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SCREENING OF PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF LEAVES EXTRACT OF *EUPHORBIA MILLI*

PRAGYA PANDEY, MAHESH KUMAR, USHA TIWARI , NEHA K. MEHRA

Institute of Biotechnology, G. B. Pant University of Agriculture & Technology, Patwadanger, Nainital, Uttarakhand, India

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Abstract: Plants contain many bioactive compounds, which are primary or secondary metabolites and have ability to cure diseases with no side effects. Present study explores the phytochemical properties of *Euphorbia milli* plant leaves extracted in petroleum ether, ethyl ether, chloroform, ethyl alcohol, and aqueous. Antimicrobial activities against *Staphylococcus aureus, Klebsilla pneumonia, Escherichia coli,* and *Pseudomonas aeruginosa* in the extracts were evaluated by well diffusion method. The Minimum inhibitory concentration (MIC) of different extracts ranged between 50 to 250 µl. Qualitative phytochemical analysis revealed presence of alkaloids, flavonoids, glycosides, reducing sugars, saponins, and terpenoids. Ethanol extract showed highest activity against all the pathogens, with the maximum zone of inhibition (25.5±0.35 mm) demonstrated against *Staph. aureus*. The results indicated that *Euphorbia milli* may be used for the treatment of various ailments of skin diseases, the treatment of warts, cancer and hepatitis.

Keywords: *Euphorbia milli*, Phytochemical, Antimicrobial activity, MIC

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Corresponding Author: MS. PRAGYA PANDEY

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INTRODUCTION

Plants contain numerous bioactive compounds as secondary metabolites (alkaloids, phenols, tannin, steroids etc.) and these are used as anti-diabetic, chemotherapeutic, anti-inflammatory, anti-arthritic agents [1,2]. The antimicrobial compounds found in plants may prevent bacterial infection by different mechanisms and may have clinical value in treating resistant microorganism strains. Many gram negative bacteria like *E. coli P. aeruginosa*, which act as opportunistic pathogens, have become resistant to most of the antibiotics [3]**.** Other bacteria like *Staphylococcus aureus* which is a commonly known pathogen that is responsible for infections like pneumonia diabetes, cancer, vascular disease, and lung disease have developed resistance to most classes of antibiotics (methicillin, etc.) [4,5].

Euphorbia milli (Euphorbiaceae) is an ornamental shrub widely distributed in tropical countries, and used as a folk medicine for the treatment of various ailments such as skin diseases, intestinal parasites and warts [6,7]. It has been reported to possesses antiarthritis, anticancer, anticonvulsant, antidiabetic, anti-eczema, anti-inflammatory, antioxidant, antispasmodic, antitumor and antitussive properties and hormonal and myelopoiesis properties [8]. Latex of the plant is used as ear drops and in the treatment of boils, sore and promoting wound healing [9, 10]. The present study was under taken to explore the phytochemical properties and antioxidant and antimicrobial activity of *Euphorbia milli* against major pathogens.

MATERIALS AND METHOD

Collection of plant leaves

The fresh leaves of *Euphorbia milli* were collected from the garden of the Institute of Biotechnology, Patwadanger, Nainital. The leaves were washed 2-3 time with distilled water and allowed to shade dried at room temperature. Dried samples were ground into fine powder using grinder.

Preparation of leaf extract

Twenty grams of fine powder was successively extracted with 500 ml of each solvent, *viz*. petroleum ether, di ethyl ether, chloroform, ethanol and water for 48 hrs using soxhlet apparatus. Each solvent extract was collected and dried in water bath to obtain the crude extracts. The yield percentage of each extract was estimated according to Parekh and Chanda (2007) as:

Dry weight / Dry material weight × 100

Qualitative Phytochemical Analysis

The phytochemical analysis of crude extract of each solvent was done to confirm the presence of alkaloids, glycoside, carbohydrates, steroids, terpenoids, tannins and phenols, saponins, flavonoids compound, according to standard protocols [11].

Antimicrobial Activity

Bacterial cultures and Media

Staphylococcus aureus (MTCC 96), *Klebsiella pneumoniae* (MTCC 432), *Escherichia Coli* (MTCC 43), *Pseudomonas aeruginosa (*MTCC 1688) were used for antibacterial assays. These selected bacteria were inoculated into nutrient broth and incubated for overnight at 37°C.

Standardization of extracts

The crude extracts were weighed and stock solution prepared at a concentration of 100 mg/ml (w/v) by diluting a 100 mg of crude extract in 1 ml of 99.5% Dimethylsulfoxide (DMSO). The stock solution was then preserved at 4°C in airtight bottle until further used.

Determination of antimicrobial activity

Antibacterial assay of the crude extracts were carried out by agar- well diffusion method. Total 20 µl of a 24 hrs fresh culture of test pathogens were evenly spread over the surface of sterile Mueller-Hinton agar plates using sterile spreader. Four wells were aseptically made in each plate using a sterile cork borer. Different amount, *viz.* 100, 150, 200 and 250 µl of prepared stock solution at a concentration of 100mg/ml (w/v) of each extract was added into the wells with a micro-pipette having sterile tips and allowed to diffuse at room temperature for 30 minutes. The plates were incubated at 37^{o}C for 24 hrs. Separate plates with seeded cultures were used as a control. Gentamicin was used as positive control for bacteria and DMSO without extract used as negative control. The plates were observed, clear zone of inhibition (mm) around each well indicating the presence of antimicrobial activity was measured. Each experiment was performed twice.

Determination of minimum inhibitory concentration (MIC)

Modified agar well diffusion method was used to measure the MIC of crude extracts of *E. milli* at concentration of 100 µg/ml. The minimum concentration of each extract showing a clear zone of inhibition was considered to be MIC [12].

Statistical Analysis

The results were analyzed by simple arithmetic mean ± SE of two replicates in each test.

RESULTS

There was maximum yield in ethanol extract (23.1%) followed by aqueous extract (15.35%), whereas minimum yield was in chloroform extract (Table 1).

The phytochemical screening of *E. milli* leaf extracts revealed the presence of secondary metabolites like alkaloids, carbohydrates, saponins, flavonoids, tannins, steroids, terpenoids, proteins, and glycosides. Its ethyl ether extract showed the absence of flavonoids, reducing sugar, tannin and glycosides (Table 1).

Antibacterial activity

The leaves extracted in different solvents had varied antimicrobial properties. All extracts had moderate activity against *K. pneumoniae.* The maximum inhibition of petroleum ether and ethyl ether extract were observed, with highest activity on *E. coli* (23.5±0.35 mm) and *P. aeruginosa* (22.5±0.34 mm), respectively as compared to the positive control (Gentamicin) showing inhibition zone (21±0.00 mm) and (20.5±0.71mm), respectively. Inhibition zone of positive control (Gentamicin) on other two pathogens were recorded as (22.5±0.71mm) and (28±1.41mm) inhibition zone against *K. pneumoniae* and *S. aureus,* respectively(Table 2,3,4 and 5). No activity of negative control (DMSO) was recorded against any bacteria.

Minimum Inhibitory Concentration (MIC)

The value of MIC of various concentrations of extracts was determined by well diffusion method. Aqueous and petroleum ether extract of *E*. *milli* showed their activity against bacteria at 150 µl concentration of 100 mg/ml (Table 7).

DISCUSSION

Activities of medicinal plants were safe as compared with costly synthetic drugs that have adverse effects [13]. Previous studies have supported that various plants and herbs are beneficial to animal and human health. In this study, the phytochemical analysis confirms that *E. milli* leaves extract contains the bioactive substances, i.e. flavonoids, which was found in all the solvent extracts except ethyl ether extract while tannins were present in ethanol and chloroform leaf extracts. Tannins and flavonoids are thought to be responsible for medical properties in the plants [14, 15]. The wound healing property of this plant can be attributed to the presence of tannins [16-18]. Terpenoids were present in petroleum ether, ethyl alcohol and aqueous leaf extracts. Terpenoids are reported to have anti-inflammatory, antiviral, anti-malarial and antibacterial activities [19,20]. Flavonoids are water soluble antioxidants that prevent oxidative cell damage, exhibits antiseptic, anticancer, anti-inflammatory effects and mild hypersensitive properties. The plant extracts showed antibacterial activity against test

pathogen at low MIC value. The ethanol extract have shown relatively greater activity with maximum zone of inhibition (25.5±0.35 mm) against *S. aureus* followed by *E. coli* (22±0.70 mm). This believed is to be due to the presence of tannins and flavonoids which have been shown to possess antibacterial properties [21,22]. Effectiveness of organic extracts demonstrates the highest activity than aqueous extract. Aqueous extract demonstrated moderate activity against all the pathogenic bacteria with maximum inhibition zone (16.5±0.354 mm) against *S. aureus*.

The present study showed that the leaf extracts of *E. milli* have considerable antibacterial activity and ethanol and chloroform extracts were effective against pathogens. These results may be useful for further research, in which the extract could possibly be exploited for pharmaceutical use.

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Table 1. Per cent(%) yield and phytochemical screening of various solvent extracts

Solvent extract	% yield of *Euphorbia milli*	Phytochemical constituents
Petroleum ether	6.55	alkaloids, flavonoids, terpenoids, steroids, proteins
Ethyl ether	2.5	alkaloids, steroids
Chloroform	2	Carbohydrates, alkaloids, flavonoids, tannin, steroids
Ethanol	23.1	Carbohydrates, alkaloids, flavonoids, steroids, tannins, proteins, saponins, glycosides, terpenoids
Aqueous	15.35	Carbohydrates, alkaloids, flavonoids, terpenoids, saponins, proteins

Table 2. Antibacterial activity of *E. milli* leaf extract of petroleum ether

Test Pathogens	Zone of Inhibition(mm)*				
	Extract concentrations(µl)				Positive control
	100	150	200	250	Gentamicin
Pseudomonas aeruginosa	-	11±0.00	13±0.71	16.5±0.35	20.5±0.71
Staphylococcus aureus	10.5±0.34	13±0.71	16.5±0.35	18±0.71	28±1.41

Klebsiella pneumoniae	-	11±0.55	12.5±0.35	16.5±0.33	22.5±0.71
Escherichia coli	12.5±1.06	14.5±0.35	18.5±0.35	23.5±0.35	21±0.00

* values of inhibition zone are in mean±SEM.

Table 3. Antibacterial activity of *E. milli* leaf extract of ethyl ether

Test Pathogens	Zone of Inhibition(mm)*				
	Extract concentrations(µl)				Positive control
	100	150	200	250	Gentamicin
Pseudomonas aeruginosa	17±0.72	19.5±0.35	20.5±0.31	22.5±0.34	20.5±0.71
Staphylococcus aureus	12.5±0.35	16±0.71	16±0.44	20±0.56	28±1.41
Klebsiella pneumoniae	6±0.00	12.5±0.35	14.5±0.34	16.5±0.33	22.5±0.71
Escherichia coli	14.5±0.35	16.5±0.31	18.5±0.31	20.5±0.33	21±0.00

* values of inhibition zone are in mean±SEM.

Table 4. Antibacterial activity of *E. milli* leaf extract of chloroform

Test Pathogens	Zone of Inhibition(mm)*				
	Extract concentrations(µl)				Positive control
	100	150	200	250	Gentamicin
Pseudomonas aeruginosa	14.5±1.00	18±1.15	20.5±0.57	20.5±1.54	20.5±0.71
Staphylococcus aureus	13.5±0.58	13.5±0.58	16.5±0.58	17±1.53	28±1.41
Klebsiella pneumoniae	10.5±1.53	11.5±0.58	16±1.15	18±1.53	22.5±0.71
Escherichia coli	12.5±0.58	15.5±1.00	17±1.00	20.5±0.58	21±0.00

*values of inhibition zone are in mean±SEM.

Table 5. Antibacterial activity of *E. milli* leaf extract of ethanol

Test Pathogens	Zone of Inhibition(mm)*

	Extract concentrations (µl)				Positive control
	100	150	200	250	Gentamicin
Pseudomonas aeruginosa	14.5±0.353	15.5±0.35	17.5±0.35	18.5±0.35	20.5±0.71
Staphylococcus aureus	13.5±0.354	17.5±0.35	21±0.71	25.5±0.35	28±1.41
Klebsiella pneumoniae	11±0.00	14±0.71	14±0.71	17.5±0.42	22.5±0.71
Escherichia coli	17.5±0.354	19.5±0.35	20.5±0.35	22±0.70	21±0.00

*values of inhibition zone are in mean±SEM.

Table 6. Antibacterial activity of *E. milli* leaf extract of aqueous

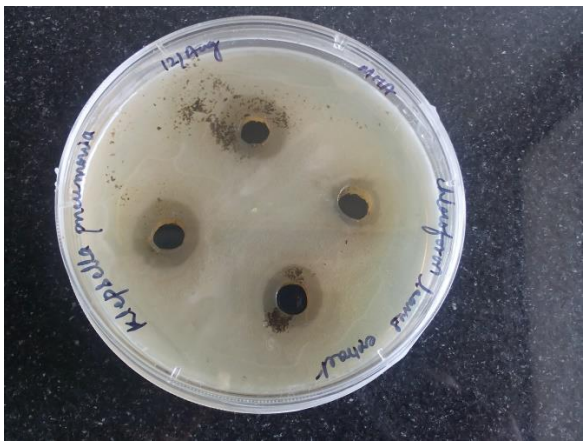
Test Pathogens	Zone of Inhibition(mm)*				
	Extract concentrations(µl)				Positive control
	100	150	200	250	Gentamicin
Pseudomonas aeruginosa	-	13.5±0.354	14.5±0.354	15±0.00	20.5±0.71
Staphylococcus aureus	11±0	13.5±1.061	15±0.707	16.5±0.354	28±1.41
Klebsiella pneumoniae	7.5±0.354	8±1.414	11.5±0.354	12.5±0.354	22.5±0.71
Escherichia coli	9.5±0.354	9±0.707	13±0.707	15.5±0.354	21±0.00

*values of inhibition zone are in mean±SEM.

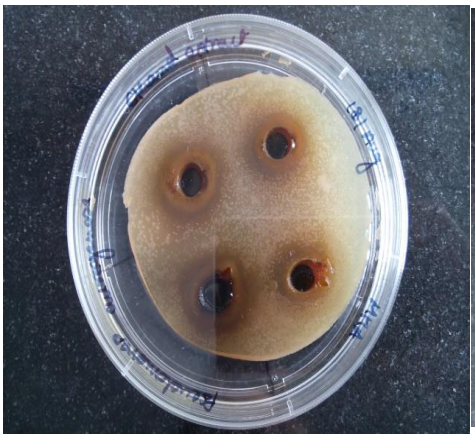
Table 7. Minimum inhibitory concentration of leaf extracts of *Euphorbia milli* in µl

Test pathogens	P.**ether**	E.**ether**	**Chloroform**	**Ethanol**	**Aqueous**
P.aeruginosa	150	100	100	100	150
S.aureus	100	100	100	100	150
K.pneumonia	150	150	150	150	150
E.coli	150	100	100	100	100

(a)Ethanol extract against *E. coli*



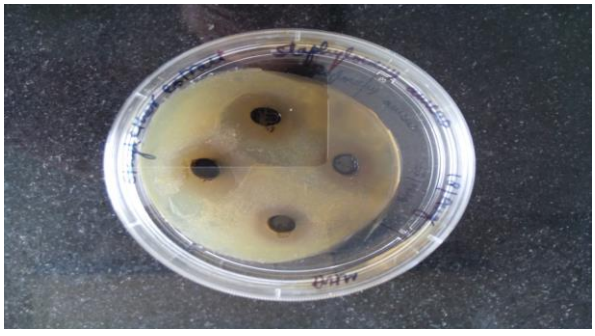
(b) Chloroform extract against *K. pneumonia*



(c)

(d)

(c) and (d): Aqueous and Chloroform extract against *P. aeruginosa*



(e) Ethyl ether extract against *Staphylococcus aureus*

Plate 1. Zone of inhibition of leaf extract on different bacteria

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