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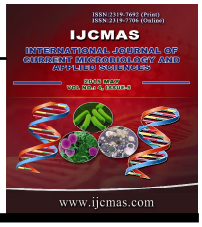

Original Research Article

Anti-microbial Activity of *Pueraria tuberosa* DC, an Economically and Medicinally Important Plant

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K e y w o r d s

Plant material, Ethanol solvent, Anti bacterial activity, Anti fungal activity, Ayurveda, Homeopathy, *Pueraria tuberosa*

A B S T R A C T

Microorganisms are present everywhere, they are to be found in air, water, soil, within the bodies of animals and plants. Some microorganisms are useful and helping many ways, while some of them are harmful. Microorganisms cause diseases in crop plants, plants and human beings. India has been used a number of recognized indigenous system of medicines for health care of human life viz, Ayurveda, Siddha, Unani, Homeopathy, Yoga and Naturopathy (Patwardhan, 2005). Medicinal plants represent a rich source of antimicrobial agents. Historically used pharmacological Screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. The tuber plant of *Pueraria tuberosa* plant material's (leaf, stem, tuber and root) extracts were tested for their antibacterial activity against *Escherichia coli*, *Bacillus cereus*, *Salmonella paratyphi*, *Staphylococcus aureus* and fungus *Candida albicans*, *Aspergillus fumigates* and *Alternaria solani* using agar diffusion assay. These both activities by used ethanolic and methanolic extract of different parts of plants were shown.

Introduction

Herbal medicines are major remedy in traditional medicinal systems for thousands of years to maintain human health and in preventing many infectious diseases. In modern times plants have been sources of analgesics, drugs, medicines for different types of diseases. Search for some new antibacterial agents should be continued by the screening of many plant families (Ekta *et al.*, 2011). The screening of many plant extracts and plant products for antimicrobial activity has shown that the higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003).

In this present investigation the antimicrobial activities of *Pueraria tuberosa* DC collected from different tribal areas of Telangana State in India. *Pueraria tuberosa* DC; is a deciduous small perennial semi woody climber with large tuberous roots and member of Papilionaceae family, is commonly known as kudzu. *Pueraria tuberosa* had various vernacular names in

different regional languages of Indian subcontinent. Kudzu is adapted to a wide range of soil types they are acidic, rocky mountainous and fertile lowland soils (Skerman, 1977; Duke, 1981). Kudzu has been used for the production of lysine enriched baker's yeast or further fermented production to ethyl alcohol. Fiber productions are used for grasscloth wallpaper traditionally to make clothing and paper, textile industries. *Pueraria tuberosa* produced valuable production of cellulose ethanol and also it is used to soaps, lotions and compost (Richard *et al.,* 1999). *P. tuberosa* is an important ayurvedic medicinal plant. Particularly it is used as a tonic (Anonym, 1978), antitumor (Jeon *et al.,* 2005), antiallergic, antirheumatic and to cure cough, malaria and typhoid fever. The aim of the present investigation is to elevate the study of antimicrobial activity effects of the extract from the tuber, leaf and root of *Pueraria tuberosa*.

Materials and Methods

Plant material: *Pueraria tuberosa* tubers were collected from different localities of the tribal areas of Warangal, Khammam and Nizamabad districts of Telangana state in India, in the beginning of rainy and winter season, and identified at Department of Botany, Kakatiya University Warangal.

Extraction of plant material: Plant **(**crude) extracts were prepared by cold percolation method. Each powder material (5gm) was soaked in 50 ml ethanol and methanol solvents kept for 48 hours with intermittent shaking. The plant extracts were filtered through Whatman no.1 filter paper, after filtration remaining plant residue was air dried and soaked again in 50 ml distill water for 48 hours and extract was collected as described earlier. These extracts were dissolved in 1.0 ml of DMSO (Dimethyl sulphoxide). Each 20 µl of sample is taken for the present experiment of antimicrobial activity of *Pueraria tuberosa*.

Antimicrobial Screening: The ethanolic and methanolic extracts of tuber plant of *Pueraria tuberosa* plant materials were screened against four bacterial and three fungal organisms. Bacterial organisms (*Escherichia coli*, *Bacillus cereus*, *Salmonella paratyphi* and *Staphylococcus aureus*) were obtained from Department of microbiology Kakatiya University, Warangal, and fungal organisms (*Candida albicans*, *Aspergillus fumigates* and *Alternaria solani*) were collected from M.G.M hospital and Department of microbiology Kakatiya University, Warangal. We have used agar disc diffusion method (Bauer and Kirby, 1966) was used to screened the antimicrobial activity. The sterile filter paper discs of 6.0 mm diameter impregnated with 20µl extract solution after evaporatation placed on the surface of the inoculated agar-agar plate and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^{o}C for 24 hours. After inoculation, zone of inhibition was observed and measured with ruler in (mm) and the same protocol was followed for the fungal activity and the study was performed in triplicate values. Ciplotoxin was used as control against all bacterial pathogens.

Results and Discussion

Antibacterial activity

The present investigation reports the antibacterial activity of tuber of *Pueraria tuberosa* plants which were collected from different tribal areas of Telangana State. The antibacterial activity was studied against four bacteria as mentioned in Table 1, which shows the name of the activity and the

results of the antibacterial activity of the investigated extracts are shown in Table 1.

The present results of Ethanolic and methanolic extracts of *Pueraria tuberosa* support the scientific basis for traditional uses. These results explain that Indian Ayurvedic medicinal plants have potential antimicrobials against *Bacillus cereus*, *Escherichia coli*, *Salmonella paratyphi* and *Staphylococcus aureus*, for the treatment of various infectious diseases. We have recorded the antibacterial activity of leaf, tuber and roots extracts of *Pueraria tuberosa*

Leaf extraction: The antimicrobial activity of ethanolic extract of leaves of *Pueraria tuberosa* on four different human pathogenic organisms have been tested by using disc diffusion method, ethanolic extract did not show any kind of activity against these bacteria.

Highest activity zone of inhibition (4.8mm) against in *Staphylococcus aureus* of leaf extract of methanol extraction of *Pueraria tuberosa* has been recorded (Table 1, Fig. 3).

Tuber extraction: The ethanol extract of tuber of *Pueraria tuberosa* was also ascertained to test the antibacterial activity. Highest activity zone (6.0mm) of inhibition against *Salmonella paratyphi* (Fig. 3) moderate activity zone against *Bacillus cereus* (4.8 mm) and *E. coli* (4.4mm) and lower zone of inhibition (3.6mm) against *Staphylococcus aureus* were recorded.

The methanol extract of tuber had showed highest zone of inhibition (7.0mm) (4.4mm) against *Salmonella. paratyphi* (Fig. 3), moderate zone of inhibition against *Staphylococcus aureus* (5.0mm) and *Escherichia coli* (4.4mm) and lower zone of inhibition (1.2mm) against *Bacillus cereus* (Figure 1).

Root extraction: Antibacterial activity of Ethanolic extract of root was also ascertained against human pathogen bacteria. The highest antibacterial activity (4.4 mm) against *Salmonella paratyphi* (Fig. 3), moderate activity zone (3.0mm) against *Staphylococcus aureus* and lower activities were recorded against *E. coli* (2.30mm) and *Bacillus cereus* (2.20mm). The methanol root extract of *Pueraria tuberosa* exhibited highest activity against *B. cereus* (6.6mm), moderate activity (4.0mm) and lowest activity against *Staphylococcus aureus* (1.8 mm).

Antifungal activity

Fungi can be pathogenic to animals, humans and plants. They cause common diseases of crop plants including smuts, rusts, mildews and blights. Smuts, leaf spots and rusts which are responsible for destroying many crops in world wide. In the present study three fungal organisms were taken into consideration to test the antifungal activity of both methanolic and ethanolic extracts from leaf, tuber and stem extracts of *P. tuberosa.*

Leaf extraction: Antifungal activity of ethanolic extract was highest against *Candida albicans* (7.2 mm) moderate activity against *Aspergillus fumigates* (4.8 mm) and least inhibition zone (3.2mm) against *Altarnaria solani* were observed from leaf extracts.

Antifungal activity of methanolic extract was highest against *Aspergillus fumigates* (18.6mm), moderate zone of inhibition (11.0mm) against *Altarnaria Solani*, lower zone of inhibition (8.6mm) against *Candida albicans* were recorded from leaf extracts of *P. tuberosa* (Table 2, Figure 2).

Tuber extraction: The ethanolic extracts of tuber of *P. tuberosa* has shown the highest zone of inhibition (11.0mm) against *Candida albicans*, followed by moderate activity zone of inhibition (8.4 mm) against *Aspergillus fumigates* and lower zone of inhibition (4.08mm) against *Alternaria solani.* Whereas methanolic extract has shown the highest activity zone of inhibition (22.0 mm) against *Candida albicans*, moderate activity zone of inhibition (16.4 mm) against *Aspergillus fumigates* and least activity zone of inhibition (10.0mm) against *Alternaria solani* (Table 2, Figure 2).

Stem extraction: Highest inhibition zone of activity (8.2mm) of ethanolic extract of stem of *P. tuberosa* has shown against *Aspergillus fumigates* moderate activity zone of inhibition (7.12 mm) against *Alternaria solani* and lowest activity zone of inhibition (4.08mm) against *Candida albicans*. Methanolic extract of *P. tuberosa* has shown the highest inhibition zone (8.0mm) against *Aspergillus fumigates* moderate activity zone of inhibition (5.2mm) against *Alternaria solani* and lowest activity zone (4.8mm) of inhibition against *Candida albicans* (Table 2).

Many number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds (Mahady, 2005; Samy and Ignacimuthu, 2000; Stepanovic *et al.,* 2003; Bylka *et al.,* 2004). Different types of medicinal plants having their different antimicrobial properties (Mothana and Lindequist, 2005; Bajpai *et al.,* 2005; Wojdylo *et al.,* 2007). Microorganisms are involved in the pathogenesis of many diseases and cause of a variety of products (Kamatou, 2006). The antimicrobial potential of the above plant extracts were seeing against the test organisms using agar gel diffusion technique. These are supported by the traditional uses of the medicinal plants in the treatment of different infections. Protection of crops from fungal diseases has been most important aspect of agriculture.

The present results therefore offer a scientific basis for traditional uses of ethanolic and methanolic extractions from different parts of *Pueraria tuberosa*. These results explain that Indian ayurvedic medicinal plants have potential antimicrobials against *Bacillus cereus, Escherichia coli*, *Salmonella paratyphi* and *Staphylococcus aureus*. Similar results were observed in *Sida cordifolia* leaf extract against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* (Mahesh and Satish, 2008). Several workers tested the methanolic extraction activity of *P. tuberosa, Withania somnifera, Gloriosa superb* and *Urgenia indica* against *E. coli* (Swarnakar and Katewa, 2009). Silva and Fernandes Júnior (2010) have observed the leaf extract (hexane) of *Croton urucurana* presented the widest spectrum against in gram-negative bacteria and strong action against *Klebsilla pneumoniae* and *Psudomonas aeruginosa.* Natural antimicrobial combinations may serve as alternative effective and cheap and safe antimicrobial agents for treatment of common microbial infections (Mathur and Goyal, 2011). In humans, immune systems are weakened in a particular way, (neutrophils count), aggressive fusarial infections penetrating into the entire body and bloodstream (disseminated infections) caused by members of *Fusarium solani* (Bruce and Miller, 2003).

We have observed the tuber extraction has shown the highest activity zone of inhibition (6.0mm) in ethanol extract against *S. paratyphi* and methanol extract with zone of inhibition (7.0mm) against *S. paratyphi.* Thus it is showing more effective antibacterial activity. Similar results were observed in tuberous medicinal plants from

aravalli hills of Rajasthan (*Crinum asiaticum, Asparagus racemosus, Gloriosa superb, Curcuma amada, Pueraria tuberosa, Urgenia indica, Sauromaticum venosum*) against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* (Swarnakar and Katewa, 2009). We have observed the ethanolic extraction of root has shown the highest activity zone of inhibition (4.4mm) against *S. paratyphi* and highest antibacterial activity was found in methanol extract with zone of inhibition (6.6mm) against *B. cereus* in root extraction of *P. tuberosa*. Plant material with antimicrobial activity to a rapid screening method was identified and compared plant material with antimicrobial activity (Sulthanbawa *et al.*, 2009). Antifungal activity zone of inhibition (18.6 mm) and (8.0 mm) against *Aspergillus flavus* in methanolic extraction of leaf and root extract of *P. tuberosa*. Whereas highest activity zone of inhibition (22.0 mm) against *Candia albicans* in methanolic extract of tuber of *P. tuberosa.*

All the anti fungal activity of the tuber was shown highest activity in methanolic extraction. Similar results were observed in antifungal activity of *Acacia nilotica* bark and leaf extract against *Aspergillus flavus* (12.0 mm) followed by leaf extraction of *Zizyphus Mauritian* (11.0 mm). *Tinospora cordifolia* and *Withania somnifera* bark extract was reported better activity against of *Dreschlera turcica* (Mahesh and Satish, 2008). We have observed the highest antifungal activity zone (22.0 mm) of inhibition against *Candida albicans*. Similarly Swarnakar and Katewa, (2009) were observed antifungal activity of some medicinal plants (*Curcuma amada, Urgenia indica and Crinum asiaticum*).

Table.1 Anti-bacterial activity in ethanolic and methanolic extraction of *P. tuberose*

Name of the Anti-bacterial Organisms	**Ethanol extraction**				**Methanol extraction**		
	Conc. (µg/ml)	**Leaf**	**Tuber**	**Root**	**Leaf**	**Tuber**	**Root**
		Zone of inhibition (mm)					
Bacillus cereus	600	NA	2.4	1.10	NA	0.6	3.3
	900	NA	4.8	2.20	NA	1.2	**6.6**
Escherichia coli	600	NA	2.2	1.15	1.5	2.2	3.2
	900	NA	4.4	2.30	3.0	4.4	6.4
Salmonella paratyphi	600	NA	3.0	2.2	NA	3.5	2.0
	900	NA	**6.0**	**4.4**	NA	**7.0**	4.0
Staphylococcus aureus	600	NA	1.8	1.5	2.4	2.5	0.9
	900	NA	3.6	3.0	**4.8**	5.0	1.8

NA= No activity

Table.2 Anti-fangle activity in ethanolic and methanolic extraction of *P. tuberose*

Name of the Anti-bacterial Organisms	Conc. (µg/ml)	Ethanol extraction			Methanol extraction		
		Leaf	Tuber	Stem	Leaf	Tuber	Stem
		Zone of inhibition (mm)					
Candida albicans	600	3.6	5.5	2.04	4.3	11.0	3.3
	900	7.2	**11.0**	4.08	8.6	**22.0**	6.6
Aspergillus fumigates	600	2.4	4.2	4.1	9.3	8.2	5.5
	900	4.8	8.4	8.2	**18.6**	16.4	10.0
Alternaria solani	600	1.6	2.04	3.06	2.4	4.00	2.6
	900	3.2	4.08	7.12	4.8	8.00	5.2

Figure.1 Anti-bacterial activity from different types of bacterial organism's with zone of inhibition in ethanolic and methanolic extraction of *Pueraria tuberosa*

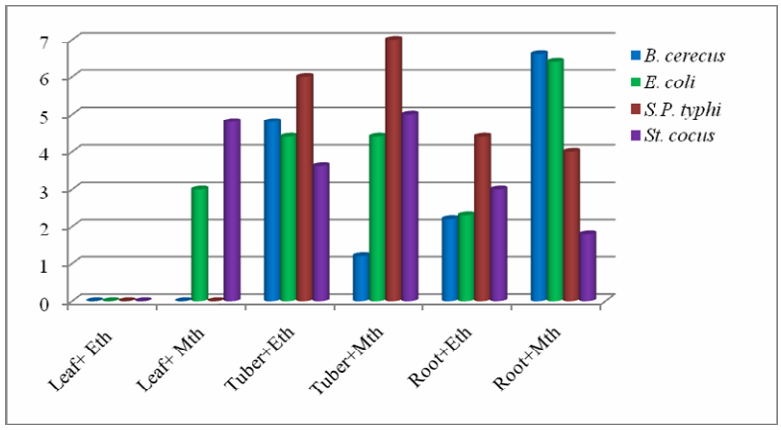


Figure.2 Anti-fungal activity from different types of fungal organism's with zone of inhibition in ethanolic and methanolic extraction of *Pueraria tuberosa*

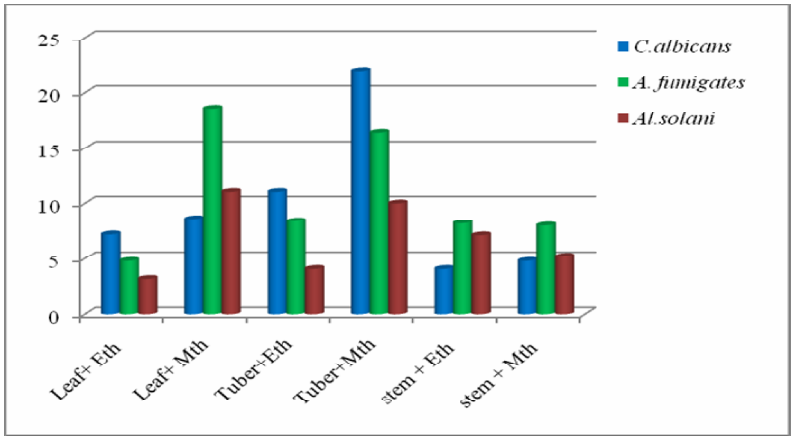
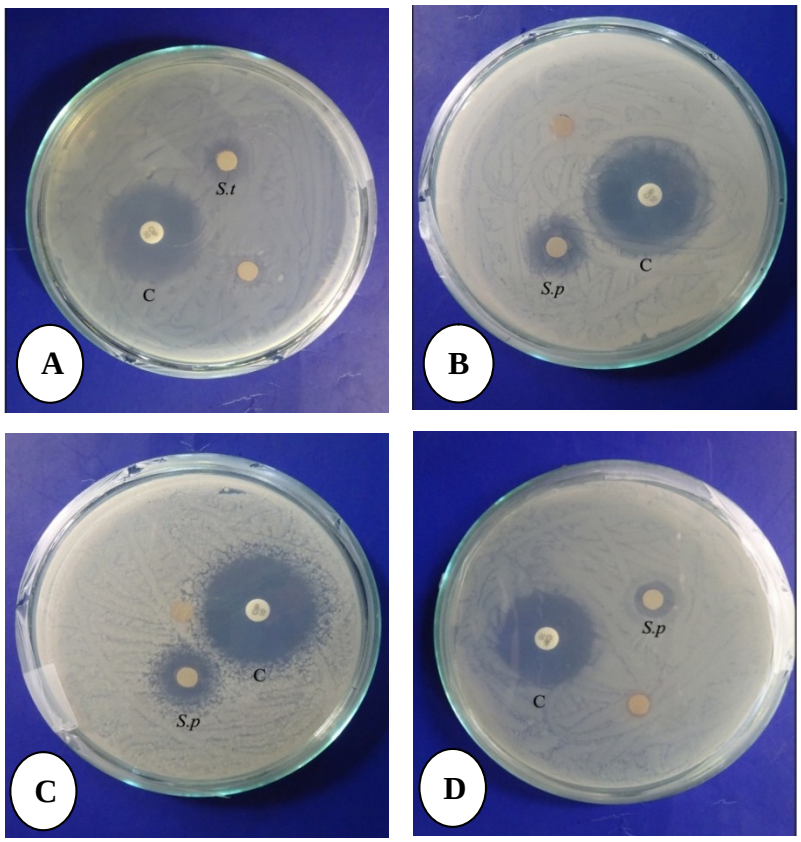


Figure.3 Antibacterial activity of ethanol and methanol extraction of *Pueraria tuberosa*



A) Methanolic extraction of *Staphylococcus aureus* in leaf
B) Ethanolic extraction of *S.paratypi* in tuber
C) Methanolic extraction of *S.paratypi* in tuber
D) Ethanolic extraction of *S.paratypi* in root

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