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Phytochemical Study and Antimicrobial Analysis of *Euphorbia milii* Extract

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Abstract: *Antimicrobial activity of Euphorbia milii extract and its subfraction against Gram-Negative , Gram –Positive bacteria and unicellular Yeast were evaluated with the help of Agar disc diffusion technique. Gram-Positive organism Staphylococcus aureus, Gram negative organism Escherichia coli (E.coli) were grown on Hexane, ethyl acetete, acetone, Methanol and water extract of Euphorbia milii (Euphorbiaceae). Hexane, Acetone and Methanol extract in the concentration of 5 µg/mL showsconsiderable inhibition zone on Staphylococcus aureus as compared to other extracts. The Hexane, Acetone and Methanol extract in the concentration of 5µg/mL shows considerable inhibition zone on Escherichia coli (E. coli) as compared to Ethyl Acetate and water extract which was compared with the inhibition zone produced by standard Amikacin Sulphate 1µg/mL .*

Phytochemical investigation of Euphorbia miliirevealed the existence of sub-ordinate metabolites like Alkaloids, Phenols, Glycocids, Saponins, Terpenoids, Tannins and Steroids. It contributes to its antimicrobial properties these compounds make the plant a potential source of natural antimicrobial agent that could be used in the development of new treatments for infections.

Keywords: *Euphorbia milii, Euphorbiaceae, Antimicrobial activity, Flavanoid and Phenol.*

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Introduction:

The family Euphorbiaceae consist of 2000 species(Davis et.al.1988). The genus *Euphorbia* is the largest genus of medicinal plants and widely cultivated in several parts of India China and Pakistan.People uses *Euphorbia milii* for the ornamental purposes. However in Nepal the latex is used to treat sprains (arsule et.al., 2017). Various species of *Euphorbia* are traditionally being used as a folk medicine for the treatment of different diseases due to its high theraputic potential such as warts, eradication of intestinal parasites and eczema (Bani et al., 2007). With no harmful side effects it's phytochemicals show protection and healing deeds to the body.

Most challenging factor during the treatment of fungal and bacterial infection is there antimicrobial resistance potential due to which it led to the upsurge of novel

antimicrobial treatment options (Nainu. et. al., 2021).Medicinal plants contain various phytochemicals and bioactive compounds which have potential to protect itself from microorganism, insects, and herbivore (Panda et.al., 2014). Vast variety of chemical compound with different structures are present in plant materials so it is necessary to determine chemical constituents of herbal samples. The viral infection in cells has been reported to induce a level of free radicals, therefore saturation of free radicals are done. The antioxidants nature of plants or herbs might play a crucial role to saturate free radicals (a Reactive Oxygen Species) and boost immunity towards the alleviation of viral infection (Hejrati et al.,2021;Peterhans,1997). For the characterization of the secondary metabolites of the plant most widely used techniques now days is Liquid Chromatography-Mass spectrometry (LC-MS). *Euphorbia* genus contains large number of active biological compounds.

Taxonomical classification of *Euphorbia milii*

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Order	:	Euphorbiales
Family	:	Euphorbiaceae
Genus	:	*Euphorbia*
Species	:	*milii*
Scientific Name	:	*Euphorbia milii.*

Some weeds of medicinal importance (Panda et al., 2014)

S. No.	Botanical name	Family	Part used	Diseases
1.	*Achyranthus aspera* L.	Amaranthaceae	Aerial part and Root	Gastro-intestinal disorders
2.	*Argemone mexicana* L.	Paperveraceae	Leaf and Flower	Eye inflammation, wounds
3.	*Cynodon dactylon* L.	Poaceae	Leaf and Root	Nasal bleeding and dysentery.
4.	*Oxalis corniculata* L.	Oxalidaceae	Leaf	Dysentery
5.	*Sida cordifolia* L.	Malvaceae	Whole plant	Fracture part.
6.	*Mimosa pudica* L.	Mimosaceae	Root	Toothache, snake bite.
7.	*Ageratum conyzoides* L.	Asteraceae	Whole plant	Kidney stones, cuts, wounds
8.	*Blume alacera* L.	Asteraceae	Leaf	Eczema, Ring worm
9.	*Vernonia cinerea* L.	Asteraceae	Whole plant	Filariasis.
10.	*Solanum nirginianum* L.	Solanaceae	Leaf, Fruit and Seed	Bronchial asthma, Cough,toothache.

Materials and Methods:

Plant Material: Fresh leaves, stems, flowers and latex were collected from healthy *Euphorbia milii* plants. The collected materials were shade dried at room temperature for 15 days. Then dried parts were crushed to make fine powder with the help of mortar and pastel and stored for chemical analysis..

Chemicals and reagents : H_2SO_4, Wagner's reagent, Ferric chloride NaOH, Dilluted HCl, Ninhydrine solution, Concentrated HCl, Acetic anhydride, Distilled water, Glacial acetic acid, Ethanol, Methanol, Peptone, Beef extract, NaCl, Potato infusion powder, Dextrose, Sulfuric acid and Acetone. All these chemicals were obtained from Botany store room .

Extraction : Powdered parts of plants materials were taken and soaked in a solvent using continuous hot extraction. The extract was filtered using Whatman Filter paper. Then the extract was concentrated using a rotatory evaporator.

Phytochemical analysis : Various phytochemical test were conducted to check the presence of different phytochemicals such as Alkaloids, Saponins, Quinones, Amino acids and Protein, Carbohydrates, Phenols, Flavonoids and Cardiac glycosides .

Test for alkaloids (Wagner's reagent): 3-5 drops of Wagner's reagent was added to the plant extract .

The reddish brown precipitate indicated the presence of alkaloids.

Test of Carbohydrates : 2ml of Benedict's reagent was added to 2ml of the test solution.The mixture was heated in a boiling water for 5 minutes.

Test for cardiac glycosides(Keller- Killani Test) : 2ml of plant extract was added to a test tube. 1ml of glacial acetic acid containing 1 drop of FeCl3 solution was added. 1ml of concentrated H2SO4 was carefully poured down the side of test tube to form a layer.

A reddish brown layer indicate the presence of cardiac glycosides.

Test of Flavonoids (Alkaline reagent test) : Few drops of FeCl3 solution were added to 2ml of the plant extract. Formation of intence green or black colour indicates flavonoids.

Test for tannins: 2ml of 5% FeCl3 solution was added to 2ml of the plant extract,. Formation of a dark blue , green, or black precipitate indicated the presence of tannin.

Test of Saponins (Foam Test): 5ml of plant extract was poured into test tube. The tube was shaken vigorously for 2-3 minutes then allowed to stand for 10-15 minutes. Persistent foam (1-2cm in height) indicated the presence of saponins.

Test of Phenol (Ferric Chloride Test) : 1ml of plant extract was added to a test tube. Then 2-3 drops of 1% $FeCl_3$ solution was added. Formation of a deep blue, green, or black colour indicated the presence of phenol.

Test of Protein and Amino acids: 2ml of plant extract was taken in a test tube. 2-3 drops of ninhydrine reagen was added. The mixture was heated in a water bath for 5 minutes. A blue, violet, or pink colour indicated the presence of protein and amino acids.

Test of Quinones: 1ml of the plant extract was added to a test tube. 1ml of 1% NaOH solution was added. The mixture was shaken well. Formation of a red, pink or violet colour indicated the presence of quinones.

Test of Coumarins : 1.5ml of 10% NaOH was added to 1ml plant exrtract. Appearance of yellow colourationconfirms the presence of coumarins (Nainu.A. Nurzadehm 2021).

Antimicrobial Study : Antimicrobial activity can be defined as a collective term for all the active principles (agents) that inhibit the growth of microbes such as Bacteria and Fungi. It prevents the formation of microbial colonies and may destroy microorganism. The antimicrobial studies of Hexane, Ethyl acetate , acetone , methanol and water extract of the plant *Euphorbia milii* (Euphorbiaceae) were performed on gram positive organism *Staphylococcus aureus* and gram negative organism namely *Escherichia coli* and *Proteus vulgarias* by using disc diffusion method.

Preparation of extract : Shade dried plant flower were chopped into small pieces and then ground to powdered form. 2 g of dried powder was kept in 5 separate round bottomed flasks and sample extraction was done for different solvents (distilled water, methanol and ethanol). Extraction was done with 20 ml of each solvent for 24 h. Crude extracts were then stored in refrigerator for further use.

Preparation of Bacterial Culture: One loopful of each bacterial strain were inoculated in 50ml of sterile nutrient broth and incubated for 24 hour in shaker incubator.

Assay of antibacterial activity: An assay of antibacterial activity is a laboratory procedure used to evaluate the ability of a substance (such as plant extracts, chemicals, or antibiotics) to inhibit or kill bacterial growth. Below is a general guide to performing an antibacterial activity assay:

Agar Disc Diffusion Method:

1. Prepared 20 ml of sterilized nutrient agar plates and allow them to solidify.
2. Spreaded 100 µL of bacterial suspension evenly onto the agar surface.
3. Placed sterile filter paper discs (6 mm diameter) on the agar plates.
4. Pipette specific volumes of the test substance onto the discs.
5. Incubated plates at 37°C for 24 hours.
6. Measured the diameter of the inhibition zones (in millimeters).

Results and Discussion:

Phytochemicals in plants play a key role in the growth and development of plants. Upon phytochemical

screening the crude extract exhibited the presence of carbohydrates, alkaloids, phenols and cardiac glycosides in aqueous, methanol and ethanol extracts whereas quinones were absent in all the extracts and sable (Table - 1).

Among all the extracts saponin has showed negative result and amino and protein has shown the presence aqueous extract . Flavonoid were present only in ethanol and methanol extracts and were absent in the aqueous extract.

Coumarins were present in the Methanol and Ethanol extracts and absent in the aqueous extract. Alkaloids basically involved in the defence mechanisms of plant against herbivorous. Pathogens plays a key role in plant metabolism and plant physiology carbohydrates in plants are great source of energy positive Phenolic compounds, Flavonoids and Saponin present in plant also help in the defence mechanism of the plant and Amino acids helps in protein synthesis.

Table 1. Result of phytochemical screening

S. No	Phytoconstituents	Leaf			Stem			Whole plant		
		Aq	E	M	Aq	E	M	Aq	E	M
1.	Alkaloids	+	+	+	+	+	+	+	+	+
2.	Carbohydrates	+	+	+	+	+	+	+	+	+
3.	Phenols	+	+	+	+	+	+	+	+	+
4.	Cardiac glycosides	+	+	+	+	+	+	+	+	+
5.	Flavonoids	_	+	+	_	+	+	_	+	+
6.	Coumarins	_	+	+	_	+	+	_	+	+
7.	Saponins	+	+	+	+	+	+	+	+	+
8.	Quinones	_	_	_	_	_	_	_	_	_
9.	Amino acids and Proteins	+	_	_	+	_	_	+	_	_

(Aq= aqueous, E= ethanol, M=methanol, + = positive result, - = negative result)

Anti-bacterial activity

Antibacterial activity is anything that destroys or kills bacteria or suppresses their growth or their ability to reproduce. Heat, chemicals such as chlorine and antibiotic drugs all have antibacterial infections. The toxicity to humans and other animals from antibacterial is generally considered low. Result of the antibacterial activity of the isolated extract by using different solvent (ethanol, methanol and aqueous) has been shown in table 2. The antibacterial activity of ethanol, methanol and aqueous extract of leaf, stem and whole plant of *Euphorbia milii* were inspected against the selected experiment pathogens such as *Proteus vulgaris, S. aureus* and *E.coli* by disc diffusion method (Fig 5). The various zone of inhibitions shown by different extracts are shown in the table 2. A significant inhibition of 15 mm was obtained in ethanol extracts and 13 mm from aqueous extract of *E. milii* against *S. aureus* (Kumari et al., 2017). In minimum inhibitory concentration (MIC), the methanolic extract exhibited maximum inhibition in comparison to ethanolic and aqueous extracts (Gupta et al., 2019). Antibacterial properties of a plant are used in the treatment of bacterial infections.

Test organism	Different types of extracts	Zone of inhibition (in mm)		
		Aqueous extract	Ethanolic extract	Methanolic extract
Proteus vulgaris	Leaf extract	8	15	14
	Stem extract	6	15	11
	Whole plant extract	-	10	11
Staphylococcus aureus	Leaf extract	13	15	17
	Stem extract	10	12	15
	Whole plant extract	6	8	10
Escherichia coli	Leaf extract	12	13	14
	Stem extract	9	10	12
	Whole plant extract	6	7	9

Conclusions:

Euphorbia milii is rich in various phytochemicals like alkaloids, carbohydrates, Flavonoids, Phenols, Saponins, Coumarins, Cardiac glycosides etc. The antimicrobial study revealed that *Euphorbia milii* show a great antibacterial activity against *Staphyloccocusaureus, Escherichia coli*, while Ethanol extract against *Proteus vulgarias*.This reveals the importance of choice of solvent for proper extract preparation.In *Euphorbia milii* flavonoid is a water soluble antioxidant and it prevents oxidative cell damage, Anticeptic, Anticancer.

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