



ORIGINAL ARTICLE

Phytochemical Screening and Antimicrobial Studies on *Plumbago zeylanica* L.

Kakad Subhash, A. S. Wabale and M. N. Kharde

Padmashri Vikhe Patil College of Arts, Science and Commerce, Pravaranagar- 413 713
dranilwabale@rediffmail.com

ABSTRACT

The present study was carried out to screen out the phytochemicals in from the roots of *Plumbago zeylanica* L. Root extract prepared in methanol, ethanol and distilled water was also tested for its antibacterial activity against bacteria i.e. *Staphylococcus aureus* and *Bacillus subtilis* by well method. Gentamycin were used as the standard drugs respectively. Phytochemical screenings revealed the presence of alkaloids, carbohydrates, flavonoids, tannins, sterioids and saponine. The maximum antibacterial activity was observed in methanol extract against the *Staphylococcus aureus* (16 mm), *Bacillus subtilis* (14mm) and minimum in ethanol (10 mm and 12mm respectively). The result suggests that methanol and ethanol extract shows moderate antibacterial activity.

Key words: *Plumbago*, Gentamycin, *Staphylococcus*, Antibacterial, Extract

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INTRODUCTION

Nature has been the source for medicinal properties from thousands of years and a remarkable number of modern drugs have been obtained from natural sources, particularly from the plants. Plant based medicines have played an important role in primary health care needs of human as well as animals.

Variety of plants exhibit antimicrobial properties, due to the presence of some active compounds like essential oils, flavonoids, terpenoids, tri-terpenoids, glycosides, alkaloids and other natural phenolic compounds. These natural energetic compounds are usually termed as secondary metabolites that are not essential for the survival of plants but act as a defensive mediator for plants. *Plumbago zeylanica* L. belonging to family Plumbaginaceae has been used in preparation of medicinal drugs. It is a perennial herb with terete, glabrous, striate and woody stems. The leaves are ovate, glaucous beneath, amplexicaul at the base and dilated into stipule like auricles. Flowers are in the spikes and are white in colour. Capsules are oblong, pointed and the pericarp is thin below, thick and hardened above [1]. Chemically the plant consists of Naptha-quinone derivatives-plumbagin, 3-chloroplumbagin, 3, 3'-biplumbgin, elliptinone, chitranone, droserone, zeylanone, iso-zeylanone, 1, 2 (3) -tetrhydro- 3, 3' -biplmbagin and plumbazeylanone are reported from roots [2].

Root paste is applied on forehead to get relief from headaches and also useful in piles, skin diseases and Vitiligo. It also relieves muscular pain. Leaves are having abortifacient properties [3]. Roots promote appetite and are useful in dyspepsia. The root paste is applied on leprosy and other skin diseases [4] Roots are useful in influenza [2]. Leaves are anti-rheumatic [5].

In present investigation an attempt is done to screen the secondary metabolites and test the antimicrobial activity against the selected pathogenic bacteria.

MATERIALS AND METHODS

A. Preparation of Extract

1.0 gm powder of root was placed in 20 ml distilled water, amyl alcohol, methanol, ethanol, ether, chloroform respectively. They were kept at room temperature for in air tight bottles for 24 hours. The content was then filtered using a separating funnel. Excess solvent was evaporated and the extract was stored in glass vials [6].

B. Agar Well Diffusion Method

Bacterial strain was inoculated on nutrient broth and allowed to form dense culture. 0.1 ml of the broth was then spread on petri-plates containing MH media using a sterile glass spreader. The wells were

prepared using alcohol sterilized borer (8 mm). Then 15 µl of plant extract was added into each well using micropipette. After 24 hours of incubation at 37°C, inhibition zone diameter was calculated. Results were compared with the standard antibiotics like Gentamycin [7].

C. Screening for Phytochemicals [8]

- 1. Alkaloids:** 1 ml plant root extract was stirred with 5 ml dilute Hydrochloric acid and was filtered. 1 ml of Wagner's reagent was added to the filtrate to observe the change in colour.
- 2. Tannins:** 1 ml plant root extract was stirred with 1 ml ferric chloride as well as with 1 ml bromine water separately.
- 3. Steroid:** 0.5ml plant root extract was mixed with 2 ml acetic anhydrous, cooled in ice and 1 ml concentrated H₂SO₄ was added to it to see change in colour.
- 4. Flavonoids:** 0.2 ml diluted NaOH was added to 0.2 ml extract with gentle shaking.
- 5. Saponine:** 0.2 ml extract was mixed with 5 ml distilled water with continuous stirring for 20 minutes.
- 6. Carbohydrate:** 1 ml extract was mixed with 2 ml Fehling solution and was boiled for 5 minutes to find out the presence of carbohydrates.

RESULTS AND DISCUSSION

A. Phytochemical Screening

Table 1: Screening of Phytochemicals from the root extract of *Plumbago zeylanica* prepared in different solvents

Solvents used for preparing extract	Amyl alcohol	Ethanol	Methanol	Ether	Chloroform	Distilled water
Phytochemicals						
Saponine	--	+	+	++	--	++
Carbohydrate	--	++	++	+	--	+
Steroid	++	+	--	+	+	+
Flavonoids	--	++	++	--	--	+
Tannin	--	++	++	+	--	+
Alkaloids	++	++	++	++	--	++

Screening of Phytochemicals from the root extract of *Plumbago zeylanica* prepared in different solvents from Table-1 reveals the presence of biochemical's alkaloids, carbohydrates, , saponins, tannins, steroids and flavonoids as

Alkaloids: 1 ml plant root extract mixed with 5 ml dilute Hydrochloric acid was filtered and 1 ml Wagner's reagent was added to 1 ml separate portion of filtrate, as a result a cloudy orange red/ slightly yellow / turbid brown color was obtained.

Tannins: 1 ml root extract was stirred with 1 ml ferric chloride. A greenish black precipitate indicated the presence of tannins. 1 ml root extract was also stirred with 1 ml bromine water and a reddish brown turbid color indicated the presence of tannins.

Steroid: 0.5 ml extract dissolved in 2 ml acetic anhydrous, cooled in ice before adding 1 ml concentrated H₂SO₄, showed reddish brown color, which indicated presence of steroid.

Flavonoids: 0.2 ml dilute NaOH was added to 0.2 ml extract and after shaking gently a dirty yellowish brown precipitate was obtained which revealed the presence of flavonoids.

Saponine: 0.2 ml extract was mixed with 5 ml distilled water and was kept on shaker for 20 minutes. Persistence of foam indicated presence of saponine.

Carbohydrate test: 1 ml extract was mixed with 2 ml Fehling solution and was boiled for 5 minutes. A red precipitate indicated presence of reducing sugars.

B. Antibacterial activities

Table 2: Antibacterial activity of *Plumbago zeylanica* L. root extract prepared in different solvents

Root extract of <i>Plumbago zeylanica</i> L. in different solvents	Zone of Inhibition (mm)	
	<i>S. aureus</i>	<i>B. subtilis</i>
Methanol	16	14
Ethanol	10	12
Amyl alcohol	--	--

Staphylococcus aureus

From Table 2, it was revealed that the root extract in methanol showed maximum zone of inhibition i.e. 16 mm and 14 mm for *S. aureus* and *B. subtilis* respectively. Ethanol prepared extract stood second in inhibiting the growth of above bacteria's viz. 10mm and 12mm respectively.

Root extract prepared in Amyl alcohol did not showed any effect on the inhibition of the above said pathogens.

Traditionally *P. zeylanica* is believed to kill intestinal parasites and is used clinically to treat rheumatism, anemia, external and internal trauma toxic swelling and malignant furunculosis Scabies. It's a remedy on fever and malaria in Ayurveda. It is also employed for their anti fertility, germicidal, anti leprosy and anti inflammatory activities.

CONCLUSION

Phytochemical screenings revealed the presence of alkaloids, carbohydrates, tri-terpenoids, flavonoids, tannins and saponine. The maximum antibacterial activity was observed in methanol extract against the *Staphylococcus aureus* (16 mm), *Bacillus subtilis* (14mm) and minimum in ethanol (10 mm and 12mm respectively). The result suggests that methanol and ethanol extract shows moderate antibacterial activity.

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