EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 1
Issue: 2
Page No: 190-198
Year: 2014

EVALUATION OF THE ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACTS OF THREE ETHNOMEDICINAL PLANTS

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Article Received on 05/08/2014 **Article Revised on 28/08/2014** **Article Accepted on 20/09/2014**

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ABSTRACT

The ethanolic extract of the leaves of *Cipadessa- baccifera, Didymocarpus humboldtiana synonym- (Henckelia humboldtiana) and Alangium salviifolium* were analysed for antioxidant activity**.** The ethanolic extract of the plants were tested for total antioxidant capacity and free radicalscavenging activity by DPPH (1, 1-diphenyl-2-picrylhydrazil). The ethanolic extract of leaf of *Alangium salviifolium* exhibited high free radical scavenging activity at concentration 0.5µg, followed by *Didymocarpus humboldtiana (Henckelia humboldtiana*) and *Cipadessa- baccifera* . The total antioxidant activity was also high in *Alangium salviifolium*, followed by *Didymocarpus humboldtiana (Henckelia humboldtiana)* and *Cipadessa- baccifera* .

KEYWORD: Antioxidant activity, DPPH assay, Total antioxidant activity, Medicinal plant.

INTRODUCTION

Plants, as the source of medicine, have been playing an important role in the health services around the globe [1]. About three quarters of the world's population rely on plant and their extracts for healthcare [2]. A good number of populations particularly those living in rural areas depend largely on herbal remedies for the treatment of different types of diseases. It indicates the importance of the individual plants in the healthcare system. The global interest in the medicinal potential of plants during the last few decades is therefore quite logical. In

the recent years interest in the study of antioxidant activity of plant extracts and isolation of the phyto compounds from plants has grown due to the fact that the free radicals have been related to degenerative diseases [3]. Therefore the free radical can be defined as any molecular species capable of independent existence. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants.

The most important oxygen-containing free radicals in many diseases are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical. These are highly reactive species, capable of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids. Free radicals attack important macromolecules leading to cell damage and homeostatic disruption. Targets of free radicals include all kinds of molecules in the body. Free radical formation occurs continuously in the cells as a consequence of enzymatic and non- enzymatic reactions [4]. Oxidative stress has been postulated in many conditions, including atherosclerosis, inflammatory condition, certain cancers, and the process of aging. This present study is one such attempt to evaluate the potential antioxidant properties present in the selected medicinal plant species which will eventually pave way to the therapeutic drug application against human diseases.

MATERIALS AND METHODS

Collection and Identification of plant material

Cipadessa- baccifera (Roth) Miq (Meliaceae) and *Didymocarpus humboldtiana – synonym Henckelia humboldtiana* Gardner (Gesneriaceae)were collected from the Palni hills of the Western Ghats, Tamilnadu on 11/05/2014 and while *Alangium salviifolium*(L.f.) Wangerin ssp. Salviif (Alangiaceae)was collected from Kollihills on 06/07/2014. The taxonomic identification of the semedicinal plants was carried out by John Britto, Director, and Head, The Rapinat Herbarium and Centre for Molecular systematic, St. Joseph's College Tiruchirappalli, India. Voucher specimens of each plant were deposited at Rapinat Herbarium (RHT 65280; RHT 65282 and RHT 65467).

Study plants and their Medicinal uses

Cipadessa- baccifera

Shrubs to 3 m tall; branchletstomentose. Leaves odd-pinnate; leaflets 3-5 pairs, opposite, 3-7 x 2-3.5 cm, narrowly oblong to ovate, base acute or rounded, margins irregularly dentate

towards apex, apex acuminate; petiolule to 8 mm long. Panicles axillary, corymbose; peduncle to 7 cm long. Calyx-lobes c. 1 mm long, triangular, spreading, pubescent. Petals 3.5-4 mm long, ovate, cream or c. 3 mm long; stamens 10; anthers yellow. Ovary 5-6-angular, ovules 2 per cell; stigma turbinate. Drupe c. 5 mm across, globose, longitudinally grooved, reddish brown. The paste of root, leaf and bark is applied topically to cure psoriasis.

Didymocarpus humboldtiana

Scapigerous herbs. Leaves to 6-7 x 6 cm, broadly ovate, obtuse, rounded or cordate at base, broadly crenate, bullate-tomentose above, tomentose beneath; nerves 5-6 pairs; petiole 5-8 cm long, wings divided. Scape few, 15-20 cm long, branched. Raceme glandular hairy; bracts and bracteoles similar, linear. Flowers blue, paired; pedicel 1.5 cm long; sepals 3.5 x 1 mm, oblong, obtuse; corolla blue, 13 mm long, thinly hairy, mouth oblique, lobes orbicular; staminodes hooked; ovary densely glandular-hairy, stigma oblique, papillose. Capsule 4 x 0.2 cm, acuminate, glandular hairy; seeds ellipsoid, rugate, brown. Stem bark of *Pongamiapinnata* and leaf of *Abutilon indicum* are ground into a paste and applied topically on affected places to heal wounds.

Alangium salviifolium

Sage-leaved *Alangium* is used in the treatment of rabies. The leaves, roots, seeds and the bark of the plant are used in the preparation of decoction and making pills. It grows to a height of about 3 to 10 meters. The bark is ash colour, rough and faintly fissured. The leaves are elliptic oblong, elliptic lanceolate or oblong lanceolate. The flowers are greenish white, fascilcled, axillary or on old wood. The berries are ovoid, ellipsoid or nearly globose. glabrous, smooth and violet to purple. The flowering season is February to June.

Plant extraction

The leaves were shade dried at room temperature and ground into powder. First 10gm of plant powder was dissolved in 70ml of ethanol solvent and then it was kept in a rotary shaker for 3 days. The suspension was filtered by using filter paper of pore size 0.2µm. The antioxidant study was carried out using the crude extract.

Evaluation of Total Antioxidant Activity

The total antioxidant capacity of the ethanol extract was evaluated by the phosphomolybdenum method according to the procedure described by Prieto*et al.,*(1999) [5]. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent

formation of green phosphate. A 10,20,30,40,50µl extract was combined with 3 ml of reagent solution and the volume was made up to 4ml with ethanol (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer (Lambda35 Perkin Elemer)) against blank after cooling to room temperature. Ethanol (2ml) in the place of extract was used as the blank. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared using ascorbic acid as the standard.

Determination of DPPH free radical scavenging activity

The procedure for estimating the antioxidant activity involved UV-Spectrophotometric determination. Three samples namely Standard, test and control were prepared. Antioxidant Activity by DPPH Scavenging 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) is free radical but stable [6, 7]. DPPH solution is initially violet in color which fades when antioxidants donate hydrogen [8]. The change in colour is monitored by spectrophotometer and DPPH free radical scavenging activity is calculated [9]. The method described by Molyneuxwas used [10]. Stock solution of 100 µM DPPH in ethanol was made. Test sample of the extract were made at 10, 20, 30, 40, 50µg for the first sample *Cipadessa- baccifera*) whereas 0.1, 0.2, 0.3, 0.4, 0.5µg were made for the rest two samples that is *Didymocarpus humboldtiana* and *Alangium salviifolium* in ethanol. Similarly, reference samples of ascorbic acid were made at similar concentration. Two milliliters of 100 µM DPPH was mixed with 2.0 ml of ethanol and kept in dark for 30 minutes. Similarly, 2.0 ml of 100µM DPPH was mixed with different concentration of test sample, volume was made upto 4ml with ethanol and kept in dark for incubation. 2.0 ml of standard ascorbic acid was also kept in dark. The absorbance was measured at 517 nm by spectrophotometer model lambda 35 Parkin Elemer (Range 190nm-1100nm), after 30 minutes the % scavenging was calculated by the equation:

$$\% \text{ of DPPH Scavenging} = \frac{(\text{Abs Control} - \text{Abs Test})}{\text{Abs Control}} \times 100$$

Where, Abs = Absorbance of Control and AT = Absorbance of test sample. The % scavenging was then plotted against concentration and regression equation was obtained to calculate IC50 (micromolar concentration required to inhibit DPPH radical formation by 50%) values. The experiments were conducted in triplicate.

RESULTS AND DISCUSSION

The DPPH assay is a very simple method for screening small anti-oxidant molecules, because the reaction can be observed visually and intensity can be analysed using common spectrophotometric assay. The stable radical DPPH has been used widely for the determination of primary anti-oxidant activity. The DPPH anti-oxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of anti-oxidants. The Fig 8 showed % DPPH scavenging activity of ethanolic extract of leaf of *Alangium salviifolium* the highest % inhibition of 40.57% at concentration of 0.5µg by DPPH assay followed by Fig 7 *Didymocarpus humboldtiana* (*Henckelia humboldtiana*) 27.96% at concentration 0.5µg. And the Fig6 *Cipadessa- baccifera* showed the lowest DPPH scavenging activity. Similarly Fig 3 showed the highest total antioxidant capacity followed by Fig 2 and Fig1 showed the least total antioxidant capacity.

Correlation analysis between the values obtained from the DPPH and TAA is presented in Figures, which indicate that the ethanolic extract of all three plant samples possess a varying radical scavenging and total antioxidant capacity.

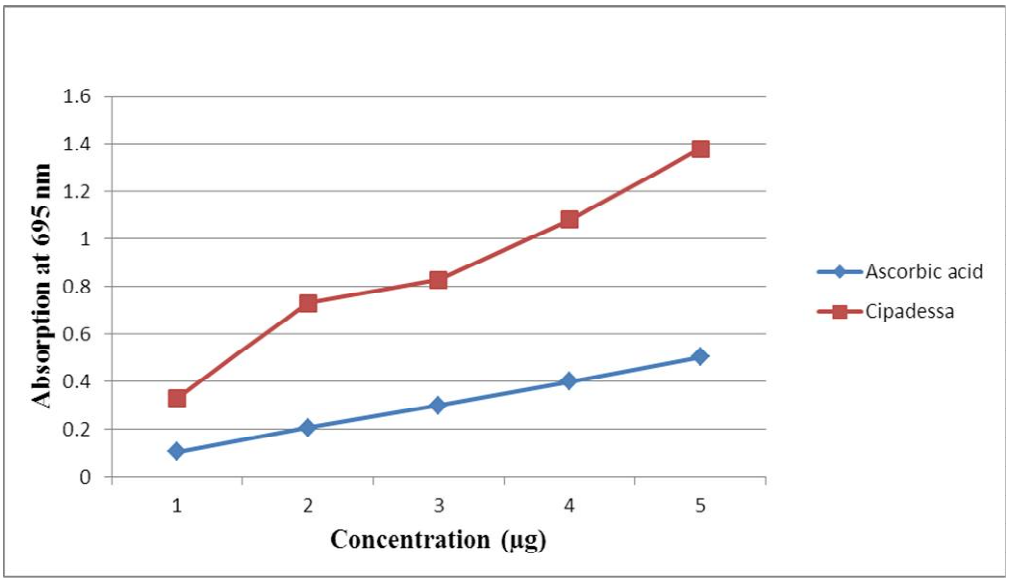

Figure 1: Total Antioxidant Activity of *Cipadessa- baccifera*

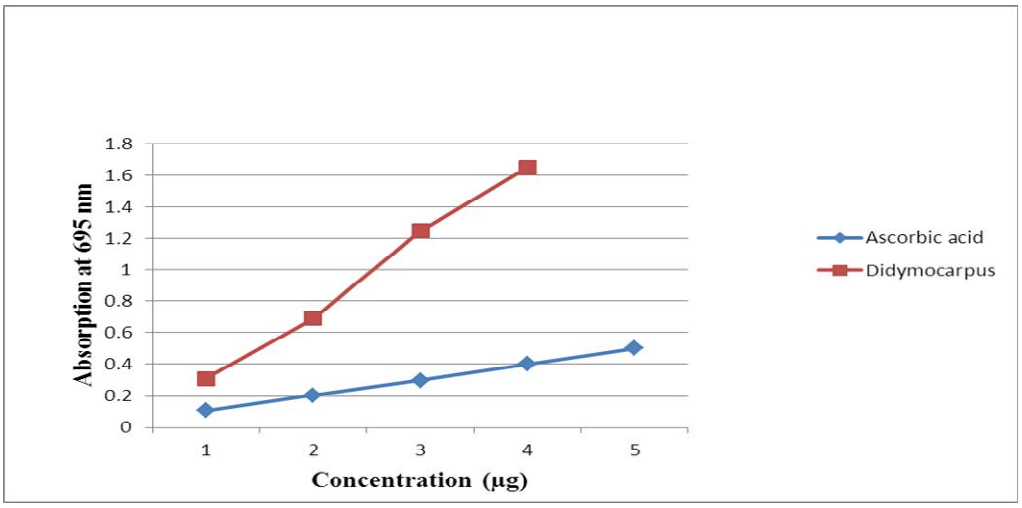

Figure 2: Total Antioxidant activity of *Didymocarpus humboldtiana*

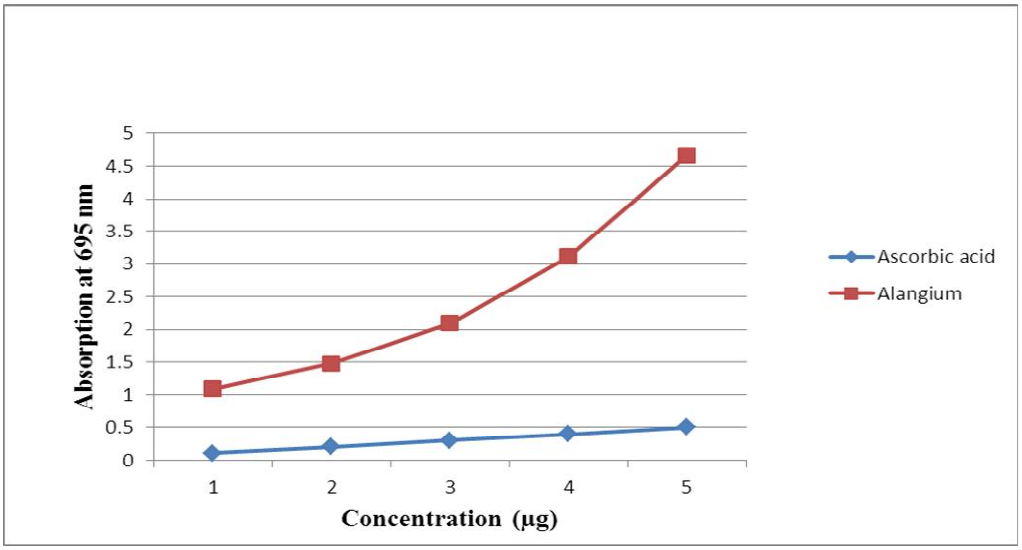

Figure 3: Total Antioxidant activity of *Alangium salviifolium*

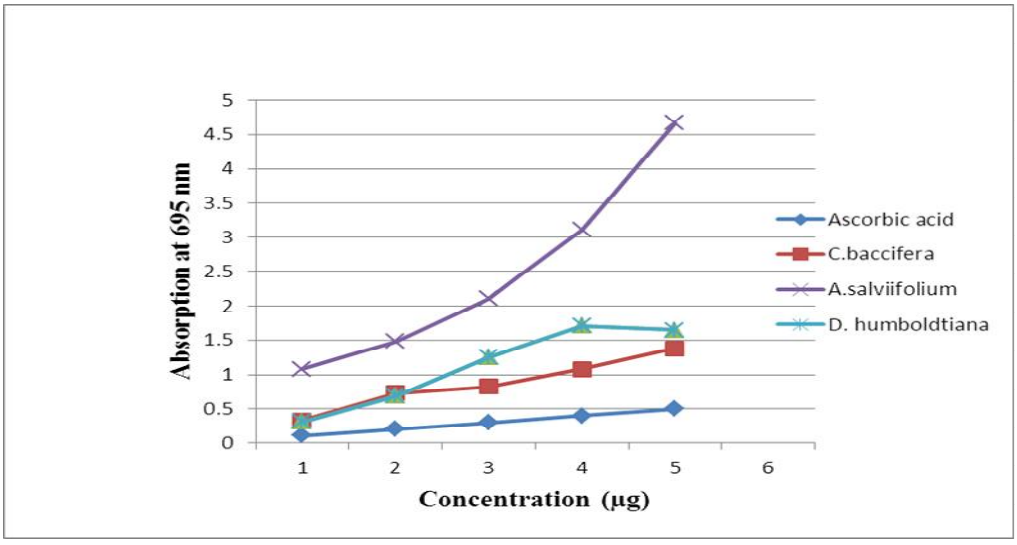

Figure 4: Comparative Total Antioxidant Activity of *C. baccifera, D. humboldtiana and A. salviifolium*

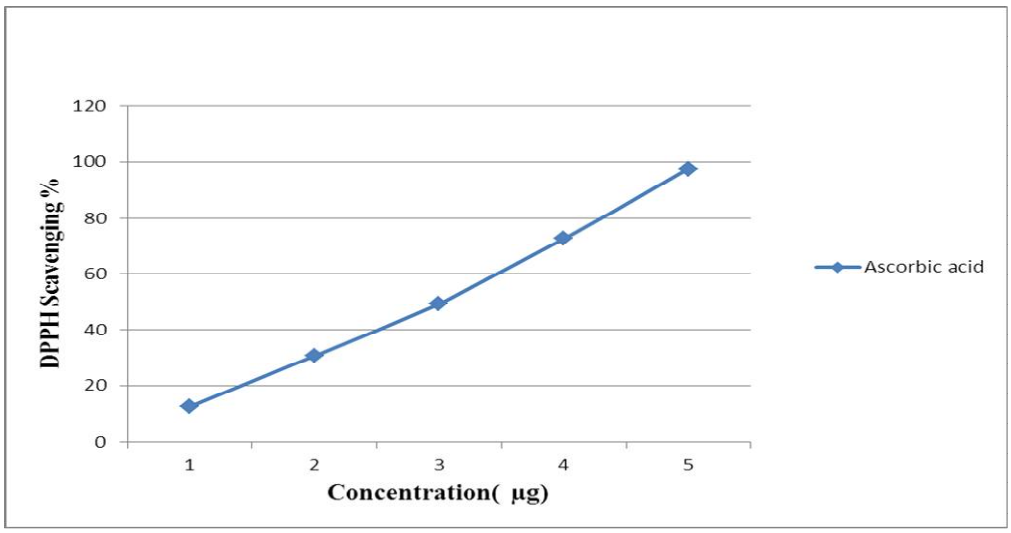

Figure5: DPPH Scavenging % of Standard Ascorbic acid

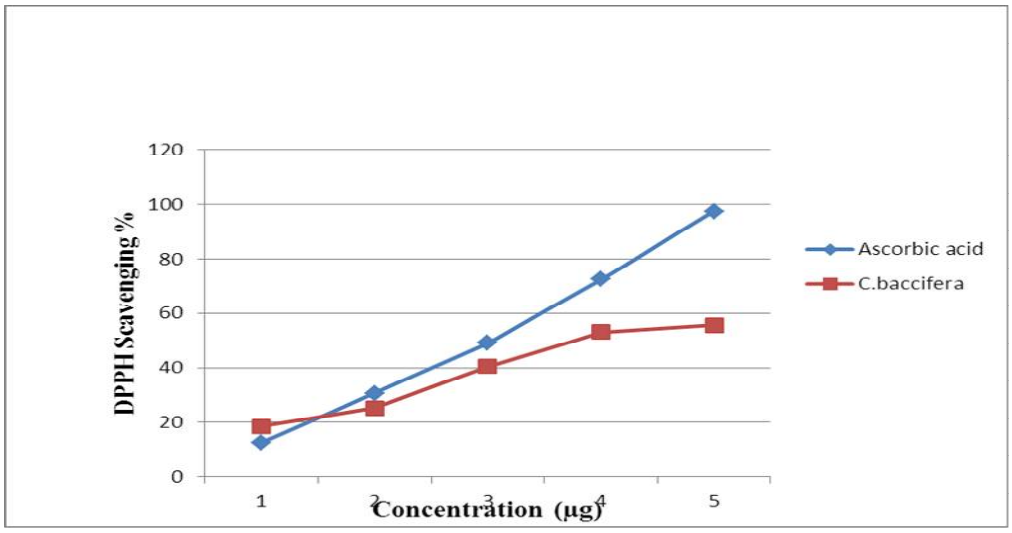

Figure 6: DPPH Scavenging % of *C.baccifera*

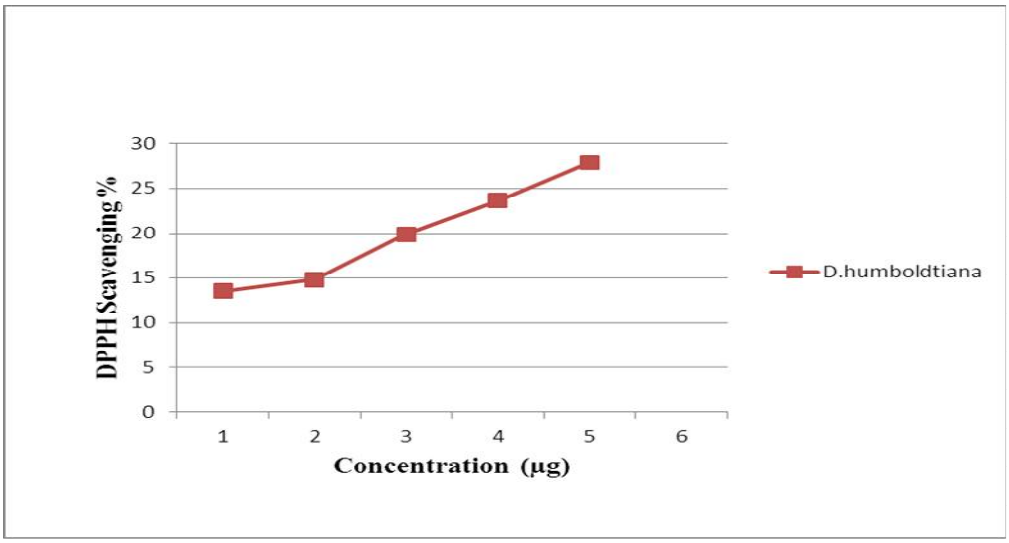

Figure 7: DPPH Scavenging % of *D.humboldtiana*

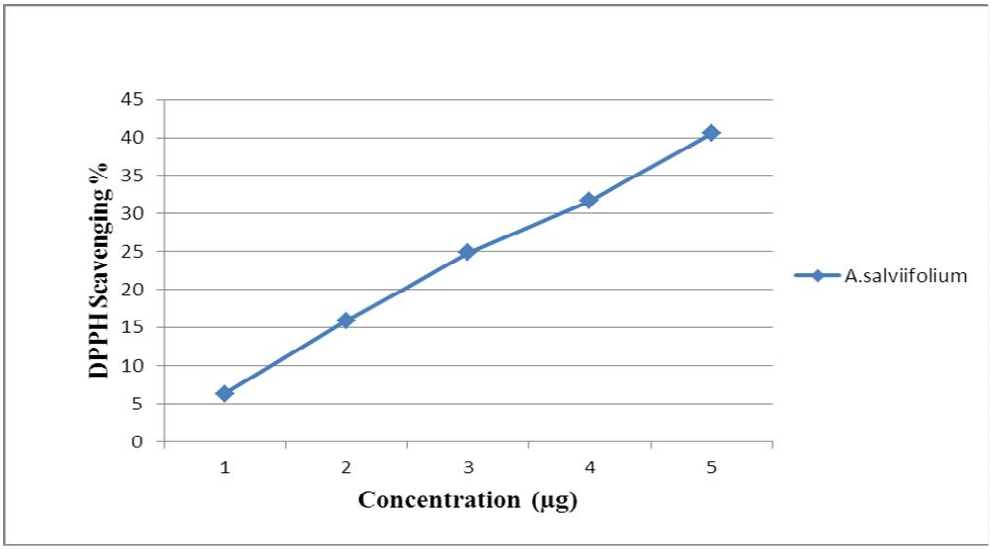

Figure: 8 DPPH Scavenging % of *A.salviifolium*

CONCLUSION

The results found from the above experiments confirm that the medicinal plants *Alangium salviifolium, Didymocarpus humboldtiana* (*Henckelia humboldtiana*) and *Cipadessa-baccifera* are potential enough to prevent oxidative stress and other diseases. This study also promotes further research in the field of Pharmacognosy and Pharmacology to extract the particular active principle responsible for various known diseases.

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