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IN VITRO ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *CALLICARPA ARBOREA* LEAVES

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ABSTRACT

Callicarapa arborea, commonly known as Beautyberry Tree belongs to Verbenaceae family. It is widely used in traditional medicine for treatment of jaundice, fever, headache, stomachache, skin and scorpion bites diseases. The fresh leaves of the plant were washed, air dried in shade, ground to powder and subjected to cold maceration using methanol as per the standard procedure, condensed by subjecting to a rotary evaporator and lyophilized. The methanolic extract prepared was analyzed for antioxidant activity by three different *in vitro* methods; namely (1) DPPH free radical scavenging activity, (2) Ferric reducing antioxidant potential (FRAP) assay and (3) Total phenolic contents by spectrophotometeric method. In the radical scavenging measurement method of DPPH and ferric iron reducing ability assay, the antioxidant activities were 9.78±0.12 mg and 12.81±0.64 mg Trolox equivalent respectively per gram of dried leaves.

KEYWORDS: *Callicarpa arborea*, antioxidant activity, DPPH, FRAP, Total phenolic.

INTRODUCTION

Callicarapa arborea, commonly known as Beautyberry Tree is a tree about 8 m tall. Branch lets, inflorescences, and flowers talks are densely velvet hairy. Leaves are elliptic, oblong elliptic, or ovate, 1337 x 713 cm, leathery, densely yellow brown velvety on the underside, dark green and shiny above, base wedge-shaped to rounded, margin entire. Purple flowers are

borne in cymes 611 cm across. Cymes are carried on 4 angled stalks. The plant is spread in India, Burma, South China, Indo-China and Malaya.[1] It is used in traditional medicine for treatment of jaundice, fever, headache, stomachache, skin and scorpion bites diseases.[2]

Reactive oxygen species (ROS) are produced inside the body as a byproduct of the reactions and also because of exogenous factors. Inside the body, some of the ROS play a positive role in energy production, phagocytosis, cell growth regulation and inter-signal or the synthesis of biologically important compounds.[3] However, when reactive oxygen species exceed the antioxidant capacity of the biological systems, oxidative stress occurs which has been associated with atherosclerotic diseases, cancer, diabetes, arthritis, reperfusion damage and inflammation etc.[4] Many natural antioxidant compounds have the ability to counter the effect of reactive oxygen species thus providing protection against degenerative diseases and infection. Recent studies have investigated the potential of plant products as antioxidants against various diseases induced by free radicals. There is an increasing interest in natural antioxidants e.g. polyphenols present in medicinal and dietary plants which might help preventing oxidative damages.[5] Realizing the fact, this research was carried out to evaluate the *in vitro* antioxidant activities of methanolic extract of *Callicarpa arborea* leaves.

MATERIALS AND METHODS

Plant Material

The plant *Callicarpa arborea* was collected from the campus of the College of Veterinary Sciences & Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram and submitted the herbarium specimens for authentication / identification to the Regional Office, Botanical Survey of India (BSI), Shillong. The BSI, Shillong has authenticated the plants and communicated the identification / authentication report vides letter reference No.BSI/ERC/Tech/2010/052, dated 27.04.2010.

The fresh leaves of the plant were collected, washed and air dried in shade. On complete drying, the dried plant material was ground to powder with Willey / Laboratory Mill and sifted through sieve number 22. The powdered leaves were then subjected to cold maceration using methanol as solvent following the procedure of Manjunatha *et al.*[6] and Harborne[7] with slight modifications. Briefly, five hundred (500g) grams of powder was soaked in 2.5 L of methanol (1:5 w/v) in a conical flask for a period of 3 days with intermittent stirring and at the end of 3rd day the content was filtered with muslin cloth followed by Whatmann filter paper No. 1. For complete extraction of the active principles, this process was repeated three

times using fresh solvent on each occasion or until the colour of the methanol becomes light. The filtrate obtained was pooled and further subjected to rotary vacuum evaporator. The material was stored at -40ºC in deep freezer in air tight containers till further use.

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8–tetramethyl chromane-2-carboxylic acid (Trolox), Gallic acid were purchased from Sigma Chemicals Co. (St. Louis, USA); Methanol, Ethanol, Sodium acetate trihydrate, ferric chloride hexahydrate (FeCl3. 6H2O), Folin-Ciocalteu Phenolic reagent, Sodium carbonate were obtained from Merck (Darmstall, Germany). Trichloroacetic acid (TCA) was obtained from Sisco Research Laboratories (SRL), Mumbai. All the chemicals used were of analytical grade.

DPPH free radical scavenging assay

The free radical scavenging activity was measured by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method proposed by Leong and Shui.[8] DPPH solution of 0.1 mM was prepared in methanol and the initial absorbance was measured at 517 nm in a UV-Visible Spectrophotometer (Thermo- Evolution 201). An aliquot (20 µl) of extract was added to 3 ml of DPPH solution and the decrease in absorbance was measured at different time intervals at 517 nm until the absorbance remained constant. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and *vice versa*. A standard curve was prepared using trolox (250 -1250µg/ml) and the free radical scavenging ability of the extracts were calculated from the decreased in the absorbance. The free radical scavenging ability of the extracts were expressed as mg Trolox equivalent (TE) per gram of dry leaves.

Ferric Reducing Antioxidant Potential (FRAP) assay

The ferric reducing antioxidant potential (FRAP) assay was carried out according to the procedure described by Benzie and Strain.[9] Briefly, 30µl of extract was added to 3 ml of FRAP reagents (10 parts of 300 mM sodium acetate buffer of pH 3.6, 1 part of TPTZ and 1 part of 20 mM Ferric chloride solution). The reaction mixture was incubated at 37ºC for 30 min and the increase in absorbance was measured at 593 nm using a UV-Visible Spectrophotometer (Thermo-Evolution 201). The standard curve was prepared using trolox (250 -1000µg/ml) and the value of FRAP was calculated from the standard curve. The results were expressed as mg Trolox equivalent (TE) per gram of dry leaves.

Total phenolic content (TPC)

The total phenolic content of the extracts were estimated by the Folin-Ciocalteau method described by Singleton and Rossi.[10] 30 microlitres of extract was added to 1ml of 1:10 Folin-Ciocalteau's reagent and incubated at room temperature for 5 min followed by addition of 970 µl of sodium carbonate (7.5%) solution. After 1 hr incubation at room temperature, the absorbance was measured at 640 nm using a UV/Visible Spectrophotometer (Thermo-Evolution 201). Different volume (20-100µl) of Gallic acid (100µg/ml) was used for calibration of a standard curve. The results were expressed as mg Gallic acid equivalent (GAE) /gm of dry leaves.

RESULT AND DISCUSSION

The antioxidant content of the leaves of *Callicarpa arborea* were evaluated by three *in vitro* assay methods *viz.* DPPH free radical scavenging, Ferric reducing antioxidant potential assay and total Phenolic content estimation. The DPPH scavenging activity and Ferric reducing antioxidant potential were expressed as mg trolox equivalent (TE). The antioxidant content observed is given in Table1. In the present investigation, considerable antioxidant activity was observed for DPPH free radical scavenging method and FRAP assay method however detectable amount was not detected in total Phenolic content assay.

Table 1: Antioxidant content of *Callicarpa arborea*

Sl. No.	Methods of estimation	Antioxidant content/ g of dry leaves
01	DPPH free radical scavenging method	9.78±0.12 mg TE
02	FRAP assay	12.81±0.64 mg TE
03	Total phenolic content	Detectable amount not present

The DPPH free radical scavenging is one of the generally accepted mechanisms against lipid oxidation. Difference between DPPH free radical binding method and other method is the short run time allowing rapid determination of the radical scavenging. The effect of antioxidants on DPPH free radical scavenging was thought to be due to their hydrogen donating ability. The DPPH free radical scavenging activity of *Callicarpa arborea* leaves was 9.78±0.12 mg TE/gm dry leaves. The presence of significant DPPH free radical scavenging activity in *Callicarpa arborea* was also reported earlier by Amin and his colleagues [11] and Kumar *et al.*[12] The ferric reducing antioxidant potential assay is based on the reducing power of a compound (antioxidant). It measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron). As the ferric to ferrous ion reduction occurs rapidly with all

reductants with half reaction reduction potentials above that of Fe^{3+}/ Fe^{2+}, the values in the FRAP assay expresses the corresponding concentration of electron donating antioxidants. The FRAP activity in the present investigation was 12.81±0.64 mg TE/gm of dry leaves.

Phenolic compounds are compounds of large groups. They are simply the compounds formed by one or more hydroxyl groups attached to the aromatic rings especially benzene. The Phenolic content in the methanolic extract in the present investigation was not detected possibly due to little amount of these compounds. Similar to our finding, Amin and his colleagues[11] also reported that the DCM and hexane extracts of stem bark was not detected.

CONCLUSION

The methanolic extract of *Callicarpa arborea* leaves have significant antioxidant activity. Thus *Callicarpa arborea* leaves can play a pivotal role in preventing the degenerative diseases and other infections. Further, it can be used as a source of the natural antioxidants.

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