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Assessment of Phytochemical, Antioxidant and Antibacterial Activity Wild Medicinal Plant *Mecardonia procumbens*

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ABSTRACT

The plant *Mecardonia procumbens* (Mill.) belongs to Plantaginaceae family, much branched herb commonly called Makardan. Leaves of *Evolvulus glomeratus* is traditionally used to heal all kinds of wounds for human and domestic animals. Now scientific research has expanded our knowledge to discovered chemical composition and active constituents present in medicinal plants. Present research work was undertaken to the phytochemical, antioxidant and antibacterial activity of M. procumbens. The antioxidant of leaves extracts was assessed based on the radical scavenging effect of the stable 1,1-diphenyl-2-picryhydrazyl (DPPH). Antibacterial activity of aqueous and ethanolic extracts M. procumbens was studies for standard bacteria one Gram-positive (Enterococcus Faecalis) and Gram-negative (Escherichia coli). The optimum inhibition zone size value for both the bacteria Enterococcus Faecalis is 14 mm and *Escherichia coli* is 10 mm in *M. emarginata*. The methanol and ethanol extracts of both plant show significant antioxidant and antibacterial activity.

Keywords: M. procumbens, Therapeutically used, Phytochemical, Antioxidant, Antibacterial Activity.

Introduction

Medicinal plants are plants used for therapeutic purposes. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are safe, efficient, easily available, less expensive, and no side effects. Then desperately need to conservation of medicinal plants and cultivation of wild medicinal plants.

Fig.1: *Mecardonia procumbens* plant

Herbal wild medicinal plants are easily available, less expensive, no side effect and more efficient make them more attractive as therapeutic agents when compared to modern medicine [1,2]. India has top ranked herbal medicinal producer because Indian plant biodiversity is the largest source of herbal plant medicine [3]. In the word there are 60 to 80 % of people in word use medicinal plants and their products for therapeutic purposes [4]. The medicinal value of these plants lies in the bioactive phytochemical constituents present in plants and that are beneficial to humans. Many active phytochemical like flavonoids, terpenoids, vitamins, alkaloids etc. were found to be responsible for these activities [5]. Present research work was undertaken on the phytochemical, antioxidant and antibacterial activity of *Evolvulus glomeratus*. The phytochemical constituent of *Mecardonia procumbens* is proteins, carbohydrate, phlobatannin, chalcone, flavonoids, glycoside and curcumin. The phytochemical flavonoids, glycoside and curcumin found in *Mecardonia procumbens* leaves could serve as a source of useful drugs for wounds healing diseases.

Material and Methods

Collection of plant materials

For the present study *Mecardonia procumbens* were collected from playground Yashwantrao Chawhan arts, commerce and science college Lakhandur. The Mecardonia *procumbens* leaves was washes under running tap water to eliminate dust and other foreign particles and then wash double distil water two or more times. Leaves were cut into small pieces were dried under shade paper towel in the laboratory and then in oven 40-50 ^{0}C, after that homogenized into a fine powder using a mortar and pestle. This fine powder was further passed through a 2 mm sieve to obtain finer particles, and then stored in airtight bottles and was used for further studies.

Preparation of Plants extracts

Process for Extraction 500 gm of each powder of the leaves were taken along with the 1000 ml of distilled water in a container. The mixture was shaken continuously with used of rotary shakers and place in a dark for 72 hour with occasional shaking. After 72 hour the mixture was filter and filtrate was concentrated to one third of the original amount. The water extract was kept in refrigerator when not in use.

Solvent extraction

Crude plant extract was prepared by Soxhlet extraction method. About 20 gm of powdered plant material was uniformly packed into a thimble and extracted with 250 ml of solvents methanol. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40^{o}C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4^{o}C. The resultant was used for phytochemical, antibacterial and antioxidant analysis [6].

Phytochemical analysis

Crude plant extract of M. procumbens was used for qualitative phytochemical analyses. Phytochemicals such as flavonoids, tannins, steroids, glycosides, saponins, phenolic compounds, terpenoids and alkaloids are analyzed [5].

Antioxidant Activity By 1, 1-diphenyl-2-picrylhydrazyl (DPPH)

The antioxidant activity of the ethanol extracts of *Mecardonia procumbens* leaves were assessed based on the radical scavenging effect of the stable DPPH [7, 8]. 0.005% of DPPH was prepared in ethyl alcohol and 4 ml of this DPPH solution was mixed with 4 ml of ethanolic plant extract solutions. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV Visible spectrophotometer. 4ml ethanol with 0.005 DPPH solutions was used as blank. The optical density was recorded in spectrophotometer and % inhibition was calculated using the following formula.

Percentage (%) Inhibition of DPPH (% AA) = A – B × 100 / A

Where A=Optical density of the blank and B=Optical density of the sample.

Extraction concentration providing 50% inhibition IC_{50} values was calculated maximum and minimum values of %AA

Antibacterial activity (well diffusion method)

Antibacterial activity was carried out to examine the sensitivity of some bacterial species against plant extracts of *Mecardonia procumbens* leaves with a comparing the antibiotics for it by Disk Diffusion Method (9). These bacteria included Gram-positive (Enterococcus Faecalis) and Gram-negative (E. coli).

Results

Phytochemical analysis

The methanol extract of was tested for the presence of bioactive compounds by using following standard methods [12,13,14].

1. Test for proteins

Ninhydrin test

1 gm crude plant extract when boiled with 2ml of 0.2% solution of Ninhydrin (tricyclic 1,2,3-trione), violet colour appeared suggesting the presence of proteins.

2. Test for carbohydrates

Fehling's test

1:1 ratio of Fehling A (cupper sulphate) and Fehling B (potassium sodium tartrate) reagents were mixed together and 4 ml of it was added to 1 gm crude plant extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of Carbohydrates.

3. **Test for phenols**

Test extract was treated with 4 drops of Alcoholic ferrous chloride solution. Formation of bluish black color indicates the presence of Phenol.

a. Phlobatannin: when extract plant sample is boiled with dilute 0.1N HCl was taken red ppt was obtained as evidence for presence of Phlobatannin.

b. Chalcone: 2ml of Ammonium Hydroxide was added to 0.5 ml ethanolic extract, the appearance of the red color showed the presence of Chalcone.

4. Test for flavonoids

Shinoda test

Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

5. Test for saponins

5 ml plants extract was mixed with 20 ml of double-distilled water then agitated in graduated cylinder For 15 min formation of foam indicates Saponin.

6. Test for glycosides

Liebermann's test

Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H2SO4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

7. Test for steroid

1ml extract was dissolved in 10 ml of $CHCl_3$ and 1ml of concentrated H_2SO_4 acid was added from the side of a test tube. The upper layer turns red and the H_2SO_4 layer showed yellow with green fluorescence. This indicates the presence of steroids

7.Test for terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H2SO4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

8. Test for alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

9. Test for Tannin

4ml extract was treated with 4 ml Ferrous chloride formation of green color indicates that presence of condensed tannin.

10. Test for Anthocyanin

2 ml of aqueous extract is added to 2 ml of 2N HCl& NH_3, the appearance of pink-red turns blue-violet indicates the presence of anthocyanin.

11. Test for Diterpine

Extract were dissolved in water and treated with 10 drops of $Cu(OAc)_2$ solution, formation of emerald green color indicates the presence of Diterpine.

Antioxidant Activity

The stock solution 1 mg/ml of ethanol extracts and DPPH solution was prepared. The required dilutions from 0.01 mg/ml to 0.1 mg/ml were prepared by appropriate dilutions (7,8). The optical densities of bank DPPH solution and sample solution can be calculate and found. With use of optical density of both solution percent antioxidant activities were calculated in table given bellow.

Table-1: Phytochemical analysis for Ethanolic Extract of M. procumbens

Phytochemical	M. procumbens	Phytochemical	M. procumbens
Saponin	+	Flavonoid	+
Steroid	+	Diterpine	+
Tannin	-	Phenol	+
Anthocyanin	+	Phlobatannin	+
Coumarin	+	Chalcone	-
Protein	+	Carbohydrate	+

Table-2: Optical Density and % Antioxidant Activity for Ethanolic Extract of M. procumbens
(O.D. of Black DPPH = 0.585)

Conc. mg/ml	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
O.D. of M. procumbens	0.415	0.335	0.272	0.217	0.203	0.183	0.123	0.109	0.105	0.062
% AA M. procumbens	26.15	39.65	53.16	63.07	65.64	68.03	77.6	80.85	82.22	87.17

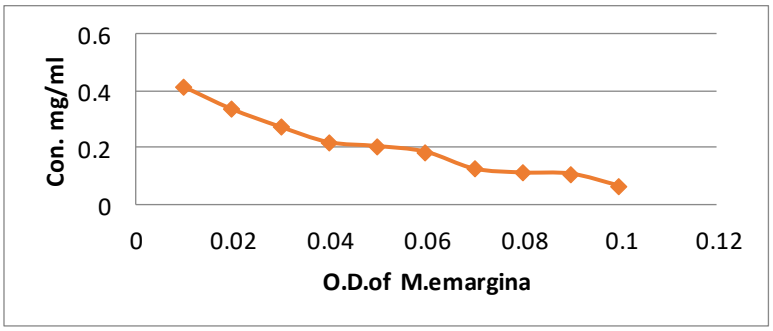

Fig.-2: Decrease in Optical Density of Sample with Increase in Concentration of Ethanolic Extracts of P. minima leaves.

Table 3:- The effectiveness of three elements

Medicinal Plants	Bacterial Species	Bacterial Species	Control
	Enterococcus Faecalis (mm)	*Escherichia coli* (mm)	Methanol extract (mm)
M. procumbens	14	10	05

Legend: (mm) = Millimeter

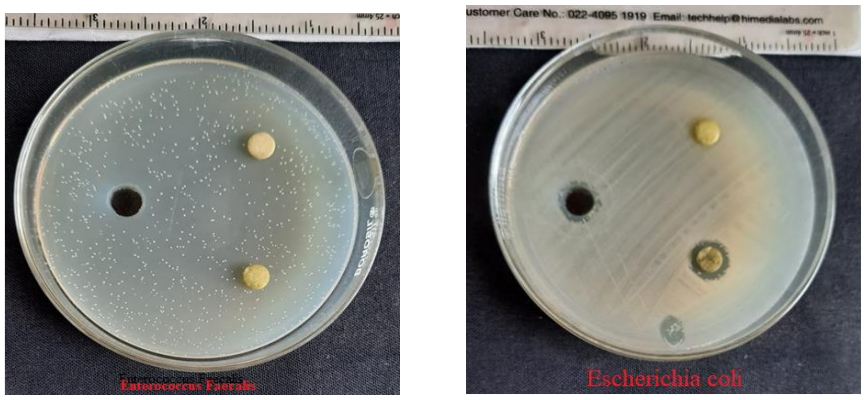

Fig.3: Antibacterial activity of the methanolic extract of M. procumbens by well diffusion method against Enterococcus Faecalis and *Escherichia coli* bacteria.

Increase in Percent Antioxidant Activity with Increase in Concentration for ethanolic extract of P. minima leaves. Calculation of IC_{50} Value for P. minima leaves : = max – ½ (max-min)

=

87.17– ½ (87.17 – 26.15) =56.66

IC_{50} value from graph corresponding ethanolic extract of P. minima leaves is 0.036 mg/ml.

Antibacterial Activity:

The examined bacterial species included Gram-positive (Enterococcus Faecalis) and Gram-negative (Escherichia coli). Sterile discs 6 mm prepared from Whatman filter paper No. 1 and well diffusion using control and barrier were made to absorb 50 µg of the test samples (13). In the solvent discs methanol were used as control (14). The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (1.5 x 108 cfu / mL)(15). Mueller-Hinton agar was prepared on the plates as the medium for the test organism (16). The bacterial inoculums were spread evenly onto the surface of the agar plate using the sterile cotton bud and then the control disc and were same amount of extract into well of medium and standard antimicrobial discs were situated on the inoculums agar superficial. The antimicrobial activity was interpreted from the size of the diameter of the zone of inhibition measured to the adjacent mm as experiential from a clear zone surrounding the disc. In case optimum inhibition zone size value of bacterial species and control were the details results for antibacterial activity are shown as shown in Table 3

Conclusion

- Phytochemical screening of selected wild medicinal plants clearly reveals that the maximum classes of photochemical are present in M. procumbens
- The M. procumbens leave extracts demonstrate good DHHP radical activity with IC_{50} value for M. procumbens is 0.036 mg/ml which show good antioxidant activity.
- Leaves extract of M. procumbens are exhibited significant antibacterial activity for bacterial species including Gram-positive (Enterococcus Faecalis) and Gram-negative (Escherichia coli). The optimum inhibition zone size value for both the bacteria Enterococcus Faecalis is 14 mm and *Escherichia coli* in 10 mm in M. procumbens methanolic extract.
- Phytochemicals in plant extract serve as a source of drugs that are useful in the medicine of some

diseases caused by bacteria and also as antioxidant agents.

Conflicts of interest: The authors stated that no conflicts of interest.

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