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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL STUDIES IN LEAF EXTRACT OF *EUPHORBIA MILII*

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ABSTRACT

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi, and herbivorous mammals. Present study deals with the phytochemical analysis and antibacterial activity of *Euphorbia milii* against methanolic extract. Phytochemical screening of methanolic extract of *E. milii* leaves had shown the presence of alkaloids, glycosides, flavonoids, phenol and tannins. The antibacterial activity of the methanolic extract of *E. milii* leaves against human pathogens like *Streptococcus pyogenes*, *Pseudomonas aerogenosa*, *Klebsiella pneumonia* and *Aeromonas Spp* were seen by using agar well diffusion method. Zone of inhibition ranged between 12-26 mm. Leaves extract inhibited the growth of all test organisms with large zone of inhibition. The spectrum activity of methanolic extract of this plant could be a possible source to obtain new and effective herbal medicines to treat various infectious diseases.

Key words: *Euphorbia milii*, Phytochemical, disc diffusion, antimicrobial activity.

INTRODUCTION

Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies. *Euphorbia milii* (crown of thorns, Christ plant, and Christ thorn) is a species of flowering plant in the spurge family Euphorbiaceae, native to Madagascar. The species name commemorates Baron Milius, once Governor of Reunion, who introduced the species to France in 1821. It is suspected that the species was introduced to the Middle East in ancient times, and legend associates it with the crown of thorns

worn by Christ. The Family Euphorbiaceae consists of 2000 species. The genus *Euphorbia* is the largest genus of medicinal plants.

It is a succulent climbing shrub growing to 1.8 m (5 ft 11 in) tall, with densely spiny stems. The straight, slender spines, up to 3 cm long, help it scramble over other plants. The leaves are found mainly on new growth,[1] and are obovate, up to 3.5 cm long and 1.5 cm broad. The flowers are small, subtended by a pair of conspicuous petal-like bracts, variably red, pink or white, up to 12 mm broad. The sap is moderately poisonous. Wat Phrik in Thailand claims to be the home of the world's tallest Christ plant. Clusters of bright green, elliptic, 5-6cm (2-2.4 inch) leaves which are produced near the growing tips of the stems, last for at least several months before dropping off, leaving the plant's spiny stems permanently bare. Old leaves are not replaced and new ones will appear only one new terminal growth.

Different species of *Euphorbia* are used as a folk medicine for the treatment of various ailments such as skin diseases, intestinal parasites and warts cures. It has been reported that it possesses antiarthritis, anticancer, anticonvulsant, antidiabetic, anti-eczema, anti-eczema, anti-inflammatory, antimicrobial, antioxidant, antispasmodic, antitumor, antitussive properties hormonal and myelopoiesis. *Euphorbia milii* plays a role in folk medicine. The Chinese use it as a cure for cancer, and some believe that it cures warts (Daniel *et.al* 1998). *Euphorbia milii* can curb the spread of schistosomiasis, a disease of the liver. Its latex has ingredients that can kill snails of the genera *Indoplanorbis* and *Biomphalaria*, which are vectors (alternate hosts) of the flatworms which cause disease (Zimmermann *et.al* 1998).

Milin, an extract of *Euphorbia milii* latex, is a glycosylated serine protease (an enzyme that breaks down protein and has a sugar attached to it) Milin will also be useful to research scientists who use serine proteases to get rid of unwanted proteins so that they can obtain the ones they want in pure forms (Sanders *FT et.al* .2003). *Euphorbia milii* is generally trouble-free. The plants will survive drought conditions. The sap of *Euphorbia milii* can irritate the sensitive skin; that of some species is poisonous and acrid. The whole plant paste of *Euphorbia milii* is used in the treatment of diabetes and ethnic practices (Seung *et.al* 1990). *Euphorbia milii* is used to treat joint pains as anti-inflammatory agent.

Whole plant paste is used to apply on bones dislocation of animals, leaves are used for snake bite and ringworm, seeds used as laxative for children (Phanikumar and Alka, 2010). Powder of *Euphorbia milii* flower and whole plant ash of *Euphorbia milii* are used for the treatment of asthma at dose of 500 mg/ 3 times and 250-500 mg twice a day orally, respectively. The calcinated product of

Euphorbia species like *Euphorbia milii* is used in the asthmatic condition (Savithramma *et al.*, 2007). Qualitative estimation of phyto constituents of ethanolic extract of *Euphorbia* was revealed the presence of bioactive compounds like alkaloids, phenols and tannins (Nagaraju *et al.*, 2012). Photochemical screening on the crude extracts of the *E. milii* contains various secondary metabolites such as alkaloids, steroids, terpenoids, flavanoids and tannins (shahid farooq *et.al* 2012).

MATERIALS AND METHODS

1. SAMPLE COLLECTION - Leaf samples of *Euphorbia milii* were collected from various area of Malappuram

2. PLANT AUTHENTICATION - *Euphorbia milii* plant authenticated from BSI (Botanical Serve of India)

3. PREPARATION OF THE PLANT EXTRACT - The plant leaves were washed for 2-3 times with tap water and finally with distilled water and air dried in shade for ten days and then dry in an oven at 60 °C for one to two days and finally milled to a coarse powder.50grams of powdered material was extracted by maceration in methanol (200ml) for 10 days agitation. The mixture was filtered through double filtration with whatman no.1 filter paper. Next the concentrate was poured into glass Petri dishes and brought to dryness in an oven at 60°C.

4. PRELIMINARY PHYTOCHEMICAL SCREENING - Leaf extract was taken in a concentration of 1mg/ml and testes were carried out for phytochemical screening (Kumar *et al.*, 2009).

5. ANTI-BACTERIAL ACTIVITY (Venkadanagarju *et al.*, 2010)

Micro organism used - 4 species of bacteria namely *Aeromaonas spp*, *Pseudomonas aerogenosa*, *Klebsiella pneumonia* and *Streptococcus pyogenes*

Preparation of Inoculums was carried out by a method of Shubashini k, 2008.

Preparation of bacterial culture

- Organisms-*Klebsiella pneumonia*, *Streptococcus pyogenes*, *Pseudomonas aerogenes*, *Aeromonas spp*.
- Media-nutrient agar media
- Agar plate-nutrient agar plate
- Incubation conditions-incubator set at 37°C for 24 hrs.

Bioassay - The standard agar well diffusion was carried out by a method of Divakar Golli *et.al* 2002. Plates were swabbed (sterile cotton swabs) with 24h old-broth culture of bacteria and fungi. Wells were made in each plate. Stock solution of plant extract was prepared at different concentration (0%, 25%, 50%, 75%, and 100%).Plant extract were added by using sterile syringe into the wells and allowed to diffuse at room

RESULTS AND DISCUSSION

PHYTOCHEMICAL SCREENING - Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism.

Table: 1 Phytochemical analysis of methanolic leaves extract of *E.milii*

| Phytochemicals | Methanol |
|--------------------|----------|
| Alkaloids | + |
| Antraquinonus | - |
| Cardiac glycosides | + |
| Carbohydrates | - |
| Flavanoids | + |
| Phenol | + |
| Proteins | - |
| Saponins | - |
| Steroids | - |
| Tannins | + |
| Terpinoids | - |

+ Presence of compounds, - Absence of compounds

ANTI-BACTERIAL ACIVITY

The inhibitory effect of *Euphorbia milii* leaves methanolic extract was evaluated against bacterial strains. Methanolic extract (100%) of the leaves displayed good antibacterial activity against all the test organisms.

Figure-1. Phytochemical screening of methanolic leaf extract of *E.milii*

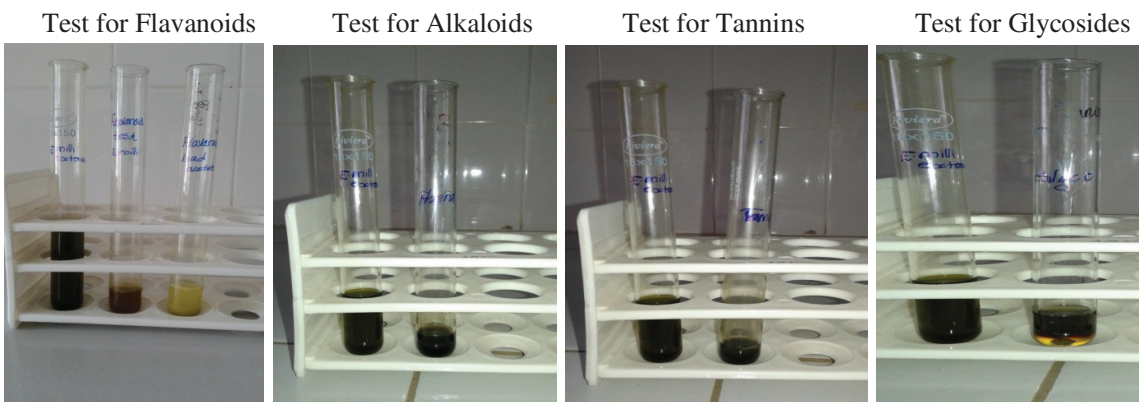


Table.2 Antibacterial activity of methanolic leaves extract of *E.milii*

| S.No | Microorganisms | Solvent (mm) | 25% (mm) | 50% (mm) | 75% (mm) | 100% (mm) | Control (mm) |
|------|-------------------------------|--------------|----------|----------|----------|-----------|--------------|
| 1 | <i>S. pyogenes</i> | 3 | 8 | 14 | 20 | 25 | 26 |
| 2 | <i>Pseudomonas aeruginosa</i> | 2 | 10 | 18 | 25 | 23 | 30 |
| 3 | <i>Klebsiella pneumoniae</i> | 4 | 15 | 18 | 20 | 25 | 30 |
| 4 | <i>Aeromonas spp.</i> | 8 | 20 | 25 | 15 | 25 | 25 |

Methanolic extract inhibited the growth of all tested microorganisms with large zones of inhibition ranged from 15-30 mm. The standard antibiotics vancomizine were found to have zone of inhibition 25-30mm. The inhibition zone of methanol (solvent) was almost less for all the test microorganisms.

In this study phytochemical screening of methanolic leaf extract of *E.milii* showed the presence of secondary metabolites such as Alkaloids, Flavanoids, Tannins, Glycoside and Phenol etc. However to confirm the phytochemical responsible for its therapeutic functions, further extensive phytochemical studies need to be carried out. The current study shows the evident that the plant extracts of *E.milii* is rich in secondary metabolites and potentially effective in wide range of disorders.

The antibacterial activity of plant extract of *E.milii* showed varied inhibitory effects on gram positive and gram negative strains in agar well diffusion method. More inhibition constant is noted in 100% plant methanolic extract.

On the basis of the present study, further pharmacological studies will be needed to isolate the bioactive compound(s) and investigate the antimicrobial activities against a wider range of pathogenic microorganisms. Antitumor studies will carried out further. Extensive study will provide a good source of medicinally important drugs in future.

Figure-2. Antibacterial activity against *Euphorbia milii*

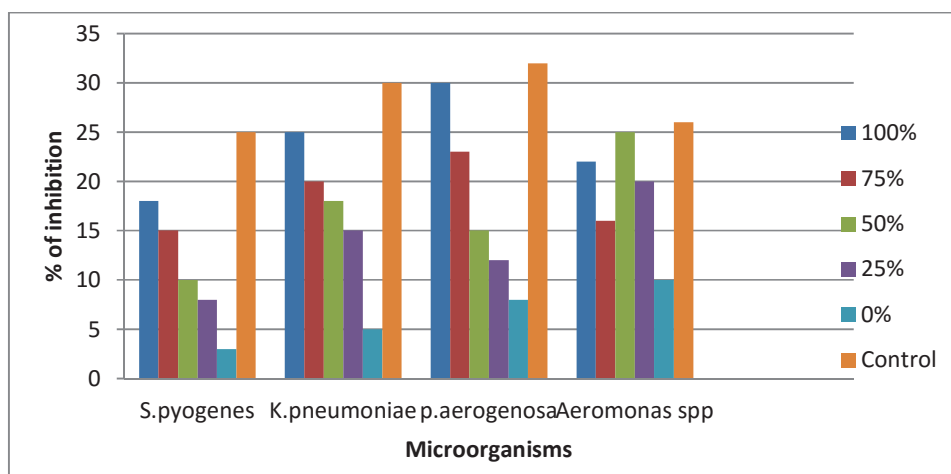
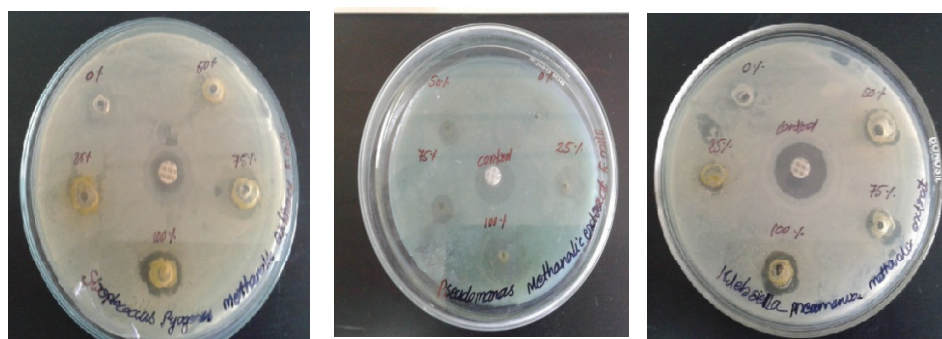


Fig. 3 Antibacterial activity of *E. milii*



Streptococcus pyogenes

Pseudomonas aeruginosa

Klebsiella pneumoniae

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