



In Vitro Antioxidant Activity of Stem Bark Extracts of *Albizia Ferruginea* Fabaceae (Guill & Perr)

Aisha Y. Lawal, Babale Abdullahi, Isah Abdulazeez

Department of Chemistry,
School of Secondary Education Sciences,
Federal College of Education, Zaria

ABSTRACT

Antioxidant activity of methanol and ethyl acetate extract of *Albizia ferruginea* (Guill. & Perr.) stem bark used in traditional medicine was determined using the free radical scavenging activity of the 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), total phenolics content and reducing power assay on the extract was also determined. The results of the DPPH scavenging activity study indicate a concentration dependent antioxidant activity with 46.90, 56.32, 68.74, 77.24 and 84.60% for methanol extract and 51.26, 68.74, 70.12, 88.55 and 90.05 for ethyl acetate extract of various concentrations of 10, 25, 50, 125 and 250 µg/ml respectively. The total phenolic content was found to be 54.37 mg/g Gallic acid equivalent (GAE). The DPPH scavenging activity of the extract was found to be promising. The extracts showed significant antioxidant activity compared with the standard Ascorbic and Gallic acids. The phytochemical screening revealed the presence of flavonoids, tannins, saponins and steroids. It indicates that the extracts of the plant has the potency of scavenging free radicals in vitro and may provide leads in the ongoing search for natural antioxidants from medicinal plants to be used in treating diseases related to free radical reactions.

ARTICLE INFO

Article History

Received: November, 2021

Received in revised form: December, 2021

Accepted: March, 2022

Published online: April, 2022

KEYWORDS

Antioxidants, Free radicals, *Albizia ferruginea*, DPPH, Reducing power, Total phenolic content.

INTRODUCTION

In the past few years, oxidative stress involving enhanced generation of reactive oxygen species (ROS) has been implicated in the etiology of several human diseases including inflammation, metabolic disorders, cellular aging and atherosclerosis, heart disease, stroke, diabetes mellitus, cancer, malaria, rheumatoid arthritis and HIV/AIDS [1,2]. Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions. Plants are potent biochemical factories and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plants based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active component. There is a scientific interest to find naturally occurring antioxidants for use in foods to

replace synthetic antioxidants, which are being restricted due to their carcinogenicity [3]. There is a resurgence of interest in the screening of plants in search for their medicinal potentials [22, 6, 8]. Thus the search for medicinal agents in plants may pave a new dimension and promising indication of drug potency of plants against pathological conditions, such as cardiovascular, arthritis, cancer and Alzheimer's diseases. The medicinal values of these plants lies in some chemical substances that produce definite physiological actions on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, steroids, flavonoids, and phenolic compounds [15].

The plant *Albizia ferruginea* (Guill.&Perr.) is a tree that grows up to 15-30 m (sometimes to 45 m) high, with unbuttressed bole clear to 20 m or more in diameter; with spreading branches to a flattened crown, of the deciduous and dry forest from Senegal to Nigeria, and



extending across central Africa to Uganda and Angola. Different communities have different names for the plant. Ibo: igu, Yoruba: Ayinre, Hausa: TsintsiyarKurmii. Akan-Asante (Ghana): awiemfo-samina, Balanta (Senegal): Pantimbol [7].

The root bark was reported to contain saponins, triglycerides and also triterpenoids [7], and is taken in Cote d'Ivoire and Senegal for dysentery and to wash sores. A decoction is used in Senegal for bronchial infections, asthma and fever pains. Root bark is powdered and taken with salt in Nigeria for constipation. In Sierra Leone, steam in which leaves are being boiled and inhaled for headaches, fevers etc. The bark is used in Cote d'Ivoire for treating gonorrhoea, burns and convulsions in infants; it is considered to be analgesic and a good remedy for intestinal pain, to be haemostatic, antiseptic, vulnerary and laxative [7].

Plants of the Fabaceae are well-recognised source of secondary metabolites. They have been studied from various points of view for the chemical components such as alkaloids, saponins, stigmaterols, steroids, glycosides, flavonoids, tannins, terpenes, etc.

Fabaceae represents the family of plants of tremendous interest in the search for active plant constituents against infectious diseases, through *in vitro* antioxidant evaluations. Previous studies on *Albizia* species have indicated the presence of phenolic compounds from *Albizia amara* with significant antioxidant activity [21]. Also, extract of *Albizia chevalieri* indicates significant antioxidant activity [2]. Methanol extract of *Sutherlandia frutescens* was reported to have antioxidant activity [9]. Furthermore, studies by [14] revealed that *Albizia lebbeck* possess remarkable anti-inflammatory properties. This work was designed to investigate the phytochemical and antioxidant properties of methanol and ethyl acetate extracts of *Albizia ferruginea* stem bark with a view to assessing the potentials of the plant as a source for antioxidants.

MATERIALS AND METHODS

Albizia ferruginea plant sample was collected along the road to Basawa barracks Zaria, Kaduna State. It was confirmed and authenticated by the curator of the Herbarium, at

the Department of Biological Sciences, Ahmadu Bello University, Zaria, a Voucher Specimen Number 2033 was deposited there.

The sample was air dried in the laboratory at room temperature and grounded to powdered using pestle and mortar.

Extraction of Plant material

Albizia ferruginea stem bark (1000g) was subjected to cold extraction (percolation) for two weeks in methanol. The percolated sample was then filtered using Whatman filter paper No. 2 on a Buchner funnel. The residue was then percolated for another two weeks, concentrated using rotary evaporator at 45°C and placed in pre-weighed container before drying.

Phytochemical screening

The extract was subjected to phytochemical screening using standard techniques reported by [12], [24] and [27], to identify the secondary metabolites such as alkaloids (Mayer's and Draggendorff's test), flavonoids (Shinoda test), terpenoids (Salkowski test), tannins (Ferric chloride test), saponins (Frothing test), cardiac glycosides (Keller-Killiani test) and anthraquinones (Borntrager's test).

Antioxidant Activity assay

Qualitative Analysis

Qualitative screening for antioxidant activity was carried out on TLC using 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) according to [25]. Thin layer chromatograms (TLC) of extracts developed in EMW (ethyl acetate /methanol/water (10:1.35 :1) solvent system was sprayed with 0.2% DPPH in methanol. Antioxidant activity was detected on the chromatogram when the initially purple DPPH background turns yellow in bands where an antioxidant is present [4].

Quantitative Analysis

Evaluation of total phenolic content

The total phenolic contents was determined using the method of [18] with slight modification. Calibration curve was prepared by mixing ethanol solution of gallic acid (1 ml; 0.025-0.400 mg/ml) with 5 ml Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate (4 ml, 0.7M). Absorbance values were measured at 765



nm and the standard curve was drawn. One milliliter of crude methanol extract (5 gl^{-1}) was mixed with the reagents above and after 30 min the absorbance was measured to determine the total phenolic contents. All determinations were carried out in triplicate. The total phenolic compound in the extracts in gallic acid equivalents (GAE) was calculated by the following formula:

Where T = total phenolic contents, milligram per gram plant extract, in GAE;

C = the concentration of gallic acid established from the calibration curve, milligram per milliliter;

V = the volume of extract, milliliter; M= the weight of methanol plant extract, gram.

EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY

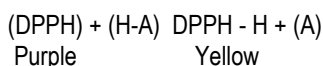
The determination of the free radical scavenging activity of the extracts was carried using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay as described by [20] with a slight modification. Various concentrations of 250, 125, 50, 25 and $10 \mu\text{gml}^{-1}$ of sample extracts in methanol were prepared. 1.0 ml of a 0.3 mM DPPH in methanol was added to 2.5 ml solution of the extract or standard, and allowed to stand at room temperature in a dark chamber for 30 min. The change in colour from deep violet to light yellow was then measured at 518 nm on a spectrophotometer. The decrease in absorbance was converted to percentage antioxidant activity (% AA) using the formula.

$$\text{AA \%} = 100 - \left\{ \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100}{\text{Abs}_{\text{control}}} \right\}$$

Blank = Methanol (1.0 ml) plus sample solution (2.0 ml),

Negative control = DPPH solution (1.0 ml, 0.25 mm) plus methanol (2.0 ml).

Ascorbic acid and gallic acid were used as standard. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as



Reducing power Assay

This was determined according to the method described by [23]. The extracts and standard of various concentrations (10, 25, 50, 125, $250 \mu\text{gml}^{-1}$) were mixed with phosphate buffer and potassium ferricyanide. The mixture was incubated at $50 \text{ }^\circ\text{C}$ for 20 mins. Trichloroacetic acid (10%, 2.5 ml) was added to the mixture.

A portion of the resulting mixture was mixed with FeCl_3 (0.1%, 0.5 ml) and the absorbance values were measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicates reductive potential of the extract.

Determination of Fe^{2+} chelating ability

The ability of the sample extracts to chelate Fe^{2+} was determined according to the method of [11]. The sample extract and standard of various concentrations (10, 25, 50, 125, $250 \mu\text{gml}^{-1}$) in 5 ml methanol were mixed with FeCl_2 (0.5 ml, 2 mM) and ferrozine (1.0 ml, 5 mM). The resulting mixture was shaken and allowed to stand for 10 min at room temperature. The Fe^{2+} was monitored by measuring the formation of ferrous iron-ferrozine complex at 562 nm in a spectrophotometer. The lower the absorbance of the reaction mixture, the higher the Fe^{2+} -chelating ability. The capacity to chelate the ferrous ion was calculated using the equation below:

$$\text{Chelating effect (\%)} = \left[\frac{(A_0 - A_1)}{A_0} \right] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of sample extract and standard.

Statistical analysis

The experiments were carried out in triplicate. The results were presented as mean standard deviation (SD). Student's t-test was used for comparison between the two means and a one-way analysis of variance (ANOVA) using computer software was used for comparison of more than two means. A difference was considered statistically significant when $p > 0.05$.



RESULTS AND DISCUSSION

Table 1: Phytochemical screening of stem bark extract of *A. ferruginea*

Phytochemicals	Result	
	Methanol	Ethyl acetate
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Steroids	+	+
Antraquinones	-	-
Cardiac glycosides	-	-

Key: + = present, - = absent

The screening of methanol and ethyl acetate extract of *Albizia ferruginea* revealed the presence of alkaloids, flavonoids, tannins, saponins and steroids. Anthraquinones and cardiac glycosides were absent in all the tested extract as shown in table 1.

ANTIOXIDANT ANALYSES

Total Phenol Content

The total phenolic content of the methanol and ethyl acetate extract *Albizia ferruginea* measured by Folin-Ciocalteu reagent in terms of Gallic Acid Equivalent (GAE) was found to be 52.83 ± 0.02 and 54.37 ± 0.45 . Phytochemicals, especially plant phenolics

constitute a major group of compounds that act as primary antioxidants [13]. They have high redox potentials which allow them act as reducing agents, hydrogen donors and singlet oxygen quenchers [16]. The delocalization of electrons over the phenolics and stabilization by the resonance effect of the aromatic nucleus prevents the continuation of the free radical chain reaction [29]. The antioxidant effects of the extract may be due to its phenolic content.

Free Radical Scavenging activity

The Free Radical Scavenging activity of the extracts from stem bark were carried out, and the result obtained are shown in Table 2.

Table 2: Result of Antioxidant activity of stem bark extract of *A. ferruginea*

Concentration ($\mu\text{g/ml}$)	AFME	AFEA %Antioxidant	Ascorbic acid Activity	Gallic acid
10	46.90 ± 0.003	51.26 ± 0.005	71.52 ± 0.002	66.26 ± 0.001
25	56.32 ± 0.006	68.74 ± 0.002	81.23 ± 0.002	75.27 ± 0.003
50	68.74 ± 0.003	70.12 ± 0.003	85.02 ± 0.001	81.81 ± 0.004
125	77.24 ± 0.003	88.55 ± 0.001	93.66 ± 0.003	91.60 ± 0.007
250	84.60 ± 0.002	90.05 ± 0.001	94.81 ± 0.001	92.59 ± 0.003

KEY: AFME; *A. ferruginea* Methanol Extract, AFEA; *A. ferruginea* Ethyl acetate extract

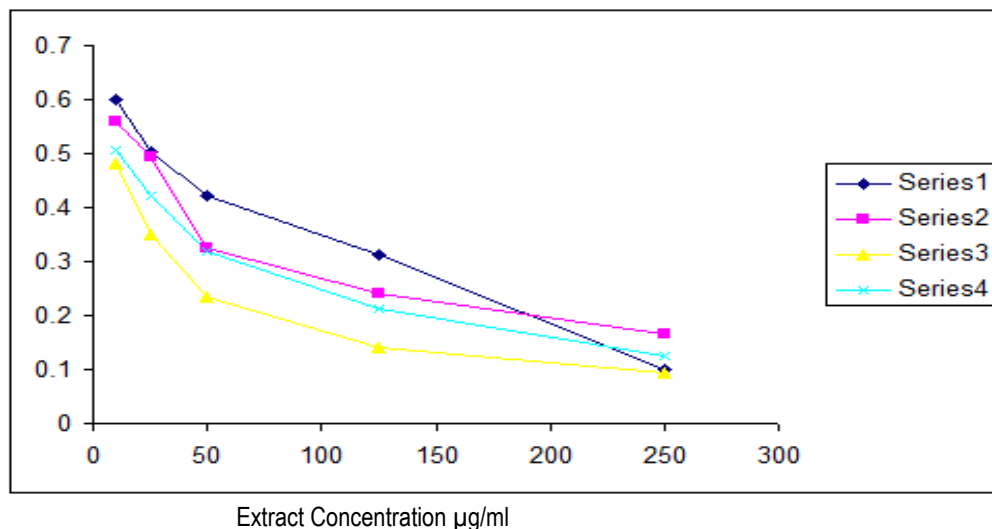
The extracts were found to highly scavenge free radicals when compared to ascorbic acid and gallic acid which are known to be standard antioxidants as can be seen in Table 2. The result indicates that there is no significant difference between the antioxidant activity of the ethyl acetate extract and those of standard ascorbic and gallic acids ($P < 0.05$) at 50, 125 and 250 $\mu\text{g/ml}$ concentrations. Also, for the methanol

extract, $P < 0.05$ at 125 and 250 $\mu\text{g/ml}$ concentrations. However, compared to the methanol extract, the ethyl acetate extract showed highly scavenging properties. The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search for the general *in vitro* antioxidant activity of pure compounds as well as for plant extracts [17, 10]. The decrease in the absorbance by the DPPH radical with increase



in concentration of the extract (Figure 9 and 10) which manifested in the rapid discolouration of the purple DPPH suggested that the methanol and

ethyl acetate extract fractions of the plant parts studied had antioxidant activities due to their proton donating abilities.



Series 1: Methanol extract; Series 2: Ethyl acetate extract; Series 3: Ascorbic acid; Series 4: Gallic acid

Figure 1: Free Radical Scavenging Activity of *A. ferruginea* stem bark

REDUCING POWER ASSAY

In the reducing power assay, presence of antioxidants in the extract reduced Fe^{+3} /ferricyanide complex to the ferrous form. The reducing capacity of compound could serve as indicator of potential antioxidant properties [19] and increasing absorbance could indicate an increase in reducing power. The results are shown in Figure 2.

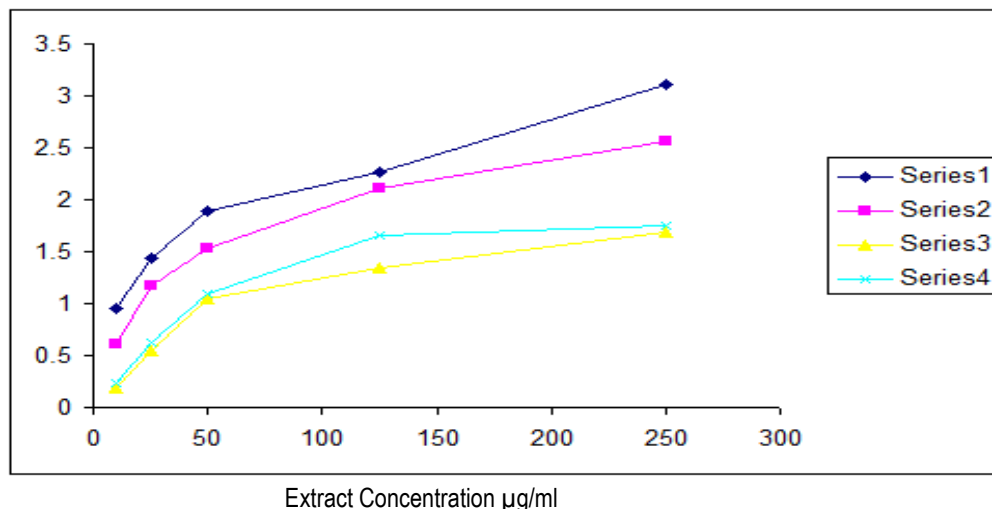


Figure 2: Reduction potential of *A. ferruginea* stem bark

Series 1: Methanol extract; Series 2: Ethyl acetate extract; Series 3: Ascorbic acid; Series 4: Gallic acid



Fe Chelating Ability

The ability of the extract to chelate Fe²⁺ was also determined according to standard [11]. The result of the chelating property was found to be within the range of 18.58% to 50.00% for the extracts from the stem bark. As a transition metal, the capability of iron to generate free radicals from peroxides by Fenton reactions has been shown to reduce oxygen radicals' production and lipid peroxidation. Thus, *A. ferruginea* extracts offers protection against oxidative damage through its chelating properties.

Albizia ferruginea extracts demonstrated moderate antioxidant activity, reducing power, as well as chelating effects. The purification of the extract may lead to increased activity in its bioactive compounds. Recent studies have shown that plants and plant based formulations can reduce the risk of chronic diseases associated with oxidative stress.

The antioxidant activities of *Albizia ferruginea* extract may be due to its proton donating capability as shown in DPPH radical scavenging results. Acting as an electron donor that can react with free radicals, it converts them to more stable products and terminates radical chain treatment. This mechanism may explain its use for the treatment of wounds and sores. Considering the phytochemical screening, total phenolics, reducing capacity and the DPPH radical scavenging activity as indices of antioxidant activity of the extract, these findings revealed the potential of *Albizia ferruginea* as a source for natural antioxidants. Although no correlation study was carried out, literature reports showed that the reduction mechanism of DPPH correlated with presence of hydroxyl groups on the antioxidant molecule [4], which can be inferred that the very good antioxidant activity of this polar extract is probably due to the presence of substances with an available hydroxyl group. This structural requirement could be linked to the presence of flavonols or condensed tannins, which are known to occur in plant species belonging to the Fabaceae family [20] to which *Albizia ferruginea* belongs to. This indicates that the plant could be a promising agent in scavenging free radicals and treating diseases related to free radical reactions. Furthermore, detailed studies on the isolation and

characterization of the plant extract as well as studies with other models such as lipid peroxidation and in vivo assays will be interesting in discovering new biological antioxidants.

CONCLUSION

Albizia ferruginea (Guill&Perr) is an extremely valuable plant with both economic and potential therapeutic values. The plant has diverse ethno medical uses in Africa and the world at large. *Albizia ferruginea* stem bark was extracted exhaustively. Preliminary phytochemical analysis conducted reveals the presence of some natural products such as alkaloids, tannins, flavonoids, steroids and saponins.

The antioxidant analyses were also carried out on the plant extracts in order to confirm the ethno medicinal claims for the plant. It had been reported that the antioxidant activity of plant materials is well related to the content of their phenolic compounds, their reductive potentials, so also their ability to chelate Fe. The scavenging effects on DPPH radical were determined measuring the decrease in absorbance at 518nm, due to DPPH radical reduction, indicating the antioxidant activity of the extracts in a short time. Total phenolic content was determined using Folin and Ciocalteu's phenol reagent. The reducing power was also evaluated, measuring absorbance at 700nm, higher absorbances of the samples indicated higher reductive potential. The presence of antioxidants in *Albizia ferruginea* justified its usage by local medical practitioners in the treatment of ailments such as heart diseases, joints pains, skin diseases, malaria, etc.

REFERENCES

- Alho H. and Leinonen J. (1999). Total Antioxidant Activity measured by Chemiluminescence method. *Methods Enzymol*, 299: 3-15.
- Aliyu, A. B., Musa, A. M., Ibrahim, M. A., Ibrahim, H., Oyewale, A. O. (2009). Preliminary Phytochemical screening and Antioxidant Activity of Methanol leave extract of *Albizia chevalieri* Harms (leguminosea Mimosoideae). *Bayero Journal of Pure and Applied Sciences*, 2(1):149-153.



- Atiqur Rahman, M., Mizanur Rahman, M., Mominullamsheik, M., Mashiar Rahman, Shabah, M. S., and Alam, M. F. (2008) Free radical scavenging activity and phenolic content of cassiasophera L. *African Journal of Biotechnology*, 7(10) : 1591 – 1593.
- Basnet, P., Matsumoo, T. and Neidlein, R. (1997). Potent free radical scavenging activity of propolis isolated from Brazilian propolis. *Z. Naturforsch [C]* 52(11-12):828-833.
- Bors, W.S. and Eltsner E.F. (1992). Screening of plant antioxidants. *Modern Methods of Plant Analysis*, 13:277-295. *Free Radical Biology and Medicine*, 3:379-387.
- Bruits, M., Asres, K. and Bucar, F. (2001). The antioxidant activity of the essential oils of *Artemesia afra*, *Artemesia abyssinka* and *Juniperus procera*. *Phytotherapy Research*, 15:103 -108.
- Burkill, H. M. (1995) the useful plants of West Tropical Africa vol. 3 Families. *Journal- Royal Botanical Gardens, Kew*. 210 -211.
- Dessi, M. A., Deiana, M., Rosa, A. Pirredda, M., Cottiglia, F., Bonsignore, L. Deidda, D., Pompei, R., and Conronglu, F.P. (2001) Antioxidant activity of plants growing in Sardinia. *Phytotherapy Research*, 15: 511 – 518.
- Fernandes, A.C., Cromarty, A.D., Albrecht, C., Constance, E. and Rensburg, J. V. (2004). The antioxidant potential of *Sutherlandia frutescens*: *Journal of Ethnopharmacology*, 95(1): 1-5.
- Gonçalves, C. Dinis, T. and Batista, M.T. (2005). Antioxidant properties of proanthocyanidins of *Uncariatomentosa* bark decoction: a mechanism for anti-inflammatory activity. *Phytochemistry*, 66:89-98.
- Gulcin, I., Buyukokuroglu, M.E., Kufrevioglu, O.I. (2003). Metal chelating and hydrogen peroxide scavenging effects of melatonin. *Journal of Pineal Research*, 34:1-10.
- Harborne, J. B., (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plants Analysis*. 3rd Edition, Chapman and Hall, London, ISBN: 0-412-57270-2, 302.
- Hatano, T., Edmatsu, R., Mori, A., Fujita, Y. and Yasuhara, E. (1989). Effects of tannins and related polyphenols on superoxide anion radical and on 1, 1-diphenyl-2-picrylhydrazyl radical. *Chemical and Pharmaceutical Bulletin*, 37:2016-2023.
- Henok, A. & Biniam, P. (2016). In vitro Antioxidant and Antibacterial Activity of *Albizia lebbek*. *Journal of Science, Technology and Arts Research*, 4(2):204.
- Hill, A. F. (1952): *Economic Botany. A text books of useful plants and plant products*; edition McGraw Hill Book Company Inc. New York, 1-38.
- Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P. Pihlaja, K., Kujala, T. S. and Heinonen, M. (1999). Antioxidant activity of plant extract containing Phenolic compound. *Journal of Agricultural and Food Chemistry*, 47(10): 3954-3962.
- Koleva, I. I., Van Beek, T., Linssen, J. P. H., de Groot, A. and Evstatieva, L. N. (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical Analysis*, 13:8-17.
- McDonald S, Prenzler P.D., Autolovich M. and Robards K. (2001). Phenolic content and Antioxidant activity of olive oil extracts. *Journal of Food Chemistry*, 73:73-84.
- Meir, S., Kanner, J., Akiri, B. and Hada, S. P. (1995). Determination and Involvement of aqueous reducing compounds in oxidative defense system of various senescing leaves. *Journal of Agricultural and Food Chemistry*, 43:1813-1819.
- Mensor, L. I., Menezes, F. S., Leitao, G. G., Reis, A. S., Dos Santos, T., Coube, C. S. and Leitao, S. G. (2001). Screening of Brazilian Plants extracts for antioxidants activity by the use of DPPH free radical method. *Phytotherapy Research*, 15: 127-130.



- Muchuweti, M., Nyamukonda, L., Chagonda, L. S., Ndhkala, A. R; Mupure, C. and Benhura, M. (2006). Total phenolic content and antioxidant activity in selected medical plants of Zimbabwe. *International Journal Food Science and Technology*, 41(1): 33-38.
- Nardi, G. M., Felippi, R., Dalbo, S., Siqueir-Junior, J. M., Arudda, D. C., Delle, M., Monache, F., Timbola, A. K. Pizzolatti, K. Antioxidant effects of *Crotonceldidifolius* bark. *Phytomedicine*, 10:176-184
- Oyaizu M (1986). Studies on the product of browning reaction prepared from glucose amine. *Japan Journal of Nutrition*, 44:307-315.
- Sofowora, E. A. (1982). *Medicinal Plant and Traditional Medicine in Africa*, 1st Edition Published by John Wiley and Sons, 6-160.
- Takao, T., Kitatani, F., Watanabe, N., Yagi, A. and Sakata, K. (1994). A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. *Bioscientific, Biotechnology Biochemistry*, 58:1780-1783.
- Trease, G.E. and Evans, W.C. (1979) *Phenols and Phenolic Glycosides, Pharmacognosy*, 11th Edition, Bailliere Tindall Ltd. London: 374-375.
- Trease, G.E. and Evans, W.C. (1989). *A Text Book of Pharmacognosy* 13th Edition, Publishes Elsevier Ltd. Edinburgh London: 20-305.
- Trease, G.E. and Evans, W.C. (2002) *Pharmacognosy* 15th Edition, Publishes Elsevier Ltd. Edinburgh London: 20-305.
- Tsao, R. and Akhtar, M.H. (2005). Nutraceuticals and functional foods I: Current trend in Phytochemical Antioxidant Research. *Journal of Agricultural and Food Chemistry*, 3:10-17.
- Yalko, M., Leibfritz, D., Moncol, J., Cronin, MTD., Mazur, M. and Telser, J. (2006). Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology*, 7 (1): 45-78.