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Antioxidant and Antibacterial Activity of Methanolic Root Extract of *Decalepis hamiltonii* Wight & Arn.

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ABSTRACT

The aim of the research study was to screening the antioxidant and antibacterial activity of methanolic root extract of *Decalepis hamiltonii*. 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* has higher percentage of inhibition for DPPH (93%), ABTS (90%), superoxide radical (80%)and hydroxyl radical (62%) which is comparable with respective standards. Antibacterial activity against some gram positive and gram negative were tested using disc diffusion method. The methanolic root extract possessed relatively higher antibacterial activity against gram positive (15.2 to 21.0 mm) than gram negative bacteria (13.5 to 16.5mm). The antioxidant and antibacterial activity was due to higher percentage of phenolic and flavonoid constituents in the methanolic root extract.

Keywords: Antioxidant; Antibacterial; ABTS; Ascorbic acid; DPPH; O_2^-

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INTRODUCTION

Nature has been a source of medicinal agent for thousands of years generally produces many secondary metabolites which constitute important leads for development of new environmental friendly microbes, herbicides and many pharmaceutical drugs [1]. World health organization has estimated that 80% of the earth's inhabitant rely on traditional medicine for primary healthcare needs and most of the therapy involve 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 *Decalepishamiltonii*, 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, antibacterial 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 *Decalepishamiltonii* 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 *Decalepishamiltonii*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.

Antioxidant activity

DPPH 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 °C for 1 h. At the end of the incubation period, 1.0 ml of TBA was added and heated at 95 °C 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.

Antimicrobial Screening

Pure culture of *Escherichia coli* (ESBL-3984), *Staphylococcus epidermidis* (MTCC 96), *Streptococcus pyogenes* MTCC 102), *Bacillus subtilis* (MTCC 441),*Clostridium bifermentans*MTCC 165), *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella pneumoniae* (ESBL-3971) of bacteria.

The agar diffusion method [14] was followed for antibacterial susceptibility test. Petri plates were prepared by pouring 20ml of Muller Hinton Agar allowed solidifying for the use in susceptibility test against bacteria. Plates were dried and uniformly spread. The excess inoculum was drained and the plates were allowed to dry for 5min. After drying the discs with extract were placed on the surface of the plates with sterile forceps and gently pressed to ensure the contact with the incubated agar surface. Ciprofloxacin (10µg/disc) was used as positive control. 5 percent DMSO was used as blind control in these assays. The incubated plates were incubated at 37ºC for 24hrs. The zone of inhibition was observed and measured in millimeters. Each assay in this experiment was repeated for three times.

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] Flavanoids, on the other hand, suppress reactive oxygen formation, chelate trace elements involved in free radical production, scavenge reactive species and potent antioxidant defenses [17] From the results obtained, it was evident that methanolic root extract possessed very good reductive ability, which indicated its potent antioxidant capability.

Antioxidant Assay

DPPH free radical scavenging activity

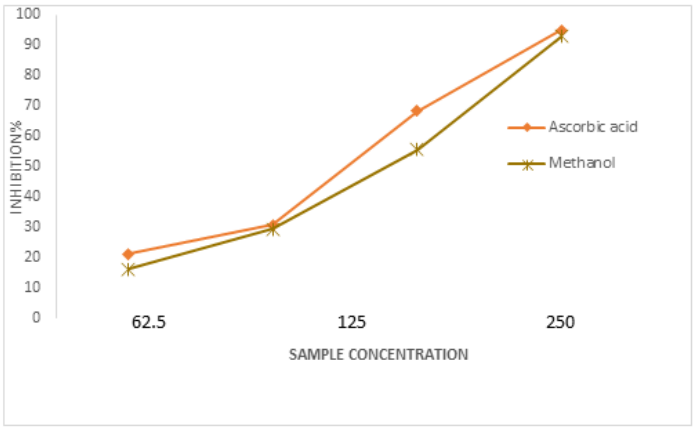

Figure 1: DPPH radical scavenging activity of methanolic root extract and standard ascorbic acid Values are mean of three replicate (n = 3), ±Standard deviation

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 Fig.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* [18] 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 radical 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 (Fig.2). Therefore, the ABTS radical scavenging activity of methanolic root extract of *Decalepishamiltonii* 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]

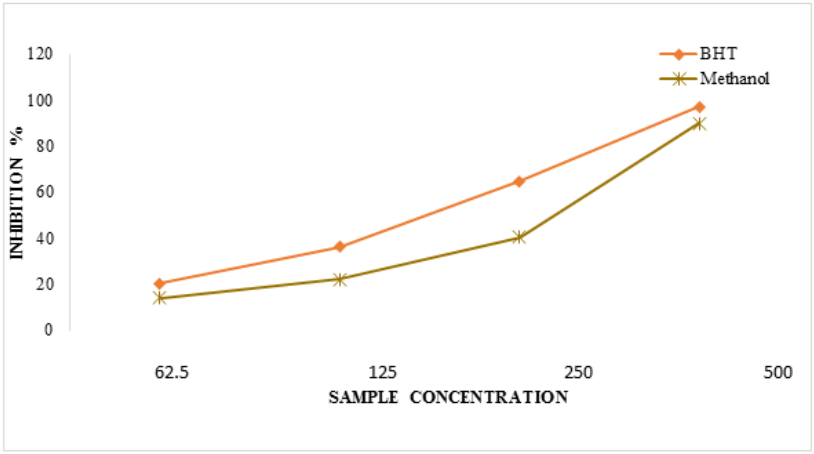

Figure 2: ABTS radical scavenging activity of methanolic root extract and standard BHT Values are mean of three replicate (n = 3), ± Standard deviation

Superoxide radical scavenging activity

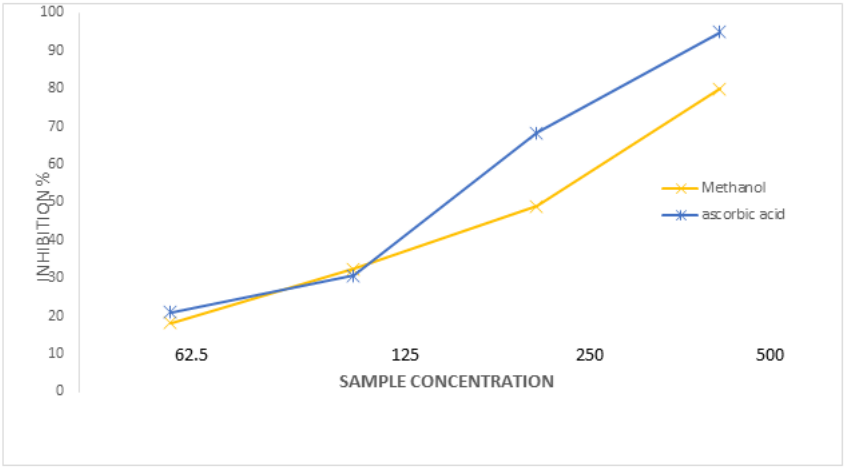

Figure 3: Superoxide radical scavenging activity of methanolic root extract and standard ascorbic acid Values are mean of three replicate (n = 3), ±Standard deviation

Fig .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 superoxide radical 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 effect of *Caesalpinasappan* extract on DNA damage induced by hydroxyl radical at the same concentration tested.

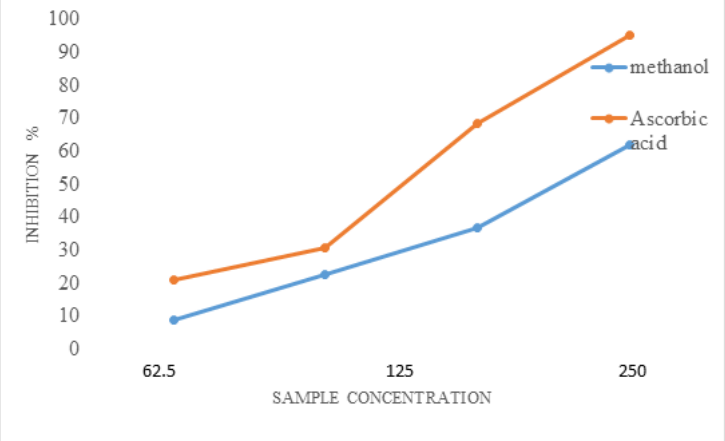

Figure 4: Hydroxyl radical scavenging activity of methanolic root extract and standard ascorbic acid Values are mean of three replicate (n = 3), ±Standard deviation

Antibacterial activity

The antibacterial activities of methanolic root extract of Decalepis hamiltonii are presented in table.1. The methanolic root extract exhibited pronounced activity against all the bacterial strains tested. *Staphylococcus epidermidis* showed maximum sensitivity (21.0mm/1000µg/disc) which comparable to standard antibiotic and *Klebsiella pneumoniae* showed least activity (13.5mm) at the same concentration. The MIC and MBC value of methanolic root extract ranged from 15.6 to 62.5 and 125 to 62.5µg/ml respectively. Our results indicate that methanolic root extract possessed relatively higher antibacterial activity against gram positive than the gram negative bacteria. It may due to their differences in cell membrane consustitents. In gram positive bacteria there is absence of lipopolysaccharide, that might function as a barrier to bioactive compounds that are reason for antibacterial activity [27]. The antibacterial activity of plant extract is related to the richness of phenolic compound such as phenols and flavanoids. These secondary metabolites found to form irreversible complexes with proline–rich proteins [28] resulting in inhibition of the cell protein synthesis. Thus the antibacterial activity of methanolic root extract of *Decalepis hamiltonii* is attributed to the presence of phenols and flavanoids.

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Table: 1 Antibacterial activity of methanolic root extracts of *Decalepis hamiltonii*

S.No	Bacterial	Zone of Inhibition(mm)	MIC (µg/ml)	MBC (µg/ml)	Zone of inhibition (mm) Ciprofloxacin
1	*Escherichia coli*	14.6 ± 0.61	31.2	62.5	18±0.55
2	*Staphylococcus epidermidis*	21.0 ± 0.77	15.6	31.2	30.7±0.60
3	*Streptococcus pyogenes*	20.4 ± 0.95	15.6	31.2	27.7±0.30
4	*Bacillus subtilis*	19.6 ± 0.20	15.6	31.2	22.6±0.55
5	*Clostridium bifermentans*	15.2 ± 0.50	15.6	31.2	17.4±0.35
6	*Pseudomonas aeruginosa*	16.5± 0.45	62.5	125	22.9±0.35
7	*Klebsiella pneumoniae*	13.5 ± 0.50	62.5	125	16.6±0.7

Mean of three assays; ± standard deviation

CONCLUSION

On the basis of the results obtained in the present study, it was concluded that the methanolic root extract of *Decalepis hamiltonii* possess significant antioxidant and antibacterial activity. Presence of adequate amount of phenol and flavonoid compound may account for this. So the finding 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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