



Research Article

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EVALUATION OF PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF *PHYTOLACCA OCTANDRA*

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Received on: 12/10/18 Accepted on: 19/11/18

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DOI: 10.7897/2277-4343.096181

ABSTRACT

Pharmacological and antibacterial properties of *Phytolacca octandra* leaf extracts were investigated against significant bacterial pathogens in the present research. *Phytolacca octandra* leaf powder was extracted with water, acetone and petroleum ether separately using Soxhlet extraction protocol. The phytochemical screening of *Phytolacca octandra* extracts was investigated for the presence or absence of different phytoconstituents like alkaloid, tannin, flavonoid, phenol, steroid, saponin, glycoside, amino acid, terpenoid and carbohydrates. The antibacterial activity of the acetone extracts were evaluated against the test organisms by well diffusion method. Acetone extracts revealed the presence of different pharmacological compounds like alkaloid, tannin, flavonoid, phenol, steroid, saponin and glycoside. MICs of *Phytolacca octandra* extracts ranged from 3.13mg/ml to 12.5mg/ml against all the test organisms. Antibacterial activity of *Phytolacca octandra* showed maximum inhibitory zones of 21mm, 18mm, 19mm, 19mm and 20mm against respective organisms for 25mg/ml of acetone extracts. Three types of phytochemical compounds (phenol, tannin and flavonoids) analyzed to identify the bioactive compounds from the column purified fractions using TLC. Green colour spots with Rf value of 0.78 was observed indicating the presence of phenol. The presence of tannin indicated by grey colour spots with Rf value of 0.83. And flavonoids showed orange colour spots when exposed to aluminum chloride reagents. The Rf value was calculated as 0.86. The obtained results corroborate the importance of ethno-pharmacological surveys in the selection of *Phytolacca octandra* plants for bioactivity screening against significant bacterial pathogens.

Keywords: *Phytolacca octandra*, Phytochemical compounds, Minimum inhibitory concentration, Antibacterial activity, Acetone extracts.

INTRODUCTION

Knowledge of the plant chemistry is very much essential for the development of useful plant products. The expanding knowledge of phytochemical screening has revealed the existence of close relationship between constituents of plants and their taxonomical status. The characters more often studied in chemotaxonomy are secondary metabolites of pharmaceutical significance such as alkaloids, glycosides, flavonoids etc¹. Many of the weeds found here, are reported to have important medicinal values in their native homes. One such example is the members of the family Phytolaccaceae².

According to Lawrence³ the family Phytolaccaceae consists of 17 genera and 125 species. *Phytolacca* is the largest genus of the family with 35 species. *Phytolacca octandra* L. is a native of tropical America. It has been introduced long ago to Kodaikanal and Nilgiri Hills in India. Now it seems to be restricted to Kodaikanal, Ooty and Munnar in South India, as a common and abundant wayside weed. It also grows along water-sides, field border, waste places etc. at higher elevations along Western Ghats. Flowering and fruiting occur mostly throughout the year but preferably in late summer, i.e., May to August.

Phytolacca octandra L. is a glabrous perennial herb of about 1.5 to 2.0m height. Leaves are long lanceolate or ovate-lanceolate and cuneate at the base. The leaf margins are slightly pinkish when mature. Evidence on the phytochemical, pharmacological and clinical studies of the species *Phytolacca octandra* L., were available during the literature survey. Since only very few data were available, reports on closely related taxa points to the possibility for the presence of similar properties in this species were presented below. Different phytochemical compounds

screened from the closely related species of *Phytolacca* spp were described. Analysis of aqueous extracts of *Phytolacca americana* L. showed the presence of seven phenolic compounds namely, gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, m-hydroxybenzoic acid, coumaric acid and cinnamic acid⁴. A glycoside compound named 'Esculentoside S', a phytolaccagenin, was reported from the leaves of *Phytolacca acinosa* Roxb.⁵

Phenolic compounds and other elements in leaf extracts were analysed and reported by Yong et al⁶. Strong anti-inflammatory saponins were isolated from callus mass derived from stems and roots of *Phytolacca americana* L. Triterpenoid saponins such as Phytolaccoside A, B and D were also reported⁷. *Phytolacca dodecandra* L. (Endod) is a proven botanical pesticide. It has been shown that due to its antifungal property, Endod is effective against dermatophytes and is recommended for the preparation of a natural antimycotic ointment.

The present study is concentrated on the phytochemical, antibacterial and biochemical properties of *Phytolacca octandra* L. plant extracts. The study is aimed to evaluate the biochemical characters and Pharmacognostic peculiarities of *Phytolacca octandra* giving a special emphasis on their antimicrobial activities.

MATERIALS AND METHODS

Herbal powder procurement

Phytolacca octandra herbal leaf powder was commercially procured from a local supplier at Coimbatore, Tamil Nadu, India.

Bacterial cultures used

Two bacterial cultures, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Klebsiella pneumoniae* which have the ability to cause hospital-acquired infections were commercially procured from a diagnostic laboratory at Coimbatore, Tamil Nadu, India.

Preparation of Leaves extract

About 50g of *Phytolacca octandra* herbal leaf powder was extracted with 125mL of acetone separately using Soxhlet extraction apparatus for 3days. The extract was evaporated to dryness using rotary flash evaporator. Similarly, petroleum ether and aqueous extracts were also prepared. From the resultant herbal extract, 5 different concentrations (5mg/l, 10mg/ml, 15mg/ml, 20mg/ml and 25mg/ml) were prepared to evaluate the antibacterial and antifungal efficacy. All the five concentrates of herbal extract was stored in the refrigerator prior to use.

Qualitative phytochemical analysis of *Phytolacca octandra* extracts

The phytochemical screening of the acetone, petroleum ether and aqueous extracts of *Phytolacca octandra* was performed using standard methods for the presence or absence of different phytoconstituents like alkaloid, tannin, flavonoid, phenol, steroid, saponin, glycoside, aminoacid, terpenoid and carbohydrates.

For the detection of saponin, 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion. Tannin was detected using 200 mg of plant material in 10 ml distilled water. The solution was filtered and about 2 ml filtrate was added to 2 ml of FeCl₃. Appearance of blue-black precipitate indicated the presence of Tannins.

Terpenoids was identified with 2 ml of plant extract added to 2 ml of acetic anhydride and concentration of H₂SO₄. Formation of blue green rings indicates as the presence of terpenoids. Steroids was detected using 1 ml of the extract dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids. Presence of glycosides was revealed with 0.5 g of solvent extract dissolved in 2.0 ml of glacial acetic acid containing one drop of FeCl₃ Solution. This was then under laid with 1.0 ml of concentrated H₂SO₄. A brown ring obtained at the interface indicated the presence of glycosides. Amino acids was detected using One ml of the extract was treated with few drops of Ninhydrin reagent. No appearance of Purple colour shows the absence of amino acids. Alkaloids were identified by using 200mg plant material in 10 ml methanol. The suspension was filtered and the obtained 2 ml filtrate was added with 1% HCl. To this mixture, 1 ml filtrate and 6 drops of Dragendroff reagent was added. Creamish precipitate and brownish-red precipitate indicated the presence of respective alkaloids. Flavonoid which is considered as an important antipsoriatic compound was identified using one ml of the extract and few drops of dilute sodium hydroxide. In this mixture, an intense Yellow colour was produced which later become colorless on addition of a few drops of dilute acid indicates the presence of Flavonoids. Carbohydrates were detected using 1 ml of Fehling's A and 1ml of Fehling's B solution added and boiled

with plant extracts for few minutes. The appearance of yellow or brownish colour indicates the presence of sugars

Qualitative antibacterial assessment of *Phytolacca octandra*

The antibacterial activity of *Phytolacca octandra* leaves extracts were evaluated against the significant organisms (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*) by well diffusion method. About 0.1% inoculum suspensions of five bacterial cultures were swabbed uniformly over each agar plate surface. Under sterile conditions, 6mm wells were cut on the agar surface of each Nutrient Agar (NA) plates. About 50µl each of *Phytolacca octandra* leaves extracts, in 5% dimethyl sulfoxide (DMSO) were loaded into the well and the plates were incubated at 37°C for 24 - 48h. The antibacterial activity was evaluated in terms of zone of inhibition around the wells of each extract in all the inoculated NA plates. The inhibition clear zones were measured and recorded in millimeter.

Determination of minimum inhibitory concentration (MIC) of *Phytolacca octandra*

Minimum inhibitory concentration (MIC) of *Phytolacca octandra* leaves extracts were determined by standard broth macrodilution method. Each plant extracts were initially dissolved in 5% dimethyl sulfoxide (DMSO) prior to experiments. All the test cultures were inoculated in a sterile Nutrient broth (Composition g/L: Peptone: 5g; Yeast extract: 5g, Beef extract: 3g, Sodium chloride: 5g; Final pH (7.0 ± 0.2) and allowed to attain the growth for 24 to 48 hours. To determine the MIC of the plant extracts, a set of tubes with 1ml of Nutrient broth was added under sterile conditions. About 150µl of each plant extracts at different concentrations ranging from 200ug/ml to 12.5mg/ml was added to the tubes containing Nutrient broth. To this above mixture, 500µl inoculum of each test cultures (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*) was added to their respective tubes. All the inoculated tubes were incubated at 37°C ± 0.2°C for 24 to 48hours. Organic solvent, acetone was used as negative control and antibacterial drug Gentamicin (100µg/ml) was used as positive control.

RESULTS AND DISCUSSION

Phytochemical profiles of *Phytolacca octandra* solvent extracts

Phytochemical compounds present in different solvent extracts of *Phytolacca octandra* was identified in the present research. Among the three solvent extracts used, acetone extracts revealed many of the phytochemical compounds like, alkaloid, tannin, flavonoid, phenol, steroid, saponin, glycoside and amino acid. Terpenoid and carbohydrates were found absent from the acetone extracts. The acetone extracts revealed that, the compounds attributed to antimicrobial properties like phenol and saponin were found higher.

When compared to acetone extracts, the compounds derived from petroleum ether extracts and aqueous extracts were significantly found at lower rates. Therefore, the acetone extracts were subjected to antibacterial and antifungal activity. The petroleum ether extracts exhibited the phytochemical compounds like alkaloid, flavonoid, phenol, steroid, terpenoid, saponin and glycosides. The aqueous extracts showed compounds like alkaloids, tannins, flavonoids, phenol and glycosides at very less concentrations (Table.1).

Table 1: Phytochemical profiles of *Phytolacca octandra* solvent extracts

S. No.	Phytochemicals	Acetone extract	Water extract	Petroleum ether extract
1	Alkaloids	+	++	++
2	Tannins	++	+	-
3	Flavonoids	++	+	+
4	Amino acids	+++	-	+
5	Carbohydrates	-	+	-
6	Phenols	++	+	+
7	Steroids	+	-	+
8	Terpenoids	-	-	+
9	Saponins	++	-	+
10	Glycosides	++	+	+

Table 2: Biochemical tests of the test cultures

S.No	Test	Bacterial cultures				
		<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterobacter aerogenes</i>	<i>Klebsiella pneumoniae</i>
1	Microscopy	Gram positive cocci	Gram negative rods	Gram negative short rods	Gram negative rods	Gram negative rods
2	Selective media	Golden yellow colonies in MSA	Green metallic sheen in EMB	Bluish green pigment in CAM	Purple colonies in EMB	Pink colonies in MacConkey
3	Indole	+	+	-	-	-
4	Methyl red	-	+	-	-	+
5	Voges - Proskauer	-	-	+	+	-
6	Simmon citrate	+	+	-	-	+
7	Coagulase	+	-	-	-	-
8	Oxidase	+	-	+	-	-
9	TSI	AK/A	A/A ^G	AK/AK	A/AK	A/AK
10	Hemolysis	Beta hemolytic	Non hemolytic	Alpha hemolytic	Non hemolytic	Non hemolytic

Table 3: Minimum inhibitory concentration (MIC) of *Phytolacca octandra* acetone extracts against bacteria

S. No	Test Organisms	MIC Values of <i>Phytolacca octandra</i> (mg/ml)
1	<i>Staphylococcus aureus</i>	6.25
2	<i>Escherichia coli</i>	3.13
3	<i>Pseudomonas aeruginosa</i>	12.5
4	<i>Enterobacter aerogenes</i>	3.13
5	<i>Klebsiella pneumoniae</i>	6.25

Table 4: Qualitative analysis of Antibacterial activity of *Phytolacca octandra* acetone extracts

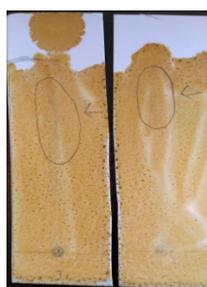
S. No	Test Organisms	Zone of Inhibition (in mm)				
		5mg/ml	10mg/ml	15mg/ml	20mg/ml	25mg/ml
1	<i>Staphylococcus aureus</i>	-	-	13	17	21
2	<i>Escherichia coli</i>	-	-	12	14	18
3	<i>Pseudomonas aeruginosa</i>	-	-	12	15	19
4	<i>Enterobacter aerogenes</i>	-	-	11	17	19
5	<i>Klebsiella pneumoniae</i>	-	-	13	15	20

Table 5: Phytochemical and bioactive compounds of *Phytolacca octandra* extract using Thin Layer Chromatography

Phytoconstituents	Mobile phase	Spraying reagent	Spot colour	Rf value (standard)	Rf value (Test)
Phenol	Ethyl acetate:Toluene:Formic acid	10% Ferric chloride	Green	0.80	0.78
Tannin	Methanol:Water	10% Ferric chloride	Grey	0.83	0.83
Flavonoid	Ethyl acetate:Toluene:Formic acid	11% Aluminium chloride	Orange	0.87	0.86



Phenol



Tannin



Flavonoid

Fig.1: TLC Analysis of column fractions for the phytoconstituents

Minimum inhibitory concentration (MIC) of *Phytolacca octandra*

The MICs of *Phytolacca octandra* extracts ranged from 3.13mg/ml to 12.5mg/ml against the five test bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*. Prior testing the cultures were confirmed using biochemical tests as per Microbiological standards. The results for the biochemical tests were tabulated in Table.2. The obtained MICs determined that all the bacterial species were found to be sensitive to the acetone extracts of the plants used. Among the different acetic extract concentrations, *Escherichia coli* and *Enterobacter aerogenes* were found very sensitive. MIC for these two organisms was found to be 3.13mg/ml, the least concentrate among the three used in the study. For *Klebsiella pneumoniae* and *Staphylococcus aureus* the MIC was observed as 6.25mg/ml. MIC for *Pseudomonas aeruginosa* was revealed as 12.5mg/ml which is the higher among the three concentrations (Table.3)

Antibacterial activity of *Phytolacca octandra*

The antibacterial activity of *Phytolacca octandra* extracts against five test bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Klebsiella pneumoniae* was evaluated by agar diffusion method. Five different concentrations of acetic extracts of *Phytolacca octandra* was used to determine the antibacterial activity against each organism. The results obtained in this study indicated that *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Klebsiella pneumoniae* tested against the acetone extract of *Phytolacca octandra* showed maximum inhibitory zones of 21mm, 18mm, 19mm, 19mm and 20mm for 25mg/ml concentration respectively. The acetic extract of subsequent concentrations 20mg/ml and 15mg/ml also showed promising inhibitory zones during the analysis against all the test organisms. Inhibitory zones measuring 17mm, 14mm, 15mm, 17mm and 15mm were obtained for 20mg/ml of plant extracts against the respective organisms as mentioned above. Similarly, for 15mg/ml of plant extracts, 13mm, 12mm, 12mm, 11mm and 13mm of inhibitory zones were obtained. Interestingly, for the other two concentrations viz., 10mg/ml and 5mg/ml no inhibitory zones were observed against any test organism (Table.4).

Antibacterial inhibitory zones obtained during the analysis revealed that the pharmacological active compounds present in the herbal extracts disrupt the cell membrane of the microbes through the physical and ionic phenomenon. The compounds present in the extract thus inhibited growth of test organisms by using an electrochemical mode of action to penetrate and disrupt their cell walls. When the cell walls are penetrated, leakage of metabolites occurs and other cell functions are disabled, thereby preventing the organism from duplication⁸. The significant activity exerted in this present research was due to the fact that the natural bioactive compounds responsible for the antibacterial activity are mostly extracted in the solvents and these active compounds may be able to penetrate the thick cell walls through general diffusion channels formed by the bacterial porins present therein, and affect the bacterial enzymes that are responsible for survival and virulence of organisms resulting in cellular lysis⁹.

Bacterial reduction percentage obtained during the study also attributed that the compounds such as alkaloid, tannin, flavonoid, phenol, steroid, saponin and glycoside in *Phytolacca octandra* extract are the responsible bioactive agents. The antibacterial activity of such phytochemicals was reported by earlier studies and revealed that they inhibited the growth of microbes in many ways such as by inhibiting protein synthesis, interfering with nucleic acid synthesis, breaking the peptide bonds, acting as

chelating agents, inhibiting metabolic pathway, interfering with cell wall synthesis or by preventing utilization of available nutrients by the microorganisms¹⁰. However these extracts do not affect the non-pathogenic bacteria which may be due to hindrance of penetration through the outer cell wall and absence of specific enzyme in the bacteria. This mode of action of the plant extract against the specific bacteria may be due to its secondary mode of action against the bacterial enzymes instead of acting on the cell wall of the bacteria¹¹.

Bioactive compounds of *Phytolacca octandra* extract using Thin Layer Chromatography

Three types of phytochemical compounds analyzed in the present research to identify the bioactive compounds from the column purified fractions using TLC were presented in Table.4. For phenol, ethyl acetate:toluene:formic acid was used as mobile phase with ferric chloride as spraying agent. Green colour spots were observed (Fig.1). For tannin, methanol:water was used as mobile phase with ferric chloride as spraying agent. Grey colour spots were observed. For flavanoids, ethyl acetate:toluene:formic acid was used as mobile phase with aluminium chloride as spraying agent. Orange colour spots were observed. The Rf values were evaluated by comparing with the standards. The Rf value for phenol, tannin and flavonoids were calculated as 0.78, 0.83 and 0.86 respectively.

CONCLUSION

These results corroborate the importance of ethno-pharmacological surveys in the selection of plants for bioactivity screening against significant bacterial pathogens. The results obtained represent a worthwhile expressive contribution to the characterization of the anti-bacterial activity of plant extracts of traditional medicinal plants from Indian flora. Subsequently, bio-guided fractionation shall be conducted as future perspective on plants showing potential antibacterial activity to identify the active compounds. Also, the ethano-pharmacological survey shall be made to investigate the other biological properties of *Phytolacca octandra* like, anti-oxidant activity, anti-viral activity, anti-larvicidal activity of *Aedes aegypti*, anti-cancer activity etc.

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Cite this article as:

Vithya Eswari. D et al. Evaluation of phytochemical and antimicrobial properties of *Phytolacca octandra*. *Int. J. Res. Ayurveda Pharm.* 2018;9(6):111-115 <http://dx.doi.org/10.7897/2277-4343.096181>

Source of support: Nil, Conflict of interest: None Declared

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