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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL EVALUATION OF *EUPHORBIA MILII* LEAF EXTRACTS

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ABSTRACT

The present work explores the Phytochemical analysis and Antibacterial activity of leaves of *Euphorbia milii* plant. Leaf extracts of *E.milii* were prepared in Hexane, Chloroform, Ethyl acetate, Acetone, Ethyl alcohol, Methanol and Water solvents. The resulted extracts of the plants were screened for Phytochemicals like carbohydrates, Tannins , Steroids, Terpenoids, Saponins, Flavanoids, Alkaloids and Soluble starch and for antibacterial activity against *Micrococcus luteus MTCC 106, Arthrobacter protophormiae MTCC 2682, Rhodococcus rhodochrous MTCC 265, Alcaligens faecalis MTCC 126, Proteus mirabilis MTCC 425, Enterobacter aerogenes MTCC 10208 , Proteus vulgaris MTCC 426, Bacillus megaterium MTCC 428, Enterococcus faecalis MTCC 439, Streptococcus mutans MTCC 497, Salmonella enterica MTCC 3858, Staphylococcus aureus MTCC 737, Pseudomonas aeruginosa MTCC 1688 and Bacillus subtilis MTCC 441.* Of the seven solvent extracts of leaves of *E. milii*, ethyl alcohol, ethyl acetate and aqueous extracts were found to be more positive towards the presence of maximum phytochemical constituents. Phytochemicals like Flavonoids and alkaloids were present in all the solvent extracts. Ethyl alcohol and ethyl acetate extracts of *E. milli* were found potential and displayed very good antibacterial activity against both Gram positive and Gram negative bacteria. The extracts that exhibited antibacterial activity were further subjected to the determination of the MIC and MBC using different concentrations. The MIC values ranged from 25mg/ml to 75mg/ml and MBC values from 50mg/ml to 100mg/ml. The results of this study revealed the antibacterial potential of the *E.milii* leaf material.

KEYWORDS: *E.milii*, Phytochemicals, Antibacterial activity, MIC, MBC.

INTRODUCTION

Medicinal plants are gifts of nature used to cure number of human diseases. To promote the proper use and to determine their potential as sources for new drugs, it is essential to study the details of the medicinal plants (Paresh *et al*., 2007). Antibiotics have saved the lives of millions of people and have contributed to the major gains in life expectancy over last centuries. However, the clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistance (MDR) pathogens (Bandow *et al*., 2003). Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases (Kumaraswamy *et al*., 2003). Hence, there is a need for novel natural compounds that can be obtained from the plants or microorganisms (Sharief Md and Umamaheswara rao, 2011).

The genus *Euphorbia* of the euphorbiaceae family is the largest one of medicinal plants widely distributed in most parts of China and Pakistan (Banis *et al*., 2007). *E.milii* is used for ornamental purpose in many countries, however in Nepal, the latex is used to treat sprains (Manandhar *et al*., 2011). While in China, it is used for the treatment of cancer and hepatitis. *E. milli* crude latex showed potent molluscicide action due to its toxic effect to mammals (Vasconcelos *et al*., 1986). *E milii* crude extract and various fractions contained excellent analgestic activity on dose dependant manner (Rauf *et al*., 2012).This work was aimed at screening of the plant, *E.milii* for its phytochemical constituents and antibacterial activity and to look in to the possibility to explore it as a potential source of bioactive principle.

MATERIALS AND METHODS

Chemicals

Nutrient agar medium, Dimethyl sluphoxide, Organic solvents i.e Hexane, Chloroform, Ethyl acetate, Acetone and Ethyl alcohol were obtained from Himedia, Mumbai.

Preparation of leaf extracts

The *E.milli* plants were collected from different places of Hyderabad and authenticated by a plant taxonomist from the Department of Botany, Osmania university. The leaves were shade dried at room temperature and ground into powder using grinder. The powder was extracted with hexane, chloroform, ethylacetate, acetone, ethylalcohol, methanol and water. All the extracts were evaporated using rotary evaporator. The resulted crude extracts were preserved in tightly packed containers for further analysis.

Tested Bacteria

The antibacterial activity of the crude extracts was determined on nine Gram positive viz., *Micrococcus luteus* MTCC 106, *Arthrobacter protophormiae* MTCC 2682, *Rhodococcus rhodochrous* MTCC 265, *Bacillus subtilis* MTCC 441, *Bacillus megaterium* MTCC 428, *Enterococcus faecalis MTCC 439*, *Streptococcus mutans* MTCC 497, *Staphylococcus aureus* MTCC 737, *Lactobacillus acidophilus* MTCC 10307 and six Gram negative bacteria namely *Alcaligens faecalis* MTCC 126, *Proteus mirabilis* MTCC 425, *Enterobacter aerogenes* MTCC 10208, *Proteus vulgaris* MTCC 426, *Salmonella enterica* MTCC 3858 and *Pseudomonas aeruginosa* MTCC 1688.

Phytochemical Screening

The crude extracts of the leaves of *E.milli* were subjected to different kinds of chemical tests to investigate the presence or absence of secondary metabolites such as flavanoids, alkaloids, saponins, terpenoids, steroid, carbohydrates, tannins by using standard protocols from Pharmacopia.

Antimicrobial activity

The agar well diffusion method was used to determine the antibacterial activity of the extract (Nagababu and Umamaheswara Rao, 2014). Bacterial suspensions of different bacteria were prepared by using 24hours old bacterial cultures. About 0.3 ml of the each bacterial suspension was mixed in separate 15 ml aliquots of sterilized molten state nutrient agar medium and poured into oven sterilized petridishes to prepare nutrient agar plates. After solidification, wells were bored in each plate by using sterile cork borer of 6mm diameter. A minute quantity of sterile agar suspension was added to the well to seal the base of each well, before dispensing 100µl of the sample which was prepared by dissolving 100mg of sample in 1ml of DMSO. In a separate well, DMSO was also dispensed to maintain the control. The plates were incubated at 37^{o}C for 24 hrs. After incubation, the diameter of the zone of inhibition was measured. For each sample and bacterial species, triplicates were maintained. Streptomycin standard antibiotic was used as positive control in the concentration of 10µg/ml DMSO.

Determination of MIC and MBC

Using broth dilution method subsequently followed by plating technique, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were

determined at different concentrations viz., 12.5mg, 25 mg, 50mg, 75 mg and 100mg per ml on those bacterial strains which showed Zone of inhibition against the plant extracts.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical component data of *E.milii* leaf extracts is presented in table-1. Results obtained from the qualitative phytochemical tests carried out on the leaf extracts revealed that the leaf extracts of *E.milli* contained a wide array of phytochemicals. Flavanoids were present in all the solvent extracts, except the chloroform. Flavanoids have been reported to have antibacterial and antimicrobial properties (Tsuchiya *et al*., 1996). Flavanoids were found in *E.milii* which are water soluble antioxidants that prevents oxidative cell damage, exhibits antiseptic, anticancer, anti inflammatory effects and mild hypersensitive properties (Okwu, 2004). Ethyl alcohol, ethyl acetate and aqueous extracts of *E.milli* leaf were more positive towards maximum phytochemicals like Flavanoids, Terpenoids, Alkaloids, Saponin and Steroids. Alkaloids were found in all the solvent extracts. Soluble starch was not found in any solvent extract, except the methanol. Terpenoids were present in hexane, ethyl alcohol, ethyl acetate and methanol extracts. Terpenoids are reported to have anti-inflammatory, antiviral, anti-malarial, antibacterial activities (Mahato *et al*., 1997). Except the hexane and acetone extracts, tannins were positive in the other solvent extracts. Tannins have been reported for their wound healing, anti inflammatory and analgestic properties (Ayinde *et al*., 2007). The presence of these phytochemicals in the *E.milli* leaf extracts suggest that the plant is pharmacologically potential.

Table 1: Phytochemical analysis of *E. milii* leaf extracts in different solvents

S. No.	Phytochemicals	H	C	E	A	EA	M	Aq
1	Carbohydrates	+	+	+	-	+	+	+
2	Monosaccharides	+	+	+	-	+	+	+
3	Free reducing Sugars	+	+	+	-	+	-	+
4	Combined reducing sugars	-	-	-	-	+	-	+
5	Tannins	-	+	+	-	+	+	+
6	Steroids	+	-	+	-	+	+	+
7	Cardiac glycosides	-	-	+	-	+	+	+
8	Terpenoids	+	-	++	-	++	++	-
9	Saponins	-	-	+	-	+	-	++
10	Flavanoids	+	-	+	+	+	++	+
11	Soluble starch	-	-	-	-	-	++	-
12	Alkaloids	+	+	+	+	+	+	+

H - Hexane; C - Chloroform; E - Ethyl acetate; A - Acetone; EA - Ethyl alcohol; M - Methanol; Aq – Water.

Antibacterial activity

The antibacterial activity results of different solvent extracts of leaves of *E.milli* on different Gram positive and Gram negative bacteria are given in figures 1 and 2, respectively. Ethyl acetate extract of *E.milii* leaf exhibited the most pronounced activity on all the test organisms except the *Salmonella enterica*. All the solvent extracts exhibited the antibacterial activity against the Gram positive *Bacillus megaterium* and Gram negative *Alcaligens faecalis*. Chloroform extract on *Streptococcus mutans* , ethyl acetate extract on *Staphylococcus aureus, Poteus vulgaris and Pseudomonas aeruginosa*, ethyl alcohol extract on *Poteus vulgaris* and aqueous extract on *Bacillus megaterium, Staphylococcus aureus* and *Salmonella enterica* showed larger inhibition zones than the positive control. The positive control (Streptomycin) had no significant activity against *Rhodococcus rhodochrous and Enterobacter aerogens.* But, these two organisms were found sensitive to some of the solvent extracts of *E. milii*. Of all the extracts, hexane extract was found to be effective against only seven of the fifteen bacteria tested. The observed antibacterial effects on the isolates are believed to be due to the presence of tannins and flavonoids which have been shown to possess antibacterial properties (Awoyinka *et al*., 2007; Cowan, 1999). Some pictures exhibiting the zone of inhibitions are given in Plate -1.

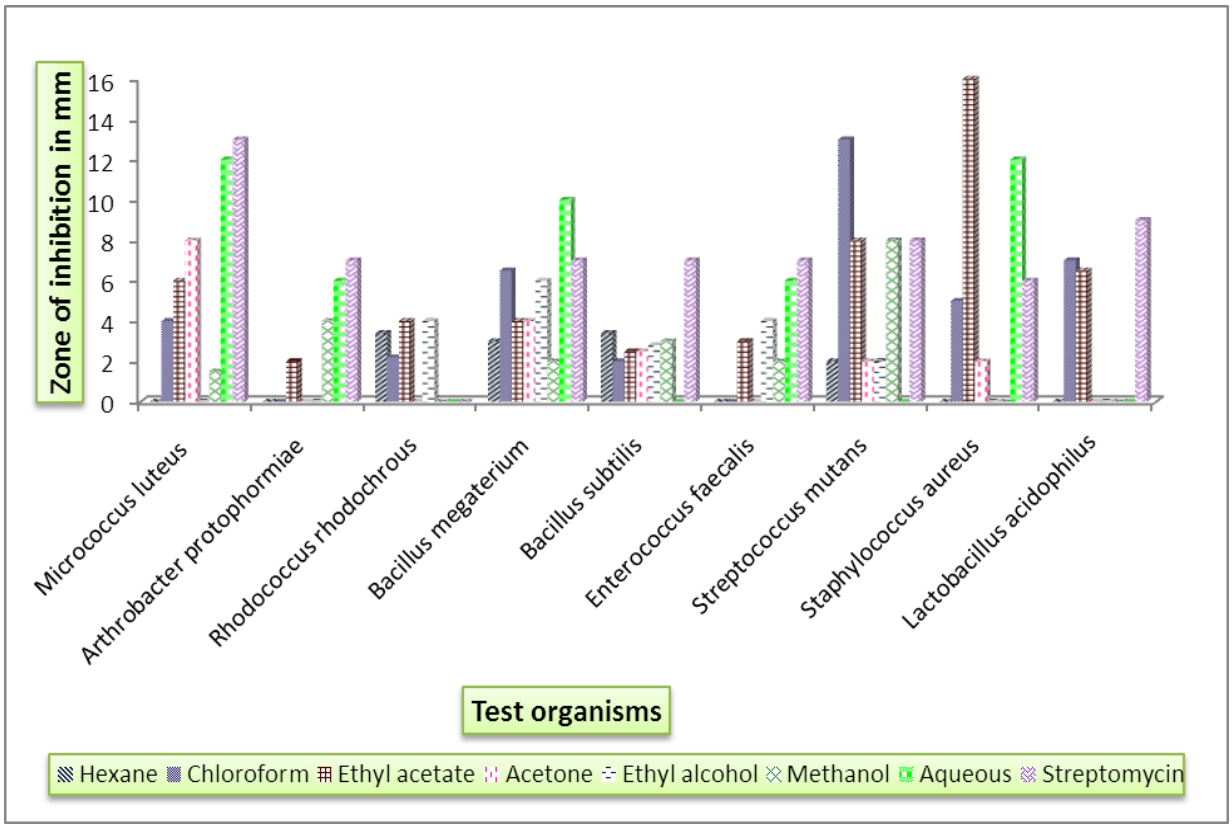


Fig 1: Antimicrobial activity of *Euphorbia milii* leaf extracts on Gram positive Bacteria

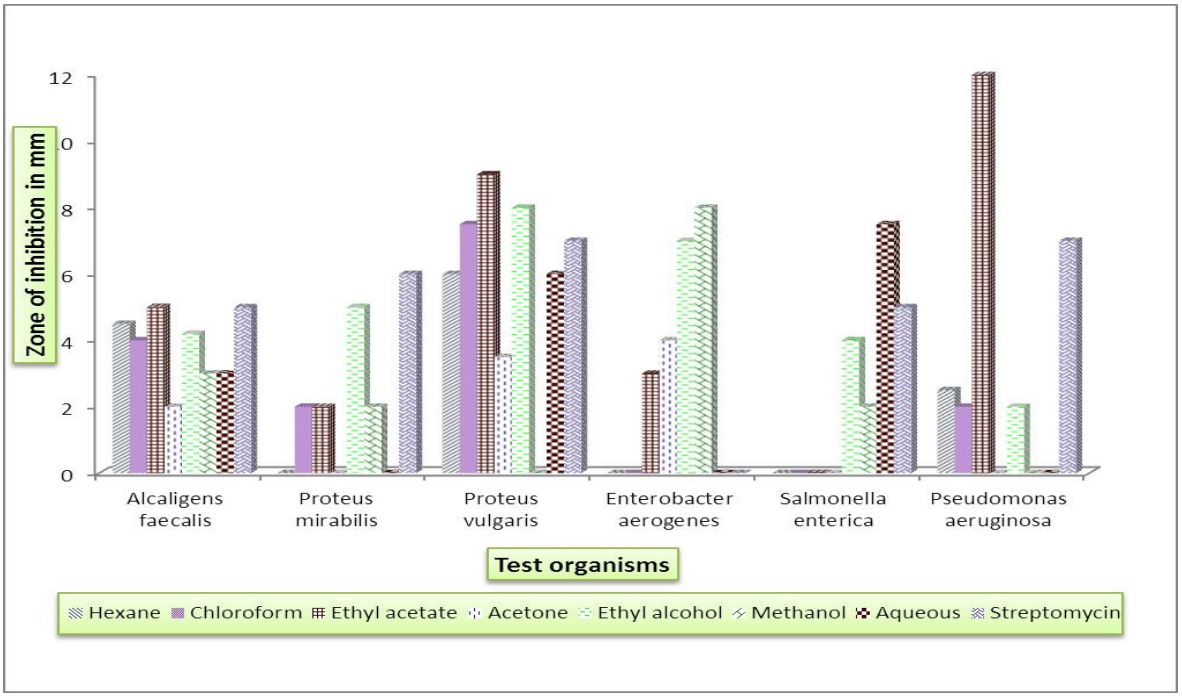
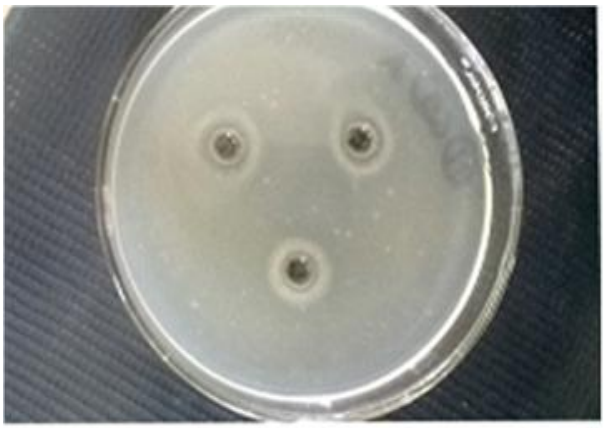
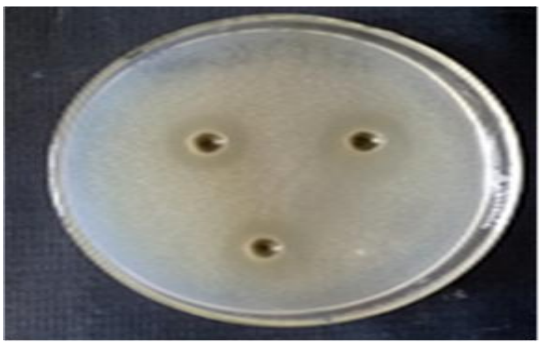


Fig 2: Antimicrobial activity of *Euphorbia milii* leaf extracts on Gram Negative Bacteria

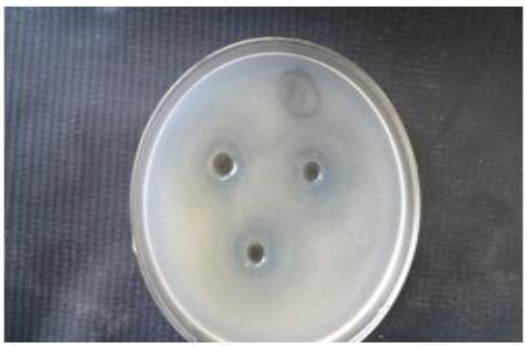
a) Aqueous extract against *Staphylococcus aureus*



b) Chloroform extract against *Bacillus megaterium*



c) Acetone extract against *Micrococcus luteus*



d) Ethyl acetate extract against *Proteus vulgaris*

Plate 1: Antibacterial activity (zones of inhibition) of *Euphorbia milii* leaf extracts

MIC and MBC

The MIC and MBC studies were done for the Chloroform, Ethyl alcohol, Ethyl acetate and Aqueous extracts and the values are tabulated in table-2. Based on the preliminary screening, chloroform, ethyl alcohol, ethyl acetate and aqueous extracts that revealed the potent antibacterial activity were further tested. The MIC data obtained revealed the variability in the inhibitory concentrations of each extract. The value of MIC was found to be in the range of 25 to 75 mg/ml.The MIC of Chloroform and Ethyl acetate extracts was found to be 25 mg/ml for *Lactobacillus acidophilus* and *Pseudomonas aeruginosa*. The MIC values of Ethyl alcohol and Aqueous extracts were found to be in the range of 50 to 75 mg/ml. The MBC values of Ethyl acetate and aqueous extracts ranged between 50-100mg/ml.

Table 2: MIC and MBC(mg/ml) values of *E. milii leaf extracts*

Test organisms	Chloroform		Ethyl acetate		Ethyl alcohol		Aqueous	
	MIC	MBC	MIC	MBC	MBC	MIC	MBC	MBC
Micrococcus luteus MTCC 106	--	--	--	--	--	--	50	75
Arthrobacter protophormiae MTCC 2682	--	--	--	--	--	--	75	100
Bacillus megaterium MTCC 428	75	100	75	100	--	--	75	100
Enterococcus faecalis MTCC 439	--	--	--	--	--	--	75	100
Streptococcus mutans MTCC 497	75	100	75	100	--	--	--	--
Staphylococcus aureus MTCC 737	--	--	50	75	--	--	50	75
Lactobacillus acidophilus MTCC 10307	25	50	25	50	75	50	--	--
Alcaligens faecalis MTCC 126	--	--	50	75	--	--	--	--
Proteus vulgaris MTCC 426	25	50	75	100	50	75	75	100
Enterobacter aerogenes MTCC 10208	--	--	75	100	--	--	--	--
Pseudomonas aeruginosa MTCC 1688	--	--	25	50	--	--	75	100

CONCLUSION

The results justify the use of this plant in traditional medicine for the treatment of various kinds of infectious diseases. From the above study, it can be concluded that *E.milii* plants has great potential as antimicrobial agent against Gram positive and Gram negative bacteria. Hence, this study would lead to the development of some stable, biologically active compounds which can be employed in the formulation of antibacterial agents.

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