

Phytochemical and biological evaluation of roots of *Plumbago* Linn.

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Received: July 19, 2018

Accepted: October 02, 2018

ABSTRACT

Roots of two species of *Plumbago* Linn. viz. *P. zeylanica* Linn. and *P. auriculata* Lam. were used for present study with aim to suggest whether roots of *P. auriculata*, a widely available ornamental plant with least medicinal value can be used as substitute for roots of *P. zeylanica*, a threatened multivalent medicinal plant for therapeutic purpose based on phytochemical and antimicrobial studies. Petroleum ether, chloroform, ethanol, aqueous extracts of roots were used for preliminary phytochemical screening and antimicrobial studies against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*. Roots of both plants are ample source of phytoconstituents namely Carbohydrates, Proteins, Amino Acids, Lipids, Alkaloids, Tannins, Flavonoids, Steroids, Glycosides. Maximum antimicrobial activity (17.45mm) was observed in chloroform extract of root of *P. auriculata* against *C. albicans*. Minimum antimicrobial activity (14mm) was observed in ethanol extracts of roots of both plants against *A. niger*. Both the plants have more or less similar phytochemical profile and antimicrobial activities with slight differences.

Keywords: *P. zeylanica*, *P. auriculata*, preliminary phytochemical screening, antimicrobial activity.

Introduction

Ayurveda has deep root in our cultural heritage and cater to medicare of huge section of our population. The earliest mention of use of medicinal plants is found in *Rigveda* (one of the oldest repositories of human knowledge) which enlists 67 plants of therapeutic use. The *Yajurveda* enlists 81 medicinal plants whereas *Atharvaveda* describes 290 medicinal plants. *Vedas* were followed by '*Charak Samhita* (in which 341 medicinal plants are mentioned) and *Sushrut Samhita* (in which 395 medicinal plants are mentioned) which added valuable knowledge to science of medicinal plants. The World Health Organization (WHO) is encouraging developing countries to use herbal remedy which they have been used traditionally for centuries. In recent time there has been shift towards herbal cure because of pronounced side effects of allopathic drugs (Agrawal and Paridhavi, 2007). Herbal renaissance is happening all over the World. Globalization of Ayurveda has gained momentum. Unani system of medicine also uses herbal drugs (Agrawal and Paridhavi, 2007).

P. zeylanica Linn. (wild variety) is very popular plant in ayurveda because of its good medicinal potential. Roots of this plant have more medicinal value as compared to other parts. Roots are used to treat piles, anaemia, ring - worm, scabies, jaundice, cough, asthma, epilepsy, hysteria etc. It is also abortifacient, aphrodisiac, appetizing, antiseptic, febrifuge, cardiogenic, neuroprotective, restorative, etc. (Chaudhari and Chaudhari, 2015) This plant has been continuously collected from the wild without replanting. It is listed as threatened medicinal plant (Parthipan, 2006; Mittal and Sharma, 2010). If *P. zeylanica* is not conserved and propagated properly, it is likely to be endangered in future. Another species *P. auriculata* Lam. is ornamental plant with least medicinal use. It is cultivated variety and widely available. It is need of hour to find whether the phytochemical constituents and antimicrobial activities of both the plants are the same or not which is helpful to know whether the medicinal potential of both the plants is the same or not. Antimicrobial activities of both plant extracts was evaluated against four pathogenic microorganisms viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*.

The objective of this study was to suggest whether root of *P. auriculata* can be used as substitute for root of *P. zeylanica* for therapeutic purpose.

Materials and Methods

1. Collection of plant Material

Roots of healthy plants of *P. zeylanica* Linn. and *P. auriculata* Lam. were collected. Freshly collected roots were washed thoroughly with water. Roots were shade dried separately at room temperature. Dried roots were cut into pieces. Dried plant parts were pulverized separately using electric grinder and stored in airtight container for further use

2. Preparation of extracts

Extracts of shade dried roots were obtained by using four solvents – petroleum ether (60-80°C), chloroform, ethanol, water.

Petroleum ether, chloroform and ethanol extracts were obtained by successive solvent extraction method using Soxhlet apparatus and aqueous extract by maceration (Khandelwal, 2011).

Petroleum ether, chloroform and ethanol extract was concentrated by distilling off the solvent (Khandelwal, 2011) Aqueous extract was concentrated under reduced pressure.

Concentrated extracts were dried on water bath. Colour and consistency of extracts were noted. The residue obtained from each solvent was weighed and its percentage successive solvent extractive values (Kumar et al, 2011) (%w/w) were calculated with reference to weight of air dried plant material.

3. Screening tests for detection of organic constituents:

Test for carbohydrates

1. Molish's Test (General Test)

Test - To 2-3ml extract, few drops of alpha-naphthol solution in alcohol was added, shaken and conc. H₂SO₄ was added from sides of test tube (Khandelwal, 2011; Roseline, 2011)

Observation – Formation of violet ring at the junction of two liquids (Khandelwal, 2011; Roseline, 2011) or the whole reaction mixture showed same color after shaking (Roseline, 2011)

Inference - Presence of carbohydrates

2. Fehling's Test (Khandelwal, 2011)

Test - 1ml Fehling's A and 1ml Fehling's B solution were mixed, boiled for 1 minute, Equal volume of extract was added. Heated in boiling water bath for 5-10 minutes

Observation – First yellow then brick red precipitate

Inference - Presence of reducing sugars

3. Benedict's Test (Khandelwal, 2011)

Test - Equal volume of Benedict's reagent and extract were mixed in test tube. Heated in boiling water bath for 5 minutes

Observation – green, yellow or red coloured solution

Inference - Presence of reducing sugars

4. Barfoed's Test (Khandelwal, 2011)

Test - Equal volume of Barfoed's reagent and extract were mixed. Heated for 1-2 minutes in boiling water bath and cooled

Observation – Formation of red precipitate

Inference - Presence of monosaccharide

5. Iodine Test (Khandelwal, 2011)

Test - Extract + few drop of dil. iodine solution

Observation – Blue colour appeared, it disappeared on boiling and reappeared on cooling

Inference - Presence of starch (polysaccharide)

6. Test for Mucilage (Khandelwal, 2011)

Test – The powdered plant material was soaked in water

Observation - Plant material swelled in water

Inference - Presence of mucilage

Determination of swelling factor/ swelling index

(Khandelwal, 2011)

1 gram of pulverized plant material was taken in 25ml stoppered measuring cylinder

2. Water upto 25ml marking was added

3. The mixture was shaken occasionally during 23 hours

4. It was allowed to stand for one hour

5. The volume in ml occupied by the plant material was measured

Test for Proteins (Khandelwal, 2011)

1. Biuret Test (General Test)

Test - Extract + 4 % NaOH + few drops of 1% CuSO₄ solution

Observation – Violet or pink colour

Inference - Presence of proteins

2. Millon's Test (Khandelwal, 2011)**Test** - 3ml Extract + 5ml Million's reagent**Observation** - Formation of white precipitate, warmed, precipitate turned brick red or the precipitatedissolved giving red coloured solution**Inference** - Presence of proteins**Test for Amino Acids****Ninhydrin Test** (Khandelwal, 2011)**Test** - 3ml of extract and 3 drops of 5% Ninhydrin solution was heated in boiling water bath for 10 minutes**Observation** - Purple or bluish colour**Inference** - Presence of amino Acids**Test for lipids** (Patil, 1990)**Test** - Extract + sudan III, mixture was shaken well**Observation** - Red color**Inference** - Presence of lipids**Tests for Alkaloids** (Khandelwal, 2011)

Extract was treated with dilute HCl and filtered. Following tests were performed with filtrate

1. Wagner's test**Test** - 2-3ml filtrate treated with Wagner's reagent**Observation** - Reddish brown precipitate**Inference** - Alkaloids present.**2. Mayer's Test****Test** - 2-3ml filtrate treated with Mayer's reagent**Observation** - Creamy precipitate**Inference** - Alkaloids present.**3. Hager's Test****Test** - 2-3ml filtrate treated with Hager's reagent**Observation** - Yellow precipitate**Inference** - Alkaloids present.**4. Tannic acid Test****Test** - 2-3ml filtrate treated with Tannic acid solution**Observation** - Buff colouredprecipitate**Inference** - Alkaloids present.**5. Dragendroff's Test****Test** - 2-3ml filtrate treated with Dragendroff'sreagent**Observation** - Orange brown precipitate**Inference** - Alkaloids present.**Tests for Tannins and Phenolic compounds****1. FeCl₃ test** (Khandelwal, 2011; Roseline, 2011)**Test** - Extract treated with 5% FeCl₃ solution**Observation** - Deep blue - black, green colour.**Inference** - Presence of tannin.**2. Lead acetate Test** (Khandelwal, 2011)**Test** - Extract treated with lead acetate solution**Observation** - White precipitate**Inference** - Presence of tannin.**3. Potassium permanganate Test** (Khandelwal, 2011)**Test** - Extract + dilute potassium permanganate solution**Observation** - Decolourisation**Inference** - Presence of Tannin.**4. Bromine Water** (Khandelwal, 2011)**Test** - Extract + bromine water**Observation** - Decolourisation.**Inference** - Presence of tannin.

Test for Flavonoids**1. Shinoda Test** (Khandelwal, 2011)**Test** - Extract + 5ml 95% alcohol, few drops of conc. HCl and 0.5 gram magnesium turnings**Observation** - Orange, pink, red to purple colour**Inference** - Flavonoids present**Test for glycosides****a) Test for Cardiac glycosides****1. Legal Test** (Khandelwal, 2011; Roseline, 2011)**Test** - Extract + 1ml pyridine + 1ml sodium nitroprusside, then made alkaline**Observation** - Pink or red color.**Inference** - Cardiac glycosides present**2. Keller- Killiani Test** (Khandelwal, 2011)**Test** - To 2ml of extract, glacial acetic acid was added, then one drop 5% FeCl_3 and conc. H_2SO_4 was added**Observation** - Reddish brown color appeared at the junction of the two liquid layers and upper layer appears bluish green.**Inference** - Cardiac glycosides present**3. Libermann's Test** (Khandelwal, 2011)**Test** - 3ml of Extract + 3ml of acetic anhydride. Heated, cooled, few drops of conc. H_2SO_4 was added**Observation** - Blue colour.**Inference** - Cardiac glycoside present**b) Test for anthraquinone glycosides** (Khandelwal, 2011; Roseline, 2011)**1. Borntrager's Test.****Test** - 3ml of Extract + dilute H_2SO_4 , boiled and filtered. To cold filtrate, equal volume of benzene or chloroform was added, shaken well. Lower organic layer was separated and to this ammonia was added slowly.**Observation** - Pinkish red color to ammoniacal layer**Inference** - Anthraquinone glycosides present**c) Test for Saponin glycosides** (Khandelwal, 2011)**1. Foam Test****Test** - Extract was shaken vigorously with water**Observation** - Persistent foam.**Inference** - Saponin glycosides present**2. Haemolytic Test****Test** - Extract was added to one drop of blood placed on glass slide**Observation** - Appearance of haemolytic zone**Inference** - Saponin glycosides present**d) Test for Coumarin glycosides****1. Test** (Evans, 2009)**Test** - Extract made ammoniacal**Observation** - Blue, blue-green or violet fluorescence**Inference** - Coumarin glycosides present**2. Test** (Khandelwal, 2011)**Test** - Extract + 1N NaOH**Observation** - Blue or green Fluorescence**Inference** - Coumarin glycosides present**e) Test for cyanogenetic glycosides****1. Grignard reaction or Sodium picrate Test****Test** - Filter paper strip was soaked in 10% picric acid and then in 10% sodium carbonate and dried. Moistened powdered plant material was placed in a conical flask. The flask was stoppered with a cork containing aforesaid filter paper strip hanging down from the stopper through the slit (Khandelwal, 2011) and the flask was incubated at 40°C for two hours. No color change in impregnated paper was observed, the conical flask was left at room temp for further 24-28 hours for re-examination (Harborne, 2010)**Observation** - Change in colour of paper from yellow to brown red within 2 or 48 hours

Inference – Presence of cyanogenetic glycosides

Tests for Steroid (Khandelwal, 2011)

1. Salkowski Test

Test - 2ml extract + 2ml chloroform + 2ml conc. H₂SO₄, shaken well

Observation - Chloroform layer appeared red and acid layer showed green yellow fluorescence

Inference – Presence of steroid

2. Liebermann- Burchard Test

Test - 2ml extract was mixed with chloroform. 1- 2ml acetic anhydride was added and 2 drop conc. H₂SO₄ was added from the side of test tube

Observation - First red then blue and finally green color appeared.

Inference – Presence of steroid

3. Liebermann's Test

Test - 3ml extract with 3ml acetic anhydride was mixed heated and cooled. Few drops conc. H₂SO₄ was added

Observation- Blue colour

Inference – Presence of steroid

4. Antimicrobial susceptibility testing

For determining antimicrobial activity, plant extracts were assessed qualitatively by disc diffusion assay. (Munro, 2007; Mahmoud et al, 2007; Cappuccino and Sherman, 2012; Lalitha)

All micro-organisms used for experiments were procured from National Collection of Industrial Micro-organisms (NCIM), National Chemical laboratory (NCL), Pune, India. Antimicrobial activity of different plant extracts was tested against following bacteria and fungi

Microorganism	Strain Name	Strain Reference
Gram positive bacteria	<i>Staphylococcus aureus</i>	NCIM 2079
Gram negative bacteria	<i>Pseudomonas aeruginosa</i>	NCIM 2036
Fungi(Yeast)	<i>Candida albicans</i>	NCIM 3471
Fungi (Mould)	<i>Aspergillusniger</i>	NCIM 545

1. Inoculum preparation:

Abacterial inoculum was prepared using sterile saline (0.85% NaCl). For this, well-isolated colonies of the respective bacterial strains were selected from an agar plate culture and dispensed in sterile saline. The turbidity of bacterial suspension was adjusted to O. D. of 0.1 at 600 nm (Cappuccino and Sherman, 2012) using sterile saline. Similarly, an inoculum of *Candida albicans* was prepared.

Fungal inoculums were prepared using sterile saline (0.85% NaCl) with 1% Tween 80. For this, fungal spores of the respective fungal strain were obtained from an agar plate culture and dispensed in sterile saline containing Tween-80. The suspension containing 1×10⁵ spores per ml was used for the assay.

2. Media preparation:

The bacterial strains (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) were cultured using a nutrient agar medium. The yeast *Candida albicans* was cultured using a Malt extract glucose yeast extract peptone (MGYP) medium. For fungi (*Aspergillusniger*), Potato dextrose agar medium was used.

3. Sterilization:

All media were sterilized at 121°C, 15 lbs pressure for 15 minute in autoclave. After autoclaving, respective media were allowed to attain temperature 50°C. Then corresponding agar medium was poured into sterile petri-plate which formed uniform layer of agar medium (~ 4 mm). Then petri plates were allowed to cool to room temperature and stored in a refrigerator (2 to 8°C) until further use. Forceps, glasswares like petri dishes, test tubes, etc. were also sterilized in autoclave

4. Preparation of test solutions and standard:

A stock solution of concentration 1000 microgram/ml of each dried plant extract was prepared in dimethyl sulfoxide (DMSO). Antimicrobial activity was evaluated by taking concentration 100 microgram per disc. For this, HiMedia sterile discs (6 mm diameter) were used. HiMedia antibiotic standard disc containing Ciprofloxacin (10 microgram per disc) was used as a standard against bacteria and Amphotericin- B (100 units per disc) was used as a standard against fungi. The standard disc was moistened with DMSO before use.

5. Evaluation of antimicrobial activity:

The corresponding nutrient agar plate was inoculated by streaking the swab over the entire agar surface. HiMedia sterile discs (diameter 6 mm) previously loaded with plant extract in DMSO (100 microgram per disc) were placed on inoculated plates. Each disc was gently pressed with sterile forceps to

ensure complete contact with agar surface. Inoculated petri plates were kept in the refrigerator. After 30 minutes, petri-plates with bacterial and yeast strains were incubated at 37°C while petri plates inoculated with fungal strain were incubated at 28°C.

6. Interpretation of results

After appropriate (for bacteria 18-24 hrs, for yeast 24 and for fungi 3-7days) incubation period, each plate was examined. The diameters of the zones of inhibition were measured.

Results

Physicochemical characters like colour, consistency and successive solvent extractive values of different extracts of roots are presented in table 1.

The results of preliminary phytochemical screening of petroleum ether, chloroform, ethanol and aqueous extracts of roots of both plants are given in the table 2. The results of screening for cyanogenic glycosides, mucilage and swelling index of roots of plants are tabulated in table 3.

The antimicrobial activity of plant extracts was detected by indication of zone around the disc. (Fig 1, 2, 3, 4) The diameters of zones of inhibition obtained in biological evaluation are tabulated in the following table 4.

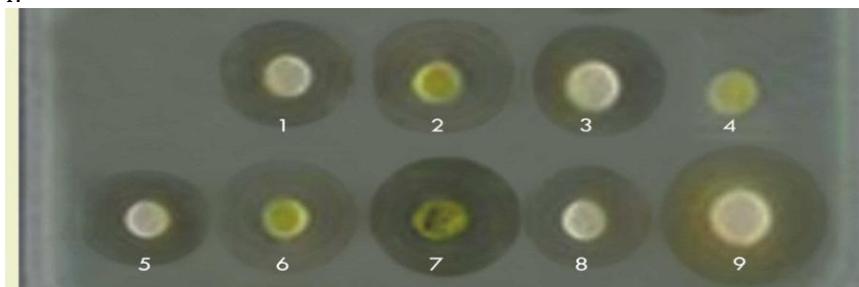


Fig. 1. Antibacterial activity against *Staphylococcus aureus*



Fig. 2. Antibacterial activity against *Pseudomonas aeruginosa*

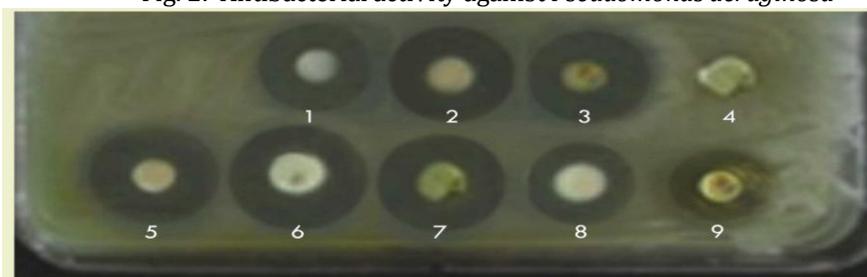


Fig. 3. Antifungal activity against *Candida albicans*

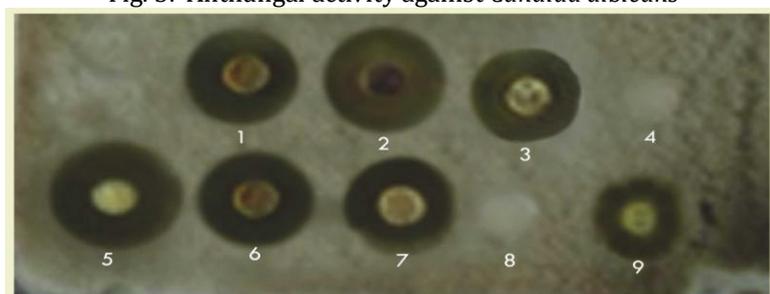


Fig. 4. Antifungal activity against *Aspergillus niger*

(In all Figures 1 = Pet. ether extract of root of *P. z.*, 2 =Chlo. extract of root of *P. z.*, 3=Alco. extract of root of *P. z.*, 4 =Aq. extract of root of *P. z.*, 5 =Pet. ether extract of root of *P. a.*, 6=Chlo. Extract of root of *P. a.*, 7=Alco. extract of root of *P. a.*,8=Aq. extract of root of *P. a.*, 9 =Standard.)

Discussion

The colours of different extracts of roots of *Plumbago* were found in different shades of brown. The consistency was found to be sticky in petroleum ether and chloroform solvent extract and more sticky in ethanol extract and non sticky in aqueous (polar solvent) extract. The successive extractive values for root of both plants are in the order- ethanol soluble successive extract value > water soluble successive extract value > chloroform soluble successive extract value > petroleum ether soluble successive extract value.

On the basis of phytochemical screening of different extracts of roots of both plants we concluded that roots of these plants are ample source of phytochemicals like carbohydrates, proteins, amino acids, lipids, alkaloids, tannin, flavonoids, cardiac glycosides, anthraquinone glycosides, saponin, coumarin glycosides cyanogenic glycosides, steroids. Ethanolic extracts have shown to possess maximum number of phytochemicals.

Many previous workers studied preliminary phytochemical screening of *P. zeylanica*. Preliminary phytochemical screening of root was carried out by Agbaje and Adeniran, 2009 (aqueous extract); Gupta et al, 2010 (petroleum ether, chloroform, ethanol extract); Ajayi et al, 2011 (ethanol extract); Rao et al, 2012 (petroleum ether, benzene, chloroform, ethanol, aqueous extract); Kakad et al, 2013 (Amyl alcohol, ethanol, methanol, ether, chloroform, aqueous extract); Jain et al, 2014 (methanol extract); Borhade et al, 2014 (petroleum ether, chloroform, methanol, water extract); Dohare et al, 2015 (petroleum ether, ethanol, aqueous extract).

Our results show some variations with records of previous work of preliminary phytochemical screening of *P. zeylanica*.

This is because there are several factors which affect presence and amount of secondary metabolites in plants. Secondary metabolites show considerable variation in plants depending on environmental, ontogenetic and genetic factors. Environmental factors affecting secondary metabolites are temperature, rainfall, length of the day, quality of light, altitude, type of soil, fertilizers used etc. Other factors affecting secondary metabolites are collection and storage of seeds for sowing, time of seed sowing, season and age of plant at the time of collection of drug, method of drying of drug (Evans, 2009).

The ability of some plants to synthesize cyanogenic glycosides is Cyanogenesis. When cyanogenic glycosides are enzymatically hydrolysed, they release cyanohydric acid (HCN) which is extremely toxic to a wide spectrum of organism. Cyanogenesis is one of the mechanisms that protect plants against predators such as herbivores. In intact plant, enzyme and cyanogenic glycosides remain separated [as these glycosides are stored in vacuole (<http://en.wikipedia.org/wiki/Glycoside>)] but if plant tissue is damaged, both are put in contact and toxic HCN is released (Francisco and Pinotti, 2000). Roots of both plants show presence of cyanogenic glycosides. The colour change of picrate paper in the present investigation was observed within 24 hours. A brown red colouration of picrate paper within 2 hours indicates the presence of cyanogenic glycosides and the respective hydrolytic enzyme and the plants are considered cyanogenic in the field. A brown red coloration of picrate paper within 48 hours indicates that the cyanogenic glycosides spontaneously released HCN without the action of enzyme and plants are not cyanogenic in the field because evolution of HCN is very slow (Francisco and Pintoti, 2000). The intensity of colour change of picrate paper was more in *P. zeylanica* root than in *P. auriculata* root. This indicates presence of more amounts of cyanogenic glycosides in *P. zeylanica* root than in *P. auriculata* root. Francisco and Pintoti (2000) also reported that wild plant species are more resistant to predators. We do not find previous reports of phytochemical screening for cyanogenic glycosides in roots of *Plumbago*.

In relation to *Staphylococcus aureus* antibacterial activity was shown by petroleum ether, chloroform, ethanol extract of root of *P. zeylanica* while in *P. auriculata* antibacterial activity against *Staphylococcus aureus* was shown by all the four extracts of root.

None of the extract of both plants showed antibacterial activity against *Pseudomonas aeruginosa*.

In relation to *Candida albicans*, antifungal activity was shown by petroleum ether, chloroform, ethanol extracts of root of *P. zeylanica* while in *P. auriculata*, this antifungal activity was shown by all the four extracts of root. In relation to *Aspergillus niger*, antifungal activity was shown by petroleum ether, chloroform, ethanol extract of roots both species of *Plumbago*.

Renuga and Thandapani (2013) got positive results of antibacterial activity of ethanol extract of *P. zeylanica* root (100 µg/ml) against *Pseudomonas aeruginosa* and *Staphylococcus aureus* by disc diffusion

method but we got positive result against *Staphylococcus aureus* only and not against *Pseudomonas aeruginosa*.

More or less similar phytochemical profile and antimicrobial activities with slight differences suggested that *P. auriculata* can be used as substitute for *P. zeylanica* but further characterization is required.

Table 1: Physicochemical characters of different solvent extracts of roots of two species of *Plumbago* Linn.

Part of plant	Character	Extract							
		Petroleum ether		chloroform		Ethanol		Aqueous	
		<i>P. z.</i>	<i>P. a.</i>	<i>P. z.</i>	<i>P. a.</i>	<i>P. z.</i>	<i>P. a.</i>	<i>P. z.</i>	<i>P. a.</i>
Root	Colour	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown	Dark brown	Dark brown	Faint brown	Brown
	Consistency	Sticky	Sticky	Sticky	Sticky	More sticky	More sticky	Non sticky	Non sticky
	Successive extractive value	1.137	0.549	1.562	1.157	7.411	5.585	3.130	2.564

Acknowledgements

We are grateful to Principal, M. J. College, Jalgaon and Principal, Dr. A. G. D. Bendale Mahila Mahavidyalaya, Jalgaon for providing necessary facilities. We are thankful to Dr. Ulhas Patil, Head, Department of Microbiology, R. C. Patel Arts, Commerce and Science College, Shirpur for help during antimicrobial testing

Table 2: Phytochemical evaluation of root extracts of two species of *Plumbago* Linn.

Phytochemical Tests	Result							
	Petroleum ether Extract		Chloroform Extract		Ethanol Extract		Aqueous Extract	
	<i>P.z.</i>	<i>P.a.</i>	<i>P.z.</i>	<i>P.a.</i>	<i>P.z.</i>	<i>P.a.</i>	<i>P.z.</i>	<i>P.a.</i>
Tests For Carbohydrates								
1. Molish's Test	+	+	+	+	+	+	+	+
2. Fehling's Test	+	-	+	+	+	+	+	+
3. Benedict's Test	+	-	+	+	+	+	+	+
4. Barfoed's Test	-	-	-	-	+	+	+	-
5. Iodine Test	-	-	-	-	-	-	-	-
Tests For Proteins								
1. Biuret Test	+	+	+	+	+	+	-	+
2. Million's Test	-	-	-	-	+	+	-	+
Tests For Amino Acid								
Ninhydrin Test	-	-	-	-	-	+	+	-
Tests For Lipids								
Extract + Sudan III	+	+	+	+	+	+	-	-
Tests For Alkaloids								
1. Wagner's Test	+	+	+	+	+	+	+	+
2. Mayer's Test	+	+	+	+	+	+	-	+
3. Hager's Test	+	+	+	+	+	+	-	+
4. Tannic Acid Test	+	+	+	+	+	+	+	+
5. Dragendorff's Test	+	+	+	+	+	+	+	+
Tests For Tannin								
1. FeCl ₃ Test	-	-	-	-	+	+	+	+
2. Lead acetate Test	-	-	-	-	+	+	+	+
3. Potassium permanganate Test	-	-	-	-	+	+	+	+
4. Bromine water	-	-	-	-	+	+	+	+
Tests For Flavonoids								
1. Shinoda Test	-	-	-	-	+	+	-	-
Tests For Glycosides								
1. Test for cardiac glycosides								

Legal Test	+	+	+	+	+	+	-	-
Keller-killiani Test	+	+	+	+	+	+	+	-
Liebermann's Test	+	+	+	+	-	-	-	-
2. Test for anthraquinone glycosides								
Borntrager's Test	+	-	+	-	+	+	+	+
3. Test for saponin								
Foam Test	-	-	-	-	+	+	+	+
Haemolytic Test	-	-	-	-	+	+	+	+
4. Test for coumarin glycosides								
Ammonical solution show blue, blue green or violet inflorescence	+	+	+	+	+	+	+	+
Extract+1N NaOH	+	+	+	+	+	+	+	+
Tests For Steroids								
1. Salkowski Test	+	+	+	+	+	+	-	-
2.Liebermann-Burchard Test	+	+	+	+	+	+	-	-
3.Liebermann's Test	+	+	+	+	-	-	-	-

+ = Present, - = Absent

Table3: Results of test for Cyanogenic Glycosides and mucilage

Test	P. z.	P. a.
Grignard reaction for cyanogenic glycoside	+	+
Test for mucilage	+	+
Swelling Index	1.67	4.33

Table4: Antimicrobial activity of extracts of *Plumbago* conc. 100 µg per disc

Type of root extract	Diameter of zone of inhibition (mm)							
	<i>S. aureus</i>		<i>P.aeruginosa</i>		<i>C. albicans</i>		<i>A. niger</i>	
	P. z.	P. a.	P. z.	P. a.	P. z.	P. a.	P. z.	P. a.
Petroleum ether	17.02	16.23	-	-	16.23	16.33	16.23	16.85
Chloroform	17.10	17.11	-	-	16.89	17.45	16.35	16.11
Ethanol	16.89	17.25	-	-	16.87	17.05	14	14
Aqueous	-	14.10	-	-	-	14.02	-	-
Ciprofloxin (10 µg per disc)	19.26		19.2		NA		NA	
Amphotericin - B (100 units / disc)	NA		NA		13.05		11.92	

- means no zone of inhibition

NA means not applicable

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