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Research Article
Phytochemicals Distribution and Antioxidant Potential of *Bauhinia monandra* (Linn.) Leaves Extract

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Abstract

Background and Objective: *Bauhinia monandra* leaves are prepared as poultices and used in many Brazilian tribes and some Nigerian communities for their antidiabetic, antioxidant and anti-inflammatory effect. Therefore, these folkloric claimed benefits inspired this study. The aim of this study was to evaluate the antioxidant potential of methanolic extract of *Bauhinia monandra* leaves (MEBmL) *in vitro*. **Materials and Methods:** In the present study, a preliminary phytochemical screening of MEBmL was done. Determination of the antioxidant activities was conducted using the 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay, the hydrogen peroxide scavenging assay and the ferrous ion chelating ability test. **Results:** Phytochemical screening revealed the presence of flavonoids, saponins, tannins, alkaloids and proteins as major constituents and reducing sugars, steroids, cardiac glycosides and amino acids as minor constituents. The EC_{50} for MEBmL and L-ascorbic acid (positive control) in DPPH assay was 126 and 8.7 μg mL^{-1}, respectively, while that of hydrogen peroxide scavenging assay and ferrous ion chelating ability was 155.86, 14.81, 128 and 32.10 μg mL^{-1}, respectively. From the findings, it was observed that the methanolic extract *B. monandra* possessed significant antioxidant potential. **Conclusion:** The antioxidant activity of methanolic extract of *B. monadra* could have been due to the various phenolic phytoconstituents present in the extract. Thus, the findings suggests that the methanolic extract of *Bauhinia monadra* leaves could be a potential source of bioactive compounds with antioxidant properties and can be further exploited in further researches as potential antioxidants of therapeutic benefit in free radical induced diseases.

Key words: Reactive oxygen species, antioxidants, DPPH, hydrogen peroxide, methanolic extract of *Bauhinia monandra* leaves (MEBmL), L-ascorbic acid, phytochemicals

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Phytochemicals consists of a diverse group of natural bioactive molecules vastly distributed in plants[1]. These bioactive molecules have gained attention in recent times due to their diverse effects on human physiology and human health and their economic importance. Secondary metabolites are basically classified into three classes namely alkaloids, phenolics and terpenoids[2]. The molecules in these classes are further grouped due to their numerous modifications, thus emphasizing the diversity of secondary metabolites. Considering this, reasonable attention has been focused on the identification of plants rich in bioactive phytochemicals and characterization of their medicinal and economic values. Regarding this concept, *Bauhinia monandra* is a promising ethnomedicinal plant due to documented studies.

Bauhinia monandra is a species of leguminous trees that belongs to the Caesalpiniaceae family and also known as cow's foot, orchid tree, Napoleon plume, Flamboyant, St. Thomas tree[3]. *B. monandra* is an evergreen shrub or tree with a rounded crown and which can grow up to 3-15 m tall. It is often cultivated for its elegant flowers and ornamental foliage and as such popularly planted as a garden and street tree in the tropical regions of Australia, America, Asia and West Indies[3]. *B. monandra* seeds are natural rich sources of vitamin A and also contain high quantity of linoleic and fatty acid and low quantity of myristic acids[3]. Research studies have shown that *B. monandra* have antidiabetic potential against experimentally induced diabetes in rats[4] and this antidiabetic potential has been linked to the presence of antioxidant compounds[5]. Native *Bauhinia* species (Leguminosae) have been vastly used in traditional medicine for the treatment of diabetes. In Brazil, leaves of three *Bauhinia* species namely, *B. purpurea* Linn., *B. forficata* Link. and *B. monandra* Kurz. are commonly used for treatment of diabetes[6]. Owing to the elevated costs of commercial diabetes medication, most Brazilian folks often prefer the use of these infusions as alternative treatment of diabetes and other ailments[6-8]. Several other studies have also shown a variety of pharmacological activities from different species of this genus[6,7], but very few have reported on the antioxidant potential of *B. monandra*. Bioactive compounds isolated from this genus include flavonoids, tannins, lactones, steroids, terpenoids and glycolipids[6,8].

Despite these observations, experimental studies pertaining to the phytochemical profile of *B. monandra* are limited. There is a lack of comparative and qualitative literature regarding the phytochemical distribution in the leaves of *B. monandra*. Thus, the scope of the present study was to identify the phytochemicals in methanolic extracts obtained from dried leaves of *B. monandra* and evaluation of antioxidant potential of the methanolic plant extract.

MATERIALS AND METHODS

Research duration: The research was conducted in Department of Pharmacology and Toxicology Laboratory, University of Nigeria, Nsukka for duration of three months (January, 2018-March 2018).

Chemicals: 1, 1-diphenyl-2-picrylhydraxyl (DPPH) and ascorbic acid (Vitamin C) were obtained from Sigma-Aldrich (Germany). Methanol, acetic acid, lead acetate, sodium hydroxide, ethyl acetate, hydrochloric acid (HCl), tetraoxosulphate (VI) acid (H_2SO_4), chloroform ($CHCl_3$), ethanol (C_2H_5OH) and ferric chloride were obtained from the chemical store of the Department of Chemistry, University of Nigeria, Nsukka. Solvents were redistilled before use while reagents were used without further purification. All chemicals and reagents were of analytical reagent grade.

Collection and identification of plant sample: The aerial parts of *B. monandra* were collected from the Botanical garden, University of Nigeria, Nsukka. The plant was taken to the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka where it was appropriately identified and authenticated with specimen voucher number, UNH 1559. The leaves obtained were subsequently air-dried and used for the experimental studies.

Preparation of plant sample: Fresh aerial leaves of *B. monandra* were washed properly to remove dust and dirt. The leaves were then air-dried under shade for a period of three weeks and pulverized into powder and stored in plastic air-tight container for further processing.

Extraction of plant sample: About 200 g of the pulverized powdered plant was macerated in 1 L of methanol and shaken properly. The mixture was allowed to stand for 72 h, after which it was then filtered using filter paper. The filtrate obtained was concentrated in a rotary evaporator and the resultant extract was preserved in air-tight glass containers and stored at 4°C in a refrigerator for subsequent use.

Phytochemical analysis of leaf extract of *B. monandra*: Some chemical tests were conducted on the methanolic leaf extract of *B. monandra* using standard procedures to identify the phytoconstituents as described by Ramamurthy and Sathiyadevi[9].

Antioxidant activities of *B. monandra* methanolic extract: The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, hydrogen peroxide (H_2O_2) scavenging assay and the ferrous ion chelating ability models were used to determine the antioxidant activities of methanolic extract of *B. monandra* leaves[10-12].

DPPH free radical scavenging assay: The free radical scavenging activity of methanolic leaf extract of *B. monandra* was determined using the *in vitro* DPPH free radical scavenging assay method described by Shimada *et al.*[10] with few modifications. Briefly, 1.0 mL of 0.8 mM solution of DPPH in 50% methanol (v/v in water) was added to 1.0 mL of various concentrations of sample extract solution at concentrations of 25, 50, 100, 200 and 400 μg mL^{-1}. The absorbance of the mixture was measured at 517 nm against the corresponding blank solution (50% v/v methanol in water) in a Jenway UV/Vis-1800 series spectrophotometer. Ascorbic acid was used as the standard test sample in all the tests which were carried out in triplicates. The inhibition (%) for scavenging DPPH free radical was calculated using the equation:

$$\text{Inhibition (\%)} = \frac{A_{control}-A_{test}}{A_{control}} \times 100$$

where, $A_{control}$ and A_{test} are absorbance of control and test sample, respectively.

Hydrogen peroxide (H_2O_2) scavenging assay: The hydrogen peroxide scavenging activity of the methanolic leaf extract of *B. monandra* was determined according to the method described by Ruch *et al.*[11] with few modification. Briefly, a solution of 43 mM hydrogen peroxide was prepared in 1 M phosphate (pH 7.4). 1.0 mL of different concentration of extract sample (25, 50, 100, 200 and 400 μg mL^{-1}) was added to 0.5 mL of 43 mM hydrogen peroxide solution. The absorbance of the mixture was measured at 230 nm against a blank solution containing phosphate buffer. The free radical scavenging activity was calculated as percentage inhibition as noted above.

Chelating ability on ferrous ions: The ferrous ion chelating potential of the extract was evaluated by method described by Sasikumar *et al.*[12] with few modifications. In summary, 1 mL of different extract sample at different concentrations (25, 50, 100, 200 and 400 μg mL^{-1}) was added to 0.05 mL of 2 mM $FeCl_3$. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine. The absorbance was measured at 562 nm against a blank reagent. The ferrous ion chelating potential was calculated as inhibition (%) using the formula noted above.

Statistical analysis: The recorded values were expressed as Mean±SEM of three replicate determinations and subjected to independent t-test analysis using the IBMSPSS statistics, version 20.0 software. The values of $p<0.05$ were considered statistically significant.

RESULTS

Phytochemical profile of methanolic extract of *Bauhinia monandra* leaves: The phytochemical screening presented in Table 1 showed the presence of different biochemical constituents from methanolic extract of *B. monandra* leaves obtained from various chemical tests. The results revealed the presence of flavonoids, terpenoids, alkaloids, tannins, saponins, reducing sugars, carbohydrates, proteins, cardiac glycosides and amino acids. Most of the classes of compounds identified in the *B. monandra* extract were phenolic in nature (flavonoids, terpenoids, alkaloids, tannins and saponins).

Table 1: Preliminary phytochemical screening of methanolic extract of *Bauhinia monandra* leaves

Constituents	Name of test	Color for positive test	Inference
Reducing sugar	Fehling's solution test	Brown precipitate	+
Proteins	Biuret	Violet	+++
Carbohydrates	Molisch test	Reddish-brown ring	+
Flavonoids	Alkaline reagent test	Yellow	++
Saponins	Froth test	Froth formation	++
Steroids	Acetic anhydride+H_2SO_4	Blue	+
Terpenoids	Salkowski	Reddish-brown	+
Alkaloids	Mayer's reagent test	Creamy precipitate	++
Tannins	Ferric chloride	Blue-black/green	+++
Cardiac glycosides	Keller-Kiliani's	Brown-red ring	+
Amino acids	Ninhydrin	Purple	+

+: Present, ++: Moderately present, +++: Highly present, -: Absent

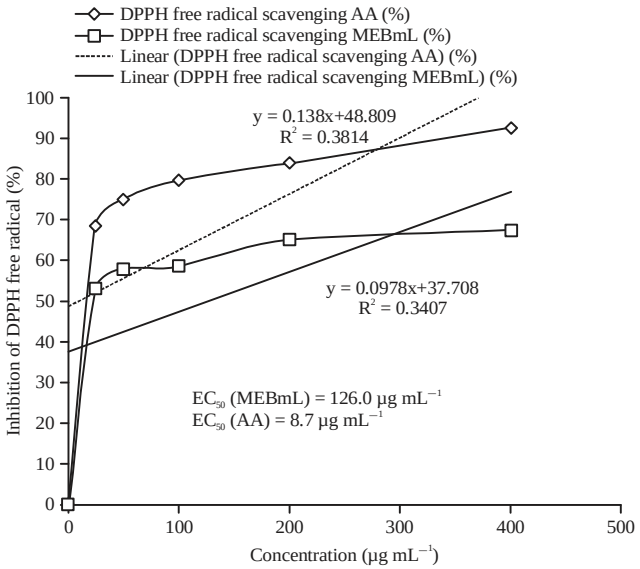

Fig. 1: Linear regression graph of inhibition (%) of DPPH free radical by L-ascorbic acid (AA) and *Bauhinia monandra* (MEBmL) against concentration (µg mL^{-1})

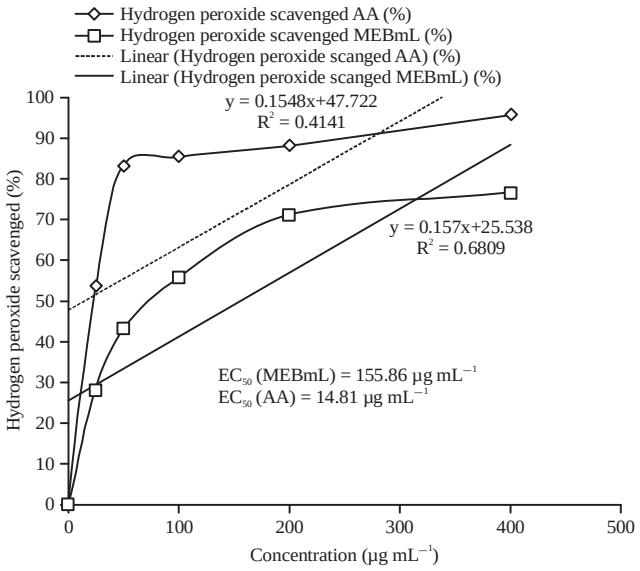

Fig. 2: Linear regression graph (%) hydrogen peroxide (H_2O_2) scavenged by L-ascorbic acid (AA) and *Bauhinia monandra* (MEBmL) against concentration (µg mL^{-1})

DPPH free radical scavenging assay of methanolic extract of *Bauhinia monandra* leaves: The results of the DPPH assay were presented in Fig. 1. A dose-dependent decrease in absorbance was noted which in turn reflected an increase in percentage scavenging of DPPH free radical for both methanolic extract of *B. monandra* leaves (MEBmL) and

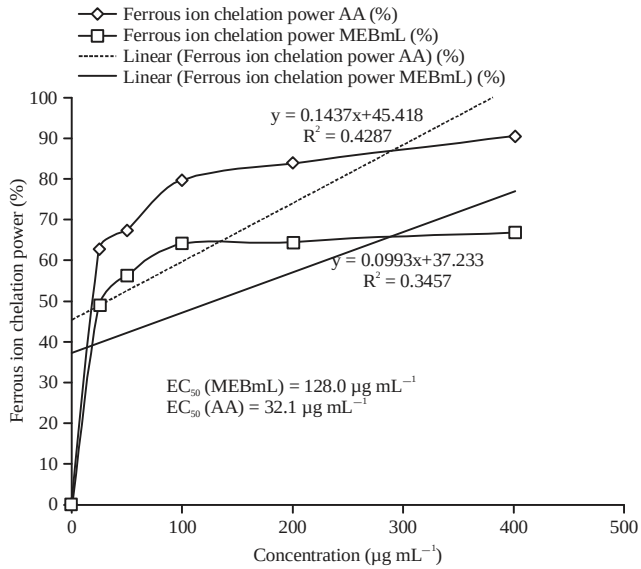

Fig. 3: Linear regression graph (%) ferrous ion chelation power by L-ascorbic acid (AA) and *Bauhinia monandra* (MEBmL) against concentration (µg mL^{-1})

L-ascorbic acid (positive control). The highest percentage inhibition (67.45 and 92.63%) of DPPH free radicals were observed at the highest concentration (400 µg mL^{-1}) for both MEBmL and L-ascorbic acid, respectively. The EC_{50} value for MEBmL was found to be 126 µg mL^{-1} calculated from the linear regression equation $y = 0.097x+37.70$, $R^2 = 0.340$, while EC_{50} value for L-ascorbic acid (positive control) was 8.70 µg mL^{-1}, calculated from the equation $y = 0.138x+48.80$, $R^2 = 0.381$ as shown in Fig. 1.

Hydrogen peroxide (H_2O_2) scavenging assay of methanolic extract of *Bauhinia monandra* leaves: The results of the hydrogen peroxide scavenging assay were presented in Fig. 2. The highest inhibition (%) of hydrogen peroxide scavenging of MEBmL was 76.46%, while that of L-ascorbic acid (positive control) at the same concentrations was 95.69%. The EC_{50} for MEBmL was found to be 155.86 µg mL^{-1} and calculated from the equation $y = 0.157x+25.53$, $R^2 = 0.680$, while that for L-ascorbic acid (AA) was found to be 14.81 µg mL^{-1} and calculated from the equation $y = 0.154x+47.72$, $R^2 = 0.414$ as shown in Fig. 2.

Metal chelating ability of methanolic extract of *Bauhinia monandra* leaves: In Fig. 3, the results of the metal chelating assay were presented. Dose-dependent increase in metal chelating ability of MEBmL was observed. The highest percentage inhibition (66.78 and 90.39%) of DPPH free radicals

were observed at the highest concentration (400 µg mL^{-1}) for both MEBmL and L-ascorbic acid, respectively. The EC_{50} for MEBmL was 128 µg mL^{-1}, calculated from the equation $y = 0.099x+37.23$, $R^2 = 0.345$ and the EC_{50} for L-ascorbic acid (AA) was 32.10 µg mL^{-1}, calculated from the equation $y = 0.143x+45.41$, $R^2 = 0.428$ (Fig. 3).

DISCUSSION

Antioxidants reduce cell damaging effect of free radicals; therefore, a number of scientific studies are ongoing on varied health benefits of natural antioxidants supplements towards addressing processes like stress, ageing and apoptosis, autoimmune and neurological diseases[13]. The preliminary phytochemical screening of crude methanolic extract of *Bauhinia monandra* leaves (MEBmL) showed the presence of various classes of phytoconstituents (Table 1). According to Vaghasiya *et al.*[14], these phytoconstituents such as tannins, terpenoids, carotenoids, flavonoids and reducing carbohydrates constitute the antioxidant properties of natural medicinal products. Therefore, the antioxidant properties exhibited by methanolic extract of *Bauhinia monandra* leaves could be attributed to the antioxidant-related phytochemicals found in the *Bauhinia monandra* leaf extract.

In the present study, Fig. 1-3 revealed that concentration-dependent inhibitory effect of DPPH, hydrogen peroxide and ferrous ion free radicals, respectively was observed at various concentrations of the extract and L-ascorbic acid (positive control) used. The mean effective concentration (EC_{50}) of the MEBmL on DPPH and hydrogen peroxide free radicals was estimated to be 126 and 155.86 µg mL^{-1}, while that of L-ascorbic acid was 8.7 and 14.81 µg mL^{-1} (Fig. 1, 2). In the ferrous ion chelating ability assay, the EC_{50} for MEBmL and L-ascorbic acid was 128 and 32.1 µg mL^{-1} (Fig. 3). The low EC_{50} values (Fig. 1, 2 and 3) implies that the MEBmL are potent inhibitors of free radicals. The potent antioxidant effect observed in the present study is in agreement with the report of Argolo *et al.*[15], which reported that the ethyl acetate extracts of *B. monandra* exhibited an IC_{50} value of 2 mg g^{-1} using DPPH scavenging assay. Similarly, Ajiboye *et al.*[16] reported that the IC_{50} value of ethyl acetate and n-hexane fractions of *B. monandra* evaluated using DPPH scavenging assay was 0.01 and 5.56 µg mL^{-1}, respectively. In another study, Chaires-Martinez *et al.*[17] reported that extracts from two *Bauhinia* species (*B. divaricata* and *B. bougainvillea*) showed potent DPPH free radical scavenging activity with IC_{50} of 0.03 and 33.33 µg mL^{-1}, respectively. Saraswathy *et al.*[18] reported that IC_{50} value of *Bauhinia variegata* in DPPH radical scavenging assay was 38.5 µg mL^{-1}. Kumar *et al.*[19] also demonstrated that IC_{50} value of *Bauhinia racemosa* in the DPPH radical scavenging assay was 152 µg mL^{-1}. These previous studies are in agreement with the present study and demonstrate a good potential of *Bauhinia monandra* and *Bauhinia* species as free radical inhibitors.

Recently, much attention is being focused on various phytochemicals (plant derived non-nutritive compounds) as substitutes for synthetic antioxidants. Although the present study did not relate any of the observed phyto-chemicals with the antioxidant activities recorded, numerous phyto constituents and their derivatives have been reported to possess antioxidant properties. For instance, flavonoids and related compounds isolated from a wide range of plants have been reported by numerous studies to have significant antioxidant activities[20-23]. Antioxidants have been employs in many pharmaceutical applications and play various roles in pathological conditions. They have wide application as additives in fats and oils and in food processing industries to rancidity and prevent food spoilage[24]. Consumption of natural antioxidants through fruits and vegetables are considered useful sources of antioxidants, which are useful in preventing many diseases. Therefore, the up-rising application of natural antioxidants in food industries and medical and therapeutics field indicates its potential as promising alternative for synthetic antioxidants owing to its low cost and minimal adverse effects.

CONCLUSION

Natural sources of antioxidants have become a great interest due to their possible usage to replace synthetic ones. Findings from the present study demonstrated the antioxidant potential of *Bauhinia monandra* leaves extract via its *in vitro* inhibitory actions on DPPH free radicals, hydrogen peroxide radicals and ferrous ion free radicals. Therefore, *Bauhinia monandra* leaves extract could potentially be used for its antioxidant activity as natural remedy for diseases associated with oxidation stress and further isolation and characterization of the bioactive compounds are encouraged.

SIGNIFICANCE STATEMENT

The research discovered the use of *Bauhinia monandra* as a significant natural antioxidant which can be beneficial as a possible remedy for diseases. Therefore, physio-pharmacological assays of this plant could reveal new therapeutic approach with emphasis on diseases where oxidative stress is identified as the major causative factor in their development and progression.

REFERENCES

1. Saxena, M., J. Saxena, R. Nema, D. Singh and A. Gupta, 2013. Phytochemistry of medicinal plants. J. Pharmacogn. Phytochem., 1: 168-182.
2. Kennedy, D.O. and E.L. Wightman, 2011. Herbal extracts and phytochemicals: Plant secondary metabolites and the enhancement of human brain function. Adv. Nutr.: Int. Rev. J., 2: 32-50.
3. Keay, R.W.J., 1989. Trees of Nigeria. Clarendo Press, Oxford, UK., ISBN: 9780198545606, pp: 74-78.
4. McCune, L.M. and T. Johns, 2002. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. J. Ethnopharmacol., 82: 197-205.
5. Anosike, C.A., O. Obidoa and L.U.S. Ezeanyika, 2012. The anti-inflammatory activity of garden egg (*Solanum aethiopicum*) on egg albumin-induced oedema and granuloma tissue formation in rats. Asian Pac. J. Trop. Med., 5: 62-66.
6. Da Silva, K.L., M.W. Biavatti, S.N. Leite, R.A. Yunes, F.D. Monache and V.C. Filho, 2000. Phytochemical and pharmacognostic investigation of *Bauhinia forficata* Link (Leguminosae). Zeitschrift Naturforschung C-J. Biosci., 55: 478-480.
7. Holdsworth, D.K., 1990. Traditional medicinal plants of Rarotonga, cook Islands part I. Int. J. Crude Drug Res., 28: 209-218.
8. Mendes, B.G., M.J. Machado and M. Falkenberg, 2006. [Screening of glycolipids in medicinal plants]. Rev. Bras. Farmacogn., 16: 568-575.
9. Ramamurthy, V. and M. Sathiyadevi, 2017. Preliminary phytochemical screening of methanol extract of *Indigofera trita* Linn. J. Mol. Histol. Med. Physiol., Vol. 2.
10. Shimada, K., K. Fujikawa, K. Yahara and T. Nakamura, 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40: 945-948.
11. Ruch, R.J., S.J. Cheng and J.E. Klaunig, 1989. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10: 1003-1008.
12. Sasikumar, V. and P. Kalaisezhiyen, 2014. Evaluation of free radical scavenging activity of various leaf extracts from *Kedrostis foetidissima* (Jacq.) Cogn. Biochem. Anal. Biochem., Vol. 3. 10.4172/2161-1009.1000150.
13. Ramassamy, C., 2006. Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: A review of their intracellular targets. Eur. J. Pharmacol., 545: 51-64.
14. Vaghasiya, Y., R. Dave and S. Chanda, 2011. Phytochemical analysis of some medicinal plants from Western region of India. Res. J. Med. Plant, 5: 567-576.
15. Argolo, A.C.C., A.E.G. Sant'Ana, M. Pletsch and L.C.B.B. Coelho, 2004. Antioxidant activity of leaf extracts from *Bauhinia monandra*. Bioresour. Technol., 95: 229-233.
16. Ajiboye, A.T., M.D. Musa, K.O. Otun, A.A. Jimoh, A.T. Bale, S.O. Lawal and M.T. Arowona, 2015. The studies of antioxidant and antimicrobial potentials of the leaf extract of *Bauhinia monandra* plant. Nat. Prod. Chem. Res., Vol. 3. 10.4172/2329-6836.1000180.
17. Chaires-Martinez, L., E. Monroy-Reyes, A. Bautista-Bringas, H.A. Jimenez-Avalos and G. Sepulveda-Jimenez, 2009. Determination of radical scavenging activity of hydroalcoholic and aqueous extracts from *Bauhinia divaricata* and *Bougainvillea spectabilis* using the DPPH assay. Pharmacognosy Res., 1: 238-244.
18. Saraswathy, A., G. Gunalan and V. Krishnamurthy, 2011. *In vitro* antioxidant activity of *Bauhinia variegata* Linn. leaves. J. Pharm. Res., 4: 3364-3367.
19. Kumar, R.S., T. Sivakumar, R.S. Sunderam, M. Gupta and U.K. Mazumdar *et al*., 2005. Antioxidant and antimicrobial activities of *Bauhinia racemosa* L. stem bark. Braz. J. Med. Biol. Res., 38: 1015-1024.
20. Kefayati, Z., S.M. Motamed, A. Shojaii, M. Noori and R. Ghods, 2017. Antioxidant activity and phenolic and flavonoid contents of the extract and subfractions of *Euphorbia splendida* Mobayen. Pharmacog. Res., 9: 362-365.
21. Jiang, H., W.Q. Zhan, X. Liu and S.X. Jiang, 2008. Antioxidant activities of extracts and flavonoid compounds from *Oxytropis falcate* Bunge. Nat. Prod. Res., 22: 1650-1656.
22. Badmus, J.A., O.E. Ekpo, F. Rautenbach, J.L. Marnewick, A.A. Hussein and D.C. Hiss, 2016. Isolation and antioxidant activity of flavonoids from *Holarrhena floribunda* (G. don) leaves. Acta Biochim. Pol., 63: 353-358.
23. Bernardi, A.P.M., C. Lopez-Alarcon, A. Aspee, S. Rech, G.L. von Poser, R. Bride and E. Lissp, 2007. Antioxidant activity of flavonoids isolated from *Hypericum ternum*. J. Chil. Chem. Soc., 52: 1326-1329.
24. Hinneburg, I., H.J.D. Dorman and R. Hiltunen, 2006. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem., 97: 122-129.