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Research Article

ASSESSMENT OF *IN VITRO* ANTI-OXIDANT AND ANTI-MICROBIAL EFFICIENCIES OF ENDANGERED MEDICINAL PLANT *FICUS DALHOUSIAE* MIQ.

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ABSTRACT

Ficus dalhousiae Miq, is sparely populated endangered plant of Nilagiri hills and eastern ghats of India. It was reported to have prominent medicinal activities in the traditional medicinal system. The successive soxhlet extraction method was carried out using Hexane, Acetone, Methanol and Aqueous solvents. The quantitative estimation of total phenol content was 94µg GAE/µg by folin ciocalteu method. The antimicrobial activity was moderate and dose dependent. Polarity of the solvent was directly proportional to the zone of inhibition. Antioxidant activity of the four extracts was calculated and their IC_{50} values were compared with the standard ascorbic acid. Methanol extract was found to have 91.36 µg/ml inhibitory concentrations. The IC_{50} values were significantly increased for the four extracts and the percentage of inhibition was directly proportional to the concentration of the extract. Methanol extract has the effective antimicrobial activity and antioxidant activity.

Keywords: *Ficus dalhousiae* Miq, antimicrobial activity, antioxidant activity, total phenol content

INTRODUCTION

Medicinal plants play a prominent, pivital role in the health of individual and communities. The medicinal properties of plant are due to some active chemical constituents that produce a definite physiological action on human body[1]. Many indigenous medicinal plants are used for therapeutic purposes for curing large number of diseases, illness and ailments [2]. Natural products, major, reliable constituents isolated from medicinal plants acts as therapeutic agents and used extensively for pharmaceutical research and for drug development. The most important active constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. At present, the demand for herbal or medicinal plant products has increased significantly. In the recent past, there has been growing interest in exploiting the biological activities of different Ayurvedic medicinal herbs, owing to their natural origin, cost effectiveness and minimal side effects [3].

Increase in microbial resistance to the existing antibiotics has lead researchers to scrutinize the antimicrobial compounds from different sources and the use of medicinal plants as an alternative form for health care proved medicinal plants as the best remedy for curing diseases especially by controlling the microbes [4]. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate [5].

India is the richest source of medicinal plants and it has the genetic diversity among the wide range of topology and climate. Moreover the agro climatic conditions are conducive for introducing and domesticating new exotic plant varieties [6]. Several plants have been used in folklore medicine. The rational design of novel drugs from traditional medicine indeed offers new prospects in modern healthcare.

Ficus dalhousiae Miq. Is evergreen, pubescent with soft young branches and is a small spreading tree, leaves are in ovate manner, pubescent is white and apex is accumulated shortly, more or less 30cms long. Origin of species is in peninsular area [7], now it is endemic[8]. In india it was named as *Urostigmadalhousiae* later based on Miquel named as *Ficusdalhousiae*[9, 10]. This plant has been distributed in Nilagiri Mountains at a height of 1200m [11, 12, 13]. This plant is found in a smaller population so it falls under very rare category in threatened plant list.

Pathogenic microorganisms are growing filthier than earlier, to eradicate these organisms there is a need to develop new drugs which are cost effective, kills multi drug resistant pathogens and drugs which are side effects free. Plants are the reliable resources that produce effective chemical constituents to resist the microbial infections and also for the other diseases. The antioxidant activities of secondary metabolites from the medicinal plants have the multipurpose usage in drug discovery and pharmacological preferences. In the present study we contemplated to study the efficiency of antimicrobial and antioxidant activities of *Ficus dalhousiae* Miq. and also to estimate the phenol content quantitatively.

Classification
Domain: Eukaryota
Kingdom: Plantae
Sub kingdom: Viridaeplantae
Phyllum: Tracheophyta
Sub Phylum: Euphyllophytina
Class: Spermatopsida
Sub Class: Rosidae
Family: Moraceae

Genus: *Ficus*
Specific epithet: *dalhousiae*-Miq.

MATERIALS & METHODS

Collection of Plant Material

The plant material was collected from the Talakona Forest of Andhra Pradesh, India. The plant material was washed under running tap water and shade dried. Powder was packed in an air tight bag and preserved in a cool dry place until further used.

Collection of microorganisms

Microorganisms used for the experiments were procured from MTCC, IMTECH, Chandighar and they were reconfirmed by gram staining, sub culturing in appropriate selective media and biochemical tests.

Gram-positive organisms

Staphylococcus aureus(MTCC 3160)
Streptococcus mutans(MTCC497)
Lactobacillus casei(MTCC1423)
Lactobacillus acidophilus (MTCC495)
Bacillus megaterium(NCIM2187)

Gram-negative organisms

Enterococcus faecalis (MTCC439)
Xanthomonascampestris (MTCC2286)
Escherichia coli (ATCC35218)
Pseudomonas aeruginosa (ATCC 9027)

Fungal strains

Candida albicans(ATCC10231)
Aspergillusniger(ATCC1015)
Rhizopusoryzae (MTCC262)
Candida rugosa (ATCC 96275)

Extraction Method: (Successive Extraction Method)

Plant powder was extracted by using four solvents (Hexane, Acetone, Methanol and Aqueous) in Soxhlet apparatus. The extract was condensed with the Rotary evaporator to obtain concentrated crude extract.

Yield of Extract

Yield of extract was obtained by calculating the amount of plant material obtained before and after the extraction process.

Anti-Microbial Activity:[14, 15]

Agar well diffusion method

Nutrient broth (NB) was prepared and inoculated with the respective microorganism. They were incubated for 24Hrs with constant shaking by orbital shaker. The nutrient agar (NA) plates were cooled to room temperature and to these plates 10µl of cultured nutrient broth was added. Four wells of 10mm diameter were prepared using a cork borer. 50µl of plant extract at a concentration of 10mg/ml were added to the each well by using the sterile micro pipette and they are allowed to diffuse at room temperature for 2 Hrs. These plates were incubated at 37^0C for 18-24Hrs. Anti- biotic sensitivity was also studied with different concentrations of the extracts to find out their effective dosage response.

Sabouraud Dextrose Agar (SDA) plates were swabbed (sterile cotton swabs) with 24 hours old - broth culture of respective fungi. Four wells (10mm diameter) were made in each of these plates using sterile cork borer. About 50µl of different concentrations (25, 50, 75, 100mg/ml) of plant extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 28°C/72 hrs for the growth of fungal pathogens. The respective extracts were maintained and solvents were used as control. The experiment was repeated thrice, and average values of zone of inhibition were recorded in mm for analyzing the antimicrobial activity. The antibiotic compound Streptomycin (10mg/ml) was used as a Standard for the antibacterial study.

Quantitative Analysis[16, 17]

Skerget (2005) proposed Folinciocalteu method for calculating total phenol content. Different concentrations of extracts (100 to 500µg) were taken and to that 0.1ml of Folin-ciocalteu reagent and 0.2N Na_2CO_3 of 2.5ml was added. Incubation period of 30 min were stabled at room temperature. Absorbance was measured at 760nm using Thermo Fisher double beam spectro photometer. Gallic acid was used as standard and the results were expressed as µg of gallic acid equivalents per gram dry mass of extract (µg GAE gDM).

Antioxidant Properties [18]

1, 1- Diphenyl-2-Picrylhydrazyl radical (DPPH) scavenging activity

The free radical scavenging activity of the extract was measured by using 1, 1- Diphenyl-2-Picrylhydrazyl radical (DPPH) as described by Brand- Williams, W (1995) with some modifications. The methanol crude extracts were prepared at 1mg/ml concentration with DMSO solution. The mixture was made uniform and working solutions were prepared at different concentrations. 0.004% (W/V) solution of DPPH in methanol was added to the solution. The mixtures were shaken and incubated for 60 min in the dark at room temperature. Absorbance was measured at 517 nm against a blank (Distilled water). The DPPH scavenging activity (I %) was calculated as follows:

$$I\% = \left[\frac{(Ao - As)}{Ao}\right] \times 100$$

Where **Ao** is the absorbance of the DPPH solution without sample extract and **As** is the absorbance of sample with DPPH solution.

RESULTS AND DISCUSSION

Physico-Chemical Evaluations

The physical properties of plants have a significant role in the extraction process. The yield of extract was determined by the weight of the plant material and the colour of the extract gives its bioactive nature. The methanol extract has showed significant high yield of extract (Graph 1).
The qualitative analysis gives an idea on the activity of the plant. Hexane and chloroform extract doesn't have any significant bioactive components and the acetone extract contained alkaloids, flavonoids, phenolics, tannins, coumarins and reducing sugars. The methanol extract showed positive result for the tannins, saponins, reducing sugars, steroids, flavonoids and phenolics. Aqueous extract has reducing sugars, saponins, phenolics and steroids[19]. The phytochemical compounds are collectively responsible for the antimicrobial activity by their nature. Mostly phenolic compounds have strain sensitivity and tannins, flavonoids also have the strong anti-bacterial and fungal activity [20,21,22.] The components which have basal components like flavonoids also act against wide range of microbial infections [23]. With reference to the qualitative analysis *Ficus dalhousiae* Miq has wide range of phytochemical constituents and their biological activities have been determined by the *in vitro* analysis.

Antimicrobial Activity of Whole Arial Part Extracts of *Ficus dalhousiae* Miq

Plant based medicine is predominant against microbes. Plant based antimicrobials have lesser side effects and multi drug potential often they associate with the synthetic antimicrobials for the effectiveness[24].

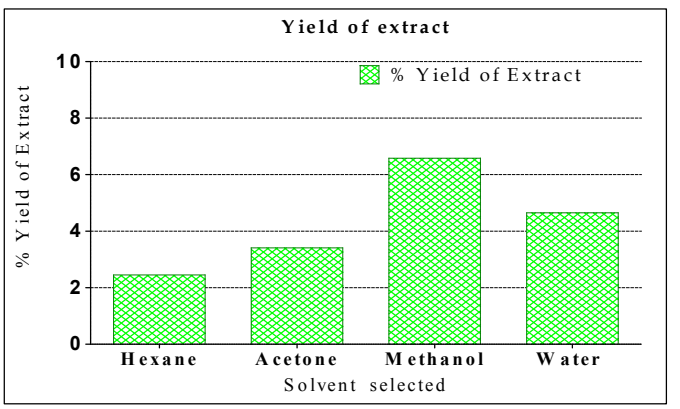
The antimicrobial activity includes antibacterial and antifungal activities. The antibacterial activity was determined for the nine organisms and antifungal activity was determined for the four organisms. The methanol extract has shown good activity against all the organisms and *Enterococcus foecalis*, *Pseudomonas auriginosa* were highly inhibited by methanol extract (Graph 2, 3, 4).

Total Phenol content of Methanol extract

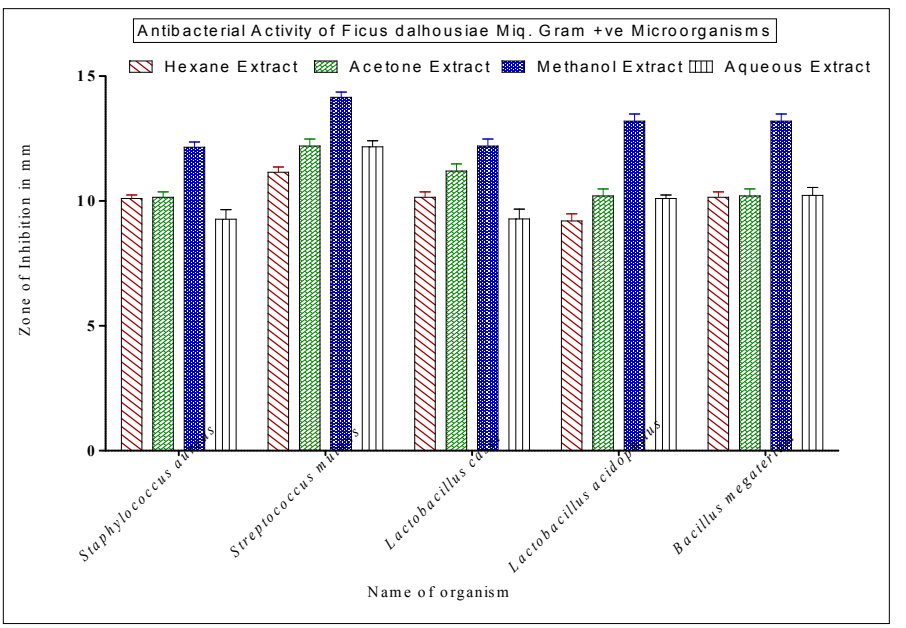
The quantitative analysis for phenol content was determined by the folin ciocaltue method. Phenols are the compounds that are most commonly present in the plant kingdom and have multiple biological effects.
The total phenol content was more relevant with the antioxidant activity and the content of this was dose dependent manner. Total phenol content was measured for the methanol extract as it contains higher amount of bioactive compounds. Phenol content of methanol extract was 24µgGAE/µg shown in the Graph 5.

DPPH Antioxidant activity of *Ficusdalhousiae* Miq

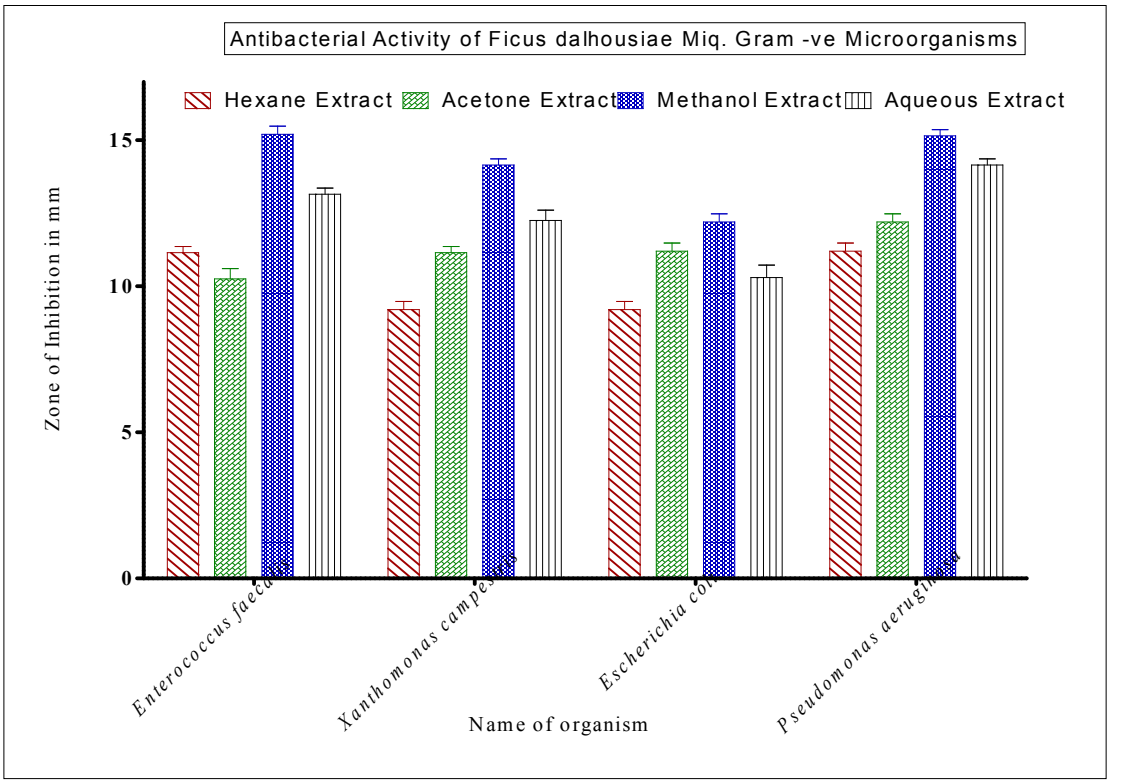
Antioxidant activity of the plant was determined by the DPPH antioxidant assay[25]. The graph 5 showed that antioxidant activity of the four extracts of *Ficus dalhousiae* Miq. The highest scavenging activity was found in methanol extract, and lowest in hexane extract. Aqueous and acetone extracts showed low to moderate scavenging activity when compared to ascorbic acid. Lowest IC_{50} value (91.36%) was found in methanol extract followed by aqueous (185.68%), acetone (157.88%) and hexane extracts (242.27%) of *Ficus dalhousiae.* An IC_{50} value is the concentration of the sample required to scavenge 50% of the free radicals present in the system. The antioxidant activity of above said extracts were found to be concentration dependent. From the results obtained, the methanol, aqueous and acetone extracts (polar solvent extracts), were more effective antioxidants to the non polar hexane extract (Graph 6).



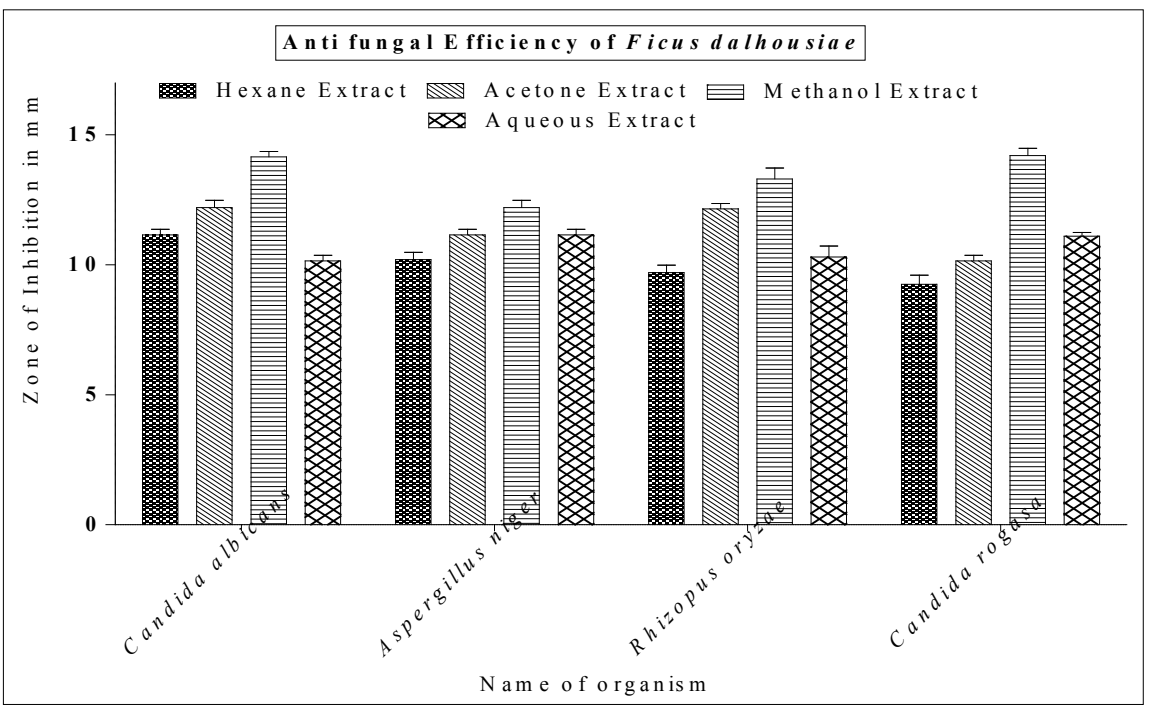
Graph 1: Yield of Extract



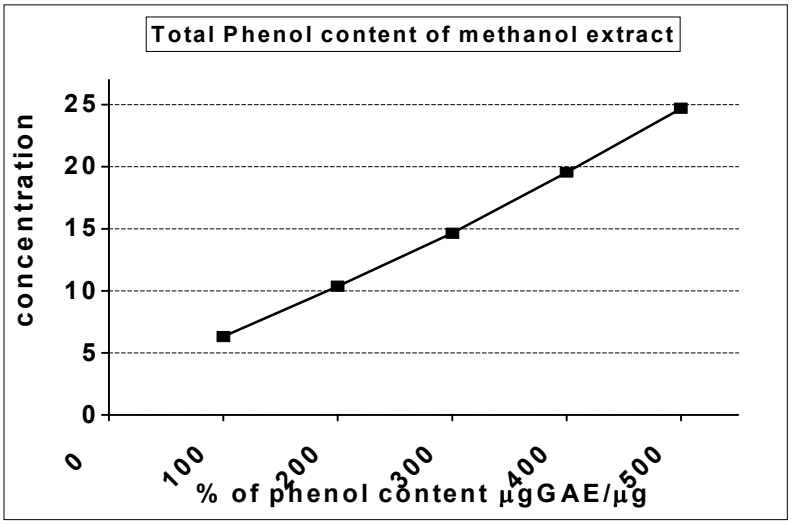
Graph 2: Anti-Bacterial Activity of Gram +ve Microorganisms



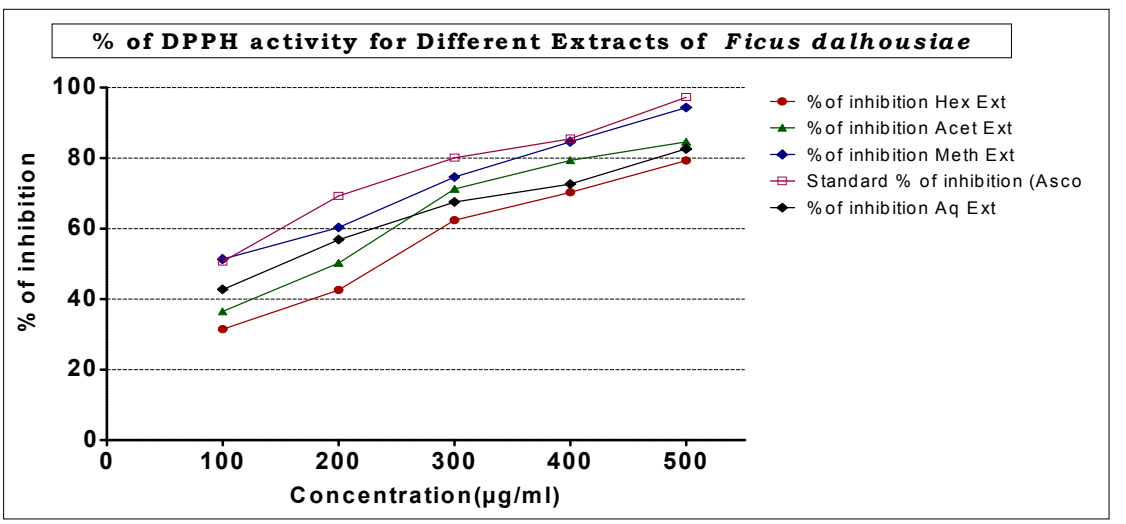
Graph 3: Anti Bacterial Activity of Gram -ve Microorganisms



Graph 4: Anti- fungal efficiency



Graph 5: Total Phenol Content



Graph 6: DPPH Activity of different extracts

CONCLUSION

Ficus dalhousiae Miq. has the ability to inhibit the growth of pathogenic organisms and have the ability to scavenge the free radicals. It is having significant bioactive constituents in the extracts. The total phenol content was measured and methanol extract showed dose dependent response equalling to gallic acid standard. The antioxidant activity of *F. dalhousiae* was found to be more significant than standard ascorbic acid. Further biological and structural analysis has to be carried out for the novel drug discovery.

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