACh thereby maintaining normal memory function [6]. Epidemiological survey provides a direct correlation between lower incidence of AD and high consumption of dietary antioxidants [7]. Natural compounds with high levels of antioxidants have been proposed as an effective therapeutic approach for AD [7]. Therefore, the search for natural antioxidants with AChE-inhibitory activity brought about this study.

Nauclea latifolia belongs to the Rubiacea family. It is also called *Sarcocephalus latifolus*. In Nigeria, the leaf extract could be used as an anthelmintic agent in farm animals [8]. The fruit contains adequate amounts of vitamins A, B, B2, C and E and the phytochemical elements in the plant include oxalate, phytate, saponins, tannins and alkaloids [8]. Biochemical evaluation indicates that it has anti-diabetic, anti-hypertensive, anti-palpitation, anti-convulsant, anti-depressant and anti-abortifient properties [9-11].

Cocos nucifera L belongs to the family of the Arecaceae (Palmae). It is cultivated for its multiple utilities, mainly for its nutritional and medicinal values. It is a source of various natural products for the development of medicines against various diseases and also for the development of industrial products. Its fruit like coconut kernel and tender coconut water have numerous medicinal properties such as antibacterial, antifungal, antiviral, anti-dermatophytic, antioxidant, hypoglycemic, hepatoprotective and immune-stimulant [12-15].

Cymbopogon citrates stapf, known as lemon grass or oil grass, is a widely used herb in tropical countries, especially in Southeast Asia. Its fragrant leaves are traditionally used in cooking, particularly for lechon and roasted chicken [16]. This plant contains phyto-constituents such as flavonoids and phenolic compounds [17]. Studies indicate that the plant possesses anti-amoebic, antibacterial, antidiarrheal, antifilarial, antifungal, anti-inflammatory and other properties [18-23]. However, there is a dearth of information on the AChE-inhibitory activities of these plants. Therefore, this study was designed to evaluate the antioxidants and AchE-inhibitory activities of the plants.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Leaves of *Nauclea latifolia* and *Cymbopogon citratus* were collected from University of Ibadan Botanical garden while *Cocos nucifera* husk fiber was bought from Ojoo market in Ibadan, Nigeria. These were identified at herbarium in the Department of Botany, University of Ibadan where voucher specimens already exist (NL= UI 00813, CC= UI 00306, CN= UI 09876). Plant materials were washed separately with tap water to remove dirty materials and were air dried in the laboratory for several weeks. The dried materials were ground into coarse powder.

2.2 Preparation of Extracts

About 1 kg of each powdered plant materials was soaked with 2.5 L of methanol for 96 ‘h’with occasional stirring for cold extraction. The extracts were filtered through muslin cloth and evaporated to dryness at 40ºC with rotary evaporator. The yields of NL, CC and CN were 4.7%, 5.8% and 6.2%, respectively. The dried extracts were stored in an air-tight container inside refrigerator until use.

2.3 Experimental Animals

Male Wistar rats (12 weeks old) weighing between 180 - 200g were obtained from Animal House of the Faculty of Basic Medical Sciences, University of Ibadan, Nigeria. They were housed in steel metal cages and fed on rodent pellets and given drinking water *ad libitum*. The rats were allowed to acclimatise for seven days before the commencement of the experiment and kept at 12 h light/ dark cycle and temperature of 29+2ºC. The animals were fasted 24 h before they were sacrificed by cervical dislocation and dissected to obtain the liver and brain used in this study. The Faculty of Basic Medical Sciences, University of Ibadan Animal Ethics Committee approved this study.

2.4 Chemicals and Reagents

2,2–diphenyl-1-picryhydrazyl (DPPH), 2-deoxy-D-ribose, Folin- Ciocalteu–reagent, Catechin, 2-thiobarbituric acid (TBA), Ethylenediaminetetraacetic acid (EDTA), Trichloroacetic acid (TCA), Ascorbic acid, Ferrous Ammonium Sulphate, Hydrochloric acid, Sodium nitroprusside, Sulfanilamide and naphthylene diamine dihydrochloride were purchased from Sigma Chemical Co., Saint Louis, MO USA. Phosphoric acid, Sodium hydroxide, Aluminium chloride, Sodium nitrite and Potassium ferricyanide were procured from British Drug House (BDH) Chemical Ltd., Poole, UK. Other chemicals were of analytical grade and purest quality available.

2.5 Biochemical Analysis

2.5.1 Total phenolic content

The total phenolic content of the extracts were determined using the method of Singleton and Rossi [24] with slight modifications. Briefly, 1 mL of Folin C reagent was added to 1 mL of sample. After 3 min, 1 mL of 15% Na_2CO_3 was added and the solution was made up to 5 mL with distilled water. The reaction mixture was placed in a water bath at 40ºC for 20 min.The absorbance was measured with a spectrophotometer at 760 nm while catechin served as standard to prepare calibration curve.

2.5.2 Total flavonoids content

The total flavonoids content was determined with a colorimetric method described by Jia et al [25] and Zucca et al. [40]. Briefly, between 10-1000 µg of the extract in 1 mL of distilled water was added to75 µl of 5% $NaNO_2$. After 5 min, 150 µl of 10% $AlCl_3.6H_2O$ was added, followed by 500 µl of 1 M NaOH and 275 µl of distilled water. The solution was properly mixed and the colour intensity of the mixture read at 510 nm after 15 min while catechin served as standard to prepare calibration curve.

2.5.3 Reducing power assay

This was determined according the method of Oyaizu [26]. The extract or standard (100 µg/mL) was mixed with phosphate buffer (pH 6.6) and potassium ferricyanide. The mixture was incubated at 50ºC for 20 min and trichloroacetic acid (10%, 2.5 mL) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1%, 0.5 mL) and the absorbance was measured at 700 nm in a spectrophotometer. Higherabsorbance of the reaction mixture indicated reductive potential of the extract.

2.5.4 DPPH radical scavenging assay

The DPPH radical scavenging activity of plant extracts were measured as described by Mensor et al. [27]. A portion (1 mL) each of the different concentrations (40-2500 µg/ mL) of the extracts or standard in test tubes was added to 1 mL of 1 mM DPPH in methanol. The mixtures were vortexed and incubated in a dark chamber for 30 min after which the absorbance was measured at 517 nm against a DPPH control containing only 1 mL of methanol in place of the extract.
The inhibition of DPPH was calculated as a percentage using the expression:

$$\% I = \frac{A_{control} - A_{sample} \times 100}{A_{control}}$$

Where % I is the percentage inhibition of the DPPH radical; $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the test compound.

2.5.5 Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of extracts was carried out by the methods described by Halliwell [28]. The assay was performed by adding 0.1 mL of EDTA, 0.01 mL of $FeCl_3$, 0.1 mL of H_2O_2, 0.36 mL of deoxyribose, 1.0 mL of plant extract (10-1000 µg/mL), 0.33 mL of phosphate buffer (50mM, pH 7.4) and 0.1 mL of ascorbic acid in sequence. The

mixture was then incubated at 37ºC for 1 h. About 1.0 mL of 10% TCA and 1.0 mL of 0.5% TBA were added to develop the pink chromogen which was measured at 532 nm in a spectrophotometer.

$$\text{Hydroxyl Radical Scavenging Activity (\%)} = \frac{\text{Acontrol} - \text{Asample} \times 100}{\text{Acontrol}}$$

Where Acontrol is the absorbance of control and Asample is the absorbance of sample solution.

2.5.6 Scavenging of hydrogen peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined according to the methods of Nabaviet al. [29,30]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extracts (0.1-1 mg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by the extracts and standard was calculated as follows:

$$\%\ \text{Scavenged}\ [H_2O_2] = \frac{Ao - A1 \times 100}{Ao}$$

Where Ao was the absorbance of the control and A1 was the absorbance in the presence of the extracts or standard.

2.5.7 Nitric oxide radical scavenging assay

The scavenging effect of extract on nitric oxide radical was measured according to the method of Ebrahimzadeh et al. [31]. Briefly, 1 mL of sodium nitroprusside (5 mM) in phosphate buffered saline (PBS) was mixed with different concentrations of extracts and distilled water. This was incubated at room temperature for 150 min after which 0.5 mL of Griess reagent was added. The absorbance of the pink chromophore formed was read at 546 nm in a spectrophotometer. Catechin was used as positive control.
The percentage inhibition was calculated as:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2.5.8 Lipid peroxidation inhibition assay

The lipid peroxidation inhibition assay was determined according to the method described by Liu et al. [32]. The reaction mixtures contained 0.2 mL of rat liver or brain homogenate in varying concentration of 30 mMtris-buffer, 0.38 mL of 0.16 mM ferrous ammonium sulphate, 0.06 mL ascorbic acid and different concentration of the extracts (10-1000µg) and were incubated for 1 hat 37ºC. The resulting thiobarbituric acid reacting substance (TBARS) formed was measured as followed; briefly, an aliquot (0.4 mL) of the reaction mixture was mixed with 1.6 mL of 0.15 M Tris-KCl buffer and 0.5 mL of 30% TCA (to stop the reaction), and placed in a water bath for 45 min at 80ºC. After which it was cooled in ice and

centrifuged at room temperature for 15 min at 3000 rpm to remove precipitates. The absorbance of the clear pink coloured supernatant was measured against blank at 532 nm.

2.5.9 Acetylcholinesterase-inhibitory assay

Acetylcholinesterase inhibitory activity of the extracts was determined using the method of Ellman et al. [33]. Aliquots of homogenates from rat brain were taken and used to measure acetyl cholinesterase activity, a marker for cholinergic neurotransmission. Briefly, acetylcholinesterase activity in the homogenate was measured by adding 2.6 mL of phosphate buffer (0.1 M, pH 7.4), 0.1 mL of 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) and 0.4 mL of the homogenate. Then 0.1 mL of acetylthiocholine iodide solution was added to the reaction mixture. The absorbance was read using a spectrophotometer at 412 nm and, the changes in absorbance for 10 min at 2 min interval was recorded.

2.6 Statistical Analysis

Experimental results were expressed as Mean ± Standard Deviation (SD). All measurements were replicated four times. The results were analyzed using one – way analysis of variance (ANOVA). The level of significance used was P=0.05.

3. RESULTS

3.1 Reducing Power, Total Flavonoids and Phenolic Contents

In table 1, total phenolic content of NL, CC and CN increased with increase in concentration of extracts. At 1000 µg/ml, total phenolic contents of NL, CC and CN were 1.83±0.02, 0.95±0.03 and 1.86±0.01 (µgCatechin Equivalent per mg dry weight) relative to catechin (2.21±0.01). Similarly, the flavonoids content of the extracts was concentration dependent. Extract of NL had the highest amount of flavonoids at 1000 µg/ml (1.18±0.07) followed by CN (0.72±0.01) while CC has the lowest (0.57±0.04) when compared with catechin (2.08±0.01) (Table 2). In table3, the reducing power increased with increase in concentration of extracts in the following order; CN>NL>CC.

Table 1. Total phenolic content of extracts of *Nauclea latifolia, Cymbopogon citrtus* and *Cocos nucifera In vitro*

CONC (µg/ml)	Total Phenolic Content (CE µg/ mg)		
	NL	CC	CN
10	0.12±0.01*	0.07±0.01*	0.09±0.02
50	0.22±0.02*	0.11±0.01*	0.24±0.01*
100	0.37±0.01*	0.17±0.01*	0.34±0.02*
250	0.73±0.03*	0.53±0.01*	0.77±0.02*
500	1.21±0.04*	0.32±0.01*	1.24±0.05*
750	1.58±0.01*	0.73±0.03*	1.59±0.05*
1000	1.83±0.02*	0.95±0.03*	1.86±0.01*

*Data are expressed as mean ± SD (n=4), * Dose dependent and significantly increase from 10 µg/ ml (P =0.05), NL = Nauclea latifolia, CC = Cymbopogon citratus, CN = Cocos nucifera, CE= Catechin equivalent*

Table 2. Total flavonoids content of extractsof *Nauclea latifolia, Cymbopogon citrtus* and *Cocos nucifera In vitro*

CONC (µg/ml)	Total Flavonoids Content (CE µg/ mg)		
	NL	CC	CN
10	0.01±0.00	0.00±0.00	0.02±0.00
50	0.06±0.01*	0.02±0.00*	0.08±0.01*
100	0.13±0.01*	0.05±0.01*	0.14±0.02*
250	0.28±0.03*	0.14±0.00*	0.28±0.02*
500	0.68±0.01*	0.30±0.04*	0.64±0.04*
750	0.92±0.07*	0.37±0.05*	0.74±0.03*
1000	1.18±0.07*	0.57±0.04*	0.72±0.03*

*Data are expressed as mean ± SD (n=4), * Dose dependent and significant increase from 10 µg/ml (P=0.05), NL=Nauclea latifolia, CC=Cymbopogon citratus, CN=Cocos nucifera. CE=Catechin equivalent*

Table 3. The reducing abiliity of *Nauclea latifolia, Cymbopogon citrtus, Cocos nucifera* extracts *In vitro*

CONC. (µg/ml)	CATECHIN	NL	CC	CN
Control	0.03±0.01	0.03±0.01	0.03±0.01	0.29±0.02
10	0.17±0.00	0.07±0.03	0.03±0.01	0.32±0.03
50	0.44±0.07*	0.11±0.01	0.04±0.00	0.40±0.81
100	0.82±0.01*	0.17±0.01*	0.05±0.01	0.48±0.01
250	1.69±0.05*	0.69±0.06*	0.07±0.01*	0.61±0.01*
500	2.21±0.05*	0.68±0.03*	0.13±0.01*	1.09±0.04*
750	2.22±0.01*	1.02±0.06*	0.21±0.01*	1.41±0.03*
1000	2.22±0.03*	1.19±0.14*	0.37±0.02*	1.72±0.02*

*Data are expressed as mean ± SD (n=4), * Significantly different from control and 10 µg/ml (p<0.05), NL=Nauclea latifolia, CC=Cymbopogon citratus, CN=Cocos nucifera.*

3.2 DPPH, Hydrogen Peroxide and Hydroxyl Radicals Scavenging Activities

Extract of CNhas the highest scavenging activity against DPPH (45.1±1.53%) followed by NL (43.6±4.22%) and CC (39.3±0.57%) at 750 µg/ml (Table 4). Fig. 1 showed the $OH^{\cdot}$ radical scavenging activities of CN, NL and CC. CN has the highest $OH^{\cdot}$ radical scavenging activity (87.33±0.61%) while catechin has 85.20±0.26%. All the extracts showed $OH^{\cdot}$ radical scavengingactivity in a dose- dependent manner at concentrations 10 - 1000 µg/ml in order CN> CC> NL. The extracts exhibited very low H_2O_2 scavenging effect (Fig. 2). The H_2O_2 scavenging effect followed the order NL> CN> CC at 1000 µg/ml.

3.3 Nitric Oxide Scavenging and Fe^{2+}/Ascorbate-induced Lipid Peroxidation in Brain and Liver Homogenates

The percentage inhibition of lipid peroxidation (LPO) in rat liver for three extracts increased with increase in concentration. The percentage inhibition of hepatic LPO for CN, CC and NL were 55.6%, 26.5% and 24.7%, respectively while catechin was 61.8% at 250 µg/ml (Table 5). There were dose-dependent increases in percentage inhibition of LPO in rat brain. At 250 µg/ml, the percentage inhibition of rat brain LPO for CN, CC and NL were 48.2%, 29.4%

and 37.0%, respectively while catechin was 43.4% (Table 6). In Table 7, only CN (Out of the three extracts) produced appreciable scavenging activity against nitric oxide radical.

Table 4. The DPPH scavenging activities of extracts from *Nauclea latifolia, Cymbopogon citrtus and Cocos nucifera In vitro*

CONC. (µg/ml)	CATECHIN	DPPH SCAVENGING ACTIVITY (%)		
		NL	CC	CN
Control	**0.000**	**0.000**	**0.000**	**0.000**
40	32.2±0.46*	0.62±0.68*	5.17±0.36*	17.3±2.40*
100	40.0±5.37*	11.01±2.21*	7.26±0.16*	27.1±1.47*
250	31.7±0.27*	35.13±3.42*	11.5±0.59*	40.8±4.86*
500	40.8±6.31*	56.7±9.13*	24.4±0.70*	39.9±1.55*
750	53.5±3.62*	43.6±4.22*	39.3±0.57*	45.1±1.53*
1000	88.0±2.80*	88.7±1.04*	56.4±8.84*	40.7±1.16*
1500	93.8±0.40*	88.1±0.08*	80.3±0.17*	64.1±2.60*
2000	91.8±0.14*	83.9±0.75*	77.9±0.42*	88.7±0.26*
2500	91.3±0.34*	82.4±0.49*	76.0±0.66*	88.1±0.18*

*Data are expressed as mean ± SD (n=4), * Significantly different from control (P=0.05), NL=Nauclea latifolia, CC=Cymbopogon citratus, CN=Cocos nucifera.*

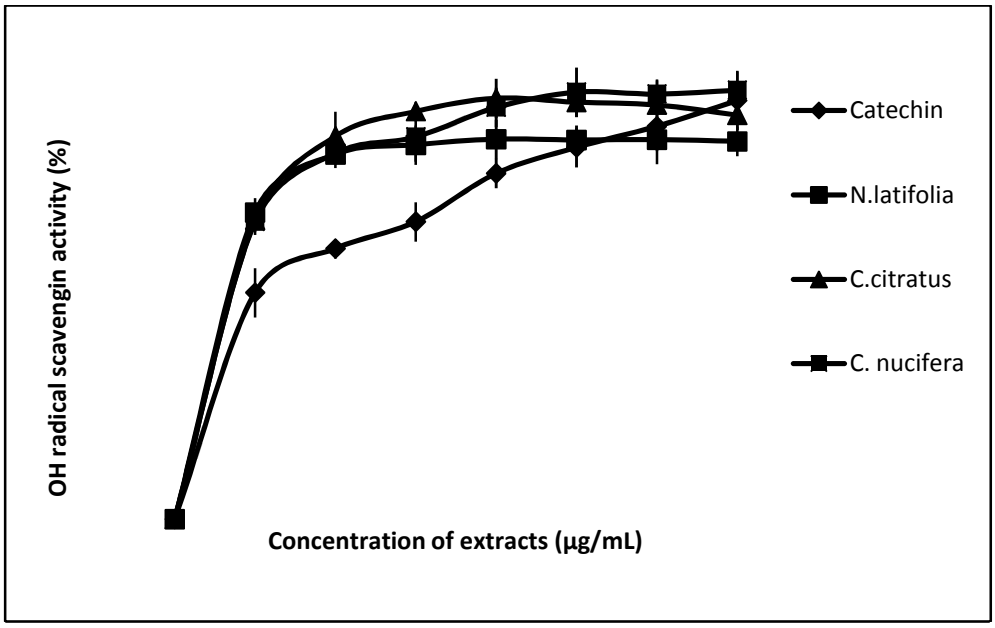

Fig. 1. The hydroxyl radical scavenging activity of extracts of *Nauclea latifolia, Cymbopogon citratus* and *Coco snucifera In vitro*

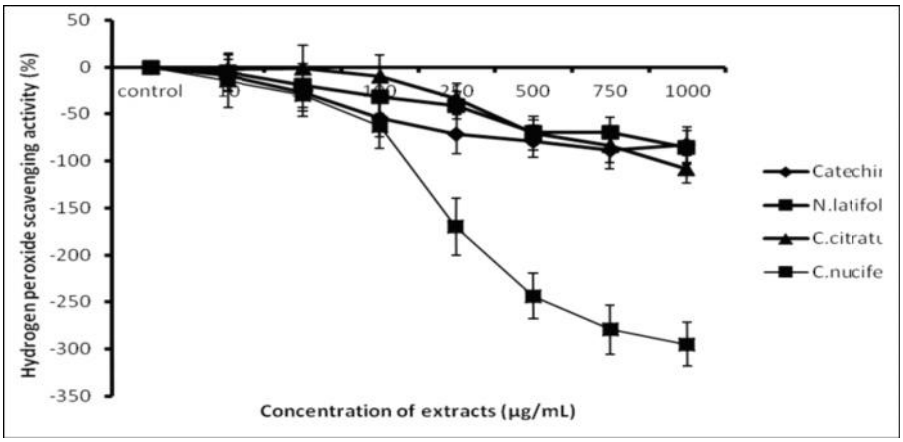

Fig. 2. The H_2O_2 scavenging activity of extracts of *Nauclea latifolia*, *Cymbopogon citrates* and *Cocos nucifera Iv vitro*

Table 5. Inhibition of fe^{2+}/ascorbate-induced lipid peroxidation in the liver by extracts of *Nauclea latifolia, Cymbopogon citrtus and Cocos nucifera In vitro*

CONC. (µg/ml)	CATECHIN	% INHIBITION		
		NL	CC	CN
Control	**0.0±0.00**	**0.0±0.0**	**0.0±0.00**	**0.0±0.00**
10	34.1±3.78*	22.4±4.40*	10.8±4.59*	27.2±27.4*
50	50.4±3.58*	23.4±2.83*	12.1±2.21*	39.3±22.7*
100	51.2±2.95*	28.8±0.23*	22.2±15.6*	41.3±15.2*
250	61.8±7.57*	24.7±1.78*	26.5±0.30*	55.6±45.5*

*Data are expressed as mean ± SD (n=4), * Dose-dependent and significant increase from control (P=0.05), NL=Nauclea latifolia, CC=Cymbopogon citratus, CN=Cocos nucifera.*

Table 6. Inhibition of Fe^{2+}/ascorbate-induced lipid peroxidation In the brain by extracts of *Nauclea latifolia, Cymbopogon citrtus and Cocos nucifera In vitro*

CONC. (µg/ml)	CATECHIN	% INHIBITION		
		NL	CC	CN
Control	**0.0±0.00**	**0.0±0.00**	**0.0±0.00**	**0.0±0.00**
10	6.5±1.17*	15.4±1.05*	10.4±1.05*	10.6±1.89*
50	7.4±0.50*	19.2±3.51*	18.2±3.37*	23.2±2.05*
100	29.0±1.12*	26.3±3.30*	24.2±1.46*	25.7±2.31*
250	43.4±20.3*	37.0±3.71*	29.4±3.49*	48.2±3.57*

*Data are expressed as mean ± SD (n=4), * Dose-dependent and significant increase from control (P=0.05), NL=Nauclea latifolia, CC=Cymbopogon citratus, CN=Cocos nucifera*

Table 7. The scavenging activity of extracts Of *Nauclea latifolia, Cymbopogon citrtus* and *Cocos nucifera*on Nitric oxide Radical *In Vitro*

CONC. (µg/ml)	CATECHIN	SCAVENGING ACTIVITY (%)		
		NL	CC	CN
Control	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
10	7.75±1.75*	1.19±1.96	2.7±0.35	16.3±0.35*
50	10.4±2.57*	2.14±4.47	2.3±0.18	23.8±1.55*
100	7.84±0.18*	4.25±2.62	1.2±0.07	19.9±0.81*
250	7.92±0.79*	4.63±4.12	1.1±0.05	22.7±2.55*

*Data are expressed as mean ± SD (n=4), * Significantly different from control (P =0.05), NL = Nauclea latifolia, CC = Cymbopogon citratus, CN = Cocos nucifera*

Table 8. Effect of extracts of *Nauclea latifolia, Cymbopogon citrtus* and *Cocos nucifera*on the activities of brain acetylcholinesterase *In vitro*

CONC. (µg/ml)	CATECHIN	AchE activities (nmol/mg protein)		
		NL	CC	CN
Control	0.12±0.02	0.12±0.02	0.12±0.02	0.12±0.02
10	0.17±0.03	0.15±0.01	0.14±0.03	0.13±0.01
50	0.13±0.01	0.12±0.01	0.13±0.02	0.15±0.03
100	0.13±0.03	0.15±0.02	0.11±0.03	0.10±0.02
250	0.14±0.01	0.14±0.03	0.13±0.02	0.08±0.01*
500	0.13±0.02	0.13±0.02	0.12±0.03	0.03±0.00*

*Data are expressed as mean ± SD (n=4), * Significantly different from control (P =0.05) NL=Nauclea latifolia, CC=Cymbopogon citratus, CN=Cocos nucifera, AchE= Acetylcholinesterase*

4. DISCUSSION

Scientific reports have supported the use of antioxidant supplementation in reducing the levels of oxidative stress and in slowing or preventing the development of complications linked to the stress [34]. Many synthetic antioxidants have shown toxic and/or mutagenic effects, which have shifted the attention towards naturally occurring antioxidants. Numerous plant constituents have proven to show free radical scavenging or antioxidants activities [35]. Results of this study showed that plant extracts (NL, CC and CN) have appreciable and known amount of phytochemicals, especially phenolic compounds. The high phenolic contents of CC and CN observed in this study is in agreement with the findings of Soares et al [36] and Oliveira et al [37]. The NL extract, out of the three plant extracts has the highest flavonoids and is expected to exhibit the greatest electron transferring ability in order to quench free radicals. It is known that flavonoids may reduce free radicals levels by quenching, up-regulating or protecting antioxidant defences and chelating radical intermediate compounds [38]. The flavonoids and phenolic contents of NL extract are high and comparable to the standard. In this study, the reducing power of the extracts measures the reductive ability in converting Fe (III) to Fe (II) in the reaction mixture [39]. The data showed that the reducing ability of the extracts increased with increase in concentration of the extracts. The results showed that the antioxidants contained in the extracts acted as reductants in a redox-linked colorimetric reaction. In this study, CN extract has greater reducing power than NL and CC which showed that the extract has higher ability to reduce Fe^{3+} to Fe^{2+}, the results is consistent with the findings of Oliveira et al. [37] and Zucca et al. [40].

The reaction of DPPH with numerous antioxidants is known and the stoichiometry characterized [41]. The DPPH antioxidant assay is based on the principle that 2,2-diphenyl-1-picryl-hydrazyl (DPPH) is able to decolourise in the presence of free radical scavengers (antioxidants). The colour turns from purple to yellow as the molar absorptivity of DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The odd electron in the DPPH radical is responsible for the absorbance at 517 nm and also for the visible deep purple colour [42]. Antioxidants in the three different extracts (CN, NL and CC) reacted with DPPH which is reduced to the DPPH-H. Consequently, the absorbancies decreased from the DPPH radical to the DPPH-H formed. The degree of discoloration indicated the scavenging potential of the extracts in terms of electron transferring potential [43]. The scavenging ability of CN was the highest at 500µg/ml among the three extracts. It is, therefore, reasonable to conclude that high antioxidants in the extracts of CN, NL and CC have resulted in the high level of DPPH radical scavenged in this study.

The hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell [44]. The hydroxyl radical is regarded as a detrimental species in pathophysiological processes and capable of damaging almost every molecule of biological system and contributes to carcinogenesis, mutagenesis and cytotoxicity [44]. In this study, hydroxyl radicals were generated by reaction of H_2O_2 and ferrous that later react with 2-deoxyribose. The reaction was stopped by adding TBA reagent to give a red colour if the malondialdehyde was formed as the result of the reaction between the radical and 2-deoxyribose. In this study, CN and CC at 250-750 µg/ml actively scavenged the hydroxyl radicals and prevented the degradation of 2-deoxyribose. The mutagenic capacity of free radicals is due to the direct interactions of hydroxyl radicals with DNA and therefore playing an important role in cancer formation. Hydroxyl radicals can be generated by biochemical reaction when superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which can subsequently produce extremely reactive hydroxyl radicals in the presence of divalent metal ions. The results demonstrated that CN and CC had appreciable hydroxyl radical scavenging activity when compared with standard antioxidant (catechin) and could serve as anticancer agent by inhibiting the interaction of hydroxyl radical with DNA. The ability of the extracts (CN and CC) to quench hydroxyl radicals might directly relate to the prevention of lipid peroxidation.

It is known that ROS may induce membrane damage by peroxidising lipid moiety, specially the polyunsaturated fatty acids in a chain reaction known as lipid peroxidation. The initial reaction generates a second radical, which in turn react with a second macromolecule to continue the chain reaction leading to functional abnormalities of cells. In this study, lipid peroxidation of rat liver and brain homogenates were induced by ferric ion and ascorbic acid. Lipid peroxidation inhibitory activity of CN was found to be the highest among the three extracts, and was comparable to catechin in the rat brain. These results indicated that CN extract has potential to be used in treating brain diseases caused by oxidative disorder. Hence, it is not surprising that CN at 250-500µg/ml significantly inhibited brain acetylcholinesterase (AChE) activities, whereas there were no significant differences between AChE activities of control and groups treated with CC and NL. This result showed that CN may enhance the acetylcholine levels in the brain thereby counteracting the acetylcholine deficit found in brain diseases induced by oxidative stress [45].

The nitric oxide (NO) radical inhibition assay showed that the extract of CN is a potent scavenger of NO in a dose-dependent manner from 10-250 µg/ml, while CC and NL are poor NO scavenger *in vitro*. The NO generated from sodium nitroprusside during this reaction may react with oxygen to form nitrite. The CN extract therefore inhibits nitrite formation by competing with NO for oxygen. It is known that scavengers of NO must compete with oxygen to reduce production of nitrite [46].

5. CONCLUSION

Enough facts have revealed greater advantages of the used of natural antioxidants over synthetic ones. Findings from this study showed that *Nauclea latifolia*, *Cocos nucifera* and *Cymbopogoncitratus* are good natural sources of antioxidants. In addition, *Cocos nucifera* exhibited potent inhibitory effect on rat brain acteylcholinesterase activities.

CONSENT

Not Applicable

ETHICAL APPROVAL

Authors declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985), as well as University of Ibadan ethics committee rules on the use of laboratory animals in research were followed in the collection of rat brain and liver used in this study.

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COMPETING INTERESTS

Authors hereby declare that there are no competing interests.

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