Antioxidant, Total Phenolic and Flavonoid Content of Selected Nigerian Medicinal Plants

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Received: November 13, 2014; Accepted: February 25, 2015; Published (web): February 26, 2015

ABSTRACT: Plant phenolics and flavonoids play a great role in scavenging free radicals in the body and act as antioxidants. Thus their determination is sometimes nedded. Total antioxidant capacity, total phenolic and flavonoid contents of the extracts of 10 medicinal plants (*Lochnera rosea*, *Allamanda cathartica*, *Asplenium platyneuron*, *Euphorbia prostrate*, *Baphia nitida*, *Crotolaria retusa*, *Zapoteca portoricensis*, *Platycerium bifurcatum*, *Mussaenda afzelii* and *Craterosiphon scandens*) from 7 botanical families growing in the tropical rainforest of Nigeria were included in this study. The total antioxidant capacity of the extracts was assessed by using the phosphomolybdate method. The phenolic content was determined by using Folin-Ciocalteau assay, while the total flavonoid was determined by the aluminium chloride colorimetric assay. The results obtained showed that the total antioxidant capacity for all the extracts were in the range of 0.888 ± 0.75 to 0.938 ± 0.00 mg EAA/g. The results showed that *E. prostrate, P. bifurcatum* and *A. platyneuron* were found to be the richest source of phenolic (97.77 ± 0.77, 87.62 ± 1.22 and 82.33 ± 0.30 mg GAE/g) while *B. nitida* and *M. afzelii* had the least total phenolic content *(*11.67 ± 0.09 and11.18 ± 0.30 mg GAE/g). The highest total flavonoid content was revealed in *P. bifurcatum* (648.67 ± 12.3 mg QE) while *M. afzelii* also had the least total flavonoid content (3.67 ± 0.00 mg QE/g). The ratio of flavonoid to the phenolic in each extract was also determined to ascertain extracts that are rich in flavonoids.

Key words: Total antioxidant capacity, total phenolic, total flavonoid, Folin-Ciocalteau, aluminium chloride, gallic acid

INTRODUCTION

Free radicals are generated *in vivo* by living cells from the cell metabolism. However, excess free radicals are responsible for some degenerative diseases like atherosclerosis, ischemic heart disease, ageing, diabetes mellitus and cancer.[1] Free radicals cause oxidative damage to macromolecules in the body, such as lipids, proteins and nucleic acids. Antioxidants prevent such free radicals from oxidative damage to DNA, proteins, and cells by donating electrons to stabilize and neutralize the harmful effects of the free radicals. Plant-derived antioxidants have received greater attention since they act as radical scavengers. Natural antioxidants are less toxic as compared to their synthetic analogues like butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT), which have been reported to be carcinogenic and cause liver damage.[2] Plant phenolics are compounds derived from phenylalanine and tyrosine and are among the widely distributed secondary metabolites in the plant kingdom.[3] Plant phenolics include flavonoids, condensed tannins, coumarins and stilbenes.[4]

Phenolics are regarded as the molecules with the highest potential to neutralize free radicals. These compounds act mainly as antioxidants due to their ability to scavenge free radicals and chelate metals *in vitro* and *in* vivo.[5] Therefore, high consumption of phenolic compounds leads to reduce risk of cardiovascular diseases and cancers. Plant phenolics have been reported to have a lot of biological activities including anti-carcinogenic, antioxidant and anti-mutagenic.[6] The present study involves

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quantitative determination of total phenolic, total flavonoid content and total antioxidant capacity from ten Nigerian medicinal plant extracts.

MATERIALS AND METHODS

Chemicals and reagents. Folin-Ciocalteu reagent was obtained from Lobal Chemie (India). Methanol was bought from Sigma-Aldrich (Germany). Sodium hydroxide (NaOH), quercetin, sulphuric acid (H_2SO_4) and ascorbic acid were purchased from BDH (England). Sodium nitrite ($NaNO_2$) and gallic acid were procured from Qualikems (India). Sodium carbonate (Na_2CO_3), aluminium chloride ($AlCl_3.6H_2O$), sodium phosphate (NaH_2PO_4) and ammonium molybdate were bought from JHD (China). All chemicals used were of analytical grade.

Plant material. The plants *Lochnera rosea* (Apocynaceae), *Allamanda* cathartica (Apocynaceae), Asplenium *platyneuron* (Aspleniaceae), *Euphorbiaprostrate* (Euphorbiaceae), *Baphia nitida* (Fabaceae), *Crotolaria retusa* (Fabaceae), *Zapoteca portoricensis* (Fabaceae), *Platycerium bifurcatum* (Polypodiaceae), *Mussaenda afzelii* (Rubiaceae) and *Craterosiphon scandens* (Thymelaeaceae) were collected from Orba in Enugu State Nigeria, in the month of March 2014 and authenticated by Mr. Alfred O. Ozioko at the International Centre for Ethnomedicine & Drug Development (InterCEDD), Nsukka, Nigeria.

Preparation of plant extracts. The air dried plant parts were pulverized and each of the powdered material (20.0 g) was macerated with 200 mL of methanol at room temperature for 48 h with agitation. The filtrates were concentrated *in vacuo* at reduced pressure and temperature (40°C) to obtain the extracts. The dried extracts were lyophilized to make them crispy.

Total phenolic content (TPC) determination. Folin-Ciocalteau method was used for the determination of the total phenolic content of the plant extracts using gallic acid as an internal standard with slight modification as previously reported.[7]

Briefly, 1 ml of the extract (1 mg/ml) was mixed with 9 mL of distilled water in a 25 mL volumetric flask. Two and half milliter (2.5 mL) of a 10 fold dilute Folin-Ciocalteau phenol reagent (FCPR, 1:10) was added. After 5 min, 10 mL of 7.5% Na_2CO_3 solution was added to the mixture and made up to the mark with distilled water. The mixture was incubated in the dark for 90 mins at room temperature. A set of standard solutions of gallic acid (200, 175, 150, 125, and 100 μ*g*/mL) were prepared in the same manner as described for the extracts. The absorbances of the extracts and standard solutions were read against the reagent blank at 760 nm with a UV/Visible spectrophotometer (UV-1800, Shimadzu, Japan). The total phenolic content was determined from the calibration curve (Figure1) and expressed as milligram of gallic acid equivalent (GAE) per gram of the extracts.[8] The determination of the total phenolic in the extract was carried out in triplicate.

Determination of total flavonoid content (TFC). Aluminium-chloride colourimetric assay was used to determine the total flavonoid content in the extracts as previously reported.[6] Briefly, 1 ml of the extract (1 mg/mL) was mixed with 4 mL of distilled water in a 10 mL volumetric flask. 0.30 mL of 5% sodium nitrite was added to the flask. After 5 min, 0.30 ml of 10% $AlCl_3.6H_2O$ solution was added to the mixture, followed by addition of 2 mL of 1.0 M NaOH after another 5 min and diluted to the mark with distilled water. A set of standard solutions of quercetin (100, 80, 60, 40 and 20 μ*g*/ml) were prepared in the same manner as described for the extracts. The absorbances of the extracts and standard solutions were measured against the reagent blank at 510 nm with a UV/Visible spectrophotometer. The total flavonoid content was determined from the calibration curve (Figure 2) and expressed as milligram of quercetin equivalent (QE) per gram of extracts.[9] The determinations of total flavonoid in the extracts and standards were carried out in triplicates.

Qualitative phytochemical analysis of the extracts. Phytochemical analysis of the plant extracts *Antioxidant, Total Phenolic and Flavonoid Content* were done to determine the presence of flavonoids, tannins and phenolics according to standard methods.[10]

***In vitro* antioxidant assay.** The *in vitro* antioxidant assay of the extracts was carried out by

dissolving 100 mg of the extracts in 100 mL of distilled water to form stock solutions (1 mg/mL or 1000 µg/mL). Serial dilutions (300, 250, 200, 100, 50 and 25 µg/mL) of each extract were made from the stock solution. Ascorbic acid (ASA) was used as standard for the assay.

Total antioxidant capacity (TAC). The total antioxidant capacity of the extracts was determined by the phosphomolybdate method.[11-13] Briefly, 0.1 ml aliquot of various concentration of the plant extracts (300, 250, 200, 100, 50 and 25 µg/ml) was mixed with 1 ml of reagent solution (600 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate, 1:1:1). The test tubes were covered with aluminium foil and incubated in a water bath at 95^{o}C for 90 min. Then the extracts were cooled to room temperature and the absorbance of the mixture was determined at 765 nm against a blank containing 1 ml of the reagent solution. Ascorbic acid was used as standard. The assay was carried out in triplicate. The total antioxidant capacity (TAC) was expressed as mg equivalents of ascorbic acid per gram (EAA/g). The antioxidant capacity was estimated by using the following formula:

$$\text{Total antioxidant capacity (\%)} = \frac{\text{Absorbance of ascorbic acid} - \text{Absorbance of extract}}{\text{Absorbance of ascorbic acid}} x\ 100$$

RESULTS AND DISCUSSION

Extraction yield and phytochemical screening. The percentage yield values of the extracts are shown in Table 1. The percentage yield of the extracts varied from 9.52 to 1.42. *M. afzelii* had the highest yield while *Z. portoricensis* stem extract gave the lowest yield. Phytochemical screenings of the extracts showed presence of flavonoids and tannins/phenolics.

Table 1. Percentage yield of different extracts.

Plant Extracts	Part's investigated	% yield
Lochnera rosea	Leaf	2.8
Allamanda cathartica	Leaf	6.6
	Stem	1.8
Asplenium platyneuron	Leaf	5.38
Euphorbia prostrata	Leaf	3.65
Baphia nitida	Leaf	8.26
Crotolaria retusa	Leaf	4.00
Zapoteca portoricensis	Leaf	4.0
	Stem	1.4
Platycerium bifurcatum	Leaf	4.41
Mussaenda afzelii	Leaf	9.52
Craterosiphon scandens	Leaf	4.48
	Stem	1.93

Percentage yield of the extracts is based on dried plant material.

Total phenolic content. The total phenolic content (TPC) of the different plant extracts measured according the Folin-Ciocalteau method is shown in Table 2. The total phenolic contents measured by the Folin–Ciocalteau method varied between 11.18 ± 0.30 and 97.77 ± 0.77 mg gallic acid equivalent per gram of extract.

Total flavonoid content. The content of total flavonoid of the different plant extracts measured spectrophotometrically by using the aluminium chloride colorimetric assay is shown in Table 2. The flavonoid content of the extracts was expressed as mg quercetin equivalent per gram of the extract.

Total antioxidant capacity (TAC). The total antioxidant capacity of the extracts was determined by the phosphomolybdate method. The results obtained showed that the total antioxidant capacity for all the extracts were in the range of 0.888 ± 0.75 to 0.938 ± 0.00 mg EAA/g (Table 3, Figure 3).

The percentage yield values of the methanol extracts of the plants are shown in Table 1. Amongst all the extracts, *M. afzelii* showed the highest yield (9.52%), while *Z. portoricensis* stem extract showed the minimum (1.42%). Plants rich in phenolics are being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food.[7] Total phenolics content of the plant extract is determined either by chemical method or quantitatively by spectrophoto-meteric method using Folin-Ciocalteau reagent.[14] This method measures the amount of the extract needed to inhibit the oxidation of the Folin–Ciocalteau reagent.[15] Folin-Ciocalteau reagent is sensitive to reducing compounds including

polyphenols. Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce a blue coloured complex (Molybdenum blue). This blue colour complex can be quantified spectrophoto-metrically, thus total phenolic content can be determined.[16] The total phenolics content (TPC) of the extracts was determined from the regression equation of the calibration curve (y = 0.0046x + 0.1616; R^2 = 0.9561) (Figure 1). *E. prostrata* extract had the highest total phenolics content; whereas the contents obtained from *B. nitida* was the least. Colorimetric reactions are widely used to determine total flavonoids content in food or medicinal plant samples. Colorimetric assay is easy to perform, rapid and applicable in routine laboratory use and low-cost.[4]

Table 2. Contents of total phenolic and flavonoid in selected medicinal plant extracts.

Plant	Family	Parts investigated	Total Phenolics (mg GAE/g)	Total flavonoids (mg QE/g)	Flavonoids/Phenolics (F/P ratio)
Lochnera rosea	Apocynaceae	Leaf	56.61 ± 3.26	95.35 ± 1.65	1.68
Allamanda cathartica	Apocynaceae	Leaf	53.35 ± 1.87	170.30 ± 0.10	3.19
		Stem	38.78 ± 0.00	140.30 ± 0.10	3.62
Asplenium platyneuron	Aspleniaceae	Leaf	82.33 ± 0.30	135.0 ± 0.00	1.64
Euphorbia prostrata	Euphorbiaceae	Leaf	97.77 ± 0.77	167.0 ± 0.00	1.71
Baphia nitida	Fabaceae	Leaf	11.18 ± 0.30	36.35 ± 0.17	3.25
Crotolaria retusa	Fabaceae	Leaf	15.00 ± 0.00	10.33 ±0.00	0.69
Zapoteca portoricensis	Fabaceae	Leaf	27.66 ± 1.97	63.67 ± 0.20	2.30
		Stem	23.90 ± 1.70	25.30 ± 0.22	1.06
Platycerium bifurcatum	Polypodiaceae	Leaf	87.62 ± 1.22	648.67 ± 12.3	7.40
Mussaenda afzelii	Rubiaceae	Leaf	11.67 ± 0.09	03.67 ± 0.00	0.32
Craterosiphon scandens	Thymelaeaceae	Leaf	16.43 ± 1.32	07.00 ± 0.00	0.43
		Stem	19.06 ± 1.02	05.34 ± 1.66	0.28

Values are expressed as mean ± SD (n=3). The absorbance against the reagent blank was determined at 710 nm and 510 nm with an UV-Visible spectrometer for phenolics and flavonoids, respectively. Total phenolics content was expressed as mg gallic acid equivalents (GAE) and total flavonoid content expressed as mg quercetin equivalents (QE)

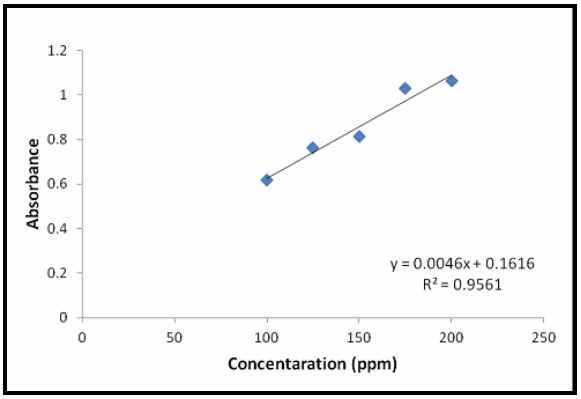

Figure 1. Calibration curve for gallic acid.

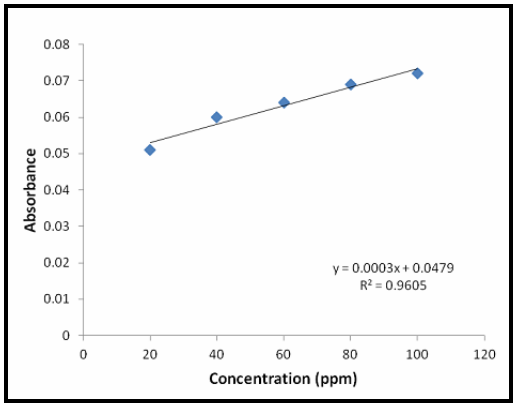

Figure 2. Calibration curve for quercetin.

Colorimetric method is based on the formation of the flavonoids - aluminium complex which has an absorptivity maximum at 510 nm. The complex formation is carried out in the presence of $NaNO_2$ in alkaline medium[17] and is based on the nitration of aromatic ring of the flavonoid with free hydroxyl groups at three or four positions. Also, aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavonoids.[15] The total flavonoids content (TFC) of the extracts was determined from the regression equation for the calibration curve (y = 0.0003x + 0.0477; R^2 = 0.9605) (Figure 2). Total antioxidant capacity (TAC) assays involve single electron transfer (SET) and hydrogen atom transfer (HAT). SET assays are easier than HAT assays and involve

the reduction of an oxidant which results in colour change.[18] Single electron transfer (SET) assay like the phosphomolybdate method was used in this study to determine the reduction capacity of the extracts. The assay is based on the reduction of Mo^{+6} to Mo^{+5} by the extract and subsequent formation of a green phosphate/Mo^{+5}complex at acidic pH. Figure 3 shows the percentage total antioxidant capacity of extracts with extracts belonging to the families of *Fabaceae*, *Thymelaeaceae* and *Apocynaceae* having the highest % TAC. The high phenolics content of the extracts indicates high antioxidant capacity because the phenolics react with active oxygen radicals such as hydroxyl radical, superoxide anion radical and lipid peroxy radical. Studies revealed that there is high correlation between antioxidant capacity

Table 3. Total antioxidant capacity of the extracts.

Plant Extracts	Parts investigated	Phosphomolybdate assay (mg EAA/g)
Lochnera rosea	Leaf	0.918 ± 0.10
Allamanda cathartica	Leaf	0.919 ± 2.50
	Stem	0.917 ± 0.80
Asplenium platyneuron	Leaf	0.906 ± 0.00
Euphorbia prostrata	Leaf	0.888 ± 0.75
Baphia nitida	Leaf	0.935 ± 0.25
Crotolaria retusa	Leaf	0.938 ± 1.00
Zapoteca portoricensis	Leaf	0.897 ± 1.10
	Stem	0.897 ± 1.10
Platycerium bifurcatum	Leaf	0.941 ± 0.00
Mussaenda afzelii	Leaf	0.888 ± 0.10
Craterosiphon scandens	Leaf	0.932 ± 0.55
	Stem	0.938 ± 0.00
Ascorbic Acid	-	-

Values are expressed as mean ± SD (n=3). The absorbance against the reagent blank was determined at 765 nm with an UV-Visible spectrometer. Total antioxidant capacity was expressed as mg equivalents of ascorbic acid per gram.

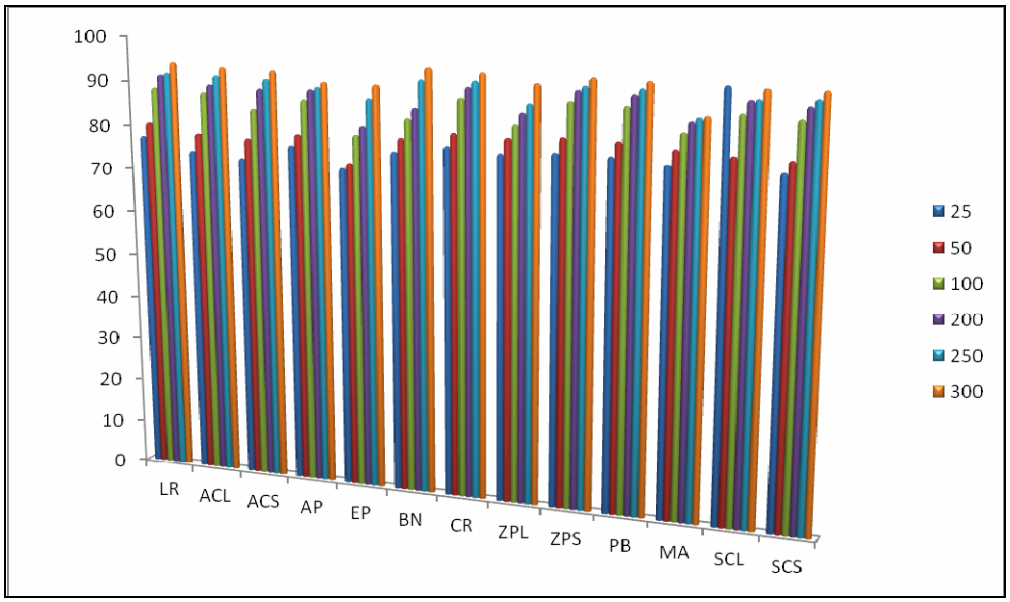

Figure 3. Percentage total antioxidant capacity (TAC) activity of investigated plant extracts.

LR = *Lochnera rosea*, ACL/ACS = *Allamanda cathartica* (Leaf & Stem), AP = *Asplenium platyneuron*, EP = *Euphorbia prostrate*, BN = *Baphia nitida*, CR = *Crotolaria retusa*, ZPL/ZPS = *Zapoteca portoricensis* (Leaf & Stem), PB = *Platycerium bifurcatum*, MA = *Mussaenda afzelii*, SCL/SCS = *Craterosiphon scandens* (Leaf & Stem).

and phenolics content.[19] Our present study showed that *P. bifurcatum*, *A. cathartica* and *B. nitida* extracts have high flavonoid/phenolics ratio indicating that the extracts have high flavonoid content. Our data also suggests high correlation between total flavonoids content and antioxidant capacity considering the high antioxidant activity with these extracts. The as methanol extract of *E. prostrata* has been reported to possess scavenging activity in DPPH model.[20] The antioxidant potential is due to the presence of phenolics and flavonoids which are known to inhibit free radicals. This result agrees with our present study in which the phenolic and flavonoid contents were observed to be high. The

isolation of polyphenolic compound- quercetin 3-O-β-D-glucopyranoside and a carboxylic acid, chlorogenic acid with antioxidant properties from *P. bifurcatum* has been studied.[21] The observed antioxidant activity could be attributed to these metabolites. Our present investigation shows that the plant extract had high phenolic and flavonoid contents. *In vivo* administration of various doses of ethanol extract of *B. nitida* to rats significantly reduced liver anti-oxidant enzymes namely super oxide dismutase (SOD), catalase and peroxidase in a dose dependent manner.[22] This is an indication that the ethanol extract of *B. nitida* possesses antioxidant activity which could be attributed to the presence of secondary metabolites like flavonoids and phenolics. The present study indicates that the flavonoid to phenolics ratio of the extract is 3.25 indicating that the extract has high flavonoid content, which could be responsible for its antioxidant activity. Study has shown that leaf extract of *Z. portoricensis* significantly increased malondialdehyde (MDA) levels and decrease superoxide dismutase (SOD) and catalase (CAT) activities in experimental rats.[23,24] This is an indication that the extract has antioxidative potential which is attributed to the presence of flavonoids in the extract, thus supports our present findings. The leaf extract of *C. roseus* (*L. rosea*) has been shown to reduce the serum cholesterol and total triglycerides levels in normal rats.[25] This reduction resulted from the presence of flavonoids in the juice, which has antioxidant activity. Our present study agrees with this report, since the extract of *L. rosea* had flavonoid/phenolics ratio of 1.68. To the best of our knowledge, no *in vitro* antioxidant assay of *A. platyneuron*, *M. afzelii* and *C. scandens* has been reported.

CONCLUSION

Extracts of ten Nigerian medicinal plants was investigated for their total antioxidant capacity, phenolics and flavonoids content. Apocynaceae and Fabaceae families were found to be rich in flavonoids, since these families had high flavonoids/phenolics ratio.

ACKNOWLEDGEMENT

The authors are grateful to Mr. Alfred O. Ozioko of International Centre for Ethnomedicine & Drug Development (InterCEDD) Nsukka, Nigeria.

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