



Research Journal of **Microbiology**

ISSN 1816-4935



Academic
Journals Inc.

www.academicjournals.com

Antioxidant and Antibacterial Properties of *Lecaniodiscus cupanioides*

¹M.O. Sofidiya, ²F.O. Jimoh, ²A.A. Aliero, ²A.J. Afolayan,
¹O.A. Odukoya and ³O.B. Familoni

¹Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria

²Department of Botany, University of Fort Hare, Alice 5700, South Africa

³Department of Chemistry, Faculty of Science, University of Lagos, Nigeria

Abstract: *Lecaniodiscus cupanioides* Planks. ExBth (Sapindaceae) is widely used in Nigerian folk medicine for the treatment of inflammatory conditions, hepatomegaly and bacterial infections. This study investigated the antioxidant and antibacterial activity of the methanolic extract of the leaves to justify its use in traditional medicine. Extract exhibited strong DPPH and ABTS radical scavenging activity greater than BHT and comparable to ascorbic acid. 0.1 mg mL⁻¹ extract inhibited DPPH and ABTS radicals up to 99.4 and 98.5%, respectively. Multiple antioxidant activity of extract was evident with moderate reducing power. TAE (37.678±1.66 mg g⁻¹ dry extract) was higher than that reported in many other plant extracts. Flavonoid and proanthocyanidin contents were 4.142±0.06 and 2.548±0.32 mg g⁻¹, respectively. Strong correlation recorded; ABTS/TAE (R² = 0.89), DPPH/TAE (R² = 0.90). Antimicrobial activity was highest on gram +ve organisms *B. cereus*, *S. aureus*, *M. kristine* and *S. pyrogens* (MIC value < 1.0 mg mL⁻¹). Gram-ve *S. pooni* and *P. aeruginosa* (MIC value = 2.0 mg mL⁻¹). Results attributed the antioxidant potential of *L. cupanioides* leaf extract to its strong proton donating ability and justified its use for the treatment of bacterial infections in ethnomedicine.

Key words: Antioxidant activity, antimicrobial effects, *Lecaniodiscus cupanioides*, leaf methanol extract, polyphenolics, wound healing

INTRODUCTION

Oxygen centered free radicals and other reactive oxygen species are byproducts of numerous physiological and biochemical processes in the human body. Overproduction of such free radicals causes oxidative damage to biomolecules such as lipids, proteins and DNA, eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes, aging and other degenerative diseases in humans (Halliwell, 1994). Oxygen free radicals contribute to tissue damage in the events following skin injury and are known to impair healing process. It is consented that Reactive Oxygen Species (ROS) are deleterious to wound healing process due to the harmful effects on cells and tissues (Singh *et al.*, 2006). Increase production of reactive oxygen species, lipid peroxidation and ineffective scavenging play a crucial role in various skin lesions (Niwa *et al.*, 1986). Wound healing is the process of repair that follows injury to the skin and other soft tissues. It is fundamentally a connective tissue response. Initial stages of wound healing involve an acute inflammatory phase followed by synthesis of collagen and other extracellular matrix which are later remodeled to form scar. Molecular oxygen plays a central role in the pathogenesis and therapy of chronic wounds. Overproduction of Reactive Oxygen Species (ROS) results in oxidative stress thereby causing cytotoxicity and delayed wound healing. Therefore, elimination of ROS could be an important strategy in healing of chronic wounds (Dissemond *et al.*,

2002). Antioxidants are known to counter the excess proteases and ROS, which are formed by neutrophil accumulation in the wound area and protect protease inhibitors from oxidative damage. Fibroblasts and other cells may be killed by excess ROS, thus making skin lipids less flexible; however, antioxidants are known to reduce the possibility of occurrence of these adverse events (Houghton *et al.*, 2005). The search for natural remedies has drawn attention to herbals. Proanthocyanidins and other tannins facilitate wound healing. A combination of grape seed proanthocyanidin extract and resveratrol facilitates inducible VEGF expression, a key element supporting wound angiogenesis. Strategies to manipulate the redox environment in the wound are likely to be of outstanding significance in wound healing (Sen *et al.*, 2002).

In recent time, many studies have focused on medicinal plants in search for antioxidant principles. Many plants used in herbal preparation have been found to possess sufficient antioxidant compounds that can be used in the battle against cellular damage and disease.

Lecaniodiscus cupanioides Planch. ExBth (Sapindaceae) is a medicinal plant used in Western Nigeria for the treatment of wounds, boils, burns and bruises, tooth ache, fever and abdominal swelling caused by liver abscess (Gill, 1992; Iwu, 1993). Aqueous root extract of the plant has been reported to prevent strychnine-induced seizure, prolonged the latency and reduced severity of PTZ and picrotoxin induced seizure (Yemitan and Adeyemi, 2005). Pharmacological studies have also shown that the plant possessed analgesic and hepatotoxic properties (Yemitan and Adeyemi, 2004; Yemitan *et al.*, 2005). Despite the various reports on the use of the extracts from this species for the treatment of various ailments, information on the antioxidant and antibacterial activities of its leaf extract is hitherto unavailable. Such type of information is vital in the understanding of its medicinal potential. The aim of this work was to evaluate the antioxidant and antibacterial activities of *L. cupanioides* leaf extract for its relevance since antioxidants hasten the process of wound healing by destroying the free radicals.

MATERIALS AND METHODS

Plant Material

The leaves of *L. cupanioides* were collected in January 2006 in Oyo State, Nigeria. The plant was authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan courtesy Mr. Wale Ekundayo. Voucher specimens were prepared and deposited as (FHI 107345) at the herbaria of both the Institute and at the Pharmacognosy Department, University of Lagos.

Preparation of Crude Extracts

The plant material was air dried at room temperature for 21 days, pulverized into powdered and a portion (100 g) of the material was extracted in methanol at room temperature for 24 h. Extract was filtered through Whatman No.1 filter paper and the filtrate evaporated into dryness at 40°C using rotary evaporator.

Chemicals

The chemicals used in this study included 2, 2-Diphenyl-2-picrylhydrazyl (DPPH); potassium ferricyanide, catechin, butylated hydroxytoluene (BHT), Ascorbic acid and FeCl₃ and were purchased from Sigma Chemicals Co. (St Louis, MO, USA). Vanillin was from BDH; Folin-Ciocalteus's phenol reagent and sodium carbonate were from Merck Chemical Supplies (Darmstadt, Germany). All other chemicals used were of analytical grade.

Determination of the Reducing Power of the Extract

The reducing capacity of the extract was determined by the method of Oyaizu (1986). Varying concentrations of the extract in methanol (0.02-0.1 mg mL⁻¹) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%) and incubated at 50°C for 20 min.

Aliquots (2.5 mL) of 10% trichloroacetic acid were added to the reaction mixture, which was then centrifuged at 1000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%). The absorbance was measured at 700 nm. The higher the absorbance value the stronger the reducing power.

Determination of DPPH Radical Scavenging Capacity

The effect of the extract on DPPH radical was estimated adopting the method of Liyana-Pathirana and Shahidi (2005). A solution of 0.135 mM DPPH in methanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL of extract in methanol containing 0.02-0.1 mg of extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid and BHT were used as standards. The ability to scavenge DPPH radical was calculated by the following Eq.:

$$\text{DPPH radical scavenging activity (\%)} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / (\text{Abs}_{\text{control}})] \times 100$$

where,

$\text{Abs}_{\text{control}}$ = Absorbance of DPPH radical + methanol

$\text{Abs}_{\text{sample}}$ = Absorbance of DPPH radical + sample extract/standard

ABTS Radical Cation Scavenging Activity

The free radical scavenging activity was determined by ABTS radical cation decolorization assay (Re *et al.*, 1999). It involved the generation of ABTS⁺ chromophore by the oxidation of ABTS with potassium persulfate. ABTS⁺ radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature in the dark for 16 h. The solution was then diluted by mixing 1 mL ABTS⁺ solution with 60 mL methanol to obtain an absorbance of 0.7074 ± 0.001 at 734 nm using the spectrophotometer. The reaction mixture (1 mL of extract and 1 mL of ABTS⁺) was allowed to stand at room temperature for 6 min and the absorbance was immediately recorded at 734 nm. BHT standard solution was prepared and assayed at the same conditions.

Determination of Total Phenolic Content

Total phenol contents in the extract were determined by the modified Folin-Ciocalteu method of Wolfe *et al.* (2003). An aliquot of the extract was mixed with 5 mL Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75 g L^{-1}) of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40°C for colour development. Absorbance was measured at 765 nm using the Hewlett Packard UV-VS spectrophotometer. The amount of total phenolics was calculated as tannic acid equivalents (TAE mg) from the calibration curve. The experiment was replicated thrice.

Determination of Total Flavonoid

Total flavonoids were estimated using the method of Ordonez *et al.* (2006). To 0.5 mL of sample, 0.5 mL of 2% AlCl_3 ethanol solution was added. After 1 h at room temperature, the absorbance was measured at 420 nm. Total flavonoid contents were calculated as quercetin equivalent from a calibration curve.

Determination of Proanthocyanidin

Proanthocyanidins were estimated by adopting the procedure of Sun *et al.* (1998). 0.5 mL of 50 mg mL^{-1} of extract was mixed in 3 mL of 4% vanillin-methanol solution and 1.5 mL hydrochloric acid and the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm and the result expressed as catechin equivalent.

Antibacterial Testing

Laboratory isolates of 10 bacteria species, which included five Gram positive and five Gram negative strains were obtained from the Department of Microbiology, Rhodes University. They were; *Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus kristinae*, *Streptococcus pyrogens*, *Escherichia coli*, *Salmonella pooni*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. They were maintained on nutrient agar slants and recovered by culturing in nutrient broth (Biolab No. 2) for 24 h. Before use, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth (Grierson and Afolayan, 1999). The bacteria were streaked in radial patterns on the agar plates and incubated at 37°C and examined after 24 and 48 h. The extract was tested at 2.0, 1.0, 0.5 and 0.1 mg mL⁻¹ and complete suppression of growth by a specific concentration of the extract was required to be declared active. The Minimum Inhibitory Concentration (MIC) values were recorded, where no bacterial growth was observed. Blank plates containing only nutrient agar and another set containing nutrient and 2% methanol served as controls (Afolayan and Meyer, 1997).

Statistical Analysis

Data were subjected to a one-way analysis of variance as applicable and the significance of the difference between the means was determined by Duncan's Multiple Range Test ($p < 0.05$).

RESULTS AND DISCUSSION

The antioxidant potential of the extract was assessed using standard *in vitro* antioxidant methods. The reducing power of *L. cupanioides* extract and the reference compounds increased with increasing concentration (Fig. 1). No significant difference in reducing activities was detected between the extract and BHT. However, reducing activity of catechin was significantly higher than that of the extract and BHT. This indicated that the antioxidant compounds are electron donors and could reduce the oxidized intermediates of lipid peroxidation processes, thus acting as primary and secondary antioxidants (Yen and Chen, 1995; Ordonez *et al.*, 2006).

The proton-radical scavenging action has been known as an important mechanism of antioxidation (Yun-Chin *et al.*, 2005). The dose-response curves of DPPH radical scavenging activity of methanol extract of *L. cupanioides* compared with BHT and ascorbic acid are presented in Fig. 2. The activity increased with increasing concentration of the extract up to 0.04 mg mL⁻¹ and leveled off with further increase in concentration. A similar trend was observed for ascorbic acid, though the activity of the extract was higher than that of ascorbic acid. In all the concentrations tested, the extract was found to be more active than BHT. A similar result on the antioxidant potential of *Ecklonia cava* was reported

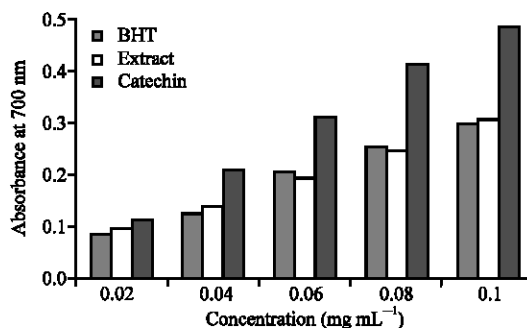


Fig. 1. Reducing power of methanol extract from *L. cupanioides*.

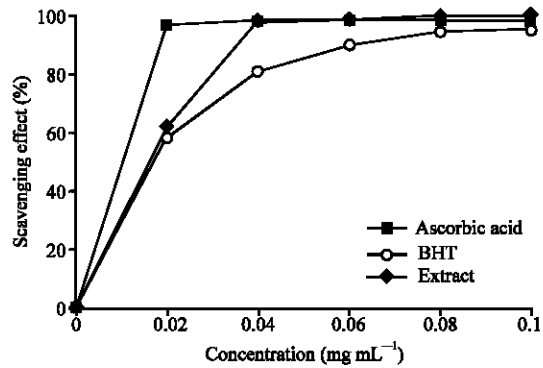


Fig. 2: Free radical scavenging capacity of methanol extract from *L. cupanioides* determined by DPPH method

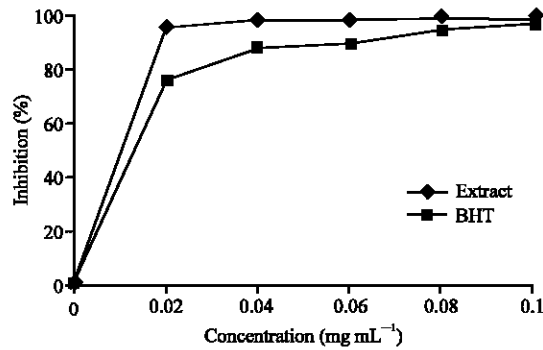


Fig. 3: ABTS⁺ scavenging effects of methanol extract from *L. cupanioides*

(Senevirathne *et al.*, 2006). These results imply that, radical scavenging activity of the extract may be attributed to its strong proton donating ability.

The ABTS scavenging activity of the extract was high (96%) at 0.02 mg mL⁻¹, which was the lowest concentration, tested. The percentage inhibition of BHT at this concentration was 75.3%, which is comparatively lower than that of the extract. The activity increased with increasing concentration and was dose dependent (Fig. 3). High concentrations of the extract have been reported to be more effective in quenching free radical in the system (Ligangli *et al.*, 2002). It is interesting to note that, the activity of *L. cupanioides* extract was higher than that of BHT.

Linear regression analysis of calibration plot gave a correlation coefficient (R^2) of 0.9277 for catechin, 0.9497 for tannic acid and 0.9812 for quercetin used as standards in the determination of proanthocyanidin, total phenol and flavonoid, respectively. The total phenolic content of the extract was 37.678±1.66 mg g⁻¹ TAE⁻¹ of dry material, which is higher than that reported in many other plant extracts, based on dry extract (Muchuweti *et al.*, 2006). The total phenolic contents of the extract showed positive correlation with antioxidant activity. The correlation coefficient between ABTS, DPPH assays and total phenol are 0.89 and 0.90, respectively. This observation is similar to the reports of several authors who worked on different plants (Sofidiya *et al.*, 2006; Odukoya *et al.*, 2005; Velioglu *et al.*, 1998). The total flavonoid and proanthocyanidin content of the extract were 4.142±0.06 and 2.548±0.32, respectively and they may contribute in a significant way to the radical scavenging activities and reducing powers of the extract. Plant phenolics constitute one of the major

Table 1: Antibacterial activity of methanol extract from the leaves of *L. cupanioides* at 2 mg mL⁻¹

Bacteria	MIC ^a (mg mL ⁻¹)		Antibiotics (mg mL ⁻¹)	
	Gram +/-	MeOH	Streptomycin	Chloramphenicol
<i>Bacillus cereus</i>	+	1.0	1.0	0.13
<i>Staphylococcus epidermidis</i>	+	^b	1.0	0.25
<i>Staphylococcus aureus</i>	+	1.0	1.0	1.00
<i>Micrococcus kristinae</i>	+	1.0	0.5	0.50
<i>Streptococcus pyrogens</i>	+	1.0	1.0	1.00
<i>Escherichia coli</i>	-	-	< 2	< 2
<i>Salmonella pooni</i>	-	2.0	< 2	< 2
<i>Serratia marcescens</i>	-	-	< 2	< 2
<i>Pseudomonas aeruginosa</i>	-	2.0	< 5	< 2
<i>Klebsiella pneumoniae</i>	-	-	< 2	< 2

^a: Minimum inhibitory concentration, ^b: Not active at concentration = 2 mg mL⁻¹

groups of compounds acting as primary antioxidants or free radical terminators (Miliaus-Kas *et al.*, 2004). Synergism of polyphenolic compounds in an extract may contribute to the overall antioxidant activity (Shahidi *et al.*, 1994). The high activity of the extract therefore, could be attributed to the presence of phenolic compounds in the extract.

The antibacterial activity of methanol extract from the leaves of *L. cupanioides* showed growth inhibition against 6 out of the 10 bacteria strains tested (Table 1). The strongest activity was found on *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus kristinae* and *Streptococcus pyrogens* with an MIC of 1.0 and 2.0 mg mL⁻¹ for *Salmonella pooni* and *Pseudomonas aeruginosa*. The growth of *Staphylococcus epidermidis*, *Escherichia coli*, *Serratia marcescens* and *Klebsiella pneumoniae* was not inhibited by the extract at the highest tested concentration. The activity of the extract was found to be higher on Gram-positive bacteria than the Gram-negative strains. The observed lower degree of activity against the Gram negative bacteria was not surprising as, in general, these bacteria are more resistant to plant extracts than the Gram positive ones (Rabe and Van Staden, 1997; Grierson and Afolayan, 1999). The antibiotic resistant patterns of *Salmonella* isolates have been reported (Gashe and Mpuchane, 2000; Urio *et al.*, 2001). The susceptibility of *Salmonella pooni* to the methanol extract of this species is an indication of the potential of the extract as a drug that can be used against this organism. Although crude plant extracts have been reported to exhibit lower antimicrobial activity than pure substances (Navarro *et al.*, 1996; Ebi and Ofoefule, 1997; Ibrahim *et al.*, 1997), the activity exhibited by the extract in this study is noteworthy and is comparable to that of streptomycin and chloramphenicol standards with MIC value of 1.0 mg mL⁻¹ on *Staphylococcus aureus* and *Streptococcus pyrogens* (Table 1). The bacterial strains used in this study, which showed susceptibility to the extract, have been implicated in some of the diseases against which this plant species is used in herbal remedy.

The significance of polyphenolic compounds of plant extracts has been recognized and is well known to exhibit a number of biological activities. They are reported to have multiple biological activities including vasodilatory, anti-inflammatory, anticarcinogenic, antiviral and antibacterial effects (Dorman *et al.*, 2004). The presence of these compounds in *L. cupanioides* could be attributed to its wound healing and other pharmacological activities associated with free radicals. Although, there could be several mechanisms of action of the extract for its effectiveness in free radical implicated diseases, the antioxidant and free radical scavenging properties of this plant seem to be highly significant. The result of this study has revealed the antioxidant potential of *L. cupanioides* and has justified its use for the treatment of infectious diseases caused by bacteria.

ACKNOWLEDGMENT

The authors are grateful to the NRF, South Africa and the University of Lagos, Nigeria for support.

REFERENCES

- Afolayan, A.J. and J.M. Meyer, 1997. The antibacterial activity of 3, 5, 7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*. J. Ethnopharmacol., 57: 177-181.
- Dissemond, J., M. Goos and S.N. Wagner, 2002. The role of oxidative stress in the pathogenesis and therapy of chronic wounds. Hautarzt, 53: 718-723.
- Dorman, H.J., O. Bachmayer, M. Kosar and R. Hiltunen, 2004. Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. J. Agric. Food Chem., 52: 762-770.
- Ebi, G.C. and S.I. Ofoefule, 1997. Investigations into folkloric antimicrobial activities of *Landolphia owerrience*. Phytother. Res., 11: 149-151.
- Gashe, B.A. and S. Mpuchane, 2000. Prevalence of salmonellae on beef products at butcheries and their antibiotics resistance profile. J. Food Sci., 65: 880-883.
- Gill, L.S., 1992. Ethnomedical Uses of Plants in Nigeria. UNIBEN Press, Benin City, Nigeria.
- Grierson, D.S. and A.J. Afolayan, 1999. Antibacterial activity of some indigenous plants used for the treatment of wound in the Eastern Cape, South Africa. J. Ethnopharmacol., 66: 103-106.
- Halliwell, B., 1994. Free radicals, antioxidants and human disease: Curiosity, cause or consequence? Lancet, 344: 721-724.
- Houghton, P.J., P.J. Hylands, A.Y. Mensah, A. Hensel and A.M. Deters, 2005. *In vitro* tests and ethnopharmacological investigations: Wound healing as an example. J. Ethnopharmacol., 100: 100-107.
- Ibrahim, M.B., M.O. Owonubi and J.A. Onaolapo, 1997. Antimicrobial effects of extracts of leaf, stem and root bark of *Anogiessus leicarpus* on *Staphylococcus aureus* NCTC 8198, *Escherichia coli* NCTC 10418 and *Proteus vulgaris* NCTC 4636. J. Pharm. Res. Dev., 2: 20-26.
- Iwu, M., 1993. Handbook of African Medicinal Plants. CRC Press, Boca Rotan.
- Ligangli, Y.H., M. Scott, M. Jonathan, W. John and Q. Minq, 2002. Free radical scavenging properties of wheat extracts. J. Agric. Food Chem., 50: 1619-1624.
- Liyana-Pathiranan, C.M. and F. Shahidi, 2005. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. J. Agric. Food Chem., 53: 2433.
- Miliaus-Kas, G., P.R. Venskutonis and T.A. Van-Beek, 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem., 85: 231-237.
- Muchuweti, M., L. Nyamukonda, L.S. Chagonda, A.R. Ndhhlala, M.M. Chipso and M. Benhura, 2006. Total phenolic content and antioxidant activity in selected medicinal plants of Zimbabwe. Int. J. Food Sci. Technol., 41: 33-38.
- Navarro, V., M.L. Villarreal, G. Rojas and X. Lozoya, 1996. Antimicrobial evaluation of some plants used in Mexican traditional medicine for treatment of infectious diseases. J. Ethnopharmacol., 53: 143-147.
- Niwa, Y., T. Kasama and S. Kawai, 1986. The effect of aging on lipid peroxide levels and superoxide dismutase activity in guinea pigs and patients with burns. Life Sci., 42: 351-356.
- Odukoya, O.A., M.O. Jenkins, O.O. Ilori and M.O. Sofidiya, 2005. The use of selected Nigerian natural products in management of environmentally induced free radical skin damage. Pak. J. Biol. Sci., 8: 1074-1077.
- Ordonez, A.A.L., V. Gomez, M.A. Vattuone and M.I. Isla, 2006. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem., 97: 452-458.
- Oyaizu, M., 1986. Studies on products of browning reactions; antioxidant activities of products of browning reaction prepared from glucose amine. Jpn. J. Nutr., 44: 307-315.
- Rabe, T. and J. Van-Staden, 1997. Antibacterial activity of South African plants used for medicinal purposes. J. Ethnopharmacol., 56: 81-87.

- Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C.A. Rice-Evans, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Bio. Med.*, 26: 1231-1237.
- Sen, C.K., S. Khanna, G. Gordillo, D. Bagchi, M. Bagchi and S. Roy, 2002. Oxygen, oxidants and antioxidants in wound healing: An emerging paradigm. *Annals New York Acad. Sci.*, 957: 239-249.
- Senevirathne, M., S. Kim, N. Siriwardhana, J. Ha, K. Lee and Y. Jeon, 2006. Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. *Food Sci. Technol. Int.*, 12: 27-38.
- Shahidi, F., U.N. Wanasundara and R. Amarowicz, 1994. Natural antioxidant from low-pungency mustard flour. *Food Res. Int.*, 27: 489-493.
- Singh, M., R. Govindarajan, V. Nath, A.K.S. Rawat and S. Mehrotra, 2006. Antimicrobial, wound healing and antioxidant activity of *Plagiochasma appendiculatum* Lehm. et Lind. *J. Ethnopharmacol.*, 107: 67-72.
- Sofidiya, M.O., O.A. Odukoya, O.B. Familoni and S.I. Inya-Agha, 2006. Radical scavenging activity of some Nigerian medicinal plant extracts. *Pak. J. Biol. Sci.*, 9: 1438-1441.
- Sun, J.S., Y.W. Tsuang, I.J. Chen, W.C. Huang, Y.S. Hang and F.J. Lu, 1998. An ultra-weak chemiluminescence study on oxidative stress in rabbits following acute thermal injury. *Burns*, 24: 225-231.
- Urio, E.M., E.K. Collison, B.A. Gashe, T.K. Sebunya and S. Mpuchane, 2001. *Shigella* and *Salmonella* strains isolated from children under 5 years in Gaborone, Botswana and their antibiotic susceptibility patterns. *Trop. Med. Int. Health*, 6: 55-59.
- Velioglu, Y.S., G. Mazza, L. Gao and B.D. Oomah, 1998. Antioxidant activity and total phenolics in selected fruits, vegetables grain products. *J. Agric. Food Chem.*, 46: 4113-4117.
- Wolfe, K., X. Wu and R.H. Liu, 2003. Antioxidant activity of apple peels. *J. Agric. Food Chem.*, 51: 609-614.
- Yemitan, O.K. and O.O. Adeyemi, 2004. Toxicity Studies of the Aqueous Root Extract of *Lecaniodiscus cupanioides*. *Nig. J. Health Biomed. Sci.*, 3: 20-23.
- Yemitan, O.K. and O.O. Adeyemi, 2005. CNS depressant activity of *Lecaniodiscus cupanioides*. *Fitoterapia*, 76: 412-418.
- Yemitan, O.K., O.O. Adeyemi and O.O. Adeogun, 2005. Analgesic activities of the aqueous root extract *Lecaniodiscus cupanioides*. *W. Afr. J. Pharmacol. Drug Res.*, 20: 10-14.
- Yen, G.C. and H.Y. Chen, 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.*, 43: 27-32.
- Yun-Chin, C., C. Shu-Ju, H. Cheng-Kuang, C. Chen-Tien and C. Su-Tze, 2005. Studies on the antioxidative activity of *Graptopetalum paraguayense* E. Walther. *Food Chem.*, 91: 415-424.