

PRELIMINARY PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF LEAF AND STEM EXTRACTS OF *MEIZOTROPIS PELLITA* (PATWA): AN ENDANGERED PLANT OF KUMAUN HIMALAYAN REGION***Renu Chandola and Dr. Anshulika Upadhyaya**

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ABSTRACT

Meizotropis pellita (Family Fabaceae) is commonly known as Patwa, an endangered plant species from Kumaun Himalaya was screened for phytochemical constituents and antimicrobial Potential using leaf and stem samples of plant. Different tests were performed in methanolic extract of leaf to find the chemical constituents that are present in order to use the plant for medicinal purposes. Dried samples were used to obtain various extracts viz. methanol, ethanol, acetone and chloroform were tested using Disc Diffusion Method against different Bacterial strains: *E. coli* (gram -ve), *Bacillus subtilis* (gram +ve), *Pseudomonas* (gram -ve) and fungal strains: *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus awamori*, *C. oryzae*, *Rhizopus oryzae* and *Trichoderma viride*. It was found out that it contain valuable bioactive constituents for further Pharmaceutical Research and this finding will help in the increase economic value of this plant and also used as a herbal medicine.

KEYWORDS: *Meizotropis pellita*, Disc Diffusion Method, Ampicillin, Phytochemical, Antimicrobial.**INTRODUCTION**

Meizotropis pellita is a native species of Patwadanger. This plant flourishing in a village named Patwadanger, situated at 12 Km from Nainital, Uttarakhand at 1500 m and 1530 m over the sea level.^[4] In nature the major reason of its extinction because of changing climatic conditions, increase deforestation, forest fires, and habitat fragmentation. The leaves of Patwa are green in colour and 45-75 cm long. In nature seeds of Patwa are "dormant".^[1] Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for society and mankind in the last few decades. The most necessary bioactive compounds of plants are tannins, alkaloids, phenolic and flavonoids compounds that cause a definite physiological response on the human body.^[5] Problems with emerging diseases, side effects of modern drugs and microorganisms resistance to drug its turned out an ultimate demand for the discovery of new novel antibiotic prototypes having great potential with least side effects.^[3] Infectious disease is increased to a great extent and became the number one cause of death worldwide, accounting for approximately 50% of the death in tropical countries.^[2] This may be due to poverty and increasing of multiple drug resistance. The present study aimed to evaluate Phytochemical and antimicrobial activity of plant parts like stem and leaf extracts of Patwa.

MATERIALS AND METHODS**Collection of plant materials**

The Fresh samples including leaves and stems of *Meizotropis pellita* (endangered plant) were collected from Patwadanger Forest {Latitude: 29.33(29° 19' 60" N) and Longitude 79.43 (79° 025' 60" E)} in the month of January 2019. The collected plant samples were taken to the laboratory for the study of antimicrobial activities and phytochemical screening.

Preparation of the extracts

The plant samples were surface sterilized and then air dried in shade at ambient temperature (31°C) and were powdered using an electronic blender. The powdered mixture was then soaked in different solvents (ethanol, methanol, acetone and chloroform) for 72 hrs. After filtering the contents using Whatman No. 1 filter paper, the filtrate was left at room temperature for 48 hrs to evaporate partially. All the extracts were dried in vacuum rotary evaporator at 40°C under reduced pressure, weighed and stored at 4°C. The filtrate was then used for phytochemical analysis and antimicrobial activity.

Qualitative analysis of phytochemical constituents

The plant extracts methanol were subjected to phytochemical analysis of the active compounds present in them. The compounds screened are Steroids, Phenols, Flavonoids, Coumarin, Saponin, Terpenoids, Alkaloids, Quinones, Resins and Tannins.

Table 1: Tests for analysis of phytochemical constituents.

S. No.	Test	Observation	Inference
1	Test solution + 2 ml of acetic anhydride + 2ml of conc.H ₂ SO ₄	Color change from violet to blue/green	Presence of steroids
2	1 ml test solution + 2ml distilled water + few drops of 10% aqueous ferric chloride solution	Formation of blue or green color	Presence of Phenol
3	Test solution + few drops of FeCl ₃	Formation of blackish green color	Presence of Flavonoid
4	Test solution + 10% NaOH	Yellow color develop	Presence of Coumarin
5	Test solution + water and shake	Foamy leather formed	Presence of Saponin
6	5 ml test solution + 2ml chloroform + 3 ml conc. H ₂ SO ₄	Layer of reddish brown coloration formed at interface	Presence of Terpenoids
7	Test solution + Wagner's reagent (Iodine in KI)	Formation of reddish brown color	Presence of Alkaloids
8	1ml test solution + Alcoholic KOH	Coloration from red to blue	Presence of Quinones
9	Test solution (1ml) + drops of acetic anhydride followed by 1ml of conc. H ₂ SO ₄	Coloration from orange to yellow	Presence of Resins
10	1ml test solution + 10ml ddw + filtered+ 1% aqueous FeCl ₃ added to filtrate	Appearance of intense green, blue, purple or black color	Presence of Tannins

Selection of microbial culture for antimicrobial screening

Bacillus subtilis (gram +ve), *Escherichia coli* (gram -ve), *Pseudomonas* (gram -ve), *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus awamori*, *C. oryzae*, *Rhizopus oryzae* and *Trichoderma viride* were selected for the study of microbial assay. The cultures were maintained on nutrient agar at 37°C for bacteria and Sabouraud dextrose agar at 30 °C for fungus. A 24 hours broth culture of the selected species was used for the antimicrobial screening.

Culture preparation and screening of antimicrobial activity-disc diffusion method (Maruzzella & Henry, 1958)

Bacterial and fungal strains were inoculated into the sterilized media. 20 ml of the medium is poured into each sterilized Petri plates and allowed to solidify at room temperature. After solidification, the disc (6 mm) of Whatman no 1 filter paper was soaked in crude extracts in different solvents (methanol, ethanol, chloroform and acetone) was placed carefully in the center of Petri-plates containing the solidified media. To compare the antimicrobial activity same concentration of the solvent using disc is placed in the plate which acts as a control to our crude solvents. The plates were incubated at 37 °C for 24 hrs for bacterial culture and 28°C for 48 hrs for fungal strains. The antimicrobial activities were assayed by measuring the resultant 'zone of inhibition' with the help of the ruler.

RESULTS AND DISCUSSION

Phytochemical analysis

Out of ten phytochemical tests, methanolic extract of Leaf showed the presence of steroids, Phenol, Flavonoid, Saponins, Alkaloids and Tannins. Negative results were recorded for Coumarin, Terpenoids, Quinones and Resins. In the case methanolic extract of stem showed the presence of Phenol, Flavonoid, Saponins, Alkaloids Coumarin, Quinones and Tannins. Negative results were recorded for steroids, Terpenoids, and Resins.

Table 2: Phytochemical analysis of stem and leaf methanolic extract.

S. No.	Test	Result	
		Stem	Leaf
1.	Steroids	-	+
2.	Phenol	+	+
3.	Flavonoid	+	+
4.	Coumarin	+	-
5.	Saponins	+	+
6.	Terpenoids	-	-
7.	Alkaloids	+	+
8.	Quinones	+	-
9.	Resins	-	-
10.	Tannins	+	+

Antimicrobial activity

In our study Antibacterial activity was recorded for three strains (*E.coli*, *B.subtilis* and *Pseudomonas*). In case of leaves out of the two leaf extracts in different solvents, methanolic extract showed potent activity against *B. subtilis* while chloroform was unable to show any antibacterial activity against *B. subtilis*. None of the crude extracts were able to inhibit the growth of *E. coli* and *Pseudomonas*. In case of stem the results revealed that the methanolic crude extract showed maximum activity followed by ethanol and Acetone While chloroform was unable to show any antibacterial activity against any bacterial culture.

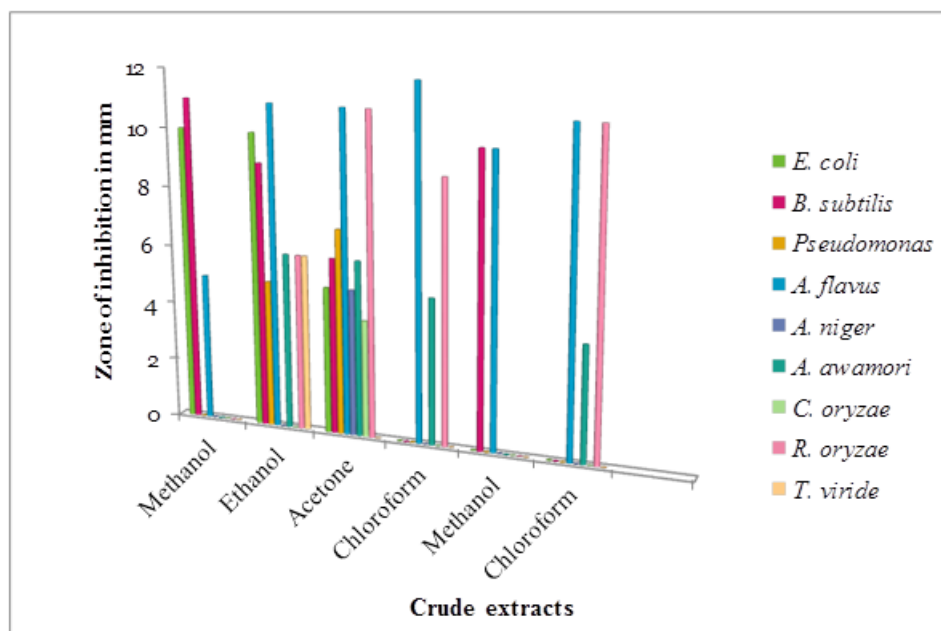
Antifungal activity was recorded for six strains (*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus awamori*, *C. oryzae*, *Rhizopus oryzae* and *Trichoderma viride*). In case of leaves out of the four leaf extracts in different solvents, crude extracts showed potent activity against *A. flavus*. None of the crude extracts were able to inhibit the growth of *A. niger*, *C. oryzae* and *T. viridae*. In case of stem Maximum antifungal activity was found against *A. flavus* (12mm) followed by *R. oryzae* (9mm) and *A. awamori* (5mm). It is also found that acetone

crude extract was the potent antifungal agent followed by chloroform and ethanol crude extract.

Table 3: Antimicrobial activity of plant extracts (Disc diffusion method).

Pathogenic microorganism	Zone of inhibition (mm)					
	Leaf crude extracts			Stem crude extracts		
	MT	CF	MT	CF	ET	AC
Bacterial cultures						
<i>E. coli</i>	-	-	10	-	10	5
<i>B. subtilis</i>	10	-	11	-	9	6
<i>Pseudomonas</i>	-	-	-	-	5	7
Fungal cultures						
<i>A. Flavus</i>	10	11	5	12	11	11
<i>A. Niger</i>	-	-	-	-	-	5
<i>A. Awamori</i>	-	4	-	5	6	6
<i>R. oryzae</i>	-	11	-	9	6	11
<i>T. viride</i>	-	-	-	-	6	-
<i>C. oryzae</i>	-	-	-	-	-	4

(MT– Methanol, CF– Chloroform, ET- Ethanol and AC- Acetone)



CONCLUSION

The conclusion revealed with phytochemical screening of leaf and stem result make a general therapeutic aspect on plant drug sample. Our study provides that the leaf and stem crude extracts of *M. pellita* has potential antimicrobial activity against some bacterial and fungal species. The present study also indicates that solvent extracts of *Meizotropis pellita* leaf and stem contains important bioactive compounds and this explores the use of this species as conventional medicine for the treatment of numerous diseases and also used as economic value.

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