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Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF METHANOLIC ROOT EXTRACT OF *DECALEPIS HAMILTONII* WIGHT & ARN

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ABSTRACT

The present study was to carry out to evaluate the antioxidant potential of methanolic root extract of *Decalepis hamiltonii*. The methanolic root extract was subjected to total phenolic and flavonoid estimation. The level of the antioxidant potentials of methanolic root extract were determined by DPPH, ABTS, Superoxide radical, Hydroxyl radical activity. The results showed that methanolic root extract of *Decalepis hamiltonii* had higher level of phenol (13.05±1.01mgGAE/g)and flavonoid content (6.4±0.70mg QE/g).The methanolic root extract showed significant antioxidant activity. The percentage of inhibition for DPPH (93%), ABTS (90%), superoxide radical (80%) and hydroxyl radical (62%) which is comparable with respective standards. The results suggested that the antioxidant activity was due to higher levels of phenolic and flavonoid contents in the methanolic root extract. Further studies along with isolation and molecular mechanism of methanolic root extract of *Decalepis hamiltonii* may lead to significant outcome.

Key words: Antioxidant; ABTS; Ascorbic acid; DPPH; O_2^-

INTRODUCTION

According to World Health Organization (WHO) up to 80% of the populations in some developing countries use traditional medicine. Traditional herbal medicine is still an important component of healthcare in India. India is the second largest country in the world with over one billion, with diverse socio-cultural backgrounds. It accounts for 16% of the world's population and holds 21% of the world's global burden of diseases. The impact of traditional systems of medicine in the public health care system of India is substantially high and medicine is intimately interwoven with religiosity and ethnicity[1]. World health organization has estimated that 80% of the earth's inhabitants rely on traditional medicine for primary healthcare needs and most of the therapy involves the use of plant extracts and their active compounds [2]. Medicinal value of these plants depends on bio active phytochemical constituents that produce definite physiological action in the human body. Some of the most important plant bioactive phytochemical constituents include alkaloids, flavonoids and phenols. The specific plants to be used and the methods of application for particular ailments were passed down through oral traditions. In this growing interest many medicinal plants have been screened extensively for their antimicrobial potential. Antimicrobials of plant origin have enormous therapeutic potential and they are effective in the treatment of infectious diseases, simultaneously mitigating many of side effects that are often associated with synthetic antimicrobials[3].The antioxidant phytochemicals from plants, particularly flavonoids and other polyphenols, have been reported to inhibit the propagation of free radical reactions, to protect the human body from disease and to retard lipid oxidative rancidity [4].The phenolics and flavonoids are also widely distributed in the plants which have been reported to exert multiple biological benefits, including antioxidants and antimicrobial activities [5].*Decalepis hamiltonii* Wight & Arn (Asclepideace) an endemic, endangered, climbing shrub and native of southern peninsula has been used in Ayurveda, the ancient Indian traditional system of medicine to stimulate appetite, relieve flatulence and as a general tonic. It is also useful as a blood purifier, preservative and as a source of bio insecticide for stored food grains[6].Through many pharmacological works have been carried out in *Decalepis hamiltonii*, systematic studies relating to free radical scavenging and antibacterial activity have not been clearly defined. Hence, the present study was to investigate the total phenol and flavonoid content, antioxidant activity of methanolic root extract of *Decalepis hamiltonii.*

MATERIALS AND METHOD

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Gallic acid (GA), Ascorbic acid, BHT, Quercetin, ABTS, and Folin–Ciocalteu's reagent and Mueller Hinton media were purchased from Himedia (Mumbai, India). All other chemical reagents used were of analytical grade.

Collection of material

The Root of *Decalepis hamiltonii* has been collected from kolli hills, Namakkal district of Tamilnadu, India. The taxonomic identification of plant was identified reference to Flora of Presidency of Madras, by [7]. Root was shade dried and it was grounded with the mechanical blender into fine coarse powder and packed in a zip lock cover and labeled.

Preparation solvent extraction

50gm of *Decalepis hamiltonii* root was packed in Soxhlet apparatus for extraction and 500 ml of methanol was used as solvent. Soxhlet was kept running for 72 hours, until the solvent color appears in the collection tube. Methanol was removed by evaporation using rotary vapor at not more than 40°C. The residue was then placed in an oven at 40°C for about 48hours to remove the moisture. The resulting dried mass was then powdered and used for further studies.

Estimation of total phenolic content

Total phenolic content was carried out following the Folin-Ciocalteu method by [8]. One ml of crude extracts solution containing (1mg /ml) was added volumetric flask. 1 ml of Folin-Ciocalteu reagent and allowed to stand at 22 ºC for 5 min; 7.5% of 0.75 ml of sodium bicarbonate solution was added and mixed thoroughly. The samples were measured spectrophotometrically (Hitachi U-20) at 765 nm using spectrometer after 90 min at 22 ºC. The amount of total phenolic was determined as Gallic acid and equivalent and expressed as mg GAE/g dry weight.

Estimation of total flavonoid content

The flavonoids content was determined by aluminum trichloride method using catechin as a reference compound [9]. This method based on the formation of a complex flavonoid-aluminum having the absorptive spectrophotometrically (Hitachi U-20) maximum at 415 nm, after remained react at room temperature for 30 min. Briefly, 0.5 mL of each extracts (1:10 g/mL) in methanol was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The amount of total flavonoids was determined as mg QE/g dry weight.

DPPH scavenging assay

The scavenging ability of the natural antioxidants of the plant extract towards the stable free radical DPPH was measured by the method [10]. Briefly, a 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. L-Ascorbic acid was used as the standard. Where $A_{C=}$ control is the absorbance of the control and $A_{S=}$ sample is the absorbance of reaction mixture (in the presence of sample). All tests were run in triplicates (n = 3), and the average values were calculated.

ABTS scavenging assay

The antioxidant effect of the leaf extracts was studied using ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourisation assay according to the method[11]. ABTS radical cations ($ABTS^{+}$) were produced by reacting ABTS solution (7mM) with 2.45mM potassium persulphate. The mixture was incubated at room temperature in the dark for 12 to 16 hrs to yield a dark-colored solution containing $ABTS^{\bullet+}$ radicals and diluted for an initial absorbance of about 0.700 (±0.02) at 734 nm. Aliquots (10µl) of the different concentrations of extract were added to 1ml of ABTS solution. The absorbance was read at 734nm after 6 minutes in a spectrophotometer. L-Ascorbic acid was used as the standard. Appropriate solvent blanks were run in each assay. All determinations were carried out in triplicate and the percent of inhibition was calculated using the formula.

Superoxide scavenging activity

The superoxide scavenging ability of the extracts was assessed by the method of [12]. Superoxide anions were generated in samples that contained in 3.0ml, 0.02ml of the leaf extracts (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.64ml of phosphate buffer. The control tubes were also set up where DMSO was added instead of the plant extracts. All the tubes were vortexed and the initial optical density was measured at 560nm in a spectrophotometer (Genesys, 10-S, USA). The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured at 560nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging from Fenton reaction was quantified using 2'-deoxyribose oxidative degradation as described by [13]. The reaction mixture contained 0.1 ml of deoxyribose, 0.1 ml of $FeCl_3$, 0.1 ml of EDTA, 0.1 ml of H_2O_2, 0.1 ml of ascorbate, 0.1 ml of KH_2PO_4-KOH buffer (125, 250, 500 and 1000 µg/ml) of plant extracts in a final volume of 1.0 ml. The mixture was incubated at 37 ºC for 1 h. At the end of the incubation period, 1.0 ml of TBA was added and heated at 95 ºC for 20 minutes to develop the colour. After cooling, the TBA formation was measured spectrophotometrically (Hitachi U-20) at 532 nm against an appropriate blank. The hydroxyl radical scavenging activities were determined by comparing the absorbance of the control with samples. The per cent TBA production for positive control vitamin C was fixed at 100% and the relative per cent TBA was calculated for the extracts. .

Statistical analysis

Data were expressed as Mean SD. Statistical analysis was performed by SPSS 16.0 One-way analysis of variance (ANOVA) was utilized to evaluate differences.

RESULTS AND DISCUSSION

Total phenolic and flavonoid content

Percentage yield of methanolic root extract of *Decalepis hamiltonii* was found to be 13.4 The total phenolic and flavonoid content of methanolic root extract were found to be 13.05± 1.01mg GAE/g and 6.4±0.70 mg QE/g dry weight respectively. Phenolics are powerful antioxidant which play vital role in the inhibition of deleterious free radical reactions[15].Total phenolic content could be regarded as an important indication of antioxidant properties of plant extract [16] Phenolic compounds are primarily responsible for scavenging for free radical donating active hydrogen iron and able to reduce oxidative stress[17]. Flavanoids, on the other hand, suppress reactive oxygen formation, chelate trace elements involved in free radical production, scavenge reactive species and potent antioxidant defenses [18]. From the results obtained, it was evident that methanolic root extract possessed very good reductive ability, which indicated its potent antioxidant capability.

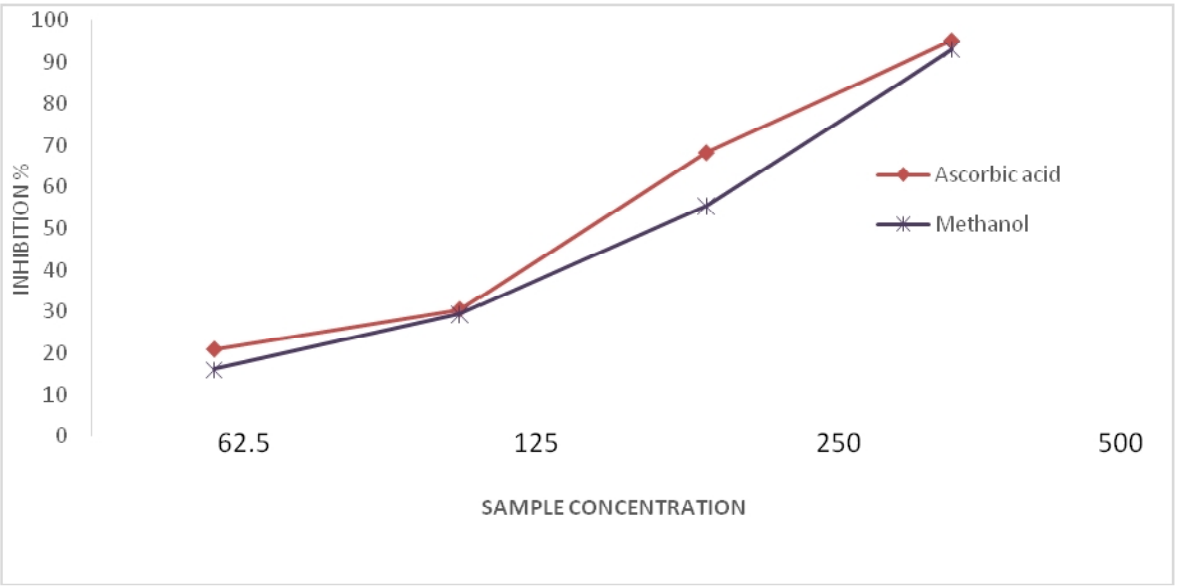

Figure 1: DPPH radical scavenging activity of methanolic root extract and standard ascorbic acid Values are mean of three replicate (n = 3), ± Standard deviation

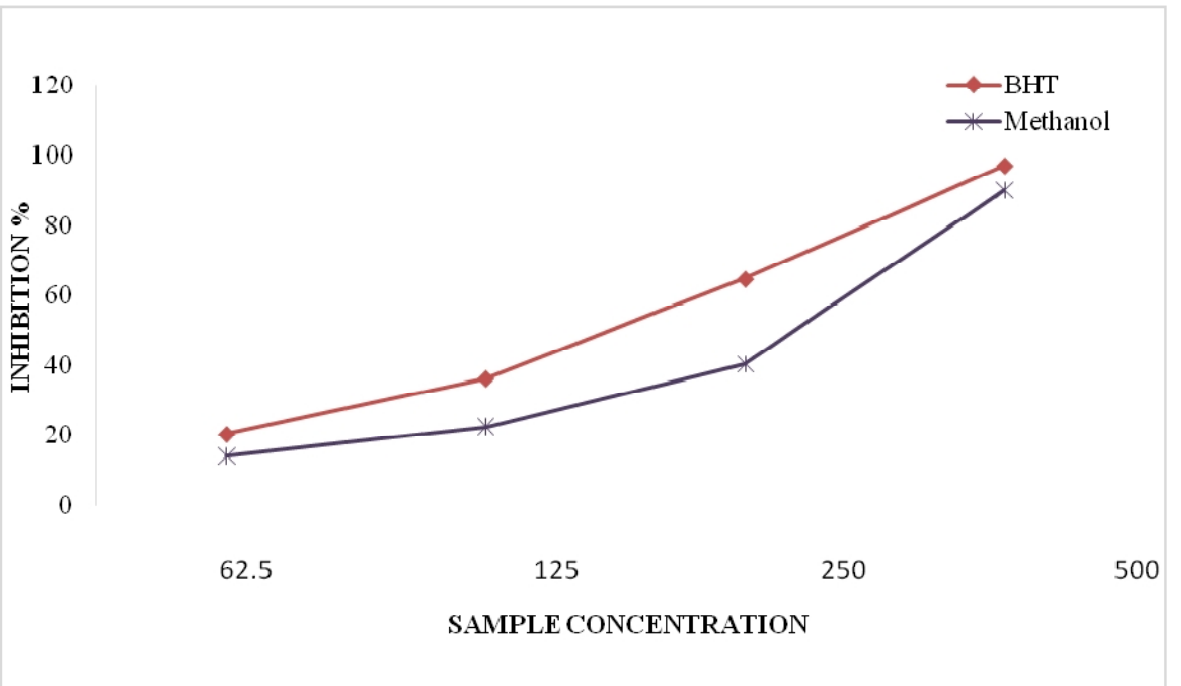

Figure 2: ABTS radical scavenging activity of methanolic root extract and standard ascorbic acid Values are mean of three replicate (n = 3), ± Standard deviation

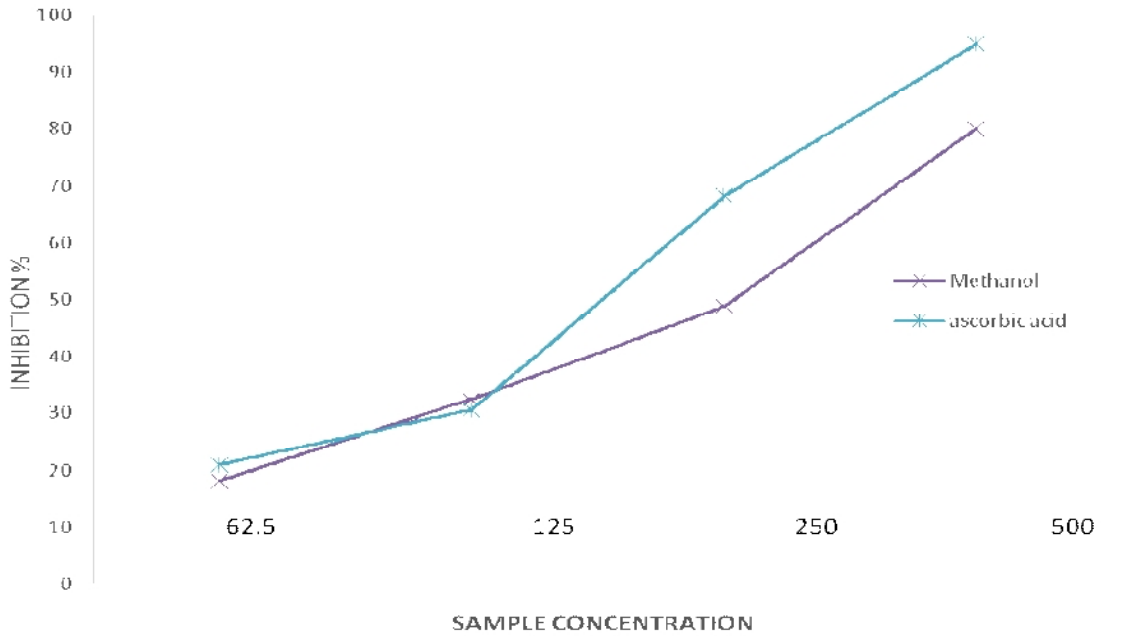

Figure 3: superoxide anion radical scavenging activity of methanolic root extract and standard ascorbic acid Values are mean of three replicate (n = 3), ± Standard deviation

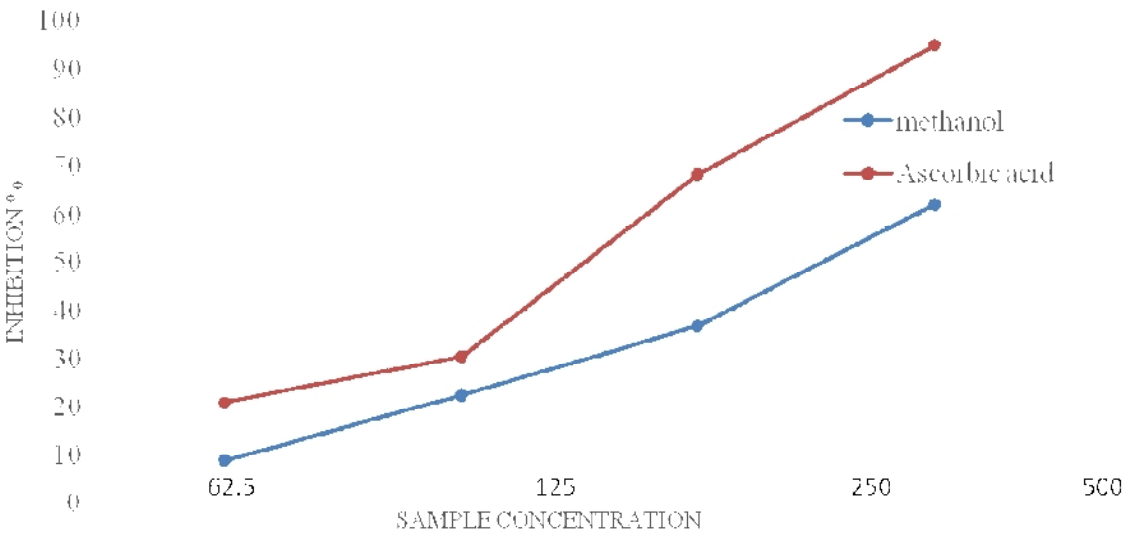

Figure 4: Hydroxyl radical scavenging activity of methanolic root extract and standard ascorbic acid Values are mean of three replicate (n = 3), ± Standard deviation

DPPH scavenging activity

Free radical scavenging potential of methanolic root extract along with the standard vitamin c at different concentration was tested by the DPPH method are shown in Figure 1. The percentage inhibition of methanolic root extract and ascorbic acid (62.5-500µg/ml) are about 16, 29, 55, 93% and 21, 30, 68 and 95% respectively and it was obvious from the results that values of the standard antioxidant were equal with our methanolic root extract. In support our work results, a similar type of work has also been carried out using the whole plants *A.benthamii* and significant DPPH activity has also been documented against *A.densiflora* root extract [19]. Methanol solvents generally used for antioxidant ability assays, are strongly hydrogen bond –accepting, therefore the hydrogen-abstracting reaction occurs very slowly[20]. The presence of acids or bases in methanol may greatly influence the ionization equilibrium of phenols and cause either a reduction or an increase of the measured rate constants [21]

ABTS scavenging activity

The ABTS radical scavenging method is one of the most extensively used antioxidant assays for plant samples. The methanolic root extract efficiently scavenged ABTS radicals, generated by the reaction between ABTS and ammonium persulfate. The activity was found to be increased in a dose dependent manner from 14 to 90% at a concentration of 62.5-500µg/ml which was comparable with the standard BHT (Figure 2). Therefore, the ABTS radical scavenging activity of methanolic root extract of *Decalepis hamiltonii* indicates its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction. Further the antioxidant activity of the extract by this assay implies that action may be by either inhibiting or scavenging properties of antioxidant towards this radical have been reported in earlier studies[22].

Superoxide radical scavenging activity

Figure 3 shows the Superoxide radical scavenging activity. The methanolic root extract was found to be more effective in scavenging superoxide radicals as compared to with the standard vitamin C. The percentage of inhibition of methanolic root extract and vitamin C were 18 to 80% and 21 to 95% respectively. It is known that the hydroxyl group of the phenolics contributes to superoxideradical scavenging ability by their electron donation [23]. The highest Superoxide radical scavenging activity of methanolic root extract of *Decalepis hamiltonii* corroborates with the results of who reported methanol to be the highest scavenging of superoxide radicals at higher concentration of plant extract. In addition it has also been established that the presence of compounds like anthroquinones, skikonins, and alkanins in the plant is a possible reason for effective scavenging or chelating of superoxide radicals [24-25].

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of methanolic root extract of *Decalepis hamiltonii* is shown in the Fig.4. High reduction of hydroxyl radical is related to the high scavenging activity performed by particular sample. In the present investigation, the hydroxyl radical scavenging activity observed was in the range of 8 to 62 % in methanolic root extract and 21 to 95 % in vitamin C which is a standard at a concentration of 62.5-500µg/ml. The hydroxyl scavenging activity increased with increasing concentration. The hydroxyl scavenging ability of methanolic root extract was comparable with the standard. Similarly,[26] reported that protective effectof *Caesalpinasappan* extract on DNA damage induced by hydroxyl radical at the same concentration tested.

CONCLUSION

On the basis of the results obtained in the present study, it was concluded that the methanolic root extract of *Decalepishamiltonii* possess significant antioxidant and antibacterial activity. Presence of adequate amount of phenol and flavonoid compound may account for this. So the findings of the study suggests that the root of the plant can be used as natural antioxidant and alternative drugs to treat the disease caused by pathogens. Further studies are underway for the isolation and characterization of antioxidant and antibiotic compounds for understanding their mechanism of action.

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