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In vitro Antioxidant Potentials of Some Herbal Plants from Southern Nigeria

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Some plants have been of interest to researchers in West Africa because of their use in the treatment of various disease conditions in Nigerian traditional medicine. The antioxidant activities of 24 herbal plants found in Southern Nigeria were investigated for *in vitro* anti-oxidant activities using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and Ferric reducing/antioxidant power (FRAP) spectrophotometric assays. Methanol extracts from *Uvaria chamae* root bark, *Bridelia micrantha* leaves, *Duranta repens* leaves, *Harungana madagascariensis* root bark and *Cassytha filiformis* stem gave high percentage antioxidant activities with DPPH assay model and significant ($p<0.05$) concentration-dependent increase in antioxidant power compared to ascorbic acid in the FRAP assay. The extracts of *U. chamae* root bark and *B. micrantha* leaves exhibited more appreciable antioxidant capacities compared to ascorbic acid at the maximal test concentration of 400 μg mL^{-1} in the DPPH assay. The results demonstrated that most extracts had high antioxidant effects and validate their folkloric use as remedies for different ailments. Even in the crude forms, the effects of the extracts were comparable and in some cases, higher than that of ascorbic acid, a compound with proven antioxidant activity. The findings suggest that the six plants named above with high antioxidant activities could be potential sources of novel antioxidants.

Key words: Anti-oxidant, DPPH, FRAP, *in vitro*, Southern Nigeria

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INTRODUCTION

The search for plant-derived medications has accelerated in recent years. Scientists especially in life sciences are greatly involved in exploring the universe for phytochemicals and ''leads'' which could be developed for treatment of diverse diseases. The vegetative kingdom is known not only to provide alternative sources of chemotherapeutic agents and drugs, but also plays important roles in human life as the main source of food, wood, oxygen producer and many more (Cowan, 1999). A large section of the world's population relies on traditional remedies to treat plethora of diseases due to their low costs, easy access and reduced side effects (Marino-Betlolo, 1980). Plants hold relevance as potential sources of potent antioxidants.

Potentially harmful reactive oxygen species are produced as a consequence of normal aerobic metabolism. Phagocytes such as neutrophils and macrophages produce Reactive Oxygen Species (ROS) during destruction of foreign bodies by engulfing (Forman and Torres, 2002). Chemicals and pollutants in food, drugs, alcohol and exposure to natural radiation, pesticides and air pollutants contribute to generation of free radicals in our body. Many physiological and pathological conditions such as ageing, inflammation, viral infections, and neurodegenerative diseases may develop through the action of reactive oxygen species and oxidative stress at large (Al-Omar *et al*., 2004). ROS can induce damage to endothelial, glomerular mesangial and tubular epithelial cells and can induce apoptosis in renal cells (Hosseinzadeh *et al*., 2005). Oxidative stress-induced tissue damage with ROS is implicated as a cause and consequence of a variety of disorders, including coronary heart disease, diabetes and cancer (Knight and Mccafferty, 1996). The body is therefore in need of a steady source of effective antioxidants to counter the destructive effects of ROS.

The antioxidant activity of drug, plants and their extracts has been studied and evaluated by various methods which include free radical scavenging, superoxide anion radical scavenging (Malencic *et al*., 2002; Parejo *et al*., 2002), hydrogen peroxide scavenging and metal chelating activities, reducing power (Oktay *et al*., 2003) and total phenolic content (Santos-Gomes *et al*., 2002). The healing potentials of the different plants are acknowledged in Nigerian traditional herbal practice but their mechanisms of actions are largely unknown. This study was therefore undertaken to evaluate the antioxidant potential of different herbal plant extracts with a view to uncovering their mechanism of actions.

MATERIALS AND METHODS

Chemicals, drugs, reagents and instruments: Freshly prepared solutions and analytical grade chemicals were used for the experiments. Methanol was obtained from Riedel-de-Haen, Germany; 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and ferric tripyridyltriazine (Fe (III)-TPTZ) from Sigma Aldrich, Germany and ascorbic acid (Sigma Aldrich, USA) were used in the study.

Collection and identification of plant material: Plant parts (leaves, stem or roots) were freshly collected from varying locations in Southern Nigeria between April and August, 2011. The different plants were identified by Mr. A. Ozioko, a taxonomist with International Centre for Ethnomedicine and Drug Development, Nsukka, Enugu state. Voucher specimen of each plant was kept in a herbarium at the department.

Preparation of extracts: The plant parts were dried at room temperature and reduced to powder using a hammer mill. The dried powder (100 g) from each plant was soaked in 80% methanol for 72 h with intermittent shaking. These were then filtered. Drying with rotary evaporator *in vacuo* at 40°C afforded each methanol extract. The extracts were stored at 4°C until used.

Evaluation of antioxidant capacity with 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) photometric assay: The free radical scavenging activity of extracts was analyzed by the DPPH assay following the method of Mensor *et al*. (2001). A given volume (2 mL) of the extract at varying concentrations ranging from 10-400 μg mL^{-1} each was mixed with 1 mL of 0.5 mM DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 30 min of incubation in the dark at room temperature. The experiment was done in triplicate. The percentage antioxidant activity was calculated as follows:

$$\text{Antioxidant Activity [AA]} = 100 - [\{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100\}/\text{Abs}_{\text{control}}]$$

Methanol (1.0 mL) plus 2.0 mL of the extract was used as the blank while 1.0 mL of the 0.5 mM DPPH solution plus 2.0 mL of methanol was used as the negative control. Ascorbic acid was used as reference standard.

Ferric reducing/antioxidant power (FRAP) assay: The total antioxidant potential of each extract was determined using the Ferric Reducing Antioxidant Power (FRAP) assay of Benzie and Strain (1996) as a measure of

"antioxidant power". FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe^{11}-tripyridyltriazine compound from colorless oxidized Fe^{111} form by the action of electron donating antioxidants. Standard curve was prepared using different concentrations (100-1000 μmol L^{-1}) of $FeSO_4$ x $7H_2O$. Freshly prepared solutions were used. In the FRAP assay the antioxidant efficiency of the extract under the test was calculated with reference to the reaction signal given by Fe^{2+} solution of known concentration, this representing a one-electron exchange reaction. The results were corrected for dilution and expressed in μM Fe^{11}/L. Vitamin C was measured within 1 h after preparation. The sample to be analyzed was first adequately diluted to fit within the linearity range. All determinations were performed in triplicate.

Calculations were made by a calibration curve. FRAP value of sample (μM):

$$\frac{\text{Change in absorbance of std 0 - 4 min} \times \text{FRAP value of std (1000 μM)}}{\text{Change in absorbance of std 0 min - 4 min}}$$

Statistical analysis: All data were expressed as Mean±SEM. Data were analyzed using one way analysis of variance (ANOVA) at 5% level of significance. Duncan New Multiple Range test was used to detect the difference among the treatment groups.

RESULTS

DPPH (1, 1- diphenyl-2-picrylhydrazyl) spectrophotometric assay): The extracts of *Uvaria chamae* root bark, *Bridelia micrantha* leaves, *Crinum jagus* bulb, *Duranta repens* leaves, *Harungana madagascariensis* root bark, *Cassytha filiformis* stem and *Mallotus oppositifolius* leaves exhibited appreciable concentration-dependent increases in antioxidant activities in both DPPH and FRAP spectrophotometric assays out of the twenty four (24) plants studied. *U. chamae* root bark and *B. micrantha* leaves possessed significantly ($p<0.05$) higher antioxidant capacities at the maximal test concentration of 400 μg mL^{-1} compared to ascorbic acid in the DPPH radical scavenging method (Table 1). The crude methanol extracts of *U. chamae* root bark and *B. micrantha* leaves had 95 and 98% antioxidant activity, respectively, at 400 μg mL^{-1} while ascorbic acid produced a comparatively reduced percentage value of 79% at the same concentration. Similarly in the DPPH assay, *C. jagus* bulb, *D. repens* leaves, *H. madagascariensis* root bark, *C. filiformis* stem and *M. oppositifolius* leaves demonstrated comparable antioxidant activities at 200 and 400 μg mL^{-1} which were not significantly ($p>0.05$) different from that of the reference (ascorbic acid). The extracts of *Citrus sinensis* leaves, *Brassica juncea* seeds, *Hymenocardia acida*

Table 1: *In vitro* antioxidant activities of some plants in Southern Nigeria using the DPPH spectrophotometric assay

	10 μg mL^{-1}	50 μg mL^{-1}	100 μg mL^{-1}	200 μg mL^{-1}	400 μg mL^{-1}
Ascorbic acid	73.64	74.1	74.6	77.16	79.21
Garcina kola (seeds)	31.27	35.76	48.97	52.74	69.65
Uvaria chamae (root bark)	71.60	72.72	73.34	86.01	95.08*
Bridelia micrantha (leaves)	4.53	32.15	63.77	77.27	97.70*
Duranta repens (leaves)	46.64	67.05	68.26	71.67	72.61
Olax varidis (leaves)	6.82	45.48	53.08	53.34	58.19
Crinum jagus (bulb)	68.30	81.45	84.08	84.13	85.78
Lapaecea (leaves)	23.81	23.92	26.30	31.21	51.15
Harungana madagascariensis (root bark)	60	75	80	84	85
Cassytha filiformis (stem)	42.7	58.5	64.9	68.10	75.80
Parkia biglobosa (stalk)	23.5	31.52	48.66	50.8	59.01
Alstonia boonei (stem bark)	18.5	21.2	48.7	51.6	68.5
Picralima nitida (mesocarp)	29.7	31.1	36.8	42.0	52.5
Picralima nitida (seeds)	30.8	42.5	45.7	48.9	55.3
Cassia sieberiana (stem)	15.5	21.2	30.4	28.1	40.1
Ricinus communis (root)	4.80	17.7	42.4	48.9	60.8
Murraya koagini (leaves)	5.20	18.3	46.7	46.8	31.30
Brassica juncea (seeds)	24.1	30.1	47.8	52.1	68.9
Citrus sinensis (leaves)	15.2	21.5	40.1	55.0	69.7
Terminalia catappa (leaves)	5.7	25.2	35.91	42.74	58.19
Hymenocardia acida (leaves)	38.7	44.1	52.6	58.7	66.9
Oxythina abyssinta (leaves)	15.6	17.2	25.4	40.9	43.1
Anarcadium occidentale (bark)	40.7	45.7	46.7	45.2	43.5
Sacrocephalus latifolius (leaves)	47.9	52.4	56.8	65.9	66.2
Mallotus oppositifolius (leaves)	38.7	41.52	55.86	62.15	78.92

Results are % anti-oxidant activity. *indicates significant increases at $p<0.05$ compared to control (ascorbic acid)

leaves and *Sacrocephalus latifolius* leaves had significantly ($p<0.05$) reduced antioxidant activities compared to ascorbic acid at all concentrations except 400 μg mL^{-1}.

Ferric reducing antioxidant power (FRAP) Assay: In the FRAP assay, *U. chamae* root bark and *B. micrantha* leaves produced high values of 1.90 and 1.39 μM respectively, at 400 μg mL^{-1} but 1000 μg mL^{-1} of ascorbic acid has a standard FRAP value of 2.0 μM. There were remarkable increases in the mean antioxidant power (FRAP values) between 200-400 μg mL^{-1} for some of the extracts that showed appreciable antioxidant activities, mentioned earlier in the DPPH assay. The FRAP value of the extract of *C. jagus* bulb increased from 1.85-1.86 μM, *D. repens* leaves (1.65-1.71 μM); *H. madagascariensis* root bark (1.58-1.95 μM); *C. filiformis* stem (1.41-1.61 μM) and *M. oppositifolius* leaves (1.50-1.69 μM) as shown in Table 2.

DISCUSSION

In vitro antioxidant activities of the various extracts were evaluated as a first step to a more detailed study of their use in Nigerian folklore medicine as remedy for diverse pathologies. As can be seen from the results (Tables 1 and 2), the methanol extract of some of the plants demonstrated significant antioxidant activities as revealed by the DPPH and FRAP spectrophotometric assays. In the DPPH assay, the crude extracts of *U. chamae* root bark and *B. micrantha* leaves produced significantly ($p<0.05$) higher antioxidant activities at 400 μg mL^{-1} when compared with the reference standard (ascorbic acid). The principle of the FRAP method is based on the reduction of a ferric-tripyridyl triazine complex to its ferrous form in the presence of antioxidants. The FRAP values of *U. chamae* root bark, *B. micrantha* leaves, *C. jagus* bulb, *D. repens* leaves, *H. madagascariensis* root bark, *C. filiformis* stem, *Citrus sinensis* leaves and *Mallotus oppositifolius* extracts at 50, 100, 200 and 400 μg mL^{-1} were significant at $p<0.05$. There was concentration-dependent increase in the FRAP values of the extracts (Table 2) which were significantly ($p<0.05$) higher than ascorbic acid.

The high antioxidant activities of these extracts could be one of the vital reasons behind their medicinal properties and folkloric uses of these plants. The leaf sap of *B. micrantha* is used locally to treat eyes sore (Smith, 1966) the bulb of *C. jagus* is found to have anti-snake venom activities (Ode and Asuzu, 2006). *Crinum jagus* bulb was also acknowledged for several medicinal purposes and in East Africa; a decoction of it is used for treatment of sores (Kokwaro, 1976). Cassytha *filiformis* is used to treat jelly fish stings in Fiji, treatment of cancers in Polynesian cultures and the juice is purported to ease birth and relieve labor pains (Nelson, 2008). Oluremi *et al.* (2010) reported that a root-infusion of *U. chamae* is noted for treating severe abdominal pains, diarrhea, cough, urinary tract infections and cerebral illnesses (Oluremi *et al.* 2010). Uvaria *chamae* also possess potent hepatoprotective activity (Madubunyi *et al.*, 1995). In Togo, a

Table 2: *In vitro* antioxidant activities of some plants in Southern Nigeria using the FRAP spectrophotometric assay

	10 μg mL^{-1}	50 μg mL^{-1}	100 μg mL^{-1}	200 μg mL^{-1}	400 μg mL^{-1}
Garcina kola (seeds)	0.45	0.52	0.88	1.11	1.33
Uvaria chamae (root bark)	1.40	1.42	1.44	1.81	1.90
Bridelia micrantha (leaves)	0.10	0.39	0.49	0.62	1.39
Duranta repens (leaves)	0.89	1.03	1.05	1.65	1.71
Olax varidis (leaves)	0.12	0.84	1.09	1.11	1.19
Crinum jagus (bulb)	1.28	1.82	1.84	1.85	1.86
Lapaecea (leaves)	0.31	0.37	0.40	0.46	1.01
Harungana madagascariensis (root bark)	0.28	0.63	0.84	1.58	1.95
Cassytha filiformis (stem)	0.73	1.13	1.20	1.41	1.61
Parkia biglobosa (stalk)	0.30	0.78	0.93	1.02	1.24
Alstonia boonei (stem bark)	0.20	0.27	0.69	1.03	1.40
Picralima nitida (mesocarp)	0.31	0.42	0.63	0.69	1.25
Picralima nitida (seeds)	0.40	0.72	0.77	0.93	1.38
Cassia sieberiana (stem)	0.17	0.33	0.59	0.42	0.83
Ricinus communis (root)	0.16	0.21	0.73	0.94	1.43
Murraya koagini (leaves)	0.10	0.19	0.76	0.76	0.58
Brassica juncea (seeds)	0.48	0.57	0.80	1.09	1.30
Citrus sinensis (leaves)	0.12	0.27	0.63	1.14	1.22
Terminalia catappa (leaves)	0.14	0.50	0.55	0.76	1.30
Hymenocardia acida (leaves)	0.40	0.62	0.93	1.23	1.48
Oxythina abyssinta (leaves)	0.19	0.20	0.52	0.76	0.79
Anarcadium occidentale (bark)	0.71	0.85	0.88	0.80	0.70
Sacrocephalus latifolius (leaves)	0.82	1.12	1.20	1.47	1.49
Mallotus oppositifolia (leaves)	0.47	0.83	1.49	1.50	1.69

Results are FRAP values expressed in μM and compared with ascorbic acid. FRAP value of ascorbic acid = 2

root-decoction of *U. chamae* is given for amenorrhea, prevention of miscarriage and pains of childbirth. An infusion of the leaf and juice of the fruit of *D. repens* was reported to have diuretic properties. The plant contains some flavones which prevent anaphylactic shock, protect against x-rays and cure from frost bite (Iqbal, 2000). Decoctions from different parts of *H. madagascariensis* were highly valued in the treatment of various human diseases including drug related renal disease. The boiled water decoction of the plant root is believed to neutralize the toxic activities of ingested poisons and restores deranged hepatic and renal functions to normal (Adeneye *et al*., 2008).

Reactive oxygen species are oxygen-centered molecules which include the non-radicals, hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), hydroxyl anion (HO) and single oxygen (O_2) and the radicals, superoxide anion (O_2^-), hydroxyl radical (HO^-) and Nitric Oxide (NO^-) (Miller, 1996). Reactive oxygen species produce changes and had been implicated as a cause and consequence of a variety of pathological conditions. The high antioxidant activities demonstrated by the methanol extracts suggest that the plants could be sources of potent antioxidant compounds. Luteolin, a flavonoid is a valuable dietary supplement partly due to its high antioxidant potentials (Igile *et al*., 1994; Reka and Ilona, 2002). Antioxidants consist of vitamins, polyphenols, flavonoids, minerals and endogenous enzymes such as superoxide dismutase, catalase and glutathione peroxidase that has the capability to neutralize unstable molecules called free radicals (Trouillas *et al*., 2003). Vitamin A (retinol), vitamin C (ascorbic acid), vitamin E (tocopherol) and selenium are valuable antioxidants. They are among the first link between chemical reactions and biological activity . Antioxidants do not completely get rid of free radicals in the body but they retard or minimize the damage caused and also block the process of oxidation by neutralizing free radicals and by this action, they themselves become oxidized. The endogenous antioxidants prevent oxidation by reducing the rate of chain initiation. The initiating radicals are scavenged and destroyed before oxidation sets in motion.

CONCLUSION

This investigation revealed that, out of the 24 plant samples studied the crude extracts of *U. chamae* root bark, *B. micrantha* leaves, *C. jagus* bulb, *D. repens* leaves, *H. madagascariensis* root bark, *C. filiformis* stem and *M. oppositifolius* leaves were found to possess significantly high antioxidant activities with DPPH and FRAP spectrophotometric assays. The observed effects were in support of the folkloric uses of preparations from the plants in diverse disease therapies.

RECOMMENDATIONS

The methanol crude extracts of *U. chamae* root bark, *B. micrantha* leaves, *C. jagus* bulb, *D. repens* leaves, *H. madagascariensis* root bark, *C. filiformis* stem and *M. oppositifolius* leaves need to undergo further studies with a view to isolating and characterizing the antioxidant principles responsible for the observed activities.

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