Original Research Article

Determination of Antioxidant activity of Leave extracts of *Albizia chevaieri* using free Radical Scavenging activity assay

ABSTRACT

Dried and powdered leaves of Albizia chevalieri were extracted using ethanol. The extract was fractionated to give methanol, chloroform and pet-ether. The four extracts obtained; ethanol, chloroform, methanol and pet-ether were evaluated for antioxidant activity using DPPH free radical scavenging activity assay. The results of the DPPH scavenging activity indicated a concentration dependent antioxidant activity. The DPPH scavenging activity of the ethanol, chloroform and methanol extracts were found to be promising. There is no significant difference in the antioxidant activity between the ethanol, chloroform and methanol extracts with that of standard Ascorbic acid at 10, 25, 250 and 500µg/ml concentrations. This showed that the ethanol, chloroform and methanol leaves 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 Nigerian medicinal plants to be used in treating diseases related to free radical reactions.

Keywords: *Albizia chevalieri, Antioxidant, Free Radical, Extract, Scavenging activity assay, DPPH.*

INTRODUCTION

The plant *Albizia chevalieri* is a tree or a shrub that grows up to 12m height under harsh conditions of the dry savannah from Senegal, Niger and Nigeria. It has an open and rounded or umbrella shaped canopy, bark pale-grayish, twigs pubescent with white lenticels, leaves with 8-12 pairs of pinnate and 20-40 pairs of leaflets each. The bark was reported to contain alkaloids and also tannis sufficient for use in tanning in Nigeria and Senegal. It is used in Borno-North eastern Nigeria as purgative, taenicide and also remedy for coughs. A decoction of leaves is used in Northern Nigeria as remedy for dysentery [1]. There are also reports on the local use of the leaves extract for cancer treatment in Zaria city, Kaduna state [2].

Previous studies on *Albizia chevalieri* have indicated the presence of phenolic compounds from *Albizia amara* with significant antioxidant activity [3] and *Albizia inundata* was reported for effective anti candida activity from Brazilian flora [4]. Liphophlic extracts of *Albizia gummifera* revealed very promising antitrypanosomal activity [5]. The extracts of *Albizia ferruginea* were also reported to have significant antimiccrobial activity on selected microorganisms [6] and *Albizia saman* was found to have good antiplasmodial activity [7]. *Albizia lebbeck* was reported to contain 3α, 5-dihydroxy-4β, 7-dimethoxy flavones and N-Benzoyl-L-phenyl alaninol [8]. As the focus of medicine shifts from treatment of manifest disease to prevention, increasing awareness on herbal remedies as potential sources of phenolic

antioxidants have grown in recent years, and several plants are being screened for their antioxidant properties using different assays [9].

DPPH, known as 2,2-diphenyl-1-picrylhydrazyl, (I.U.P.A.C name, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl with molecular formula $C_{18}H_{12}N_5O_6$ is a stable free radical that is commonly used to evaluate the ability of compound to act as free radical scavenger or hydrogen donor and to measure the antioxidant activity of tissue extract [10]. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism [11]. Antioxidants offer resistance against oxidative stress by scavenging the free radical and many other mechanisms thus preventing disease progression [12]. The reaction of DPPH with an antioxidant or reducing compound produces the corresponding hydrazine DPPH-H, which can be followed by color change from purple (absorbance at 515-528nm) to yellow. The DPPH method is widely used for measurement of free radical scavenging ability of antioxidants [13], [14]. DPPH is a rapid, simple, accurate and inexpensive assay for measuring the ability of different compounds to act as free radical scavengers or hydrogen donors, and to evaluate the antioxidant activity of foods and beverages [15].

This work was designed to investigate the antioxidant properties of ethanol methanol, chloroform and pet-ether leaves extracts of *Albizia chevalieri* with a view to assessing the potential of the plant as a source for antioxidants.

MATERIALS AND METHODS

General

The Ethanol was obtained from Sigma Aldrich, the DPPH and Ascorbic acid was obtained from chemistry laboratory Bayero University Kano. While other reagents and chemicals were of analytical grade supplied by Chemistry Laboratory, Kano University of Science and Technology, Wudil. All glass wares used were washed with detergents and oven dried before use. The leaves of the plant *Albizia checalieri* were rinsed with clean tap water to remove dust and impurities.

Collection of Plant Material

The leaves of plant *Albizia chevalieri* were collected on 4th November 2017 from Kududdufawa village Ungogo local government area of Kano State. The plant was authenticated by Baha'uddeen Said Adam from the Department of plant Biology, Bayero University Kano, with accession number BUKHAN 0378.

Extraction of Plant Material

The extraction and fractionation of air-dried and ground plant material was carried out according to the method described by [15]. 150g of the powdered plant sample was weighed and percolated in 600ml of 99% Ethanol in 2L conical flask for 14 days. It was filtered using Whattman No. 2 filter paper and the solvent (Ethanol) was completely removed using rotary evaporator. The extract obtained was dried under room temperature to get a thick paste form of the plant extract. A dark green glassy and gummy thick paste was obtained and weighed. It was labeled as AC01.

Fractionation of Crude Extract

The dried Ethanolic extract was partitioned with chloroform-water in 1:1 ratio of 100ml in a separating funnel. The mixture was shaken for 15 minutes and allowed to settle for 2 hours in separation funnel, the water and chloroform fractions were separated in glass beakers. The chloroform fraction was concentrated at 35^{o}C using rotary evaporator and drying was completed under room temperature. The chloroform extract obtained was labeled as AC02. While the water fraction was discarded.

Similarly, the dried chloroform extract was further partitioned with Methanol and Pet-ether in 1:1 ratio of 100ml in a separation funnel. The mixture was shaken for 15 minutes and allowed to settle. The methanol and pet-ether fractions were separated in glass beakers and were concentrated at 45^{o}C and 35^{o}C respectively using rotary evaporator. The extracts were completely dried under room temperature and weighed where the methanol and pet ether extracts were obtained and labeled AC03 and AC04 respectively.

DPPH Free Radical scavenging activity assay

The determination of the radical scavenging activity of the crude extract of *Albizia chevalieri* was carried out using DPPH (1,1-diphenyl-2-picrylhydrazyl) as described by [16] with slight modification. Various concentrations of 500, 250, 100, 50, 25 and 10µgml^{-1} of each of the sample extract in methanol were prepared. The commercial known antioxidant, Ascorbic acid (Vitamin C) was used for comparison or as a positive control. The DPPH in the absence of plant extract was used as control and the plant extract in the absence of DPPH was used as blank. DPPH (1mM, 200µl) in methanol was added to 100µl solution of each of the plant extract, and allowed to stand at room temperature in a dark chamber for 30min. The change in color from purple to yellow was then measured at 517 nm on spectrophotometer. Measurement was performed in triplicate. The percentage of radical scavenging activity was calculated using the following equation:

$$\%Radical\ Scavenging\ activity = 100 - \left\{\frac{Abs\ Sample - Abs\ Blank}{Abs\ Control}\right\} X\ 100$$

Abs sample = Absorbance of plant extract + DPPH

Abs blank = Absorbance of plant extract

Abs control = Absorbance of DPPH

$$(DPPH^*) + H\text{-}A \longrightarrow (DPPH\text{-}H) + A^*$$

Purple Yellow

RESULTS AND DISCUSSION

Extraction and Fractionation of Plant Material

The extraction of the powdered leaves of *Albizia chevalieri* was carried out using 99%v/v ethanol and the resulting extract was partitioned to give methanol, chloroform and pet-ether fractions (Fig. 1). 5.11% of the plant material went into the ethanol which on partitioning between chloroform/water, about 21.66% of the ethanol fraction went into chloroform. Further partitioning of the chloroform extract resulted in about 28.16% and 17.50% of the extract going into methanol and pet-ether respectively. This showed that the leaves extracts of the plant contains reasonable amount of medium polar and non-polar compounds (Table 1).

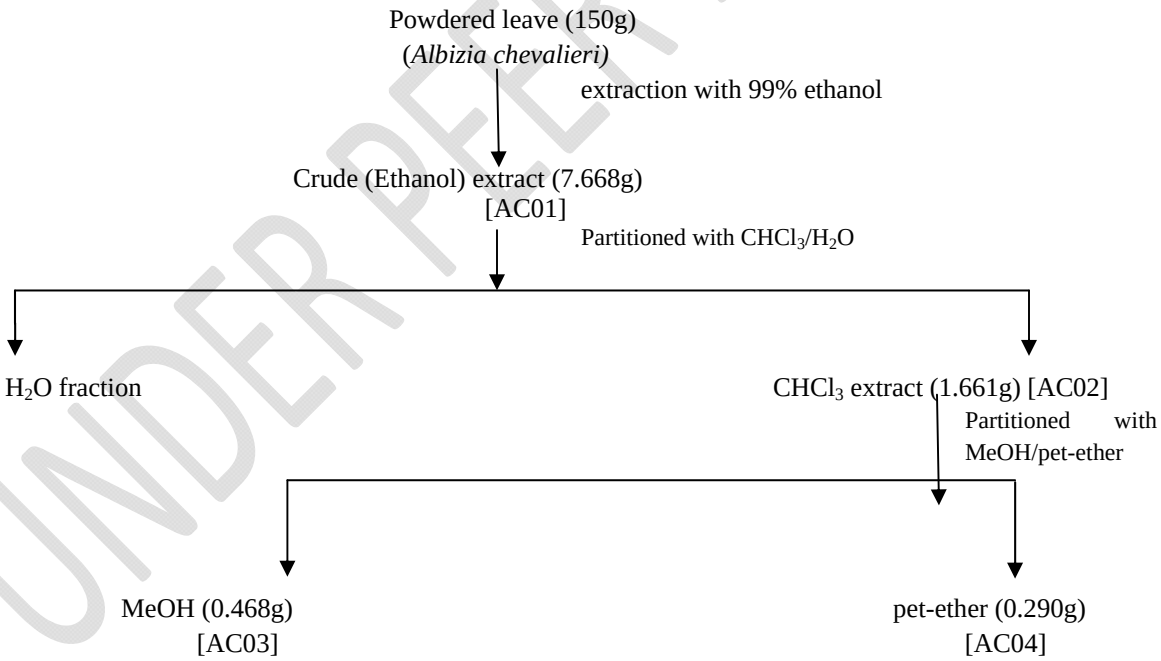

Figure 1: Extraction and Fractionation Procedure of the Powdered leave of the Plant

Table 1: Weights of Extracts Recovered and their Physical Properties

Extracts	Colour	Texture	Weight (g)
AC01 [crude extract]	Dark green	Gummy like	7.668
AC02 [$CHCl_3$ extract]	Black	Semi-solid	1.661
AC03 [methanolic extract]	Black	Semi-solid	0.468
AC04 [pet-ether extract]	Black	Semi-solid	0.290

Antioxidant activity of *Albizia chevalieri*

Antioxidant properties of leaves extracts of *Albizia chevalieri* was evaluated to find a new source of antioxidant. DPPH radical is a commonly used substrate for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay [18]. This assay is known to give reliable information concerning the antioxidant ability of the tested compounds [19]. The principle behind this assay is the color change of DPPH solution from purple to yellow as the radical is quenched by the antioxidant [20].

The leaves extracts of *Albizia chevalieri* were screened for DPPH radical scavenging activity according to the method described [17] with slight modification and the result of the screening is shown in (table 2) as compared to Ascorbic acid, a known antioxidant. Four different extracts of *Albizia chevalieri* (AC01, AC02, AC03, and AC04) showed high radical scavenging activity at various concentrations of 10, 25, 50, 100, 250 and 500µg/ml (Fig. 2).

Table 2: Result of Antioxidant activity of leave extract of *Albizia chevalieri* at different concentrations

Conc. (µg/ml)	% Antioxidant activity of A. chevalieri				
	Ethanol Extract (AC01)	**Chloroform Extract (AC02)**	**Methanol Extract (AC03)**	**Pet- Ether Extract (AC04)**	**Ascorbic Acid (A.A)**
500	**94.3745**	**88.7889**	**90.1311**	**47.8518**	**95.0026**
250	**94.5114**	**91.6722**	**93.9065**	**38.7689**	**94.4946**
100	**51.6757**	**56.9921**	**63.5448**	**44.3022**	**75.1166**
50	**50.2716**	**44.5092**	**55.8617**	**36.3448**	**61.4481**
25	**41.6788**	**43.3837**	**48.7701**	**38.0271**	**38.6072**

10	**41.7715**	**42.5928**	**51.5697**	**38.7336**	**39.9136**

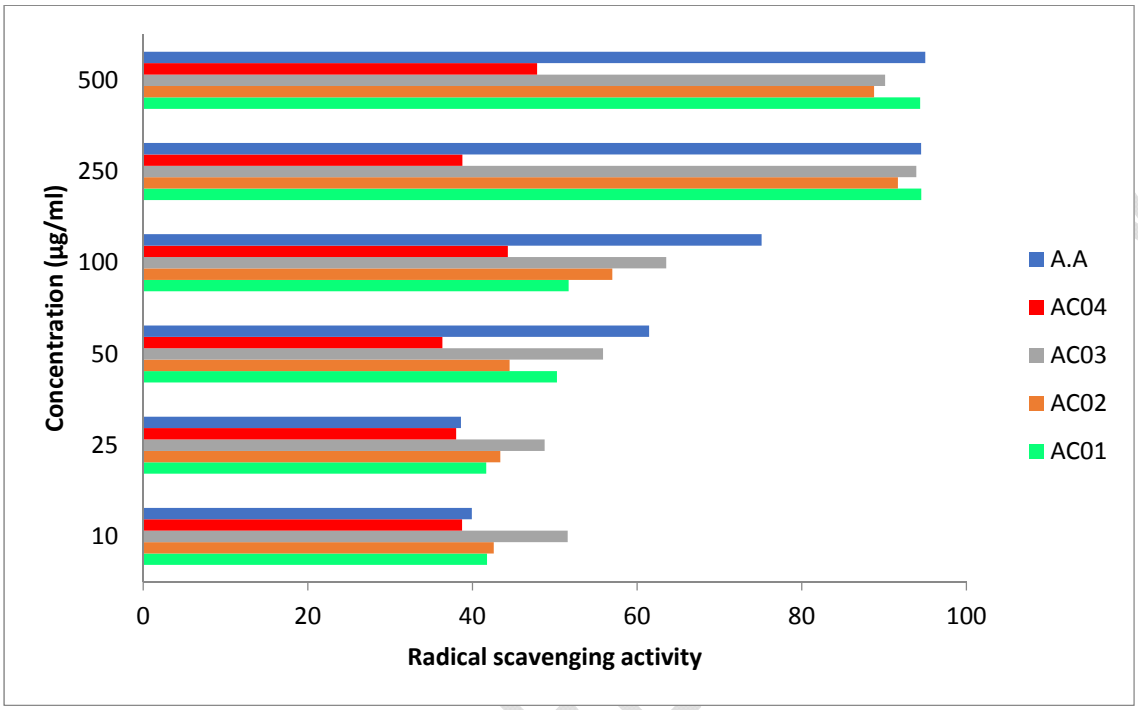

Figure 2: Free Radical scavenging activity of various extract of *Albizia chevalieri* at different concentrations

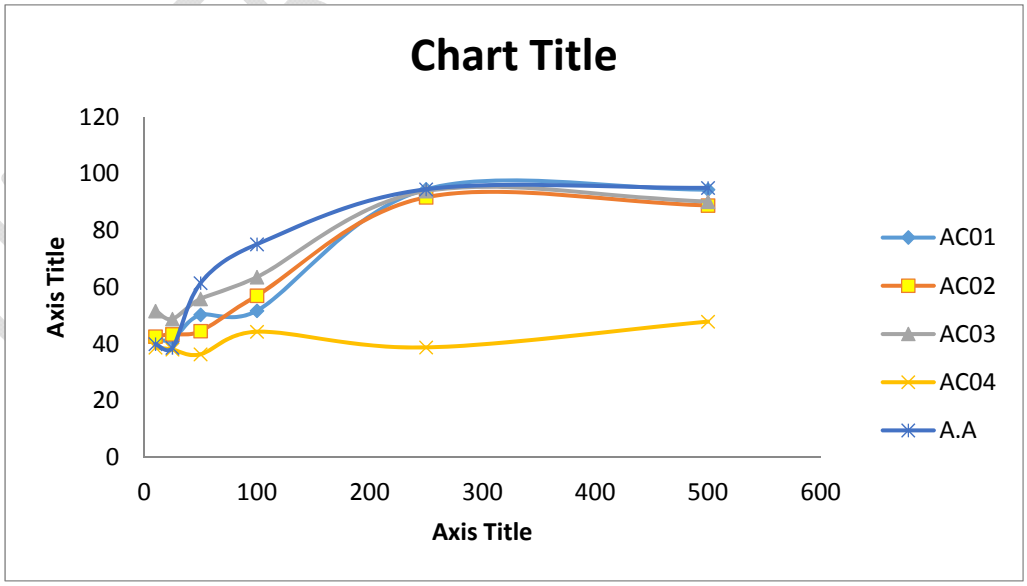

Figure 3: Free Radical scavenging activity of *Albizia chevalieri*

Inhibitory concentration at 50% (IC_{50})

The radical scavenging activity of each extract was determined by calculating the inhibitory concentration at 50% (IC_{50}), the IC_{50} of various extract of *Albizia chevalieri* (AC01, AC02, AC03, AC04 and Ascorbic acid) were 52.92, 54.53, -1.87, 71.59 and -0.81µg/ml respectively. The lower the IC_{50}, the more potent the extract, this showed that methanol extract has the highest radical scavenging activity (-1.87µg/ml) more than the standard Ascorbic acid (-0.81µg/ml), followed by ethanol extract (52.92µg/ml), Chloroform extract (54.53µg/ml) and pet-ether extract (71.59µg/ml) [fig. 4].

Table 3: Result of IC_{50} of leave extract of *Albizia chevalieri*

Plant Extracts	IC_{50} (µg/ml)
Methanol extract (AC01)	52.92
Chloroform extract AC03)	54.53
Ethanol extract (AC02)	-1.87
Pet-ether extract (AC04)	71.59
Ascorbic acid (Standard)	-0.81

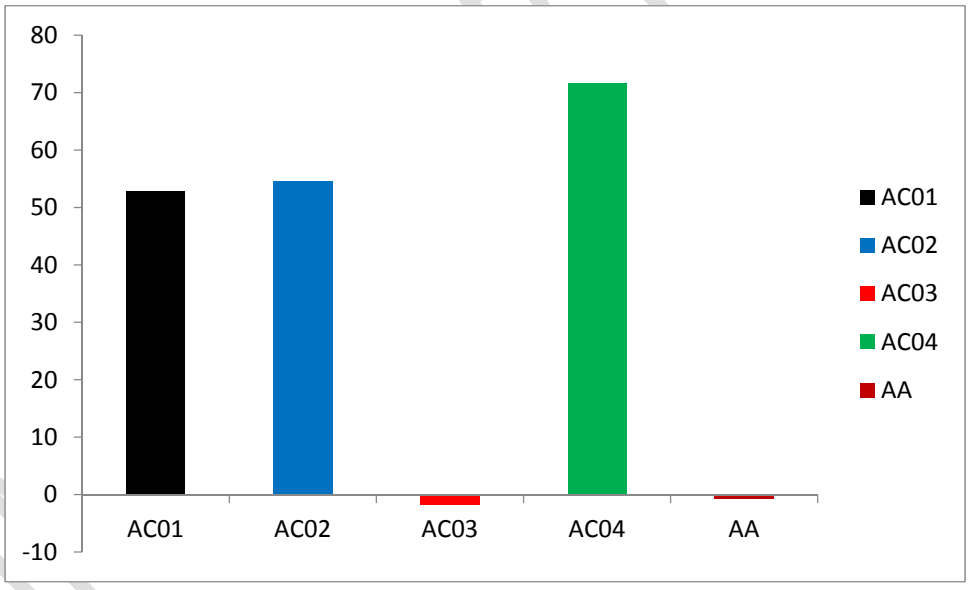

Figure 4: Inhibitory Concentration at 50% (IC_{50}) of various extracts of *Albizia chevalieri.*

CONCLUSION

These findings revealed the potential of A. chevalieiri as a source for natural antioxidants. It indicates that the plant could be a promising agent in scavenging free radicals and treating diseases related to free radical reactions. The leaves extracts of *A. chevalieri* were found to have high radical scavenging activity as compared with standard Ascorbic acid. The results of the DPPH scavenging activity study indicate a concentration dependent antioxidant activity which increases with increase in concentration of the extract.

REFERENCES

1. Burkill, H.M (1995). "The Useful plant of west Tropical Africa". *Families J – L. Royal Botanical Gardens, Kew*. **3 :** 207 – 208.
2. Aliyu, A.B., Musa, A.M., Ibrahim, M.A., Ibrahim, H. and Oyewale, A.O. (2009). "Preliminary Phytochemical Screening and Antioxidant activity of leave extract of *Albizia chevalieri* Harms", *Bayero Journal of Pure and Applied Sciences.* (**2**)1: 149-153.
3. Muchuweti, M., Nyamukonda, L., Chagonda, L.S., Ndhlala, A.R., Mupure, C. and Behura, M. (2006). "Total Phenolic content and antioxidant activity in selected medicinal plants of Zimbabwe". *Int. J. Food Sci. Tech.,* **41**: 33 – 38.
4. Tempone, A.G., Sartorelli, P., Teixeira, D., Prado, F.O. Calixto, I. A.RL, Lorenzi, H. and Melhem, M.Sc. (2008). "Brazilian flora extract as source of nivel anileishmanial and antifungal compound", *Mem, 1st Oswaldo Cruz,* **103**(5): 443 – 449.
5. Freiburghaus, F., Owgwal, E.N., Nkuny, M. H. Kaminsky, R. and Brun. R. (2007). "Invitro antitrypanosomal activity of African Plants used in traditional medicine in Uganda to treat Sleeping sickness", *Trop. Med, Int. Health,* **1**(6): 765 – 771.
6. Agyare, C., Kofuer, G.A., Mensah, A. Y.and Agyemang, D.O. (2006). "Boletin Latinoamericano Y. del Cribe de plantas Medicinales" *Y Aromatica,* **5**(2): 31 – 35.
7. Kohlera, I., Jenett – Siema, K., Siemsb, K., Herna ndezc, M.A., Ibarrac, R.A., Berendsohnd, W.G. Bienzlee, U. and Eicha, E. (2002). "In vitro Antiplasmodial investigation of Medicinal plants from El Salvador.z". *Naturforsch.* **57c**: 277 – 281.
8. Rashid, R.B., Chowdhury, R., Jabbar, A., Hassan G.M and Rashid, M.A. (2003). "Constituents of Albizia lebbeck and antibacterial activity of isolated flavones derivatives". *Saudi pharm. J.,* **11**(1-2): 52 – 56.
9. Karou, D., Ndaembega, W.M.C., Outtara, L., Ilboudo, D.P., Canini, A., Nikiema, J.P., Simpore, J., Collizi V. and Traore, A.S., (2006). "African Ethnopharmacology and New Drug Discovery". *Medicinal and Aromatic plant Science and Biotechnology*, **1**: 1 – 9.
10. Kedare, Sagar B., and R. P. Singh. (2011). "Genesis and development of DPPH method of antioxidant assay" *J Food Sci Technol.* **48**(4): 412-422.

11. Tiwari, A., (2001). “Imbalance in antioxidant defense and human diseases: multiple approach of natural antioxidants therapy”. *Curr. Sci.,* **81**: 1179-1187.
12. Braugghler, J.M., Duncan, C.A. and Chase, L.R. (1986). “The involvement of Iron in lipid peroxidation: importance of ferrous to ferric ratio in initiation”. *J. Biol. Chem.,* **61**: 102-182.
13. Perez – Jimenez, J., and F. Saura – Calixto, (2008). “Antioxidant capacity of dietry polypenols determined by ABTS assay: a kinetic expression of the results”. *International Journal of food science and Technology,* **43**: 185 -191.
14. Perez – Jimenez, J., S. Arranz., M. Tabernero, M.E. Diaz- Rubia, J. Serrano, I. Goni and F. Saura- Calixto, (2008). “Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: extraction measurement and expression of results). *Food Research International* **41**(3): 274 – 285.
15. Prakash, A., (2001). “Antioxidant Activity Medallion Laboratories Analytical Progress”, **19**(2).
16. Fatope M.O., Ibrahim H. and Takeda Y. (1993) “Screening of higher plants reputed as pesticides using brine shrimp lethality assay” *Int. J. Pharmacog*. **31**: 250-56.
17. 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 antioxidant activity by the use of DPPH free radical method”. *Phytother. Res., Tech.*, **15**: 127 – 130.
18. Bozin B, Mimica-Dukic N, Samojlik I, Goran A, Igic R (2008). “Phenolics as antioxidantsin garlic (Allium sativum L., Alliaceae)”. *Food Chem.,* **111**: 925-929.
19. Huang D, Ou B, Prior RL (2005). “The chemistry behind antioxidant capacity assays”. *J. Agric. Food Chem.,* **53**: 1841-1856.
20. Karagozler AA, Erdag B, Emek YC, Uygum DA (2008). “Antioxidant activity and proline content of leaf extracts from Dorystoechas hastate”. *Food Chem.,* **111**: 400-407.