

In-Vitro Antimicrobial Activity Phytochemical Screening of *Phytolacca acinosa* in Kashmir Valley

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Abstract: The hydroalcoholic extract of the roots of *Phytolacca acinosa* were investigated for their in vitro phytochemical, antibacterial and antifungal activities. The preliminary phytochemical screening of plant extract shows the existence of tannins, flavonoids, carbohydrates, protein sulphur, proteins, polyphenol, tannic and phenolic compounds. The extract of *Phytolacca acinosa* showed significant antibacterial and antifungal activities when tested against eight bacterial strains and four fungal strains by disc diffusion method. The results indicated that the extract inhibited the growth of selected bacteria and fungi. Thin layer chromatography performed on the root extracts confirmed the presence of various phytochemicals. The findings obtained in the present study suggest that root extract of *Phytolacca acinosa* could be useful in treating diseases caused by the tested organisms.

Keywords: Medicinal plant, Hydroalcohol, *Phytolacca acinosa*, Thin layer chromatography

1. Introduction

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total.^[1,2] Medicinal plants constitute an effective source of traditional (Ayurvedic, Chinese, Homeopathic and Unani) and modern medicine. There is a pressing need for evaluation and analysis of herbal drugs using sophisticated techniques.^[3]

Plant-based natural constituents can be obtained from any part of the plant like bark, leaves, flowers, fruits, roots, seeds, etc. Recently, the quest for the isolation of new compounds from medicinal plants has become an intriguing area of research. Plants with ethnospharmaceutical importance were being studied because of their healing properties^[4] as well as for their efficient antimicrobial and antifungal properties.

Infectious diseases caused by the microorganisms (like bacteria, fungi and virus) are responsible for large scale morbidity and mortality worldwide.^[5] The global emergence of bacterial resistance is responsible for many infectious diseases.^[6] Although many antibiotics have been introduced, the drug resistance in bacteria has continuously escalated.^[7] This situation directed the interest of researchers towards the development of novel drugs from herbal products, having antimicrobial potentials.^[8]

Phytolacca is a genus of perennial plants native to North America, South America, East Asia and New Zealand. Some members of the genus are known as pokeweeds or similar names such as pokebush, pokeberry, pokeroot or poke sallet.^[9] The generic name is derived from the Greek word (*phyton*), meaning "plant," and the Latin word *lacca*, a red dye.^[10] The genus comprises about 25 to 35 species

of perennial herbs, shrubs, and trees growing from 1 to 25 m (3.3 to 82.0 ft) tall, flowering in July to August. Habitat of the herb is Valleys, hillsides, forest understories, forest margins and roadsides at elevations of 500 - 3400 metres. It is also found in cultivated land houses, moist fertile lands and as a weed. They have alternate simple leaves, pointed at the end, with entire or crinkled margins; the leaves can be either deciduous or evergreen. The stems are green, pink or red. The flowers are greenish-white to pink, produced in long racemes at the ends of the stems. They develop into globose berries 4–12 mm diameter, green at first, ripening dark purple to black.^[11-13]

Phytolacca species roots have been comprehensively used as antiasthmatic, antibacterial, antidote, antifungal, antitussive, diuretic, expectorant, laxative and vermifuge. The plant has an interesting chemistry and has been investigated as a potential anti-AIDS drug. It contains potent anti-inflammatory agents, antiviral proteins and substances that affect cell division. The root is used internally in the treatment of urinary disorders, nephritis, oedema and abdominal distention. Externally, it is used to treat boils, carbuncles and sores. The roots are harvested in autumn and dried for later use. The chemical composition, antimicrobial and biological activities of roots of *phytolacca* and its extracts have recently been of immense interest.

The present study was designed to investigate the phytochemical, antibacterial and antifungal potentials of less explored *Phytolacca acinosa* against bacterial strains and fungal strains causing human disease.

2. Materials and Methods

2.1 Collection of medicinal plants

Fresh roots of *Phytolacca acinosa* medicinal plants were collected in the month of July 2014 from low altitude Gogaldor Tangmarg Kashmir, India. Each specimen/plant

material was labelled with the date of collection, locality, and their medicinal uses were recorded.

2.2 Processing of medicinal plants

The roots of plants were used to prepare extracts for the study. The plants collected were washed with water to remove the soil and dust particles. Then they were dried in a thoroughly shaded place, and blended to form a fine powder and stored in airtight containers.

2.3 Human pathogenic bacterial species

The human pathogenic strains: *Staphylococcus aureus*: ATCC (American type culture collection) 6538, *Enterococcus faecalis*: MTCC (Microbial type culture collection) 2729, *Streptococcus mutans*: MTCC 497, *Micrococcus luteus*: ATCC 6259, *Escherichia coli*: ATCC 2065, *Pseudomonas aeruginosa*: ATCC 741, *Pseudomonas putida*: ATCC 47054, *Proteus vulgaris*: ATCC 1335, *Candida albicans*: ATCC 10231, *Aspergillus niger*: ATCC 1197, *Herpes*: ATCC 1493, *Tinea cruris* were obtained from Institute of Microbial Technology, Chandigarh. The strains were used for evaluating antibacterial and antifungal activity. The bacterial strains were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C, whereas the yeasts and molds were grown in Sabouraud dextrose agar at 28°C. The stock cultures were maintained at 4°C until further use.

2.4 Preparation of plant extract

The hydroalcoholic extracts of the medicinal plants were prepared by dissolving 100g of the dried powder of root, extracted with hydro alcoholic soxhlation (Merck).^[14] The extraction with hydro alcoholic solvent was kept for seven to eight days to obtain extract. After that, the extract was evaporated in rotary evaporator at 55°C to obtain crude for phytochemical analysis, determination of bio-active compounds and antimicrobial susceptibility. The extract was stored under refrigeration at 4°C. This crude extract of hydroalcohol was used for antimicrobial properties.

2.5 Preparation of sterile disc

Whatman's No.3 filter paper was punched into 5 mm disc form and sterilized, each sterile disc was incorporated individually with 20–60µl of extracts using micropipette. Precautions were taken to prevent the flow of the solvent extract from the discs to the outer surface. The condensed extracts were applied in small quantities on discs and were allowed to dry in air. After sometime another dose of extract was applied on discs and stored at 4°C until further use.

2.6 Phytochemical investigation

Phytochemical analysis for Carbohydrate, Protein, Amino acid, Glycosidase, Flavonoids, Alkaloids, Tannic and phenolic compounds, Protein sulphur, Carbonate, Starch, Coumarin glycosidase, Saponin, Tannin, Terpenes, Polyphenol was conducted as per the standard protocol.^[15-17]

2.7 Antibacterial activity using disc diffusion method

100µl of fresh culture of human bacterial pathogens were swabbed on the sterilized Muller Hinton agar plates. The discs with different concentrations (mg/mL) of plant extract (25%, 50%, 75% and 100%) were inoculated over the agar plates using sterile forceps and incubated for 24 hours at 37°C. After incubation, the zone of inhibition around each disc was measured (mm) and recorded.^[18]

2.8 Antifungal activity using disc diffusion method

Fungal strains were cultured on Sabouraud's Dextrose Agar plates. The discs with different concentrations (mg/mL) of plant extract (25%, 50%, 75% and 100%) were inoculated over the SDA plates using sterile forceps and incubated for 72 hours at 28°C. After incubation, the zone of inhibition around each disc was measured (mm) and recorded.

2.9 Thin Layer Chromatography (TLC)

The solvent system was poured to a depth of 0.5 cm in a rectangular chromatographic glass chamber. The chamber was lined with a piece of filter paper to ensure proper saturation. The spots of extract were applied on a silica gel-G plate with the help of capillary tube. The distance between two spots was kept approximately 2.0 cm. The applied spots were dried at room temperature and the plate was gently placed inside the glass chamber. The angle of the plate with the vertical was kept approximately 15°. The chromatogram was developed till the solvent front migrated to about 10.0 cm. The plate was taken out and the solvent front was marked. The plate was dried at room temperature and inspected either under UV light or sprayed with the specific detecting reagent. The coloured spots were marked and the R_f value of each separated component was calculated.

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front TLC plates}}$$

3. Results and Discussion

3.1 Preliminary phytochemical screening

It was found that hydroalcoholic extracts of *Phytolacca acinosa* roots contained tannins, flavonoids, carbohydrates, protein sulphur, proteins, polyphenol, tannic and phenolic compounds shown in Table-1.

Table 1: Phytochemical investigation of hydroalcoholic root extract of *Phytolacca acinosa*

Compounds	Present/ Absent	Compounds	Present/ Absent
Carbohydrate	Present	Organic, Oxalic and Malic test	Positive
Protein	Present	Protein sulphur	Present
Amino acid	Absent	Carbonate	Absent
Glycosidase	Absent	Starch	Absent
Flavonoids	Present	Coumarin glycosidase	Absent
Alkaloids	Absent	Saponin	Absent
Tannic & phenolic compounds	Present	Tannin	Present
Terpenes	Absent	Polyphenol	Positive

3.2 Antimicrobial activity

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. The antibacterial screening of different concentrations of the extracts on the test isolates are represented in Table 2, 3 and 4. The findings show that the increase in concentration of the extracts, increased the zones of growth inhibition of the bacteria. The assessment of the antibacterial activity was based on the measurement of diameter zone of Inhibition (mm) that formed around the disc filled with the plant extract at different concentration. Maximum inhibition zone was recorded at 100 mg/ml and the minimum inhibition zone at 25 mg/ml in the bacteria.

Among the four Gram positive bacteria tested, *E. faecalis* and *M. luteus* gave the largest zone of inhibition, whereas, *S. aureus* and *S. mutans* gave the least zone of inhibition against hydroalcoholic root extract of *Phytolacca acinosa* and similarly among the four Gram negative bacteria tested, *P. aeruginosa* and *P. putida* gave the largest zone of inhibition, whereas, *E. coli* and *P. vulgaris* gave the least inhibition zone. This shows that higher concentration of hydroalcoholic root extract of *Phytolacca acinosa* had the antibacterial activity.

In the present study among the four fungal strains tested, the antifungal activity of hydroalcoholic root extract of *Phytolacca acinosa* does not show marked zone of inhibition around the disc on increase in concentration of extract.

The results indicated that the hydroalcoholic root extract of the plants inhibited the growth of the bacteria and fungi. Analysis of *Phytolacca acinosa* roots revealed that it contains wide range of chemical compounds- tannins, flavonoids, carbohydrates, protein sulphur, proteins, polyphenol, tannic and phenolic compounds. So, it can be suggested that due to the presence of these chemical compounds, the root extract possess antibacterial and antifungal properties

3.3 Thin Layer Chromatography

TLC of hydro alcoholic root extract of *Phytolacca acinosa* by using solvent system ethyl acetate: Methanol (1:1) provided Rf Value = 1.206. Figure-1

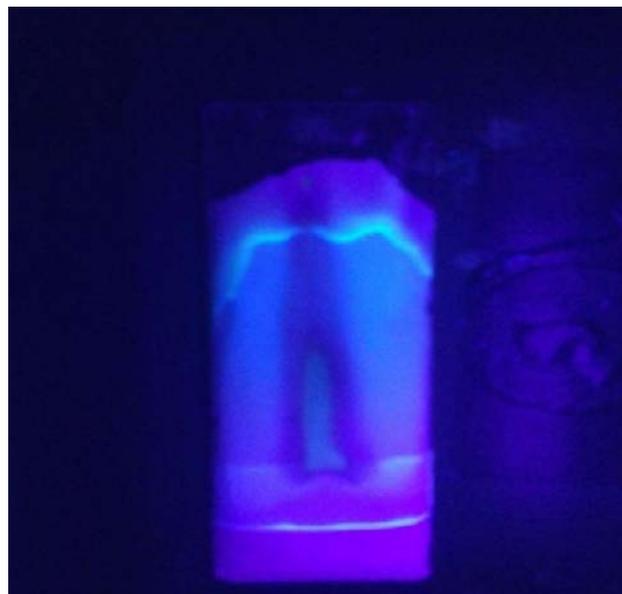


Figure 1: Qualitative analysis by Thin Layer Chromatography of root extract of *Phytolacca acinosa* using Ethyl acetate: methanol (1:1). The Rf Value of produced content of root is 1.206.

The TLC of the extracts confirms the presence of diverse potent bio-molecules in the plants. TLC analysis provides an idea about the polarity of various chemical constituents, in a way such that compound showing high Rf value in less polar solvent system have low polarity and with less Rf value have high polarity. These potent bio-molecules can be further used for development of different drugs in future.

4. Conclusion

To our knowledge, this is the first report from Kashmir valley in regards to evaluate hydroalcoholic extracts of *Phytolacca acinosa* for antimicrobial properties. The hydroalcoholic root extracts were found to be active on most of the clinically selected bacteria and fungi. The present study justifies the use of roots in the traditional system of medicine to treat various infectious disease. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents. The present study provides baseline information for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

Table 2: Antibacterial activity of hydroalcoholic root extract of *Phytolacca acinosa* in Gram positive bacteria

Conc of extracts (mg/ml)	Zone of inhibition (mm)			
	<i>S. aureus:</i> ATCC 6538	<i>E. faecalis:</i> MTCC 2729	<i>S. mutans:</i> MTCC 497	<i>M. luteus:</i> ATCC 6259
100	11	15	11	14
75	11	12	11	12
50	10	10	10	11
25	9	10	8	11

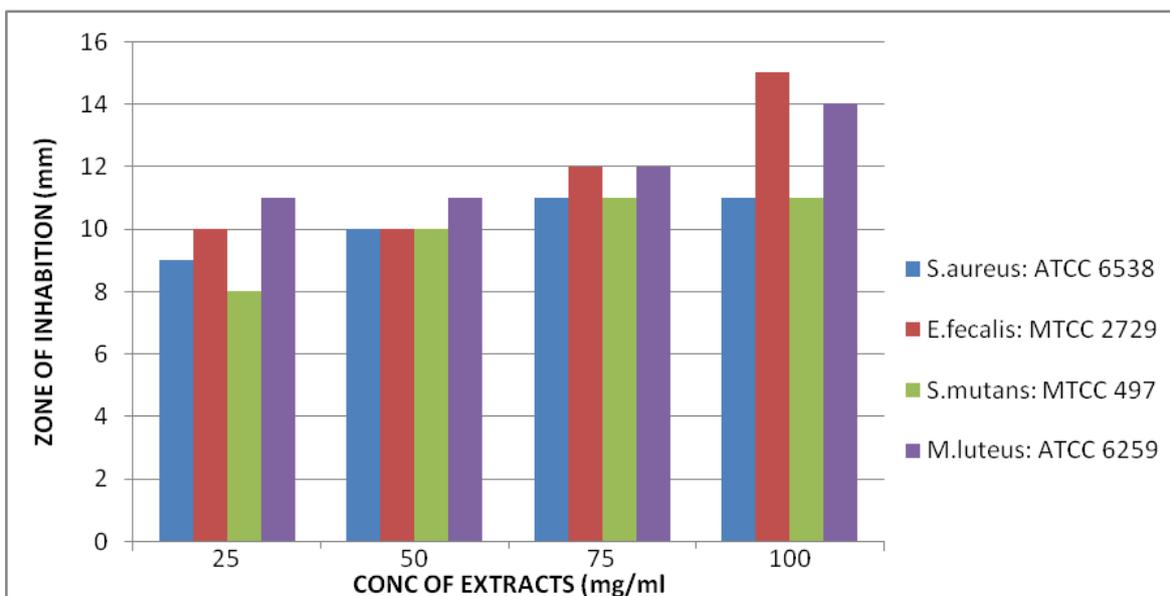


Table 3: Antibacterial activity of hydroalcoholic root extract of *Phytolacca acinosa* in Gram negative bacteria

Conc of extracts (mg/ml)	Zone of inhibition (mm)			
	<i>E. coli</i> : ATCC 2065	<i>P. aeruginosa</i> : ATCC 741	<i>P. putida</i> : ATCC 47054	<i>P. vulgaris</i> : ATCC 1335
100	12	14	12	11
75	11	11	11	10
50	10	11	11	10
25	9	10	10	8

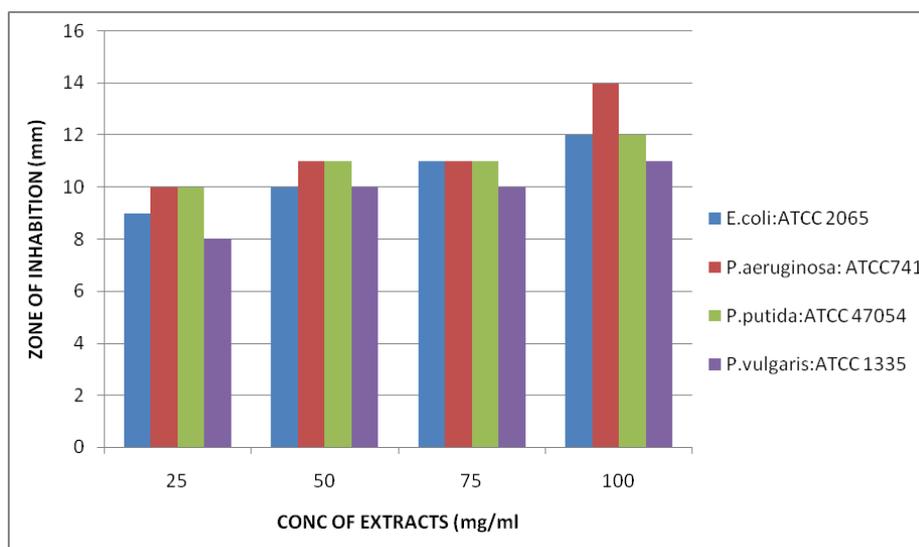
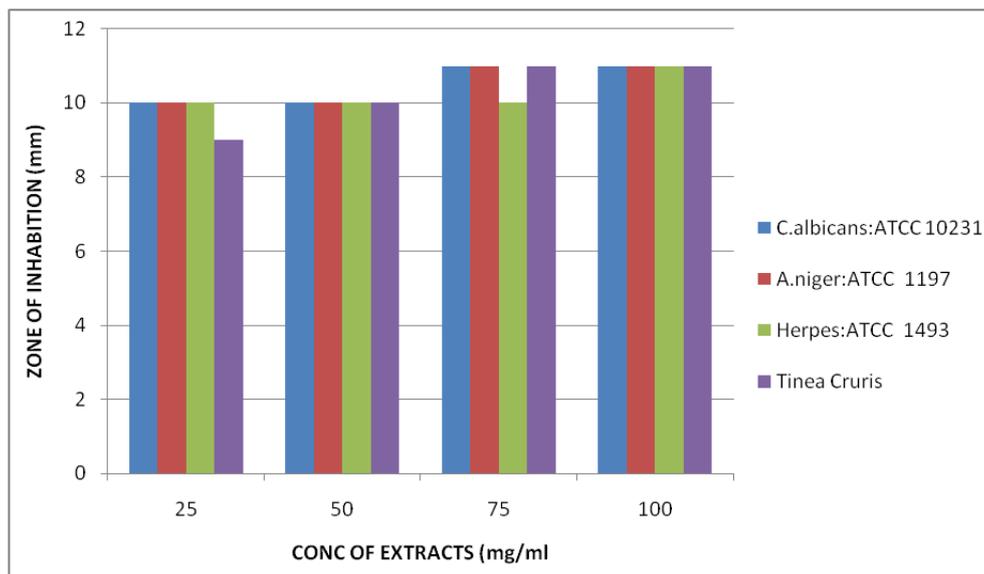


Table 4: Antifungal activity of hydroalcoholic root extract of *Phytolacca acinosa* in fungal strains

Conc of extracts (mg/ml)	Zone of inhibition (mm)			
	<i>C. albicans</i> : ATCC 10231	<i>A. niger</i> : ATCC 1197	<i>Herpes</i> : ATCC 1493	<i>Tinea cruris</i>
100	11	11	11	11
75	11	11	10	11
50	10	10	10	10
25	10	10	10	9



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