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Free radical scavenging activity, total phenolic and flavonoid constituents of medicinal plants used in Nigerian ethnobotany

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Abstract

Introduction: Plant polyphenols have the ability to protect biomolecules and cell membrane from oxidative stress thereby offering protection against the development of wide range of diseases. This study evaluated the antioxidant property of the methanol extract of twelve medicinal plants, identified and selected from a previous ethnobotanical studies conducted in South-western region of Nigeria.

Methods: 2, 2-Diphenyl 1-picryl hydrazyl (DPPH) free radical scavenging and nitric oxide inhibitory assays were used to evaluate the antioxidant activities. Total phenolic content (expressed as garlic acid equivalent), total flavonoid (expressed in terms of quercetin equivalent) and total antioxidant capacity were also determined by standard methods. Garlic and ascorbic acid were included in the study as reference standards. Results were expressed as the mean ± SEM. Differences between means were tested for statistical signiûcance using Student t- test ($p \geq 0.05$).

Results: The extracts exhibited scavenging activities against the DPPH radical. *Uvaria chamae* (stem bark) and *Thonningia Sanguinea* (whole plant) had IC_{50} values of 4.30 and 6.12 respectively, though not comparable to standard drug (ascorbic acid) with IC_{50} value of 1.4 µg/mL However, *T. sanguinea* extracts exhibited the highest nitric oxide inhibitory activity with IC_{50} values of 1.3±0.1 µg/mL significantly comparable to garlic acid with an IC_{50} of 1.1 ±0.1 µg /mL ($p \geq 0.05$). Methanol extract of *T. sanguinea* and *K. senegalensis* (stem bark) had the highest phenolic contents 343 ± 1.79 and 346 ± 0.02 mg/g respectively. *Thonningia sanguinea* had the highest total antioxidant capacity (TAC) of 374.46 ± 8.41 AAE (mg/g). *Uvaria chamae,* had the highest flavonoid content of 84.84 ± 6.26 QE mg/g of quercetin equivalent.

Conclusion: This study suggests that the screened plants, especially *Thonningia sanguinea* can serve as a valuable source of plant antioxidants.

Key words: *DPPH and Nitric Oxide Assays, fFlavonoid Compounds, Folin-Ciocalteu method, Thonningia sanguinea and Total Phenol,*

Résumé

Introduction : Les polyphénols végétaux ont la capacité de protéger les biomolécules et la membrane cellulaire du stress oxydatif, offrant ainsi une protection contre le développement d'un large éventail de maladies. Cette étude a évalué la propriété antioxydante de l'extrait de méthanol de douze plantes médicinales, identifié et sélectionné parmi des études ethnobotaniques antérieures menées dans la région du sud-ouest du Nigéria.

Méthodes : 2, 1-picryle 2-diphényl hydrazyl (DPPH) piégeage des radicaux libres et des dosages d'inhibition d'oxyde nitrique ont été utilisés pour évaluer les activités antioxydantes. La teneur en composé phénolique totale (exprimée en équivalent d'acide d'ail), en flavonoïde totale (exprimée en termes de quercétine équivalent) et la capacité antioxydante totale ont également été déterminées par des méthodes standard. L'ail et l'acide ascorbique ont été inclus dans l'étude en tant que référence standard. Les résultats ont été exprimés sous forme de moyenne ± ESM. Les différences entre les moyennes ont été testées pour la signification statistique à l'aide du test t d'Elève (p Ã 0,05).

Résultats: Les extraits ont montré des activités de balayage contre le radical DPPH. *Uvaria chamae* (écorce de tige) et *Thonningia Sanguinea* (plante entière) avaient des valeurs de CI_{50} de 4,30 et 6,12 respectivement, comparables à celles de l'acide ascorbique avec une valeur de CI_{50} de 1,4 µg / mL. *Les* extraits de *T. sanguinea* présentaient l'activité inhibitrice d'oxyde nitrique la plus élevée, avec des valeurs de CI_{50} de 1,3 ± 0,1 µg / mL comparables à celles de l'acide d'ail avec une CI_{50} de 1,1 ± 0,1 µg / mL. Les extraits au méthanol de *T. sanguinea* et *K. senegalensis* (écorce de tige)

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avaient les teneurs en phénol les plus élevées, 343 ± 1,79 et 346 ± 0,02 mg/g. *Thonningia sanguinea* avait la capacité antioxydante totale (CAT) la plus élevée, de 374,46 ± 8,41 AAE (mg / g). *U. chamea,* avait la plus forte teneur en flavonoïdes de 84,84 ± 6,26 QE mg / g de quercétine équivalent.
Conclusion: Cette étude suggère que les plantes criblées, en particulier *Thonningia sanguinea,* peuvent constituer une source précieuse d'antioxydants.

Mots-clés : *tests de DPPH et d'oxyde nitrique, composés flavonoïdes, méthode de Folin-Ciocalteu, Thonningia sanguinea et Phénol total,*

Introduction

Nature has continued to be a source of medicinal agents all through human history. A remarkable number of contemporary drugs have been isolated from natural resources which also serve as a reservoir of rare medicinal agents [1]. Plant produces various anti oxidative metabolites to counteract reactive oxygen species (ROS) in order to survive. Natural antioxidants either as crude raw herbs or their chemical constituents are very effective in preventing the destructive processes caused by oxidative stress [2, 3]. Free radical reactions have been known to be involved in many acute and chronic disorders in human beings, including diabetes, atherosclerosis, aging, immunosuppression and neurodegeneration [4]. Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells [5].

Studies on medicinal plants have indicated the presence of phenolics with their concomitant bioactivities which explains the presence of such phenolic constituents. Plant phenolics are commonly found in both edible and non-edible plants, which play important roles in plant development in addition to eliciting multiple biological properties. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers [6]. The importance of natural phenolic compounds has been on the rise among scientists, food manufacturers, and consumers [7].

The present study was designed to characterize the radical scavenging capacity, nitric oxide inhibitory activity, the total phenolic and flavonoid contents as well as total antioxidant capacity of methanol extracts of twelve selected medicinal plants. The studied plants are: *Thonningia sanguinea* Vahl (fruiting body), *Khaya senegalensis* (Ders.) A. Juss (bark & leaf), *Parquetina nigrescence* (Afzel) Bullock (root bark), *Spondia mombin* Linn (bark and leaf), *Tetrapluera tetraptera* (Schum&Thonn.) Taub. (Fruit) *Secamone afzelii* (Schult.) K. Schum, *Abutilon mauritianum* (Jacq) Medic (leaf), *Senna siamea* Linn (bark), *Chrysophyllum albidum* G. Don(seed), *Zephyranthes candida Lindl* (whole plant), *Uvaria chamae* P. Beauv (stem bark), *Lippia multiflora* Poir (leaf). They were identified and selected from the Nigeria ethno medicine, where they have been indicated for the treatment of various diseases especially inflammation, malaria and viral infections [8, 9]

Materials and methods

Drugs and chemicals

Griess reagent (Sulfanilamide, napthylethylene diamine hydrochloride), Sodium nitroprusside, Sodium nitrite, Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhdrazyl (DPPH), Sodium bicarbonate, Gallic acid, Quercetin, Ascorbic acid, Sodium phosphate ammonium molybdate, sodium acetate and aluminium chloride were obtained from Sigma Chemicals, USA. All other chemicals and reagents were of analytical grade and were used without further purification.

Plant collection and authentication

Plant materials were obtained at different times of the year. Specimens were collected from the botanical garden, University of Ibadan and seeds were purchased from Bode market, Ibadan, Oyo State, Nigeria. Plant materials were authenticated and voucher specimens were deposited at the forest herbarium, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria under the following FHI numbers; *Khaya senegalensis* (leaf) 108856, *Khaya senegalensis* (bark) 108856, *Chrysophyllum albidum* (seed) 108851, *Zephyranthes candida* (whole plant) 110045,*Abutillon mauritianum* (leaf) 109985, *Senna siamea* (bark) 110047, *Tetrapluera tetraptera* (seed)108850, *Parquetina nigrescence* (root)108852, *Secamone afzelii* (leaf)109987,*Thonnigia sanguinea* (whole plant) 108854, *Uvaria chamae* (bark) 109900, *Spondias mombin* (leaf) 109901, *Spondias mombin* (bark) 109901, *Lippia multiflora* (leaf) 108858

Extract preparation

Plant materials were air-dried for two weeks and pulverized into coarse powder with the aid of a milling machine. Pulverized plant materials (400 g each) were extracted by maceration in redistilled methanol for 72h at room temperature with occasional stirring. Methanol was used in this study because it is an amphiphilic solvent, a lot of non-polar components are also dissolved in methanol along with polar bio-active components of the plants

and also because its extracts can be concentrated easily due to its low boiling point in comparison with water and ethanol. Extracts were filtered and concentrated using a rotary evaporator at 40 °C. The dried extracts were stored in the refrigerator at 4 °C until needed for analyses.

Antioxidant assays

Each extract was dissolved in methanol to prepare a stock concentration of 1 mg/mL; serial dilutions were then prepared for various antioxidant assays. All assays were performed in triplicates. Reference chemicals were used for comparison in all assays.

DPPH free radical scavenging activity

The *in vitro* free radical scavenging activity of the fractions was assessed using 2, 2- diphenyl-1-picrylhydrazyl (DPPH) assay according to a previously described method [10]. A fresh working solution of DPPH at 0.04 mg/mL was prepared by diluting DPPH solution with methanol. The DPPH solution (150 µL) was added to 100 µL of the graded concentration of the sample (6.25 – 400 µg/mL). The reaction mixture was well-shaken and incubated in the dark for 15 min at RT and the absorbance was taken at 517 nm using a spectrometer (SPECTRA max PLUX, Analytik and Biotechnologie, Germany). The scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following equation:

$$\% \text{ Inhibition} = \left[1 - \left(\frac{A1}{A0}\right) * 100\right]$$

A_0 =absorbance of the control
A_1 =absorbance of the extracts

Concentration of sample required to scavenge 50% of free radicals (IC_{50} values) were determined using non-linear regression. Assays were performed in duplicates and repeated twice.

Nitric oxide inhibitory assay

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction as described by Janetia et al [11]. Sodium nitroprusside (10 mM) in phosphate-buffered saline (pH 7.4) was mixed with different concentrations of the extract dissolved in phosphate-buffered saline in a test tube and incubated at 25 °C for 180 minin the dark. Samples from the above (100 µL) was transferred into a 96 well plate and reacted with 100 µL Greiss reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediaminedihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamme was read at 550 nm and referred to the absorbance of standard solutions of sodium nitrite treated in the same way with Griess reagent.

Inhibition activities of nitrite formation by the plant extracts and the standard antioxidant garlic acid were calculated relative to the control. The percentage inhibition was linearized against the concentration of each extract and Gallic. The IC_{50} which is an inhibitory concentration of each extract required to reduce 50% of the nitric oxide formation was determined.

Estimation Total Flavonoid Contents (TFC)

The total flavonoids content was determined in accordance with the methods of Milauskas et al [12]. The reference drug used for the assay was quercetin. Extract (150 µL) was introduced into the wells of microtiter plate (in triplicates), 50 µL of aluminium chloride and 100 µL of sodium acetate buffer respectively were added to each of the wells. Absorbance was measured at the wavelength of 412 nm. A calibration curve was constructed and used in the estimation of the total flavonoids content.

Phosphomolybdate assay/Total Antioxidant Capacity Assay (TAC)

The total antioxidant capacity was measured according to the method reported by Prietto et al [13] with a slight modification. Extract (100 µL) and 100 µL of ascorbic acid (reference standard) were taken, placed separately in a test tube with 1mL of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. After incubation in a thermal block at 95°C for 1 hour 30 min, samples were cooled at RT and the absorbance was measured at 695 nm against a blank which was incubated at 1 mg/mL concentration under the same conditions. The experiment was conducted in triplicate and values were expressed as mg ascorbic acid equivalent per gram of extract.

Determination of total phenolic content

The concentration of phenolics in plant extracts was determined using spectrophotometric method [14]. Methanolic solution of the extract in the concentration of 1 mg/mL was used in the analysis. The reaction mixture was prepared by mixing 0.5 mL of methanolic solution of extract, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.0 mL of 7.5% $NaHCO_3$. Blank was concomitantly

prepared, containing 0.5 mL methanol, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.0 mL of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at 765 nm. The samples were prepared in triplicate for each analysis and the mean values of absorbance were obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/mL) from the calibration line; then the content of phenolics in extracts was expressed in terms of garlic acid equivalent (GAE mg/g of extract).

Table 1: DPPH Free Radical Scavenging Activity of Investigated Plant Extracts

Plant Extracts	% Yield of extract	IC_{50} (µg/mL)
Khaya senegalensis(L)	5.20	12.16 ± 0.06
Khaya segalensis (B)	14.92	10.85 ± 0.21
Chrysophyllum albidum(S)	14.00	25.34 ± 0.01
Zephyranthes candida (W)	1.84	47.43 ± 0.11
Abutillon mauritianum(L)	3.90	7.76 ± 0.68
Senna siamea (B)	4.04	15.60 ± 0.43
Tetrapluera tetraptera (S)	21.70	44.12 ± 0.44
Parquetina nigrescence(R)	5.30	71.02 ± 078
Secamone afzelii(L)	5.30	97.60 ± 0.19
Thonnigia sanguinea (W)	2.50	6.12 ± 0.11
Uvaria chamae (B)	6.96	4.30 ±0.82
Spondias mombim (L)	6.19	14.92 ± 0.03
Spondias mombim (B)	0.53	20.04 ± 0.45
Lippia multiflora	1.98	17.10 ± 0.75
Ascorbic Acid	-	1.40± 0.22

Key: L=Leaf, B=Bark, W=Whole plant, F=Fruit R=Root

Statistical analysis

Data obtained were expressed as the mean ± SEM. Means of results (data) obtained from various assays were tested for statistical signiûcance using student t- test of a commercially available GraphPad® package version 5.0 (San Diego, USA). Mean differences were considered significant at $P < 0.05$.

Result

The extracts exhibited scavenging activity against the DPPH radical and had IC_{50} values, ranging from 4.30 to 97.60 µg/mL (Table 1). *Uvaria chamae*, *T. sanguinea* and *K. senegalensis* (sb) had IC_{50} values of 4.30, 6.12 and 10.85 µg/mL respectively. Ascorbic acid used as a reference in this study, had IC_{50} value of 1.4 µg/mL *Secamone afzelii and Parquetina nigrescence* (r) and had the lowest scavenging activities against the DPPH radical among the test extracts with IC_{50} value of 71.02 and 97.60 µg/mL, respectively, Other results are displayed in Table 1.

Furthermore, the results of NO scavenging activity of the selected plant extracts are shown in Figure 1. The IC_{50} values for NO inhibition ranged from 1.3 ± 0.7 to 44 ±1.7 µg/mL (Fig. 1). *Thonnigii sanguinea*, and *K. senegalensis* (l) had the highest inhibitory activity with IC_{50} values of 1.31 ± 0.7 and 3.1 ± 0.1 µg/mL respectively. The total phenolic content of the plants extract ranged from 14.71 ± 0.05 to 346 ± 0.02 (Fig. 2). *Khaya senegalensis* (b), *T. sanguinea* and *S. mombin* (b) methanolic extracts gave high phenolic contents of 346.77 ± 0.02, 343.47 ± 1.79 and 357.4 ± 0.02 mg/g, respectively. On the other hand, *Abutilon mauritianum* had the lowest phenolic contents of 14.7 ± 0.05 mg/g as shown in figure 2

In addition, the total antioxidant capacity (TAC) of the extracts expressed as ascorbic acid Equivalent (AAE) ranged from 11.17 ± 1.37 to 374.66 ± 8.41 AAE mg/g (Fig. 3). *Thonnigii sanguinea* had the highest TAC value of 374.46 ± 8.41 AAE mg/g followed by *S. mombin* (b) with TAC value of 359 ±4.47 AAE mg/g (Fig.3). For the total flavonoid contents, *Uvaria chamea* had the highest contents of 84.84 ± 6.26 mg/g, *P. nigresence* (r), *Z. candida* and *C. albidum* had little or no flavonoid content (Fig 3)

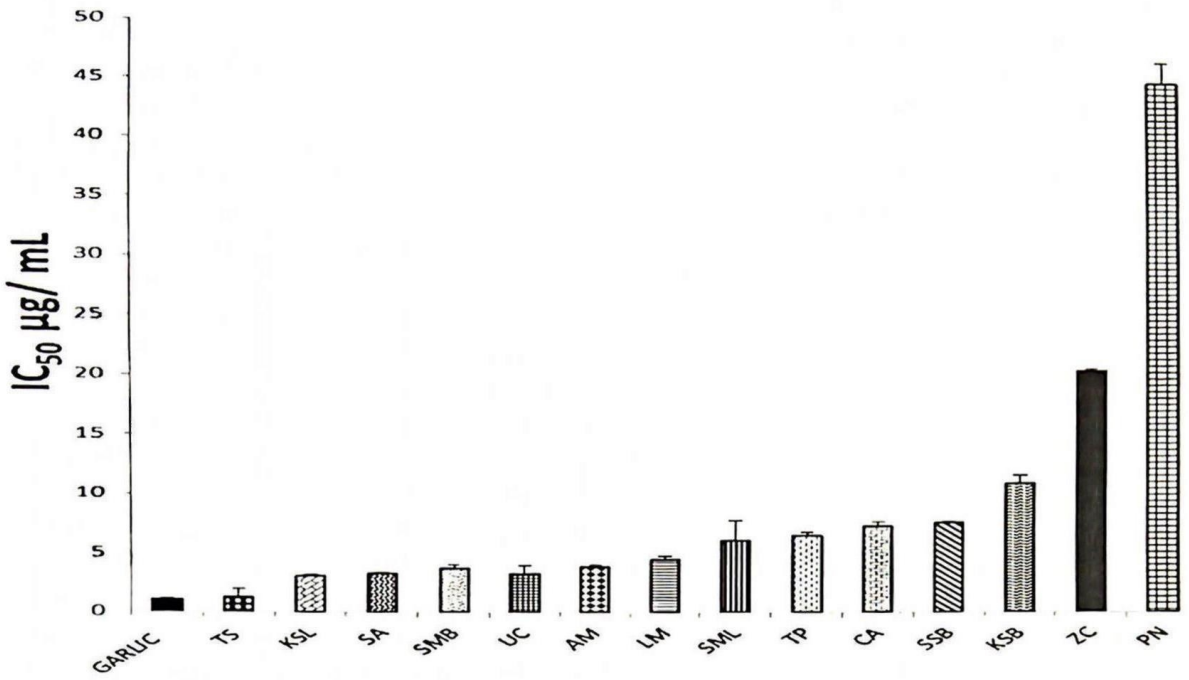

Fig. 1: Nitric oxide inhibitory activity of investigated plant extracts

Key: AM- *Abutilon mauritianum, TT- Tetrapluera tetraptera*(F) , **CA-** *Chrysophyllum albidum*(S), **PN-** *Parquetina nigresence* (R), **ZC-** *Zephyranthes candida* (W), ***SS-*** *Senna siamea* (B),**SM-** *Spondias mombin*(B&W),**SA-** *Secamone afzelii,* **TS-** *Thonnigii sanguinea* (W), **KS** *–Khaya senegalensis*(B&L), **LM** *- Lippia multiflora* and **UV-** *Uvaria chamae*(B)

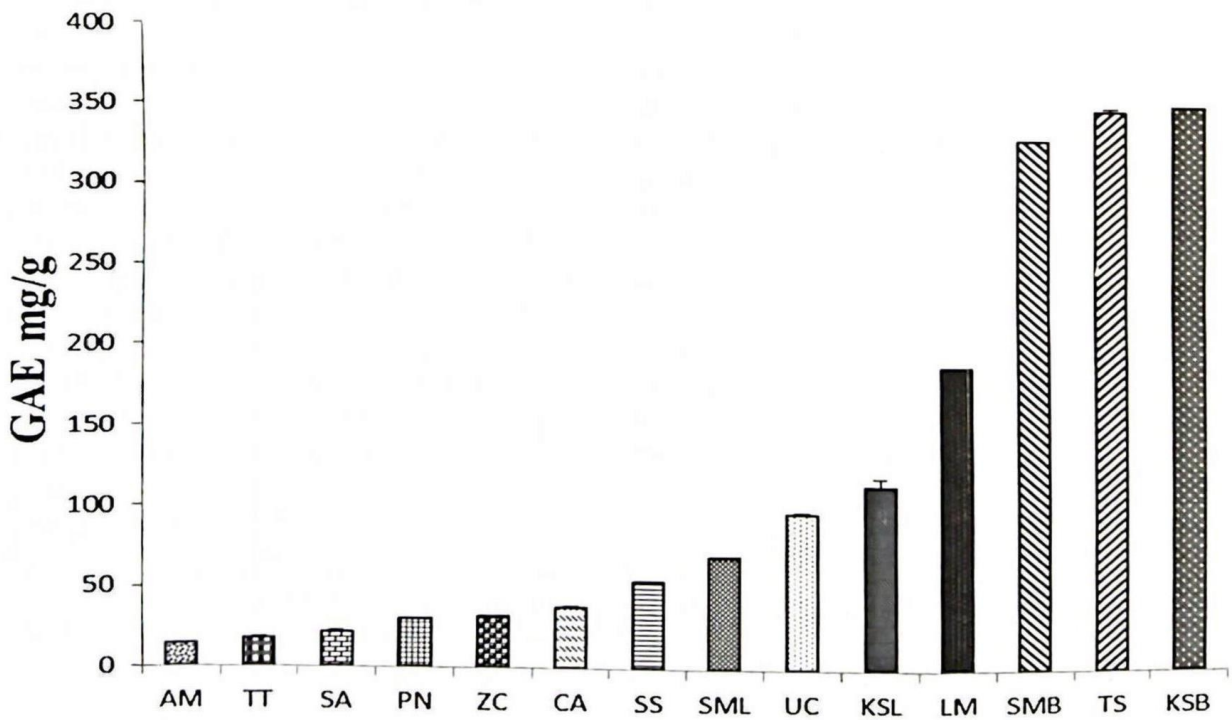

Fig. 2: Total phenolic contents expressed as Gallic Acid Equivalent (GAE mg /g)

AM- *Abutilon mauritianum,* **TT-** *Tetrapluera tetraptera*(F) , **CA-** *Chrysophyllum albidum*(S), **PN-** *Parquetina nigrescence* (R), **ZC-** *Zephyranthes candida* (W), ***SS-*** *Senna siamea* (B),**SM-***Spondias mombin*(B&W),**SA-** *Secamone afzelii,* **TS-** *Thonnigii sanguinea* (W), **KS** *–Khaya senegalensis* (B&L), **LM** *- Lippia multiflora* and **UV-** *Uvaria chamae*(B)

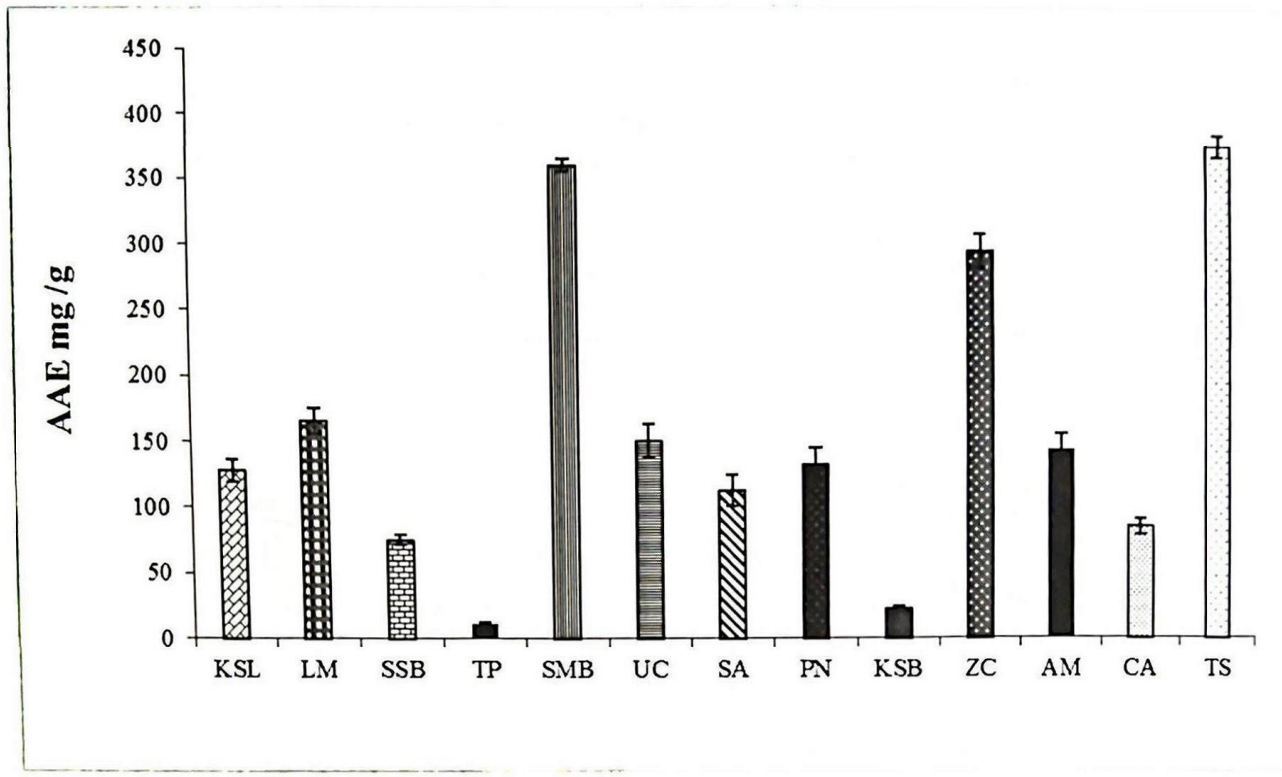

Fig. 3: Total antioxidant property of extracts expressed as Ascorbic Acid Equivalent AAE mg/g
AM- *Abutilon mauritianum*, **TT**- *Tetrapluera tetraptera* (F), **CA**- *Chrysophyllum albidum*(S), **PN**- *Parquetina nigrescence* (R), **ZC**- *Zephyranthes candida* (W), ***SS***- *Senna siamea* (B), **SM**-*Spondias mombin* (B&W),**SA**- *Secamone afzelii*, **TS**- *Thonnigiia sanguinea* (W), **KS** –*Khaya senegalensis* (B&L), **LM** - *Lippia multiflora* and **UV**- *Uvaria chamae*(B)

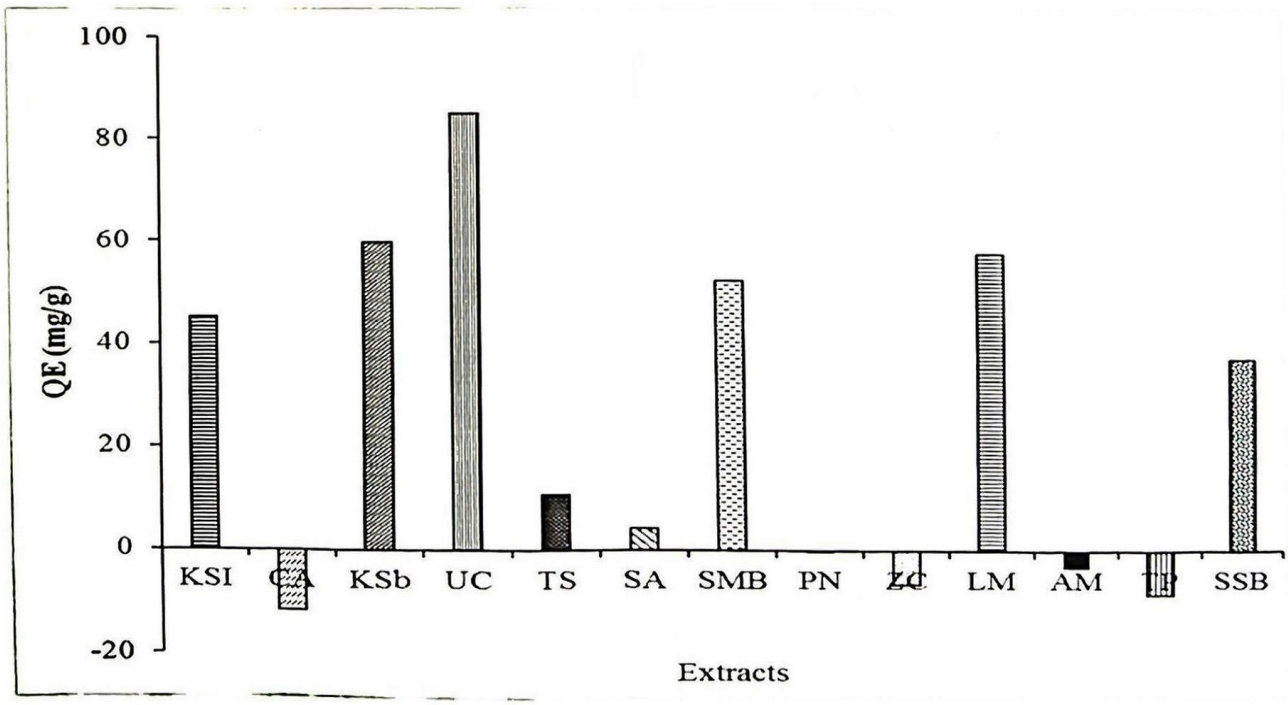

Fig. 4: Total flavonoid contents of the Extracts expressed as Quecertin Equivalent QE (mg/g)
AM- *Abutilon mauritianum*, **TT**- *Tetrapluera tetraptera* (F), **CA**- *Chrysophyllum albidum* (S), **PN**- *Parquetina nigrescence* (R), **ZC**- *Zephyranthes candida* (W), ***SS***- *Senna siamea* (B),**SM**- *Spondias mombin* (B&W),**SA**- *Secamone afzelii*, **TS**- *Thonnigiia sanguinea* (W), **KS** –*Khaya senegalensis* (B&L), **LM** - *Lippia multiflora* and **UV**- *Uvaria chamae* (B)

Discussion

Excessive production of free radicals causes decrease in membrane fluidity, loss of enzyme receptor activity and damage to membrane protein leading to cell death [15]. These free radicals are involved in different disorders like ageing, cancer, cardiovascular disease, diabetes, inflammation, rheumatoid arthritis, epilepsy and degradation of essential fatty acids [16]. Therefore, substances that can scavenge the excess free radicals are important agents that can protect the body from the deleterious effect of free radicals.

In search of new antioxidant agents from plants, the antioxidant activity of 12 medicinal plants was evaluated using the DPPH and Nitric oxide based assays. The Diphenyl-2-picryl-hydrazyl (DPPH) contains an odd number of electrons, which gives a purple colour to the compound. As an antioxidant agent donates an electron, the DPPH gets paired with the hydrogen from the antioxidant and becomes decolorized [17]. Thus, interaction of an antioxidant agent with DPPH results in a colour change that can be estimated in a colorimetric assay [18]. In this study, most of the extracts evaluated exhibited various scavenging activities against the DPPH radical. The radical scavenging activity of the five most active extracts followed this order *Uvaria chamae* (sb) > *Thonnigia sanguinea* (w) > *Abutillon mauritianum* (l) > *Khaya senegalensis* (sb)> *Khaya segalensis* (l). DPPH radical scavenging activity of different plant part of *Uvaria chamae* had been previously reported; the seed extract [19], root extracts [20,21] the leaf, the result from the stem bark in this assay established the fact that radical scavenging agent is well distributed in the various parts of the plant although the DPPH scavenging activity is good but it is does not significantly comparable to ascorbic acid in this study ($p= 0.032$). Similarly, there has been a previous report of the DPPH scavenging activity of *T. sanguinea* from Ghana [22]. Gyamfi and co-workers also reported the isolation of two antioxidant ellagitannins; thonningianins A and B from *T. sanguinea* [23, 24]. *Thonnigiia sanguinea* had the highest Total antioxidant capacity (TAC) in this study.

Nitrite ions were detected in this study using the Griess reagent. Nitrite ions react with Griess reagent to form a purple azo and in the presence of nitric oxide scavengers the amount of nitrites decreased [25]. Interestingly seven of the plant extracts scavenged nitric oxide in the following order of increasing activities *Thonningia sanguinea* >*Uvaria chamae* > *Khaya senegalensis* leaf > *Secamone afzelii* > *Spondia mombin* bark >*Abutilion mauritanium* > *Lippia multiflora*. The NO scavenging activity of *T. sanguinea* is comparable to gallic acid, a standard antioxidant agent ($p = 0.79$). *In vivo,* nitric oxide interacts with superoxide anion, another free radical to produce peroxynirite. Generation of peroxynitrite, can result in subtle modulation of cell signalling to overwhelming oxidative injury resulting in cells necrosis or apoptosis [26]. The ability of these extracts to scavenge NO will result in pharmacological inhibition of peroxynitrite induced damages thereby preventing ischaemia-perfusion injury, circulatory shock, inflammation, pain vascular and neurogenerative diseases associated peroxynitrite induced oxidative stress [27-29]

It has been reported that phenols and polyphenolic compounds, such as flavonoids possess antioxidant activity and including them in human nutrition might results in positive effect which can be of vital importance to general well-being. It has been postulated that the mechanism of action of phenolics such as flavonoids may be through scavenging or chelating process [30, 31]. Phenolic groups in polyphenols can accept an electron to form relatively stable phenoxyl radicals, thereby upsetting chain oxidation reactions in cellular components and it has been said that their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals [32]. This finding suggests that the observed antioxidant activities of *T. sanguinea* correlate with its phenolic content; *T. sanguinea* had one of the highest phenolic content in this study. Also it had the highest total antioxidant content and its NO scavenging activity is significantly greater than other extracts (pd" 0.005). *K. senegalensis* stem back had the highest TPC, but there is no significant difference between its TPC and that of *T. sanguinea* ($p > 0.05$). In addition, *K. senegalensis* had good radical scavenging activity in both DPPH and NO scavenging assays. *Uvaria chamae* with the highest total flavonoid content also had the highest DPPH radical scavenging activity. Previous studies showed that antioxidant activity directly correlates with phenolic content in medicinal plants [33-37] and this corroborates the results of our studies.

One of the major findings in this study was that *T. sanguinea* demonstrated much stronger antioxidant activity and contained significantly higher phenolic content than common vegetables and fruits earlier studied by our group [38] and are considered as good natural sources of dietary antioxidants. Thus the significance of the various activity of these medicinal plants cannot be overlooked since they have been indicated in the traditional treatment of various illness including;

antiviral, anti-inflammatory and antimalarial studies. Their ability to greatly scavenge free radicals might be one of the ways through which they militate against various disease states.

Conclusion

The results obtained in this study showed that some of the extracts especially *T. sanguinea,* and *U. chamae* exhibited potent free radical scavenging activity; they also have high total phenolic, flavonoid and antioxidant content. This suggests that the plants have potentials as sources of natural antioxidants and justified their use in the Nigerian ethno-botany as therapeutic agents in preventing or slowing the progress of oxidative stress-related degenerative diseases.

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