

Preliminary Phytochemical and Antimicrobial Properties of *Pueraria tuberosa* (Willd.) DC: A Potential Medicinal Plant

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Abstract

In vitro antimicrobial and chemical properties of petroleum ether, ethyl acetate and ethanol extracts of *Pueraria tuberosa* were evaluated. Among the test samples ethyl acetate extract showed pronounced antimicrobial activity, while ethanol extract exhibited the least activity and petroleum ether extract failed to inhibit the test pathogens. Preliminary phytochemical analysis of extracts revealed the presence of antimicrobial compounds such as alkaloids, flavonoids, coumarins, volatile oils and glycosides. The phenolic compounds and flavonoids were abundant in ethyl acetate extract when compared to other extracts. The broad spectrum of antimicrobial activity of ethyl acetate extract may be due to the presence of flavonoids. Based on the observations, *P. tuberosa* appears to be a valuable source for antimicrobial principles.

Key words: *Pueraria tuberosa*; Phytochemical; Antimicrobial activity

Introduction

Pueraria tuberosa (Willd.) DC.(Fabaceae), a perennial woody climber with large tuberous roots, grows up to 6m tall. The leaves are compound, opposite, trifoliate, ovate and coriaceous. Flowers are white with pink ting in dense panicles. Pods are flat, constricted between seeds.

P. tuberosa is an important and potential medicinal plant in traditional and folklore systems. In Ayurveda system the flowers are used as cooling agent and as aphrodisiac, while roots act as a demulcent and refrigerant in fevers. The root tuber is sweet, oily, cooling, tonic, and effectively used in aphrodisiac, galacatagogue and diuretic. It is also used to cure leprosy, diseases of blood and urinary discharges. It is employed as an emetic, tonic and also believed to be a lactagogue (Kirtikar and Basu,

1933).

In folk medicine the root tuber is applied for blood purification and to improve sperm production. The shade dried root powder controls overgrowth in stomach. The consumption of raw root for one month leads to sterilization in women (Venkata Ratnam, 2006).

Materials and methods

Materials

P. tuberosa root tubers were collected from Nallamala forest of eastern Ghats (2004). The voucher specimens were identified with the help of regional and local floras (Gamble, 1935; Venkata Raju and Pullaiah, 1995) and deposited at Sri Krishnadevaraya University herbarium (SKU), Anantapur.

Preparation of extracts

The collected root tubers were cut in to small pieces, shade dried, powdered and extracted with 250 ml of petroleum ether, ethyl acetate and ethanol using Soxhlet apparatus. The extracts were filtered and concentrated under reduced pressure below 40°C to dryness. Crude extracts were screened for their phytochemical and antimicrobial properties.

Phytochemical screening

The different solvent extracts of *P. tuberosa* root tubers were analyzed for the phytochemical composition by using standard qualitative methods (Gibbs, 1974; Harborne, 1991).

Preparation of paper discs

Fifty milligrams of crude extracts were dissolved in one ml of dimethyl sulphoxide (DMSO). Sterilized Whatmann No.1 filter paper discs of 6 mm diameter were saturated with 10 µL of the extract and allowed to dry at room temperature in a laminar air flow bench.

Microorganisms used

The microbial strains viz., *Bacillus cereus* MTCC 1429, *Staphylococcus aureus* MTCC 737, *Escherichia coli* MTTC 1687, *Micrococcus luteus* MTCC 2522, *Pseudomonas aeruginosa* MTTC1688, *Klebsiella pneumoniae* MTCC 109, *Salmonella typhimurium* MTCC 98 and yeast, *Candida albicans* MTTC 183, obtained from the Microbial Type Culture Collection Centre, Institute of Microbial Technology (IMTECH), Chandigarh, India, used in the study.

Antimicrobial assay

The antimicrobial activity of the extracts was evaluated by disc diffusion method (Cruickshank, 1968). Previously prepared paper discs containing different concentrations of extracts were placed on the surface of the petriplates, containing 20 mL of respective media seeded with 0.1 ml of previously prepared microbial suspensions (10^5 CFU/ mL). Standard antibiotics viz., ampicillin, kanamycin, tetracycline and vancomycin (30 µg/disc) obtained from Hi-media, Mumbai, were used as

positive controls. The discs containing petroleum ether, ethyl acetate and methanol served as negative controls. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The plates were incubated for 24 h at 37°C and the diameters of the inhibition zones were recorded. Three independent trials were conducted for each concentration to confirm the activity.

The minimum inhibitory concentration (MIC) was determined using a common broth micro dilution method in 96-well micro titer plates (Camporese et al., 2003; NCCLS, 1999). Two fold dilutions of each extract were carried out, starting from 5 to 0.15 mg/mL. 10 µL of the previously prepared different microbial suspensions (10^5 CFU/ mL) were added to each well. Plates were incubated for 18 h at 37° C and were examined with Elisa reader (TECAN, Sunrise, China) at 620 nm and the lowest concentration of each extract showing no growth was taken as its minimum inhibitory concentration (MIC). The solution DMSO (100 µL/mL) served as the negative control. All the samples were tested in triplicates to confirm the activity.

Results

Phytochemical analysis

The phytochemical analysis of *P. tuberosa* root tubers showed the presence of different groups of secondary metabolites viz., alkaloids, coumarins, flavonoids, terpenoids, anthocyanidins, volatile oils and glycosides, which are of medicinal importance. Of the test extracts, ethanol extract showed positive results for most of the test compounds. The phenolic group and flavonoids were rich in ethyl acetate extract when compared to other metabolites (Table 1).

Table 1: Preliminary phytochemical screening of *P. tuberosa* root tuber extracts

Compound	Extracts			
	P	EA	E	W
Alkaloids	+	+	+	+
Anthracene glycosides	-	-	+	-
Coumarins	+	+	+	+
Flavonoids	-	+++	+	-
Terpenoids	+	+	+	+
Catecholic compounds	-	-	+	-

Anthocyanidins	-	-	+	+
Volatile oils	T	-	-	-

Extracts: P: Petroleum ether; EA: Ethyl acetate; E: Ethanol; W: Water
 Phytochemical tests: +++: quantitative; +: positive; -: negative; T: trace

Antimicrobial activity

The results on antimicrobial potency of the *P. tuberosa* root tuber extracts against eight microbial strains are presented in table 2 and 3. The antimicrobial activity was assessed using the agar disc diffusion method by measuring the diameter of growth inhibition zones with 500µg/disc concentration of different solvent extracts. The results showed that the ethyl acetate extract exhibited broad spectrum of inhibition zones against *Klebsiella pneumoniae* (15 mm), *Micrococcus luteus* and *Candida albicans* (14 mm), *Salmonella typhimurium* (13 mm), *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (each 12 mm). The ethanol extract showed feeble activity against the test organisms (between 6-8 mm). However, ethyl acetate extract strongly inhibited a Gram positive bacterium (*Micrococcus luteus*) and Gram negative bacteria (*Klebsiella pneumoniae* and *Salmonella typhimurium*) at 156µg/ml (MIC value) concentration.

Table 2: Antimicrobial properties of *P. tuberosa* root tuber extracts

Organisms	Extract Concentration µg/disc	Extracts		Standards*
		EA	E	
<i>Bacillus cereus</i>	500	12	6	22 ^a
<i>Micrococcus luteus</i>	500	14	10	
<i>Staphylococcus aureus</i>	500	12	6	23 ^k
<i>Escherichia coli</i>	500	10	6	22 ^t
<i>Pseudomonas aeruginosa</i>	500	12	8	28 ^t
<i>Klebsiella pneumoniae</i>	500	15	-	23 ^t
<i>Salmonella typhimurium</i>	500	13	10	-
<i>Candida albicans</i>	500	14	8	25 ^v

Extracts: EA: Ethyl acetate; E: Ethanol
 Standards: a: ampicillin; k: kanamycin; t: tetracycline; v: vancomycin

Table 3: Minimum inhibition concentrations of *P. tuberosa* root tuber extracts.

Organisms	Extracts $\mu\text{g/ml}$	
	EA	E
<i>Bacillus cereus</i>	312	625
<i>Micrococcus luteus</i>	156	312
<i>Staphylococcus aureus</i>	312	625
<i>Escherichia coli</i>	625	625
<i>Pseudomonas aeruginosa</i>	312	625
<i>Klebsiella pneumoniae</i>	156	625
<i>Salmonella typhimurium</i>	156	625
<i>Candida albicans</i>	625	1250

Extracts: EA: Ethyl acetate; E: Ethanol

Discussion

The results from preliminary phytochemical screening revealed that ethyl acetate and ethanol extracts showed the presence of flavonoids, which may exhibit antimicrobial activity. Phenolic compounds have been reported for antioxidative, antidiabetic, anti carcinogenic, antimicrobial, antiallergic, antimutagenic and antiinflammatory activities (Arts and Hollman, 2005; Scalbert et al., 2005). Flavonoids are a major group of phenolic compounds reported for their antiviral (Barnard et al., 1993), antimicrobial (Afolayan and Meyer, 1997) and spasmolytic (Amor et al., 2005) properties.

The antimicrobial properties of *P. tuberosa* root tuber extracts were performed by disc diffusion method. The organic solvents, petroleum ether, ethyl acetate and ethanol extracts were tested against three Gram positive, four Gram negative and one fungal species. Among the test extracts, ethyl acetate extract showed pronounced antimicrobial activity. Both Gram positive (*Micrococcus luteus*) and Gram negative (*Klebsiella pneumoniae* and *Salmonella typhimurium*) bacteria were strongly inhibited at 500 $\mu\text{g/disc}$ concentration. Interestingly *Candida albicans* was also strongly inhibited at the same concentration. The variations in the effectiveness of the extract against different organisms depend upon the chemical composition of the extracts and membrane permeability of the microorganisms for the chemicals and their metabolism.

The phytochemical analysis of *P. tuberosa* revealed that, isoflavonoids isolated from stems (Zeng et al., 1999) and tuberosin from root tuber (Joshi and Kamat, 1973). Prakash et al., (1985) reported contraceptive potency of the *P. tuberosa* root tubers which supports the local folk claims. The broad spectrum of antimicrobial activity of ethyl acetate extract was reported (Aladesanmi and Odidiran 2000; Bakshu et al., 2001; Erdogrul, 2002). Present observations also showed similar results.

Results from the present study indicate that *Micrococcus luteus*, *Klebsiella pneumoniae*, *Salmonella typhimurium* and *Candida albicans* were more sensitive to ethyl acetate extract of *P. tuberosa* root tuber. Based on the above observations *P. tuberosa* appears to be a promising and valuable source for antimicrobial compounds against both bacteria and yeast and also in substantiate the folk claims on the therapeutic properties of the crude drug.

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