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Investigation on Phytochemical and Antimicrobial Properties of Tuber Extracts of *Pueraria tuberosa* Linn

M.R. Aruna[1], D.J. Mukesh Kumar[2], D. Senbagam[3] and B. Senthilkumar[1]*

[1*]Department of Biotechnology, Muthayammal College of Arts and Science, Rasipuram, TN, India.
[2]CAS in Botany, University of Madras, Guindy Campus, Chennai, TN, India.
[3]Department of Microbiology, Vivekananda College of Arts and Science for Women, Tiruchengode, TN, India.

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***Pueraria tuberosa* (Indian Kudzu) is an important medicinal herb used in Indian traditional medicine system since it is having array of medicinal values. The bioactive compounds from the tubers of the *P. tuberosa* were sequentially extracted using the range of solvents like hexane, benzene, chloroform, ethyl acetate, acetone and methanol. The phytochemical analysis revealed the presence of significant amount of total antioxidants, phenolic acids and flavonoids in the *P. tuberosa* tuber extract. Among the different solvent extracts, ethyl acetate and chloroform extracts possessed maximum total antioxidants, whereas methanol and acetone extracts showed maximum flavonoid and phenolic compounds. The antimicrobial activity of different solvent extracts of *P. tuberosa* tubers was evaluated against selected bacterial and fungal pathogens. The bacterial strains showed higher susceptible range over the fungal isolates. The findings of the present investigation revealed that the candidate plant could be further explored for possible antibiotic and antifungal agents and provides preliminary scientific validation of the traditional medicinal use of this plant.**

Keywords: *Pueraria tuberosa*, Indian Kudzu, Antioxidants, antimicrobial activity.

In the current scenario, development of drug resistance (Senthilkumar and Prabakaran, 2005; Balaji and Senthilkumar, 2011; Senthilkumar *et al*., 2014; Behailu *et al*., 2016) among the disease causing microbes to the commonly used antibiotics has provoked the research for discovery of new antimicrobial agents. Plants are considered as primary choice for dietary supplements and also for exploring for new medicines (Abdallah, 2011; Murugan *et al*., 2014). Medicinal values of many plants are still remaining unexplored for its enumerable activity of compounds responsible for their activity. Besides, plant materials remain important resources of remedies to combat against serious diseases around the world and plants are known to contain enormous biologically active compounds which possess antibacterial properties (Ahmad and Wajid, 2013). Hence, it is essential to carryout pharmogonostic investigations of plants to find novel drugs or templates for the development of new therapeutic agents (Nasir *et al*., 2015).

P. tuberosa is a perennial woody climber with large tuberous roots belongs to the family *Fabaceae*. It grows up to 6 m tall, it has opposite trifoliate compound leaves and the flowers are white. Pods are flat, constricted between seeds (Theng and Korpenwar, 2014). *P. tuberosa* is an important and potential medicinal plant in Indian traditional system of medicine that is in Siddha and Ayurveda. The Ayurvedic Pharmacopoeia of India is calling *P. tuberosa* under the name of Indian Kudzu (or) Vidari. The flowers of *P. tuberosa* are

* To whom all correspondence should be addressed.
Mob.: +91-9443286292;
E-mail: senthilmicro@yahoo.co.in;
nevithasenthil@gmail.com

used as cooling agent and as aphrodisiac (Mishra, 2012). The root tuber is sweet, oily and it has cooling effect and used in the form of tonic, demulcent and refrigerant for fever, inflammation, etc. Besides, it is effectively used in aphrodisiac, galacatagogue and diuretic, and used to cure leprosy, blood and urinary related disorders (Verma *et al*., 2012). In folk medicine, the *P. tuberosa* root tubers had been used for purification of blood and improve the sperm production. The consumption dried raw root powder had been used control the worm growth in intestine and used to keep clean the stomach (Vijay *et al*., 2013).

The medicinal value of *P. tuberosa* is extended as fertility controlling agents such as aphrodisiac, cardiotonic, diuretic and galactogogue and the experimental evidence exhibits anti-hyperglycemic, anti-hyperlipidemic, anti-fertility in male rats, hepatoprotective, and anti-implantation activities (Hsu *et al*., 2003; Tanwar *et al*., 2008). It is used in food supplements as nutritive, diuretic, expectorants and for the management of rheumatism, fever, and bronchitis (Pandey *et al*., 2013). *P. tuberosa* tubers have rich in extended vast level of bioactive phytoconstituents like isoflavonoids, puerarin, daidzein, genistein, puetuberosanol and tuberosin (Amal *et al*., 2014). During the past decade, interest in these isoflavonoids has increased considerably because of the beneficial effects proposed by epidemiologists, nutritionists, and food manufacturers since isoflavonoids could interact with milk proteins, namely, bovine serum albumin, casein micelle, β-lactoglobulin have been reported in case of certain food and antimicrobial drug preparation (Shilpashree *et al*., 2015). Against these backdrops, in the present study, the exploration of bioactive compounds of *P. tuberosa* was made using various polar and non-polar solvents, and the evaluation of antimicrobial properties of bioactive compound was made against common human pathogens.

MATERIALS AND METHODS

Collection and preparation of material

The fresh tubers of *Pueraria tuberosa* were collected from Yercaud hills, India and authenticated at Centre for Advanced Studies in Botany, University of Madras, India. The tubers were washed with mild detergents to remove the adhered dust and soils. The tubers were rinsed several times in tap water till the detergent was completely removed and were shade dried at room temperature till it is completely dried. Dried tubers were powdered with the help of a blender and fine powder was separated by sieving. The powder was tightly packed in sterile polythene bags for further experimental purpose.

Extraction of Bioactive compounds

The extraction of *P. tuberosa* tuber was made by sequential cold extraction method using the different polar and non-polar solvents such as hexane, benzene, chloroform, ethyl acetate, acetone and methanol (Sadguna *et al*., 2015). Fifty grams of *P. tuberosa* tuber powder was mixed with 4 volumes of solvent in a glass stopper flask and kept overnight at room temperature for complete extraction of phytocompounds. The mixture was agitated well and filtered through Whatman No.1 filter paper. Both filtrate and debris was air dried by evaporating the solvent in open air at room temperature till they attain complete powder form. The filtrated powder was stored in a sterile vial at 4 °C for further use and the dried debris was subjected to sequential extraction using remaining solvents. The similar extraction method was continued using other solvents and the extracted tuber debris was used as source material for other solvents.

Estimation of bioactive compounds

The different solvent extracts of *P. tuberosa* root tubers were analyzed for the phytochemicals like total antioxidants, phenolic compounds and flavonoids by using modified methods of Tripathi and Kohli (2013). The experiments for the estimation of bioactive compounds were repeated three times and the average data expressed as mean± median.

Total antioxidants

The total antioxidants of *P. tuberosa* tuber extract were estimated by ABTS (2, 2'-azino-bis 3ethylbenzenethiazoline-6-sulphonic acid) method. The reaction mixture was prepared by adding 25 ml of tuber extract, 1 ml of diluted ABTS solution and 5 ml of distilled water and the mixture was allowed to stand for 5 min. The absorbance of the mixture was read at 734 nm using spectrophotometer

against reagent blank. The total antioxidant value was estimated by comparing the absorbance with standard chart prepared using β-carotene.

Total phenolic acids

The total phenolic content of *P. tuberosa* tuber extract was determined using folin ciocalteau reagent method. The reaction mixture was prepared by adding of 1 ml of tuber extract, 3 ml of distilled water and 0.5 ml of folin ciocalteau reagent and 2 ml of Na_2CO_3 (20%). The reaction mixture was mixed well and warmed for 1 minute for the development of blue colour. The absorbance of the mixture was read at 650 nm against the reagent used as a blank. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample.

Total flavonoids

The estimation of total flavanoid was done by methanolic aluminium chloride reagent. One ml of tuber extract was added with 1 ml of 2 % of aluminium chloride reagent prepared in methanol. The mixture was agitated well for 2 minutes and absorbance was read at 430 nm against the reagent blank. Total flavonoid content of the tuber extract was expressed by comparing the absorbance within equivalent of Gallic acid standard.

Evaluation of antimicrobial activity

Microbial Strains

The microbial strains were selected accordingly, which are human pathogens that commonly cause various diseases. The pure strains were obtained from Microbial type culture collection (MTCC), Chandigarh, India. The test was performed against different strains of bacteria such as *Escherichia coli* (MTCC 590), *Klebsiella pneumonia* (MTCC 9509), *Shigella boydii* (MTCC 11947), *Salmonella typhi* (MTCC 3224), *Staphylococcus aureus* (MTCC 1430), *Pseudomonas aeruginosa* (MTCC 4673), *Proteus vulgaris* (MTCC 658), *Micrococcus luteus* (ATCC 106), *Vibrio cholera* (MTCC 3906) and fungi such as, *Candida albicans* (MTCC 183), *Candida parapsilosis* (MTCC 2509) and *Candida tropicalis* (MTCC 184).

Disc diffusion assay

The antimicrobial activity was carried out by Kirby Bauer method using Muller Hinton Agar (Senthilkumar *et al*., 2014; Sadguna *et al*., 2015). The overnight microbial cultures were adjusted to the approximately 1.5×10^8 cells/ml according to the McFarland standard. The cultures were swabbed to the sterile dry Mueller Hinton agar (Hi-media, India). Meanwhile, the tuber extract was dissolved in 40% Dimethyl sulphoxide (DMSO) and incorporated to the sterile paper discs for the concentration of 50 mg/ disc. The loaded disks were dried in sterile condition and placed on the surface of the appropriate inoculated Mueller Hinton agar medium. The sterile disc loaded with 10µl of DMSO was maintained as control. The plates were incubated at 37 °C for 24 h. After the incubation, the zone of inhibition was recorded in millimetres. The experiments for the antimicrobial activity were repeated three times and the average data expressed as mean± median.

RESULTS AND DISCUSSION

Experimental plant

As a primary part of this work, the *P. tuberosa* plant was identified and the tubers were collected. *P. tuberosa* was a climbing shrub with tuberous roots with trifoliate leaves. The inflorescence is axillary racemes with bluish-purple colour. The tubers of *P. tuberosa* exhibited different shapes like spherical, semi spherical and cuboidal

Table 1. Phytochemical analysis of *P. tuberosa* tuber extracts

Solvents	Bioactive compounds		
	Total antioxidant (mg/g)	Total flavonoid (µg/g)	Total phenolic compound (µg/g)
Hexane	1.93±0.153	105.66±5.132	103.67±4.042
Benzene	2.47±0.153	123±3.606	253.33±3.512
Chloroform	7.77±0.025	272.333±2.517	102.67±3.055
Ethyl acetate	8.14±0.025	372.33±2.517	600.33±0.577
Acetone	0.95±0.035	580.33±2.517	350.33±0.577
Methanol	1.33±0.306	873.33±4.163	906.67±2.082

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

Test organisms	Solvent extracts/ Zone of inhibition (mm)						
	Control	Hexane	Benzene	Chloroform	Ethylacetate	Acetone	Methanol
C. parapsilosis	-	-	-	-	-	9.0±0.0	-
C. albicans	-	8.27±0.252	-	-	-	7.17±0.208	8.43±0.153
C. tropicalis	-	-	-	8.33±0.252	-	-	-
E. coli		9.17±0.208	-	9.3±0.2	11.3±0.2	10.3±0.2	10.0±0.0
K. pneumoniae	-	10.3±0.3	10±0	-	9.17±0.208	11.43±0.153	-
M. luteus	-	11.3±0.265	11.17±0.208	10.43±0.153	13.3±0.252	9.1±0.58	11.27±0.252
P. vulgaris	-	10.1±0.581	-	-	11.37±0.321	10.0±0.0	9.45±0.351
P. aeruginosa	-	8.3±0.3	11.27±0.306		9.46±2.347	13.45±0.351	9.1±0.581
S. typhi	-	-	-	-	8.45±0.351	9.46±0.437	-
S. boydii	-	11.23±0.208	9.23±0.252	9.23±0.245	10.46±2.347	11.13±0.367	10.37±0.321
S. aureus	-	10±0.0	9±0.0	-	8.37±0.321	12.62±0.141	-
V. cholerae	-	-	9.3±0.3	8.56±0.343	11.17±0.208	10.25±0.345	9.25±0.351

with fibrous root. The surface has brown thick skin and inner part was whitish brown in colour. The inner flush was annulated with exudates.

Bioactive compounds

The extracts from each solvent were subjected to total antioxidant estimation. The results showed that almost all the solvent extracts showed the presence of antioxidants (Table 1). Among the solvents, ethyl acetate has extracted maximum quantity (8.12 mg/g) of antioxidants followed by chloroform extract (7.75 mg/g). The least quantity of antioxidant extraction was observed in almost all remaining solvents. The total flavonoid extraction results revealed the highest yield of 870 µg/g and 580 µg/g of flavonoids by methanol and acetone, respectively whereas, least flavonoids was extracted by hexane. In the case of total phenolic acid extraction, methanol and acetone has extracted more amount of phenolic acid as 905 µg/g and 600 µg/g, respectively. In contrast, Hexane

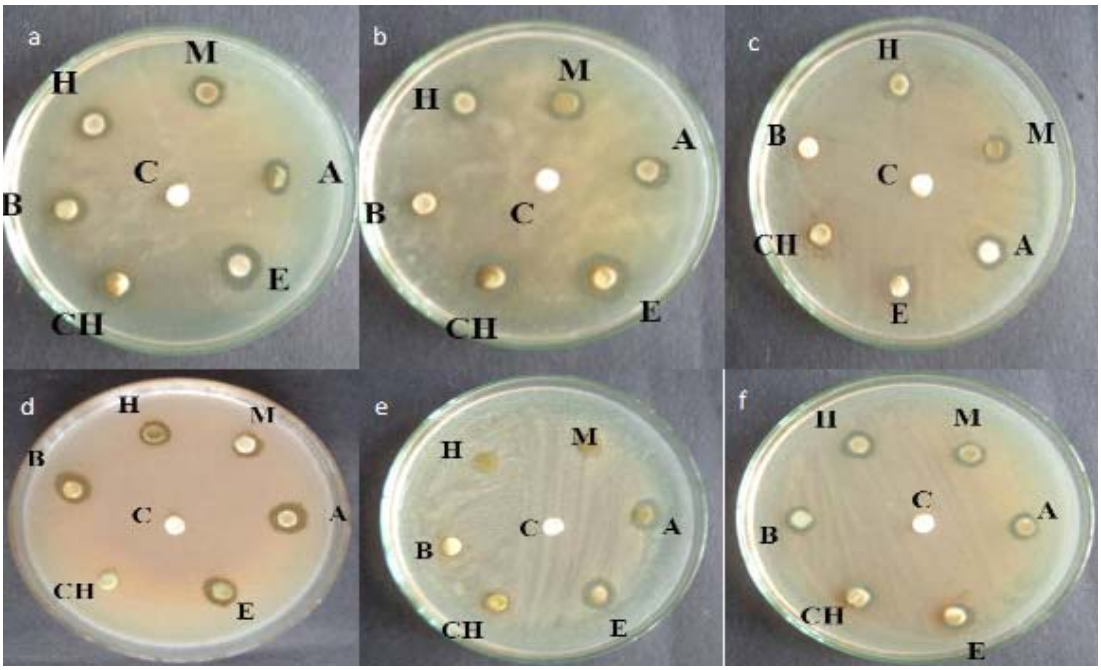

Fig. 1. Antibacterial activity of *P. tuberosa* tuber extract a) *E. faecalis* b) *E. coli* c) *P. vulgaris* d) *P. aeruginosa* e) *S. typhi* f) *S. boydii* (C – Control; H – Hexane; B – Benzene; CH – Chloroform; E – Ethyl acetate; A – Acetone; M – Methanol)

and choloroform extract yielded least quantity of total phenolic acid (100 µg/g) whereas, other solvents extracted moderate quantity. The *P. tuberosa* contains isoflavonoids in the stem and root, the tuberosin is an active flavonoid which has versatile potentials (Pandey and Tripathi, 2010). Roots of *P. tuberosa* contain β-sitosterol, stigmasterol, daidzein, puerarin and a new isoflavone C-glycoside – 4´, 6´´-diacetyl puerarin. An isoflavone glycoside has also been isolated from roots and tubers of *Pueraria alopecuroides* (Yang *et al*., 2016). It is significant to note that *P. tuberosa* which seems almost extinct in vast tracts of the Eastern and Western Ghats of India. The phytochemical analyses have reported the presence of various isoflavonoids of high antioxidant properties including daidzin, genistin, tectoridin, and puerarin in *P. tuberosa* (Sawale *et al*., 2013).

Antimicrobial activity

The antimicrobial susceptibility test of *P. tuberosa* revealed that the extracts of ethyl acetate, hexane and acetone showed broad spectrum of inhibitory activity among the tested bacteria (Fig.1). The bacterial strains showed higher susceptible range over than fungal isolates. Among the fungal strains, *C. albicans* showed higher susceptible rate than other two fungal strains (Table 2). Among the bacterial strains, *M. luteus* and *S. boydii* showed more sensitive to the compounds extracted by almost all the solvents. *E. coli* and *P. aeroginosa* showed sensitivity towards most of solvent extracts whereas, *S. typhi* showed resistance except ethyl acetate and acetone extracts. Among the different solvents used for extraction, the ethyl acetate was found to be an effective solvent that extracts the antimicrobial compounds from *P. tuberosa*. The previous literature evidences reported that that the ethyl acetate extract of *P. tuberosa* exhibited broad spectrum of antibacterial activity against *K. pneumoniae*, *M. luteus*, *S. typhimurium*, *B. cereus*, *S. aureus* and *P. aeruginosa*. The ethanol extract showed feeble activity against the test organisms (Ratnam and Raju, 2009). The presence of biologically active compounds such as phenols, polyphenols, tannins, alkaloids flavanoids and terpenoids in the plants are known to possess antibacterial activity. *P. tuberosa* is an under utilised species and it can be a valuable alternative to much sought after other species. Puerarin, highly abundant in *P. tuberosa*, has hypothermic, spasmolytic, hypotensive and antiarrhymatic activities (Vidhya and Nishteswar, 2015).

CONCLUSION

The present investigation revealed that the ethanolic tuber extract of the selected *P. tuberosa* were shown active against all the selected pathogens. The ethyl acetate tuber extracts were found to show a moderate antibacterial activity and the *P. tuberosa* in particular exhibited greater prosperity to inhibit fungal growth even at low concentration. Further studies on *P. tuberosa* will certainly aid the isolation of bioactive compounds for their pharmacological importance to check the growth of harmful microbes.

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