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Research Article

**PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES ON  
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**ABSTRACT**

The present study was carried out to analyse the preliminary phytochemical screening for ten principle bioactive compounds in petroleum ether, ethanol and aqueous extracts of stem and leaves of *Plumbago zeylanica*, collected from Anaikatti Hills, Coimbatore. Petroleum ether, ethanol and aqueous extract of leaves and stems of *Plumbago zeylanica* were also investigated against bacteria and fungi by paper disc method. Gentamycin and Amphotericin-B were used as a standard drug respectively. Preliminary phyto chemical screening revealed the presence of alkaloids, carbohydrates, triterpenoids, flavonoids, gum, mucilage, protein, fatty acids and saponine. The maximum activity was observed in ethanol extract against *Micrococcus luteus* (12mm) and minimum activity was observed in pet-ether extract against *Staphylococcus aureus* and *Micrococcus luteus* at inhibition range was observed (7mm). The result suggest ethanol and petroleum ether extract showed moderate anti-bacterial activity and aqueous extract did not active against negative bacterial species. In antifungal, it was found that among the extracts and compare with standard, ethanol extract showed a significant anti-fungal activity.

**Keywords:** Paper disc, *Staphylococcus aureus*, *Aspergillus Niger*, *Plumbago zeylanica (L)*.

**INTRODUCTION**

Historically plants have provided a source of inspiration for novel drug compounds. As plants derived medicines have made large contributions to human health and well being. Their role of two folds in the development of new drugs. They may become the base for the development of a medicine, a natural blue print for the development of new drugs. The phyto medicine to be used for the treatment of diseases<sup>1</sup>. Traditional medicine using plant extract continues provide health coverage for over 80% of the world population especially in the developing the world<sup>2</sup>. *Plumbago zeylanica.L* has been reported to be used in variety of folk medicine in Africa and Asia. It has to be used in the treatment of refractory

prostate cancer<sup>3</sup>. Anti fertility activity<sup>4</sup>. Anti hyper lipidermic activity<sup>5</sup>. Anti estrogenic activity<sup>6</sup>. Since no scientific reports are available on anti microbial properties of these medicinal plants. The present investigation is carried out to find out the anti microbial activity. The anti bacterial and anti fungal activity of the stem and leaves extracts were estimated by paper disc method.

**MATERIALS AND METHODS**

The plant *plumbago zylanica* was collected from the lands of Anaikatti hills in Coimbatore district. Tamilnadu and identified by Dr.G.V.S Murthi joint director, botanical survey of India, southern regional center, TNAU

campus, Coimbatore Tamilnadu. The stem and leaves of the plant were removed carefully. Shaken to remove unwanted particles like sand and soil drying was done under shade by spreading the stem and leaves over the paper on a wooden table for one week. The powdered stem and leaves powder was extracted with nonpolar to polar solvents like petroleum ether, ethanol and water. The yields of the different extracts are 3.46%w/w 15.02%w/w, 9.24%w/w respectively. The extracts were used to determine the phytochemical constituents and anti microbial activity.

#### Phytochemical Screening

Preliminary phytochemical screening was performed as per standardized procedure<sup>7,8</sup> the various phyto constituents in petroleum ether; ethanol and aqueous extracts were identified.

#### Antimicrobial Screening

The extracts were subjected to antibacterial (*S.aureus*, *M.luteus*, *P.mirabilis*, *Shigella boydii*) and antifungal (*Aspergillus niger*, *Candida albicans*). The anti microbial screening was performed by agar diffusion method using a paper disc<sup>9, 10</sup>. The sterilized (autoclaved at 120 °C for 30 min) medium was inoculated with the suspension of the m/o. the paper impregnated with the extracts (1200µg/ml) was placed on the solidified medium. The petridishes were pre incubated for one hour at room temperature and incubated at 37°C for 24 hrs and 48 hrs for antibacterial and antifungal activity respectively. Gentamycin (1200µg/ml) and Amphotericin B (1200µg/ml) used as a standard for anti bacterial and anti fungal activities.

#### Separation of the Compound

The compound present in *plumbago zylanica* were quantitatively analysed by using TLC which is commercially available. The aluminum sheets with silica gel -60F254 were used. The isolation and separation of steroids, carbohydrates and alkaloids was done. The result was reported in Table 1

## RESULTS

### Phytochemical Screening

The results of the phytochemical screening revealed the presence of alkaloids, carbohydrates, tannins, phenolic compounds, proteins, amino acids and flavonoids. In petroleum ether, ethanol and aqueous fractions while glycosides presence in ethanol and aqueous fractions. Saponins in ethanol and aqueous fractions. Steroids, fixed oils and fats in petroleum ether fractions (Table 2).

### Anti-Microbial Activity

Anti microbial activities of the petroleum ether, ethanol and aqueous fractions are summarized in tab3&4. The ethanol extracts showed zone of inhibition ranging from 10-12mm against all the test organisms. The zone of inhibition produced by petroleum ether fraction ranged from 7-10mm against all the test organisms. The zone of inhibition produced by aqueous fraction ranged from 9-21mm against all the test organisms except *Proteus Mirabilis*, *Shigella boydii*.

## DISCUSSION

The results of phytochemical screening of the ethanol extracts, petroleum ether and aqueous fraction revealed, the presence of alkaloids, carbohydrates, phenolic compounds, proteins amino acids and flavonoids. These metabolites have been reported to possess antimicrobial activity<sup>11</sup>.

The ethanol extracts showed significant anti bacterial activity against all the organisms. Petroleum ether fractions show moderate activity against all the organisms. Aqueous fractions did not active against negative bacterial species. It is important to note that the strong activity of ethanol extract and the petroleum ether fraction against *Candida albicans* indicate that the plant serves as a source of an anti fungal agent. The anti microbial activity of the ethanol and petroleum ether fraction suggest that , the presence of bio active compounds which serve as anti microbial agent or lead compound for the synthesis of an effective and less toxic antimicrobial agent.

## CONCLUSION

The study showed that the ethanol extract and petroleum ether from the leaves and stems of *plumbago zylanica* have anti microbial properties.

**Table 1: TLC of Petroleum ether, Ethanolic and Aqueous extracts of stem and leaves of *plumbago zeylanica***

S. No.	Extracts	Color of spots observed				Rf value		
		Day light	UV		Iodine chamber	Spot1	Spot2	Spot3
			Short $\lambda$	Long $\lambda$				
1	Petroleum ether	Green	Green	Brown	Brown	0.98	0.97	0.97
2	Ethanol	Green	Green	Brown	Brown	0.95	0.94	0.95
3	Aqueous	Green	Green	Brown	Brown	0.96	0.97	0.97

**Table 2: Preliminary Phytochemical analysis of *plumbago zeylanica* (L) with different solvents**

S. No.	Name of the compound	PE	E	W
1	Alkaloids	+	+	+
2	Carbohydrates	+	+	+
3	Glycosides	-	+	+
4	Steroids	+	-	-
5	Fixed oil&fats	+	-	-
6	Triterpenoids	-	-	-
7	Tannins	+	+	+
8	Phenolic compounds	+	+	+
9	Proteins	+	+	+
10	Amino acids	+	+	+
11	Saponins	-	+	+
12	Gums	-	-	-
13	Mucilage	-	-	-
14	Flavonoids	+	+	+

+ -present, (-) absent, PE- petroleum ether, E- ether, W- water.

**Table 3: Anti bacterial activity profile of three extracts of three extracts from the stem and leaves of *plumbago zylanica* (L)**

Test bacteria	Zone of inhibition(mm)					
	Ethanol 1200 $\mu$ g/ml	Standard gentamycin 1200 $\mu$ g/ml	Petroleum ether 1200 $\mu$ g/ml	Standard gentamycin 1200 $\mu$ g/ml	Aqueous 1200 $\mu$ g/ml	Standard gentamycin 1200 $\mu$ g/ml
Stephylococcus aureus	10	24	7	22	9	25
Micrococcus luteus	12	23	8	24	21	24
Proteus mirabilis	11	24	10	23	0	24
Shigellaboydii	9	25	9	22	0	23

**Table 4: Anti fungal activity profile of this extracts from the stem and leaves of *plumbago zaylanica*(L)**

Test fungus	Zone of inhibition(mm)					
	Ethanol 1200 $\mu$ g/ml	Standard amphotercin-B 1200 $\mu$ g/ml	Petroleum ether 1200 $\mu$ g/ml	Standard amphotercin-B 1200 $\mu$ g/ml	Aqueous 1200 $\mu$ g/ml	Standard amphotercin-B 1200 $\mu$ g/ml
Aspergillus niger	10	18	12	18	8	18
Candida albicans	12	17	13	17	9	19

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