ISSN 2348-6201

IN VITRO SCREENING OF ANTIOXIDANT PROPERTIES OF TEN CAMEROONIAN MEDICINAL PLANTS

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ABSTRACT

Background: Oxidative stress is involved in the pathogenesis of several degenerative diseases. This work studied the in vitro anti-oxidative properties of methanolic extracts of 10 Cameroonian medicinal plants including Autranellacongolensis (AC); Beilschmiediaanacardioides(BA); Crossopteryxfebrifiga (CF); Cussoniaarborea (Cu A); Cyphostema adenaucaule (Cy A); Dissotislongipetala (DL); Lonchocarpussepium(LS); Naucleapobeguinii (NP); Pycnanthusangolensis (PA); Picralimanitida (PN) used in folk medicine against various ailments associated with oxidative damages pattern. Method: The extracts were subjected to phytochemical screening to identify major classes of secondary metabolites. Their total contents in phenols, flavonoids were determined; whereas the anti-oxidative and reducing power were evaluated. These extracts were tested for their scavenging activity using DPPH, ABTS, superoxide, hydroxyl and nitric oxide radicals and hydrogen peroxide. The inhibitory effects of extracts against lipid peroxidation were also investigated on rat brain homogenates. Results: The methanolic extracts of CF, DL, LS and AC showed important antioxidant activities and scavenging properties against all radicals system used. The extract of CuA and NP were inactive against hydrogen peroxide. The most potent antioxidant activity was obtained with AC extract, which showed the lowest IC50 value as compared to the standard. The IC50 values of this extract were 7.31±0.25, 5.20±0.35, 42.17±0.60, 77.30±7.41, 32.94±1.32, 9.71±1.07 and 121.59±1.08 µg/ml against ABTS, DPPH, hydrogen peroxide, inhibition of lipid peroxidation, superoxide radical, hydroxyl radical and nitric oxide radical system respectively. These values were found to be comparable to those obtained with the standards. Significant positive correlations were observed between total antioxidant activity with total reducing power ($p<0.01$; $R2=0.8190$), total flavonoids ($P<0.05$; $R2=0.6419$) and total phenol ($P<0.01$; $R2=0.8643$); whereas non-significant correlation was obtained between other systems and the total phenolic constituents. Conclusion: The data generated from this study sustained the use of these medicinal plants in the treatment of various diseases associated with oxidative stress. The potent antioxidant activity of the Autranellacongolensis extract and its high content in phenolic constituents are to be taken into the consideration for further phytochemical and biological investigations.

Indexing terms/Keywords

Oxidative stress; phenols; medicinal plants; Autranellacongolensis; antiradical properties.

Academic Discipline And Sub-Disciplines

Phytopharmacology.

SUBJECT CLASSIFICATION

Biochemistry.

TYPE (METHOD/APPROACH)

Quasi-Experimental.

ISSN 2348-6201

INTRODUCTION

Oxidative stress contributes to the pathogenesis of several diseases including infectious and metabolic pathologies. Cancers, diabetes mellitus, liver injuries, aging, cardiovascular diseases, inflammation and neurodegenerative disorders form the majority of these pathologies [1-4]. The stress results from overproduction of reactive oxygen species (ROS) called pro-oxidant species or the failure of antioxidant defence systems leading to the destruction of biological macromolecules such as proteins, lipids and nucleic acids [5]. Antioxidants neutralize the destructive effect of ROS when they come in contact together and stop the degenerative chain reaction of free-radical oxidation, thus preventing or repairing oxidative damages of tissues [6]. If there are insufficient quantities of antioxidants to match its exposure to free radicals, then the body is said to be in a state of oxidative stress. In this state, unimpeded free radicals cause damage that can lead to inflammation, immune dysfunction, DNA damage and, potentially, a whole range of degenerative diseases [7].

A network of antioxidants known as the antioxidant defence system works together to maintain an effective protection of cells from free radical damages. This network involves biological reducing agents or scavengers which can be synthesized by the body such as coenzyme Q, glutathione, peroxidases, and superoxide dismutase and catalase which play a key role in emergency defence of cells. The extrinsic antioxidant defence system involves dietary antioxidants and drugs. Such antioxidants include vitamins E and C, alpha-lipoic acid, carotenoids, flavonoids, tannins and other dietary phenol derivatives [7]. Plants constitute the main source of extrinsic antioxidants as well as some effective drugs. It is important to investigate the antioxidant profiles of natural species used in the treatment of some diseases' complications. Several medicinal are used in the folk medicine in the treatment of various ailments associated with degenerative effects, but in most cases their antioxidant properties have not yet been established. Based on literature search, ten (10) Cameroonian medicinal plants used in the treatment of diseases whose pathogenesis involves oxidative stress were selected (table 1). The present study aimed at contributing in the evaluation of their mechanism of action in vitro.

Table1: Selected medicinal plants tested for antioxidant properties

Plants (botanic name) And Family	Voucher Number	Part used	Extraction yield (%)	Medicinal properties
Autranellacongolensis (AC) Sapotaceae	39458/HNC	Bark	11.40	Diarrhea and chronic dysentery, malaria [8, 9]
Beilschmiediaanacardioides (BA) Lauraceae	32975/HNC	Bark	12.50	Uterine tumours, rubella, rheumatisms, bacterial and fungal infections [10]
Crossopteryxfebrifuga (CF) Rubiaceae	39970/HNC	Bark	7.43	Painful inflammatory disorders, fever, malaria, trypanosomiasis[11, 12]
Cussoniaarborea (Cu A) Araliaceae	39978/HNC	Bark	13.43	Leprosy, Snakebite, malaria and constipation[13-15]
Cyphostema adenaucaule (Cy A) Vitaceae	51976/HNC	Bark	16.97	Stomachache [16]
Dissotislongipetala (DL) Melastomataceae	40925/HNC	Bark	9.00	Nerve disorders [17, 18]
Lonchocarpussepium (LS) Fabaceae	76230/HNC	Bark	8.00	Antifungal, expectorant, sedative, suppurative. Madre de cacao is a folk remedy for alopecia, boils, bruises, burns, colds, cough, debility, eruptions, erysipelas, fever, fractures, gangrene, headache, itch, prickly heat, rheumatism, skin tumours, ulcers, urticaria and wounds [19].
Naucleapobeguinii (NP) Rubiaceae	504710/HNC	Bark	10.98	Malaria [20]
Pycnanthusangolensis (PA Myristicaceae	628121/HNC	Seeds	53.33	Hypercholesterolemia, type 2 diabetes, cancer, Bile and liver complaints, Stomachache [17]
Picralimanitida (PN) Apocynaceae	565411/HNC	Bark	14.08	Analgesic, anti-inflammatory, diarrhea, fevers, gonorrhea, hypertension, intestinal worms, jaundice, malaria, trypanosomiasis and others protozoa diseases [8]

MATERIAL AND METHODS

Plant material

The plants were harvested in different localities of Cameroon and identified at the National Herbarium of Cameroon (NHC) where voucher specimens were deposited with voucher number. Detailed information on each medicinal plant is given in table 1.

Preparation of extracts

Fresh plant materials were collected and dried at room temperature in an aerated laboratory, they were ground to yield a powder; five (05) kg of the powder were soaked in 13.5 L of methanol for 48 hours to give about 7.5 L of extract after filtration. The filtrate was evaporated on a rotary evaporator to obtain the different crude extracts, and the extraction yields were recorded (table1). An aliquot of each crude extract obtained was used for antioxidant tests while the remaining fraction was kept for further studies.

Qualitative tests: phytochemical screening

Qualitative phytochemical tests were performed for methanolic extracts of all plants to identify the various classes of phyto-constituents. These tests were performed according to the standard protocol as described below [21-23].

Test for Carbohydrates: Carbohydrates were identified by Mohlisch's test. Two drops of α-naphthol solution in alcohol was added in 2 ml of aqueous extract and shaken. Concentrated H_2SO_4 was added on the side of the test tube. The presence of violet ring at the junction of two liquids indicated the presence of carbohydrates.

Tests for steroids: The Lieberman-Burchard Reaction was used for identification of steroids. A mixture of 2 ml of extract, 2 ml of chloroform and 2 ml of acetic anhydride was made. Then 2 drops of concentrated H_2SO_4 were added from the side of test tube. It was observed for first red, then blue and finally green colour.

Test for alkaloids: To 3 ml of methanolic extract, few drops of Dragendorf's reagents were added and the mixture was observed for orange brown precipitate.

Test for Glycoside: The test was divided into two parts and both observations were compared for final conclusion.

Part A: To 3 ml of extract, diluted H_2SO_4 was added and heated on a water bath for 2 min, and then the mixture was neutralized with 1N NaOH solution. The pH was checked with litmus paper and to resulting solution was added Fehling's A and B. Intense red precipitate indicated that glycosides might be present.

Part B: To 3 ml of extract, distilled water was added and heated. According to part A where NaOH was added for neutralization, an equal quantity of water was added. Then to resulting solution was added Fehling's A and B. Increased red precipitate indicated the absence of glycoside.

Test for saponin: To 3 ml of distilled water was dissolved 0.5 g of extract and the mixture was shaken vigorously for 1 min. Persistent foam indicated the presence of saponins.

Tests for coumarins: To 2 ml of extract was added 1 ml of 10% ammonia solution (NH_4OH). One drop was spotted on filter paper and observed in UV chamber for the presence of blue or green fluorescence.

Quantitative tests

Estimation of total phenol content: The content in phenolic constituents of the extracts was estimated by using Folin-Ciocalteu (FC) method [24], based on the oxidation of phenolic group with phosphotungstic acid to yield a green-blue complex.

The extracts and standard, Gallic acid (10 mg each) were dissolved separately in MeOH and the volume was made up to 10 ml. These solutions were serially diluted with methanol to obtain the lower dilutions. In a test tube were added 1 ml of FC reagent (2N) diluted (1:10) of with distilled water, 0.8 ml sodium carbonate (0.7 M) and 0.2 ml of diluted methanolic extract. After shaking, all tubes were kept for 2h at room temperature to complete the reaction. The absorbance was measured at 750 nm using a UV/visible spectrophotometer. Standard curve prepared with Gallic acid give a linear correlation ($y = 0.103x$, $R^2 = 0.996$) in the range 0.5 – 10 µg/ml. The phenolic content of extract was computed and the values were expressed in Gallic acid equivalent mg /g of the dried extract.

Estimation of total flavonoids

Flavonoid contents of extracts were determined by a modified colorimetric method described by Lin and Lee [25]. To 400 µl of distilled water was added 20 µl of extract, 50 µl of a 7.5% sodium nitrite solution and 20 µl of 15% aluminum chloride solution. After 6 min incubation, 200 µl of 1 M sodium hydroxide and 1 ml of distilled water were added to the mixture and the absorbance was read at 510 nm. Quercetin was used for as standard and from the calibration curve, the flavonoids content of different extracts were computed and expressed as quercetin equivalent in mg/g of dried extract.

Estimation of total antioxidant activity

The total antioxidant activity of extract was evaluated by phosphomolybdenum method [26].

The mixture of 1ml of the reagent (4 mM ammonium molybdate, 0.6 M sulfuric acid and 28 mM sodium phosphate) and 200 µl of diluted extract in MeOH was sealed in an Eppendorf tube and boiled at 95ºC for 90 min. The optical density was read at 695 nm using a UV/visible spectrophotometer. Ascorbic acid was used as standard and from the calibration curve ($y = 0.0043x$, $R^2 = 0.9984$) with linear correlation within 25 and 200 µg/ml; the total antioxidant of different extracts was determined and expressed in equivalent ascorbic acid in mg/g of dried extract.

Estimation of total reducing power

The reducing power was estimated by the method of Chou et al. [27]. In a test tube was added 2.5 ml of phosphate buffer (200 mM, pH 6.6), 2.5 ml of 1% potassium ferricyanide and 1 ml of diluted extract. All test tubes were incubated at 50 ºC for 20 min and 2.5 ml of 10% trichloro acetic acid (TCA) was added. They were further kept for 30 min, and then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (1 ml, 0.1%) and incubated for 5 min. The absorbance was measured at 700 nm against a blank using UV/Visible spectrophotometer. The calibration curve was obtained with ascorbic acid with linear correlation (y = 0.0043x, R^2 = 0.9984) between 25 and 200 µg/ml, and the reducing power of the extract was computed and expressed in equivalent ascorbic acid in mg/g of dried extract.

Evaluation of antiradical activity of plant extracts by scavenging systems

All extracts were screened for their antioxidant activity by determining their minimum concentration required to scavenge half (50%) of a fixed amount of each specific radical (IC_{50}) present in the medium. All experiments were designed as follow:

- Assay control which contained reagent and solvent (in which the extract is diluted) without extract;
- Sample which contain extract (at a defined concentration) and reagent;
- Sample control which contain extract and solvent (in which the radical reagent is dissolved) without reagent.

The test absorbance was computed as Absorbance sample – Absorbance sample control. The percentage of scavenging or percentage of inhibition (%I) was computed using the formula below.

$$\%I = 100 \times \frac{Abs\ assaycontrol - A有s\ test}{Abs\ assaycontrol}$$

The IC_{50}were calculated linear regression from the sigmoidal dose-inhibition curve using ED50plus (v1.0)software.**Scavenging of $ABTS^{\circ+}$ radical cation.**

All extracts were screened for their scavenging activity against $ABTS^{\circ+}$ radical cation by the method described by Re *et al.*, [28].

In a flask were mixed 0.3 ml of 17 mM Potassium persulfate and 50 ml of 2mM ABTS solution. The flask was cover with aluminum foil and kept overnight in dark till a green colour was developed. The absorbance was read at 734 nm and adjusted to 0.7 (working reagent) by diluting the solution with distilled water. In a test tube were added 1 ml of MeOH, 0.2 ml of diluted extract in MeOH and 0.16 ml of working reagent. The mixture was incubated for 20 min at room temperature. Absorbance was measured at 734 nm using a UV-visible spectrophotometer. Ascorbic acid was used as standard.

Scavenging of DPPH radical

This essay was carried out in a 96-well microtiter plate as done by many authors [29].

In each well were added 200 µl of freshly prepared 100 µM DPPH and 10 µl of diluted extract in MeOH. The plates were incubated at 37 °C for 30 min and the absorbance was read at 490 nm using an ELISA reader. Ascorbic acid was used as standard.

Scavenging of hydrogen peroxide

Scavenging hydrogen peroxides by plant extracts was evaluated [30].

Various concentrations of the samples (extracts and standard) in methanol (1 ml) were added to 2 mL of hydrogen peroxide solution in phosphate buffer saline (PBS, pH 7.4). After 10 min incubation, the absorbance was measured at 230 nm with a UV-visible spectrophotometer. Ascorbic acid was used as standard.

Anti-lipoperoxidation activity

The protective effect of extract against lipid peroxidation was evaluated on rat brain homogenates. Prior to this experiment we earlier obtained an Ethical clearance for animal experimentation

from the Institutional Animal Ethical Committee of SreeSiddganga College of Pharmacy, Tumkur, Karnataka, India (Ref:SSCPT/IAEC.Clear/141/2012-13) and the Faculty of Health Sciences Institutional Review Board (Ref. No.: 2011/058/UB/.FHS/IRB) at the University of Buea, Cameroon. To prepare the homogenates a total of 6 overnight fasted albinos rats (150-200 g) were sacrificed. The brains were removed, washed with ice cold saline and homogenized in 10 volumes of ice cold PBS (pH 7.4) using a glass homogenizer. The homogenates were cold (4ºC) centrifuged at 1000 rpm for 10 min. A stock solution of 10 mg/ml of extract was prepared in MeOH, and the lower concentrations were obtained by dilution. Tocopherol was diluted in MeOH and used as standard. To 0.25 ml of homogenate was added 0.25 ml of sample and the lipid peroxidation was initiated by adding 0.25 ml of FeCl3 (100 µM) and 0.25 ml of ascorbic acid (100 µM), followed by incubation at 37 ºC for 20 min. The reaction was stopped by adding 1 mL of 0.25N HCl containing 15% trichloro acetic acid 0.375% thiobarbituric acid and 0.05% butylatedhydroxytoluene (BHT). The mixture was boiled for 15 min then cooled, centrifuged at 3000 rpm for 5 min and the absorbance of the supernatant was measured at 532 nm.

Nitric oxide radical inhibition assay

Scavenging nitrite oxide radical of extract was done using modified previously described method [31]. The reaction mixture (6 mL) containing sodium nitroprusside (10 mM, 4 mL), phosphate buffer saline (1 mL) and the extracts, the compound and standard solutions (1 mL) were incubated at 25ºC for 150 min. After incubation, 0.5 mL of the reaction mixture was removed and mixed to 1 mL of sulphanilic acid reagent (0.33%in 20% glacial acetic acid) and allowed to stand for 5 min for completion of diazotization reaction and then 1 mL of NEDD was added, mixed and allowed to stand for another 30 min in diffused light. The absorbance was measured at 540 nm against the corresponding blank solutions in a 96-well microplate titre using ELISA reader (Epoch, USA).

Scavenging hydroxyl radical

Scavenging activity of extracts on hydroxyl radical was achieved using the deoxiribose method [32]. In a test tube was added 1 ml of phosphate buffer, 0.3 ml of 1mM FeCl3 and 0.3 ml of 1mM EDTA. After 2 min pre-incubation was added 0.3 ml of sample, 0.3 ml of 20 mM hydrogen peroxide in phosphate buffer (pH, 7.4, 20 mM), 0.3 ml of 1 mM ascorbic acid and 0.3 ml of 30 mMDeoxyribose. The reaction mixture was further incubated for 30 min at 37 ºC. Then, 1 ml of ice-cold mixture containing 2.8% trichloro-acetic acid and 0.5% thiobarbituric acid in 0.25N HCl were added. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was measured at 532 nm.

Scavenging superoxide radical

Superoxide radical anion scavenging activity was evaluated using PMS-NADH system by modified method of [33].

In each well of 96-well microtiter plate were added 50 µl of 312 µM NBT, 50 µl of 936 µM NADH and 50 µl of sample. The reaction was initiated by adding 5 µl of 120 µM PMS. All reagents were prepared in 100 mM phosphate buffer pH 7.4. The reaction mixture was incubated for 5 min at 25ºC and the absorbance was read at 560 nm using ELISA reader plate.

Statistical analysis

All experiments were run in triplicate and the results were expressed as mean ± standard deviation. Correlation analyses of antioxidant activity versus the total phenolic and flavonoid content were performed using Pearson's correlation in SPSS 17.0. Data were subjected to analysis of variance followed by Tukey's Post-test. The *p* value less than 0.05 was considered to be statistically significant.

RESULTS

Phytochemical screening

All extracts were screened qualitatively for major classes of phytochemical compounds and results are summarized in table 2. The main phytochemical classes targeted were phenolic and flavonoids because these compounds have been reported as potent antioxidants. They were quantified in extracts simultaneously with their total antioxidant activities and reducing powers.

Table2: Results of phytochemical screening for different plant extracts

Extracts	Phytochemical tests					
	Glycosides	Steroids	Saponins	Alkaloids	Carbohydrates	Coumarins
AC	+	-	+	+	+	-
BA	-	+	-	+	+	+
CF	-	-	-	+	-	+
CuA	+	-	+	+	+	-
CyA	+	-	+	-	+	+
DL	+	-	-	-	+	+
LS	+	+	+	+	+	+
NP	+	+	-	-	+	-
PA	-	+	-	+	+	+
PN	-	-	-	+	-	+

Autranellacongolensis; BA: Beilschmiediaanacardioides; CF: Crossopteryxfebrifiga; Cu A: Cussoniaarborea; Cy A: Cyphostema adenaucaule; DL: Dissotislongipetala; LS: Lonchocarpussepium; NP: Naucleapobeguinii; PA: Pycnanthusangolensis; PN: Picralimanitida

The total amount of phenol constituents of different extracts were determined and expressed as Gallic acid (GA) equivalent (mg/g of extract), and it was found that, the amount of phenolic content differed from one plant to another as illustrated in figure 1a.The significantly highest ($P<0.05$) phenolic content was obtained with AC methanolic's extract(450±7.37 equivalent GA mg/g of extract) while the highest flavonoid content was found in NP and AC with values of 144.85±5.16 and 152.15±4.15 mg equivalent ascorbic acid /g of crude extract respectively. Significant positive correlations were observed between total antioxidant activity with total reducing power ($p<0.01$; $R^2=0.8190$), total flavonoids ($P<0.05$; $R^2=0.6419$) and total phenol ($P<0.01$; $R^2=0.8643$).

Antioxidant activity of extracts by scavenging systems

These tests were performed by determining the scavenging activity of the extracts against free radicals. Hence we evaluated the IC_{50} for scavenging activity of all extracts against DPPH, ABTS, superoxide, hydroxyl and nitric oxide radicals, then hydrogen peroxide and lipid peroxidation. On the basis of the results obtained above, all extracts exhibited dose dependent inhibition of $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and hydroxyl radicals. They were also able to prevent lipid peroxidation. The methanolic extracts of CF, DL, LS and AC showed high antioxidant activities and scavenged all radicals tested, while PN, BA, CyA and PA extracts were inactive against hydrogen peroxide, hydroxyl radical and the superoxide radical. The most potent antioxidant activity was obtained with AC extract, which showed lowest significant values ($p<0.05$) of IC_{50} as compared to the standards. The IC_{50} values of this extract were 7.31±0.25, 5.20±0.35, 42.17±0.60, 77.30±7.41, 32.94±1.32, 9.71±1.07 and 121.59±1.08 µg/ml against ABTS, DPPH, hydrogen peroxide, inhibition of lipid peroxidation, superoxide radical, hydroxyl radical and nitric oxide radical respectively (Table 3).

Table 3: IC50 values of plant extracts against free radical tested (µg/ml).

Plant extracts and standards	Antioxidant tests performed						
	ABTS scavenging	DPPH scavenging	Hydrogen peroxide scavenging	Lipid peroxidation	Superoxide radical scavenging	Hydroxyl radical	Nitric oxide radical
AC	7.31±0.25	5.20±0.35	42.17±0.60	77.30±7.41	32.94±1.32	9.71±1.07	121.59±1.08
BA	28.20±0.44	74.07±1.72	-	116.84±12.24	-	255.06±9.62	-
CF	22.23±0.12	15.67±1.44	179.00±0.58	198.25±11.42	92.61±1.16	70.83±0.84	315.14±1.68
Cu A	25.50±0.68	91.93±3.10	-	172.41±6.98	462.69±9.44	100.89±0.62	511.83±4.13
Cy A	26.77±0.78	98.17±2.89	-	886.77±14.02	-	122.02±1.40	-
DL	17.93±0.12	7.73±0.35	198.67±0.88	262.52±3.66	38.72±2.84	58.50±1.96	433.95±6.37
LS	13.17±0.42	29.67±2.24	136.33±1.20	226.48±10.28	101.81±1.66	54.91±1.90	337.28±1.43
NP	28.27±0.47	51.00±1.55	131.00±0.58	260.29±5.64	-	35.53±0.37	288.34±0.08
PA	38.13±0.88	65.27±9.41	-	161.12±4.21	-	278.09±10.35	-
PN	29.27±0.19	85.17±2.89	-	428.83±8.36	-	159.85±10.24	-
Ascorbic acid*	6.92±0.07	3.60±0.23	123.00±2.65	-	98.68±4.44	49.78±5.65	117.24±0.74
α-tocopherol*	-	-	-	90.35±4.65	-	-	-

-: not determined

*standard molecule

Values are mean ± standard error of mean.

The lower the IC50 value, the higher the antioxidant activity.

Autranellacongolensis; BA: Beilschmiediaanacardioides; CF: Crossopteryxfebrifiga; Cu A: Cussoniaarborea; Cy A: Cyphostema adenaucaule; DL: Dissotislongipetala; LS: Lonchocarpussepium; NP: Naucleapobeguinii; PA: Pycnanthusangolensis; PN: Picralimanitida

Negative significant correlations (table 4) were obtained between the IC_{50} of all scavenging systems tested and the total phenol content of the extracts. Marked negative correlations ($P<0.05$) were also observed between ABTS, DPPH, superoxide, hydroxyl and nitric oxide radicals' assays with the total antioxidant activity and the total reducing powers.

ISSN 2348-6201

Table 4: Correlations between the IC_{50} of various scavenging systems and the quantification systems

Systems	Total Phenol	Total antioxidant activity	Total Flavonoids	Total reducing power
ABTS	-0,83562**	-0,86662**	-0,25865	-0,80448**
DPPH	-0,74263**	-0,75048**	-0,33214	-0,8417**
Hydrogen peroxide	-0,69477**	-0,55338*	-0,55492	-0,56711*
Hydroxyl radical	-0,65817**	-0,8244**	-0,37648	-0,66075*
Lipid peroxidation	-0,38589**	-0,14843	-0,34272	-0,43976
Nitric oxide radical	-0,87665**	-0,81035**	-0,65461	-0,63045*
Superoxide radical	-0,64351*	-0,79903**	-0,36334	-0,79399**
**: Correlation is significant at p< 0.01 level *: Correlation is significant at p< 0.05 level				

DISCUSSION

Plants contain a wide variety of free radical scavenging molecules, such as flavonoids, anthocyanins, carotenoids, dietary glutathionine, alkaloids, tannins, saponins, steroids, terpenoids and rotenoids which are rich in antioxidant activities [6]. ROS produced *in vivo* include superoxide radical (O_2^{*-}), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) can interact in the presence of certain transition metals to yield highly-reactive oxidizing species, the hydroxyl radical (HO*) [34]. Consequently, an adequate intake of polyphenols diet can help to prevent the oxidative stress generated when the production of ROS is beyond the antioxidant capacity of a biological system [35, 36]. Quantitative analyses showed that *Autranellacongolensis*' content in total flavonoids, total phenolic compounds, total antioxidant and total reducing power was important compare to other extracts (figure 1).

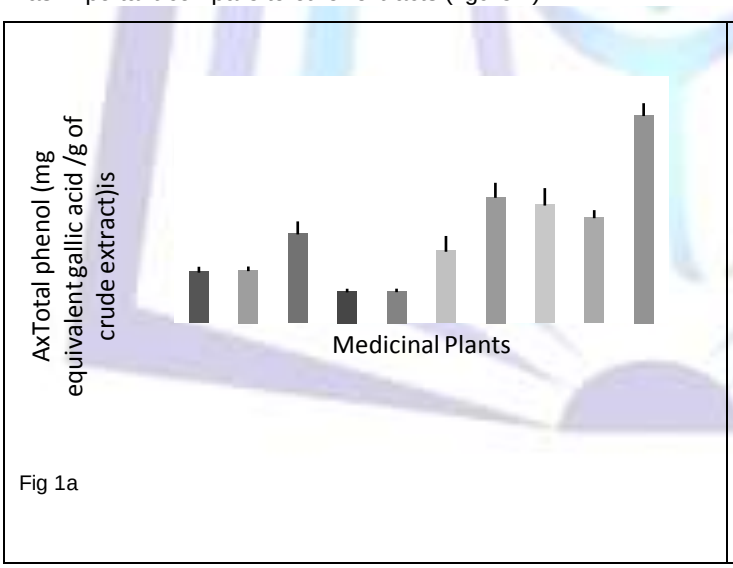

Fig 1a

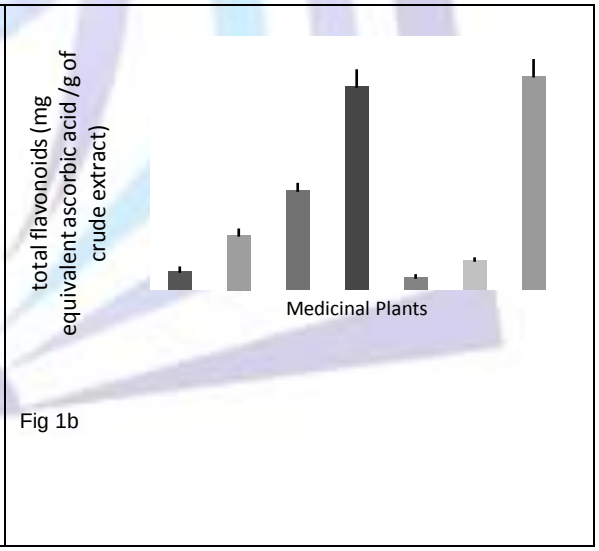

Fig 1b

ISSN 2348-6201

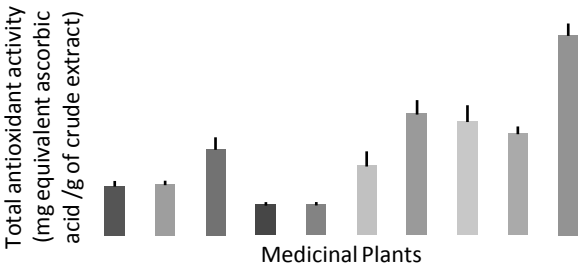

Fig 1c

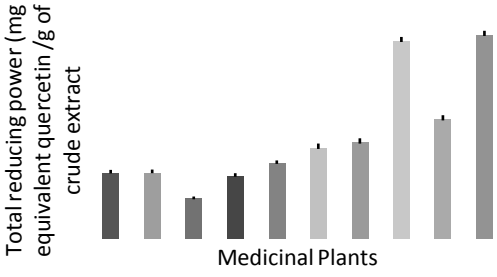

Fig 1d

Figure 1: Quantitative analysis of crude extracts. 1a: Total phenol (mg equivalent gallic acid /g of crude extract); 1b: total flavonoids (mg equivalent ascorbic acid /g of crude extract); 1c: Total antioxidant activity (mg equivalent ascorbic acid /g of crude extract);1d: Total reducing power (mg equivalent quercetin /g of crude extract.

Ethnobotanical investigations have earlier shown its importance in the treatment of diarrhoea and chronic dysentery [9]. Previous Chemical investigations of *A. congolensis* led to the isolation of six compounds of which three were pentacylictriterpenes of the taraxerane type, together with one Δ7-sterol (24R)-stigmast-7,22(E)-dien-3α-ol (chondrillasterol), and two phenolic compounds (+)- catechin and 24-feruloytetracosanoic acid [9]. The use of this plant as antiinflamatory agent could thus be linked to (+)-catechin[9]. Flavonoids reduce free radicals by quenching, up-regulating or protecting antioxidant defenses and chelating radical intermediate compounds. Alkaloids and their synthetic derivatives from this plant possess analgesic activities when administered to animals [37]. A lower IC_{50} value corresponds to a larger scavenging power and a DPPH radical scavenging activity of less than 50 µg/ml is considered significant [38]. Based on IC_{50} values, results presented in table 3 show that the methanolic extract of *Autranellacongolensis* is comparable to standard drugs ascorbic acid and alpha tocopherol and deployed the highest antiradical activity among the extracts tested. This important antiradical activity may be due to its important content in total phenol content, given that a strong negative correlation exists between total phenol content and the IC_{50} of various scavenging systems (table 4). The use of AC in treating different ailments associated with oxidative stress like Diarrhea and chronic dysentery, malaria [8, 9] is hereby justified. The potentials of this plant's extract as a source of natural antioxidants in many devastating diseases like AIDS and hepatitis should be envisaged [37]. *B. anacardioides*(BA) stem bark is used in the Noun sub-division of the West Region of Cameroon to treat uterine tumours, rubella, rheumatisms, bacterial and fungal infections [10] whereas seeds are used as spices. Another species *B. lancilimba* is used in the same region to cure skin bacterial infections whereas *B. manii* is used to treat dysentery and headache and as an appetite stimulant [10, 39]. Its phytochemistry revealed the presence of flavonoids and phenols (figure 1) which can justify its antioxidant, antiradical and reductive properties. Although its total phenol and flavonoids content appears to be lower compared to that of other extracts, its total reducing power is considerable (figure1). BA however presented IC_{50} values indicating significant antiradical properties on some radical systems notably the DPPH (28.20±0.44) and ABTS (74.07±1.72) systems. The lack of action on other systems like hydrogen peroxide, hydroxyl radical and the superoxide radical may be due to the nature of their phenol compounds. Other studies have shown that phenolic compounds isolated from *Beilschmiedia* species like Vanillin and 4-hydroxybenzaldehyde were analgesic, anti-inflammatory and antifungal [10]. Endiandric acid H used to manufacture drugs for treatment of asthmatic disorders or concomitant inflammatory symptoms was isolated from this species. Traditional preparations of *Crossopteryxfebrifuga* (Afzel.) Benth. (CF) (Rubiaceae) are widely used in Northern Nigeria in the management of trypanosomiasis, malaria and painful inflammatory disorders [11]. Previous studies have shown that the methanolic extract of the stem bark of CF possesses significant analgesic and anti-inflammatory properties possibly mediated via Non-selective inhibition of cyclo-oxygenase pathways. A recently discovered property as cytoprotective effect on the gastrointestinal tract (GIT) [11] and as antimalarial [12] may be due to its important content in phenolic, flavonoids and tannins (figure 1). Its significant antiradical and antioxidant activity on the DPPH and ATBS systems with IC_{50} less than 50 µg/ml indicates a source of potent interesting drugs against infectious diseases and debilitating effects due to opportunistic infections always associated to immunosuppressive diseases and inflammation conditions. *Cussonia species* are used in African traditional medicine mainly against pain, inflammation, gastro-intestinal problems, malaria and sexually transmitted diseases [40]. An average total antioxidant activity and total reducing power of *Cussoniaaboria*was obtained in this study. The antioxidant property of *Cyphostema adenaucaule (Orchidaceae)* is presented for the first time in this work. *Dissotislongipetala* (DL) presented a phytovchemical profile made up of glycosides, carbohydrates, phenols, tannins and coumarins. Its bark extract indicated high total phenol and low total flavonoids contents (figure 1) with very important antioxidant and reducing power. It can be postulated that these antioxidant and reducing power are due to its important phenolic content. IC_{50}≤50 µg/ml were obtain against 3 of the 6 antiradical systems notably ABTS, DPPH, and superoxide radicals. For the best of our knowledge antioxidant and reducing powerproperties are revealed here for the first time. Although the total flavonoids of *Lonchocarpussepium* content was poor (figure 1b), the extract was rich in total phenol which correlated well with its antioxidant and reductive power (figure 1a, 1c, 1d). The antiradical power of the extract was interesting on the ABTS, DPPH, and the hydroxyl radical systems. This evaluation on *Lonchocarpussepium* appears to be the first of its kind on the antiradical activity of this species. *Naucleapaubeguinii*is a *Rubiacea*and many pharmacological

studies have been reported on some family members like *Nauclealafolia* indicated the presence of flavonoids, phenols, tannins and alkaloids with anticancer and anti-inflammatory properties [41]. We detected in the methanolic extract of *Naucleapaubeguinii*the presence of these same phyto-constituents with strong antioxidant activity and reduction power correlating with its phenol and flavonoids contents. The extract was also very efficient on many antiradical systems like the ABTS, DPPH and hydroxyl radical with IC_{50} close to 50 µg/ml. *Pycnanthusangolensis,* a *Myristicaceae* is used against bile & liver complaints, stomachache, hypercholesterolemia, type 2 diabetes and cancer [17]. Its leaves' methanolic extract have been proven to possess anti- helminthic and antimicrobial properties [42].Allantoin, isoflavonoids and dihydroguaiacetic acid have been isolated from the bark while some terpenoidquinones with hypoglycaemic activity have been found in the bark and leaves [42]. *Picralimanitida* seeds' extract has been studied before; it possesses anti-inflammatory and analgesic properties. The extract in addition to its known opioid analgesic actions also has antagonistic actions on bradykinin-induced hyperalgesia. The extract also induces rat cytochrome P450 enzymes [43]. This is in accordance with our study showing an important antioxidant activity and reductive power. Mabeku*et al*. [44] detected the presence of Alkaloids, flavonoids, saponins, polyphenols in the methanolic extract of PN and described its antimicrobial properties. In general ethnobotanical investigations reported that medicinal plants studied are used for the treatment of various diseases associated with oxidative stress. The therapeutic benefit of these plants may be due to their antioxidant properties which were explored using different free-radical generating systems. A wide range of works have been done in this field to sustain the protective effect of dietary phenols in the protection against degenerative diseases [45-53]. Selective scavenging activities of AC, CF, CuA, DL and LS extracts were observed against superoxide anion. The reported activity is the scientific basis of the protective effect of these plants against oxidative damage. Because all the extracts deployed significant antioxidant activity, their protective activity on DNA strand scission is to be explored. The protective effect of the extracts on lipid peroxidation was shown in our studies. Lipid peroxidation generally initiated by pro-oxidant species is a common mechanism of tissue necrosis during oxidative damage [54, 55].

These results suggest that selected plants can be used as a source of antioxidants for pharmacological preparations. The ability of these extracts to quench free radicals using *in vivo* oxidative stress models is to be envisaged.

AUTHOR'S CONTRIBUTIONS

AJN designed the protocol, executed the laboratory work and drafted the manuscript. PEAN contributed to ethnobotanical survey, provided the plant and contributed to draft the manuscript.JRC prepared the extracts, while JCAN contributed to ethnobotanical survey and to design the protocol, read and substantially revised the manuscript. SW contributed to the protocol designing and revised the manuscript. SMB, VVV and BDT contributed to monitor the laboratory work and provided required chemicals and laboratory consumables for different analysis. All authors read and approved the manuscript.

ACKNOWLEDGMENTS

The authors thank the Centre of International Co-operation in Sciences (CICS), Chennai, India, to provide Research Training Fellowship for developing Country Scientists (RTFDCS) to one of the authors (AJN). They also thank the Research laboratory of the Sri Siddaganga College of Pharmacy, Tumkur, Karnataka, India for providing research infrastructures, the International Foundation for Science (IFS) and the African Phytomedicine Center Alango Foundation, Dschang Cameroon for the partial financial support.

COMPETING INTERESTS

The author(s) declare that they have no competing interests.

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