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Antioxidant potential of some Nigerian herbal recipes

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Abstract

Free-radicals have become a major source of health concern. The search for drug templates led to the screening of *Acalypha ciliata, Acalypha hispida*, *Acalypha indica*, *Acalypha wilkesiana* var. golden-yellow, *Acalypha wilkesiana* var. lace- acalypha, *Acalypha wilkesiana* var. red-acalypha, *Centrosema procumbens*, *Centrosema pulmieri, Cyathula prostrata, Pycnanthus angolensis* and *Tridax procumbens* for potential antioxidant activity. The plant extracts tested positive for initial rapid free-radical scavenging activity when sprayed with DPPH reagent. The extracts of *A. ciliata, A. hispida*, *A. indica*, *A. wilkesiana* var. golden-yellow, *A. wilkesiana* var. lace-acalypha and *Tridax procumbens* gave marginal antioxidant activity (IC_{50}) of 0.65, 0.61, 0.72, 0.78, 0.76 and 0.67µg mL^{-1}. However, *C. prostrata and P. angolensis* demonstrated remarkably moderate IC_{50} of 0.48 and 0.55 µg mL^{-1} respectively. The determined IC_{50} of *C. prostrata* was stronger than that of *P. angolensis* and compare favourably with the IC_{50} obtained for vitamin C at 0.45 µg mL^{-1}. Furthermore, the determined IC_{50} values of *C. prostrata* and *P. angolensis* were better than those of vitamins A and E at 0.57 and 0.59 µg mL^{-1} respectively. The antioxidant activities given by the extracts were instructive because the phytochemical screening of the plants indicated the presence of terpenes, flavonoids and tannins which have been known to exhibit antioxidant activities. The results of the antioxidant assays have revealed *C. prostrata and P. angolensis* as potential plant templates which could be further investigated in detailed *in-vitro* and *in-vivo* studies for potentially safer and cheaper antioxidant drugs for the fight against destructive free-radicals.

Keywords: Free-radicals; antioxidant activity; DPPH reagent; *C. prostrata; P. angolensis.*

INTRODUCTION

The extracts of plants have provoked interest in the search for natural products. They are being screened for their potential use as alternative remedies in the treatment and management of many disease conditions especially in free-radicals implicated ailments. Also, their use in the preservation of food, pharmaceuticals, spices and nutriceuticals from the toxic effects of the harzadous oxidants cannot be over-emphazised (Liz-Balchin and Deans, 1997). The increased attention on plants became imperative because of the toxicities encountered with the synthetic antioxidants such as butylated hydoxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butylated hydroquinone (TBHQ) (Chan, 1987; Barlow, 1990; Naimiki, 1990; Porkorny, 1991). Moreover, the trend of employing plant extracts and isolated compounds therefrom has begun to gain wide acceptabilty in the food industry and amongst consumers of health products as the problems associated with these synthetic oxidants are being

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mitigated and shelf-lives of drugs and food products are also being prolonged thereby (Dormain *et al*., 2000; Burt, 2004; Svoboda *et al.*, 2006). In the light of this reality, ten plant extracts were screened for possible antioxidant activity with the aim of obtaining templates with minimal or no toxicities. It is hoped that some if not all of these natural templates may become beneficial to man in the current fight against the destructive reactive oxygenated species (ROS) that are responsible for disease conditions such as hypertension, premature aging, cancers, respiratory dysfunctions and vitamin deficiencies amongst many others.

EXPERIMENTAL

Collection of plant materials. The plants used in this study are namely; *Acalypha ciliata*, *Acalypha hispida*, *Acalypha indica*, *Acalypha wilkesiana* var. golden-yellow, *Acalypha wilkesiana* var. lace- acalypha, *Acalypha wilkesiana* var. red-acalypha, *Centrosema procumbens*, *Centrosema pulmieri*, *Cyathula prostrata*, *Pycnanthus angolensis* and *Tridax procumbens.* The plants were collected around August, 2012 from forests, open fields and abandoned roadside farms at Itak Ikot, Ikono Local Government Area, Akwa Ibom State, Nigeria. The plants were identified in their fresh states by staff in the Department of Pharmacognosy and Natural Medicine, University of Uyo, Nigeria. Afterwards, voucher specimens labelled No H102 to No H112 were prepared and deposited in the Herbarium Unit.

Extraction and processing of plant materials. Aerial parts of plants were separately oven-dried (40 ^{0}C) and then ground into coarse powders on an electric mill (Gallenkamp, UK). The powders were extracted with cold 96 % ethanol at room temperature (27 ± 2 ^{0}C) for 72 h. The obtained filtrates were also separately evaporated to dryness *in vacuo* on a rotary evaporator (R205D, Shensung BS & T, China). The resultant dried extracts were kept in appropriately labelled amber bottles and subsequently stored in a refrigerator at -4 ^{0}C prior to the antioxidant tests.

Initial rapid thin-layer chromatographic assay

2,2-Diphenyl-1-picryhydrazyl hydrate (DPPH) assay. This assay is premised on the principle of reduction. The purple color of the methanolic solution of DPPH is bleached when it accepts hydrogen or electrons from extracts or standard antioxidant drug. The tests were done by developing the spotted samples of crude extracts in ethyl acetate: methanol (1:2) in duplicates. Ascorbic acid (Gemini Drugs, Nigeria) was spotted along to serve as positive control. The developed chromatograms were sprayed with 0.1 % w/v methanolic solution of DPPH reagent (Sigma-Aldrich, Germany). The plates were irradiated with ultra-violet light at λ_m 366 nm for 15 minutes. Spots which appeared white against a purple background (Bondet *et al.*,1997; Cuendet *et al.*,1997; Kirby and Schmdt, 1997; Burits and Bucar, 2000) were taken as evidence of positive tests indicating anti-oxidant activity.

Spectrophotometric determination of antioxidant activity using DPPH reagent. Substances capable of donating electrons or hydrogen atoms (free-radical scavengers) are able to convert the purple-colored DPPH radical (2, 2-Diphenyl-1-picrylhydrazyl hydrate) to its yellow-colored non-radical form (1, 1-Diphenyl-2-picryl hydrazine) (Guangrong *et al.,* 2008; Nagalapur and Paramjyothi, 2010). This reaction can be monitored by spectrophotometry. This is the most widely employed method of screening for antioxidant activity in plants (Hu and Kitts, 2000; Khaled *et al.,* 2002; Singh *et al*., 2002; Nia *et al*., 2003; Oladimeji *et al.,* 2007; Guangrong *et al*., 2008; Nagalapur and Paramjyothi, 2010).

Preparation of calibration curve for DPPH reagent. DPPH (4 mg) was weighed out and dissolved in methanol (100 mL) to produce the stock solution (0.004 % w/v). Serial dilutions were done to obtain the following concentrations; 0.0004, 0.0008, 0.0012, 0.0016, 0.0020, 0.0024, 0.0028, 0.0032 and 0.0036 % w/v. The absorbance of each of the sample was taken at λ_m 512 nm (Bondet *et al.*,1997; Cuendet *et al*., 1997; Kirby and Smith,1997; Burits and Bucar, 2000) using ultra-violet spectrophotometer (Jenway 6405, USA). This machine was zeroed after an absorbance had been taken by a solution of methanol without DPPH which was used as the blank. Hence, the calibration curve for the DPPH reagent was prepared.

Determination of the antioxidant activity of crude extracts. 2 mg each of crude ethanolic extract was separately dissolved in 50 mL of methanol. Serial dilutions were done to obtain the following concentrations; 0.0008 mg mL^{-1}, 0.0016 mg mL^{-1} and 0.0024 mg mL^{-1} using methanol. 5 mL of each concentration was incubated with 5 mL of 0.004 % w/v methanolic DPPH solution for optimal analytical accuracy (Bondet *et al.,* 1997). After an incubation period of 30 minutes in the dark at room temperature (25 ± 2 ^{0}C), observation was made for a change in the color of the mixture from purple to yellow (Guangrong *et al.,* 2008; Nagalapur and Paramjyothi, 2010). The absorbance of each of the test samples was then taken at λ_m 512 nm (Bondet *et al.,* 1997; Cuendet *et al*., 1997; Kirby and Smith, 1997; Burits and Bucar, 2000; Nia *et al.,* 2003). The Radical Scavenging Activity (RSA %) or Percentage Inhibition (PI %) of free radical DPPH was thus calculated:

$RSA\ \%\ (PI\ \%) = [(A_{blank} - A_{sample})/ A_{blank}] \times 100$ (Guangrong *et al*., 2008).

A_{blank} is the absorbance of the control reaction (DPPH solution without the test sample and A_{sample} is the absorbance of DPPH incubated with the extract or standard anti-oxidant drug. Extract or standard antioxidant drug concentration providing 50 % inhibition (IC_{50}) was calculated using a graph of inhibition percentage against the concentration of the extract or standard antioxidant drug (Guangrong *et al.,* 2008; Lebeau *et al.,* 2000; Leitao *et al*., 2002).

DPPH assay of standard antioxidant drugs. Standard antioxidants namely, vitamin A (Orange Drugs, Nigeria), vitamin C (Greenfield Drugs, Nigeria) and vitamin E (Neimeth, Nigeria) were used. While vitamin C was in a tablet dosage form, vitamins A and E were formulated as gelatin capsules. The estimated weight of the formulations containing 2 mg of the standard antioxidant drugs were determined by proportionality and then diluted. Methanol was used to dissolve vitamin C, while n-hexane was used to dissolve vitamins A and E because solubility problems encountered with these two vitamins. Thus, methanolic and hexane solutions of 0.004 % w/v DPPH were used for incubation of vitamin C, vitamin A and E respectively for 30 minutes. The absorbance value of each of the drugs was taken at wavelength at λ_m 512 nm and the IC_{50} determined.

RESULTS AND DISCUSSION

Collection and processing of plant materials. The plants were identified and collected observing basic rules of plant collection. Also, the principles governing extraction and processing of extracts were observed, thus preventing any changes to the chemical composition of the crude extract Odebiyi and Sofowora, 1978; Odebiyi and Sofowora, 1979). Previous studies on the crude extracts of these plants revealed the presence of saponins, tannins, flavonoids, terpenes and cardiac glycosides while alkaloids, anthraquinones and cyanogenic glycosides were absent (Oladimeji, 1997; Oladimeji and Usifoh, 2014).

Table 1: Preparation of Calibration Curve of Methanolic Solution of DPPH Reagent at λ_{max} (512 nm)

Concentration (% w/v)	Absorbance
0.0004	0.067
0.0008	0.133
0.0012	0.193
0.0016	0.226
0.0020	0.265
0.0024	0.332
0.0028	0.377
0.0032	0.445
0.0036	0.519
0.0040	0.554

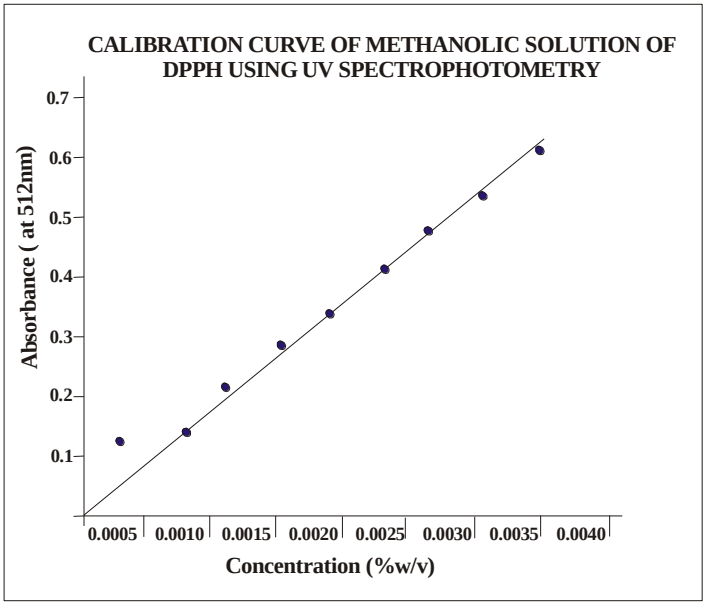

Figure 1: Calibration curve of methanolic solution of DPPH reagent.

Table 2: Absorbance of Samples Incubated with DPPH at Different Concentrations λ_{max} (512 nm)

Sample	Absorbance		
	0.0008 mg mL^{-1}	0.0016 mg mL^{-1}	0.0024 mg mL^{-1}
Acalypha ciliata	0.263	0.182	0.175
A. hispida	0.247	0.144	0.123
A. indica	0.283	0.233	0.172
A. wlkesiana var. golden-yellow	0.325	0.312	0.287
A .wilkesiana var. lace-acalypha	0.324	0.320	0.275
A. wilkesiana var. red-acalypha	0.638	0.632	0.610
Centrosema procumbens	0.681	0.672	0.641
Centrosema pulmeiri	0.352	0.347	0.331
Cyathula prostrata	0.115	0.092	0.072
Pycnanthus angolensis	0.158	0.154	0.152
Tridax procumbens	0.269	0.258	0.245
Vitamin A	0.182	0.178	0.164
Vitamin C	0.105	0.082	0.064
Vitamin E	0.188	0.173	0.159

Table 3: Radical Scavenging Activity (Percentage Inhibition) of Samples at Different Concentrations and IC_{50} of Samples (Blank Absorbance of 0.004 % w/v Methanolic DPPH Reagent: 0.684)

Sample	RSA % (PI %)			IC_{50} (μg mL^{-1})
	0.0008 mg mL^{-1}	0.0016mg mL^{-1}	0.0024 mg mL^{-1}	
Acalypha ciliata	61.55	73.43	74.42	0.65
A. hispida	63.80	78.94	82.01	0.61
A. indica	50.63	65.94	74.85	0.72
A. wlkensiana var. golden-yellow	52.49	54.39	58.04	0.78
A. wilkensiana var. lace-acalypha	52.63	53.22	59.80	0.76
A. wilkesiana var. red-acalypha	6.73	7.06	10.82	NR
Centrosema procumbens	0.44	1.76	1.86	NR
Centrosema pulmeiri	48.54	49.27	51.61	NR
Cyathula prostrata	83.19	86.55	89.47	0.48
Pycnanthus angolensis	76.90	77.49	77.78	0.55
Tridax procumbens	60.77	62,28	64.18	0.67
Vitamin A	73.39	73.98	76.02	0.57
Vitamin C	84.65	88.01	90.65	0.45
Vitamin E	72.51	74.70	76.54	0.59

Key: RSA % (PI %) = Radical Scavenging Activity (Percentage Inhibition); IC_{50} = Concentration at which 50 % of DPPH is Scavenged or Inhibited; NR = Not Regressed (values could not be regressed from the % inhibition-concentration curves).

Secondary metabolites such as saponins, cardiac glycosides, alkaloids, tannins and flavonoids have demonstrated in several previous studies (Hillar *et al*., 1990; Rios *et al.*, Lamikanra *et al*., 1990; Burapadaja *et al.*, 1995; Harouna *et al*., 1995; Aiyelaagbe *et al*., 1998; Adewunmi *et al.*, 1998; Ibewuike *et al*., 1998; Adesina *et al.*, 2000) to be responsible for the cure or management of many ailments caused by microbes and different kinds of disease conditions in the ethnomedicine of plants.

Rapid thin-layer chromatographic analysis for antioxidant activity. The extracts and ascorbic acid gave white spots on purple background when the chromatogram was sprayed DPPH reagent. The observed white spots (irrespective of initial spotted color) are evidence of the reduction of the DPPH reagent (discoloration) by the by free-radical scavenger(s) in the samples.

Spectrophotometric determination of antioxidant activity

Preparation of calibration curve. A calibration curve was prepared for the DPPH radical reagent by taking its absorbance at different concentrations. The DPPH reagent obeys the Beer-Lambert law at concentrations of 50-100 μM (Blois, 1958). The Beer-Lambert law is the basis of all absorption spectrophotometry. Therefore, a plot of absorbance against concentration for a cell of unit thickness (1 cm) should give a straight line passing through the origin (Olaniyi, 1989; Olaniyi, 2000). It was observed that a strictly direct proportionality existed between the absorbance and concentration. Hence, a regression line which passed through the origin was observed. The absorbance of the DPPH solution increased as the concentration increased as can be seen in Table 1 and Figure 1. Furthermore, the regression line buttresses this observation with a correlation factor of 0.97. Hence, the calibration curve obtained was used to correctly extrapolate subsequent concentrations of residual DPPH free radical during the antioxidant test. Thus, the curve displayed in Figure 1 confirms the purity, integrity and suitability of the DPPH reagent for the antioxidant assay.

Determination of the antioxidant activity of crude extracts, Vitamins A, C and E. The reduction of the DPPH radical was determined by taking its absorption at a wavelength of λ_m 512 nm. It was observed

that the absorbance of DPPH decreased as the concentration of added free radical scavenger (extract/standard antioxidant drug) increased which suggested that the DPPH reagent was being reduced. The results of the reduction are as presented in Table 2. The Radical Scavenging Activity (RSA %) or Percentage Inhibition (PI %) and the IC_{50} values of extract and standard antioxidant drugs were computed as Table 3 shows. The RSA % is an indicator of the antioxidant activity of extract/standard antioxidant drug (Meir *et al*., 1995; Hu and Kitts, 2000; Khaled *et al.*, 2002; Singh *et al*., 2002; Nia *et al*., 2003; Guangrong *et al*., 2008; Nagalapur and Paramjyothi, 2010). The determined IC_{50} for the extracts of *A. ciliata, A. hispida, A. indica*, *A. wilkesiana* var. golden-yellow, *A. wilkesiana* var. lace- acalypha and *Tridax procumbens* were marginal at 0.65, 0.61, 0.72, 0.78, 0.76 and 0.67 μg mL^{-1} respectively as presented in Table 3. However, *C. prostrata and P. angolensis* gave moderate antioxidant activities of 0.48 and 0.55 μg mL^{-1} respectively. The determined IC_{50} of *C. prostrata* was comparatively stronger than that of *P. angolensis* and also compare favourably with the determined antioxidant activity of vitamin C at 0.45 μg mL^{-1} (a standard antioxidant drug). Furthermore, the antioxidant activities of the extracts of *C. prostrata* and *P. angolensis* were better than those of vitamins A and E at 0.57 and 0.59 μg mL^{-1} respectively as can be seen in Table 3. The antioxidant activities demonstrated by the plant extracts were not surprising because different preparations of these plants are used in ethnomedicine to treat/manage disease conditions such as wounds, bronchitis, arthritis and cancers amongst so many others (Dalziel, 1956; Oliver, 1959; Oliver, 1960; Watt and Breyer-Brandwijk, 1962; Etukudo, 2000; Etukudo, 2003; Sofowora, 2008; Evans, 2009). Also, the phytochemical screening of the crude extracts of the plants indicated the presence of terpenes, flavonoids and tannins (Oladimeji, 1997; Oladimeji, 2012; Oladimeji *et al.*, 2012; Oladimeji and Usifoh, 2014). Interestingly, these classes of compounds have been reported in previous studies to exhibit antioxidant activities (Tsimidou and Boskou, 1994; Lagouri and Boskou, 1995; Yokosawa *et al.*, 1997; Daniel *et al*., 1998; Grassmann *et al.*, 2001; Alemika *et al.*, 2004; Malaya *et al.*, 2004; Svoboda *et al.*, 2006; Oladimeji *et al.*, 2007). Furthermore, the remarkable antioxidant activity obtained with *C. prostrata* was in order because the chromatographic separation of its ethyl-acetate fraction afforded a novel antioxidant principle (7, 9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione) which demonstrated mechanism of reaction similar to the mechanism of reaction of the antiscorbutic activity of vitamin C (Oladimeji *et al.*, 2014). In addition, the importance of the radical scavenging ability of some phytochemical compounds have found useful applications in the extension of shelf-life and control of deterioration of fatty foods, nutriceuticals and spices (Thomas and Wade, 2001; Braca *et al.*, 2003; Shahidi, 2000; Liyana-Pathirana and Shahidi, 2006).

CONCLUSION

The results of this present study have revealed plant templates such as *C. prostrata* and *P. angolensis* which could be further investigated in detailed *in-vitro* and *in-vivo* studies for the discovery of antioxidant drugs which could serve as alternatives to the ones currently in use on account of cheapness and with little or no toxicities.

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