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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *PUERARIA TUBEROSA* (ROXB. EX WILLD) DC

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ABSTRACT

The screening and study of selected Indian medicinal plant *Pueraria tuberosa* (Roxb. Ex Willd)DC., were selected for phytochemical screening and antibacterial studies. The solvents used for the extraction of plant roots were ethanol, benzene, chloroform, acetone, petroleum ether and distilled water. The Gram-Postive and Gram-negative bacteria *Yeast candida, Aspergillus niger, Staphylococcus aureus, Eschericha coli, Salmonella typhi, Bacillus subtilis, Pseudomonas fluorescence, Klebsiella pneumonia* and *Streptococus pyogenes*. were tested. The results obtained in the present study suggest that preliminary phytochemical analysis detected the presence of Alkaloids, Flavonoids, Terpenoids, Steroids, Cumarins, Carbohydrates and Tanins. The *Pueraria tuberosa* (Roxb. Ex Willd)DC. could be used in treating diseases caused by the test organisms.

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INTRODUCTION

Medicinal plants have a long-standing history in many indigenous communities and continue to provide useful tools for treating various diseases. A large number of the country's rural population depends on medicinal plants for treating various illnesses. These plants played a significant role in various ancient traditional system of medication in India. Phytochemical, Antibacterial Screening and Spectroscopic Analysis of the Crude Samples of Stem Bark Extract of *Lonchocarpus cyanescens* (Nwokonkwo *et al*., 2017). Preliminary phytochemical and Fourier Transform Infrared Spectral analysis and Antimicrobial Studies of solvents extracts of *Urginea indica* (Roxb.) Kunth (Liliaceae) and *Cyclea peltata* Arn. ex Wight (Menispermaceae), results were clearly revealed that the plant contained different bioactive compounds such as of Alkaloids, Anthoquinones, Coumarins, Steriods and Flavonoids compounds were rich in the extracts of *Urginea indica* (Liliaceae) and *Cyclea peltata* (Menispermaceae) are connected with defense mechanism against many microorganisms (Patil *et al*., 2015). Plants are a source of large amount of drugs comprising to different groups such asantispasmodics, emetics, anti-cancer, antimicrobials etc (Tiwari *et al*., 2011). Preliminary Phytochemical Screening and Evaluation of Anti-Inflammatory Activity of Methanolic Extract of *Barleriacristata* Linn. Roots in Experimental Animals (Banu *et al*., 2011). Antimicrobial activity and phytochemicalanalysis of selected Indian folk medicinal plants. Kirby-Bauer method was followed for disc diffusion assay (Shihabudeen *et al*., 2010). Preliminary studies on phytochemicals and antimicrobial activity of solvent Extracts of Eichhornia crassipes (Mart.) Solms. They had study the fresh plant contain alkaloids, flavonoids, phenols, sterols, terpenoids, anthoquinones and protein (Thamaraiselvi *et al*., 2012). Studies on the phytochemistry, spectroscopic characterization and antibacterial efficacy of salicornia brachiata (Krishnan *et al*., 2014). Preliminary phytochemical screening of different solvent extracts of stem Bark and roots of Dennetiatripetala G. Baker (Solomon *et al*., 2013). Seed ethanolic extract showed high content of phytochemicals, highest antimicrobial and antioxidant activity and results supported the usage of Vernonia anthelmintica in folk and traditional medicine (Santosh *et al*., 2013). Phytochemical screening and antimicrobial activity of medicinal plant Pergulariadaemia From Chandrapur Forest Region (Jogi *et al*., 2012). Phytochemical screening, functional groups and element analysis of Tylophora Pauciflora wight and Arn. They had concluded that traditional use of tylophorapauciflora for human ailments and partly explained its use in herbal medicine as rich sourch of phytochemicals with the precence of tanins, phenol, saponins, steroids, flavoinoids and terpenoid (Sarlin *et al*., 2012). Preliminary phytochemical screening of different solvent extracts of stem Bark and roots of *Dennetiatri petala*

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G. Baker (Ugochukwu *et al*., 2013). The most essential of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food (Amin *et al*., 2013). Medicinal plants are moving from fringe to main stream use with a greater number of people seeking remedies and health approach (Saha *et al*., 2010).

MATERIALS AND METHODS

Plant collection

The following medicinal plants were selected and collected for the study from the local area of Nimiya forest of Betul district. The Medicinal Plants *Pueraria tuberosa* (Roxb. Ex Willd) DC. was collected from follow land in and around Nimiya forest brought into the laboratory for further processes. The collected samples were carefully stored in sterile polythene bags and used for the further study.

Sterilization of Plant Materials

The disease free roots were selected for this investigation. About 2gm dried roots were taken. Then, surface sterilized with 0.1% mercuric chloride and alcohol from few seconds. Again the materials were washed thoroughly with distilled water.

Preparation of Plant Extracts

The organic solvent extract was prepared by adding 5 gm powder of ethno veterinary medicinal plants in 250 ml of organic solvent(Absolute Alcohol, Acetone, Petroleum Ether, Benzene, chloroform and Distil Water) for 6 hrs. By Soxlhet method and filtrate was evaporated in controlled conditions of temperature of active constituents of preparations. Dried extracts were stored in labelled sterile wide mouthed screw capped bottle at 40c and used for further study.

Preliminary Phytochemical screening

Phytochemical screening were performed to assess the qualitative chemical composition of different crude extracts using commonly employed precipitation and coloration reactions, the methods of Harbone, Trease and Evans were used to identify the major secondary metabolites like Alkaloids, Flavonoids, Saponins, Carbohydrate, Protein, Phenols, Steroids, Tannins, Glycosides, Terpenoids, Phlobatannins, Coumarins, Emodins, Anthoquinones, Anthocyanins, Leucoanthocyanins in the extracts.

Antimicrobial screening

All solvent extracts were screened *in vitro* growh inhibitory activity against different microbes *E. coli, Pseudomonas fluroscene, Salmonella typhi Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Streptococus*Yeast candida, *Aspergillus niger*. using disc-diffusion method. The bacteria rejuvenated in Nutrient broth (Hi-media – laboratories, Mumbai, India) at 37^0c for 18 hrs. and then stored at 40^0c on Nutrient agar subcultures were prepared from the stock for bioassay.

Phytochemical screening

From the below table no. 1 it is clear that,

Alkaloids

All extracts have shown positive test for Alkaloids with Hager's reagent.

Glycosides

All extracts have shown negetive test for Glycosides with Libermann's reagent.

Phenols

All extracts have shown negetive test for Phenols.

Saponins

All extracts ave shown negetive test for Saponins.

Tannins

All extracts have shown negetive test for Tannins with Braymer's reagent.

Flavonoids

All extracts have shown negetive test for Flavonoids.

Terpenoids

All extracts have shown negetive test for Terpenoids.

Steroids

It was found that concentration of Steroids have been extracted in Ethanol and Acetone extract. This is evident from the positive test with Salkowski reagent. Benzene, Chloroform, Petroleum ether and Distil water extract have shown negetive test for Steroids.

Phlobatannins

All extracts have shown negetive test for Phlobatannins.

Coumarins

All extracts have shown negetive test for Coumarins.

Proteins

All extracts have shown positive test for Proteins with Xanthoproteic reagent.

Emodins

All extracts have shown negetive test for Emodins.

Carbohydrates

It was found that concentration of Carbohydrates have been extracted in Benzene and Chloroform extract. This is evident from the positive test with Molisch reagent. Ethanol, Acetone, Petroleum ether and Distil water extract have shown negetive test for Carbohydrate.

Antimicrobial activity

Among all the extracts only Petroleum ether extract showed positive results against *Klebsiella pneumoniae.* The maximum zone of inhibition of 6 mm was observed in Petroleum ether extract against *Klebsiella pneumoniae.*

Table 1. Phytochemical activity of root extracts of *Pueraria tuberosa* (Roxb. Ex Willd) DC

Plant parts	Test / Reagents Used	Ethanol extract E	Benzene extract B	Chloroform Extract C	Acetone extract A	Petroleum Ether P	Distil Water extract W
Tuber	Alkaloids (Hager's Test)	+	+	+	+	+	+
	Glycosides (Libermann's Test)	–	–	–	–	–	–
	Phenols	–	–	–	–	–	–
	Saponins (Foam Test)	–	–	–	–	–	–
	Tannis (Braymer's Test)	–	–	–	–	–	–
	Flavonoids	–	–	–	–	–	–
	Terpenoids	–	–	–	–	–	–
	Steroids (Salkowski Test)	+	–	–	+	–	–
	Phobatannins (Precipitate Test)	–	–	–	–	–	–
	Coumarins	–	–	–	–	–	–
	Proteins (Xanthoproteic Test)	+	+	+	+	+	+
	Emodins	–	–	–	–	–	–
	Carbohydrates (Molisch Test)	–	+	+	–	–	–

Present -- +ve Absent -- -ve

Table 2. Antimicrobial activity of root extracts of *Pueraria tuberosa* (Roxb. Ex Willd) DC. by Disc Diffusion Method (Zone of Inhibition in mm at 100 µg/disc)

S.No.	Microorganism	Ethanol	Benzene	Chloroform	Acetone	Petroleum ether	Distil water
1	YC	0	0	0	0	0	0
2	AN	0	0	0	0	0	0
3	SA	0	0	0	0	0	0
4	EC	0	0	0	0	0	0
5	ST	0	0	0	0	0	0
6	BS	0	0	0	0	0	0
7	PF	0	0	0	0	0	0
8	KP	0	0	0	0	6	0
9	SP	0	0	0	0	0	0

*Data represented in mean of three replicates. **YC** = *Yeast candida*, **AN** = *Aspergillusniger*, **SA** = *Staphylococcus aureus*, **EC** = *Escherichia coli*, **ST** = *Salmonella typhi*, **BS** = *Bacillus subtilis*, **PF** = *Pseudomonas fluorescence*, **KP** = *Klebsiellapneumoniae*, **SP** = *Streptococuspyogenes*

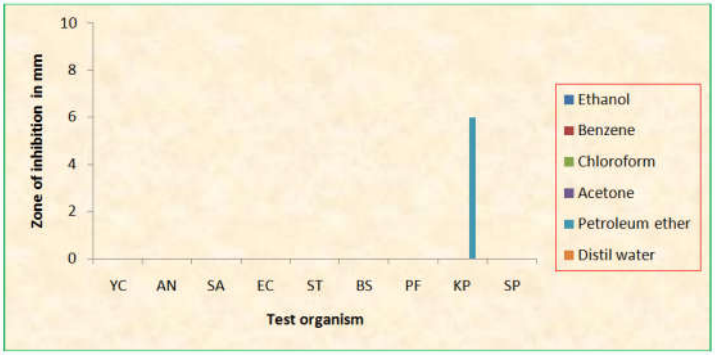

Fig. 1. Analysis of antimicrobial sensitivity of root extracts of *Pueraria tuberosa* (Roxb. Ex Willd)DC

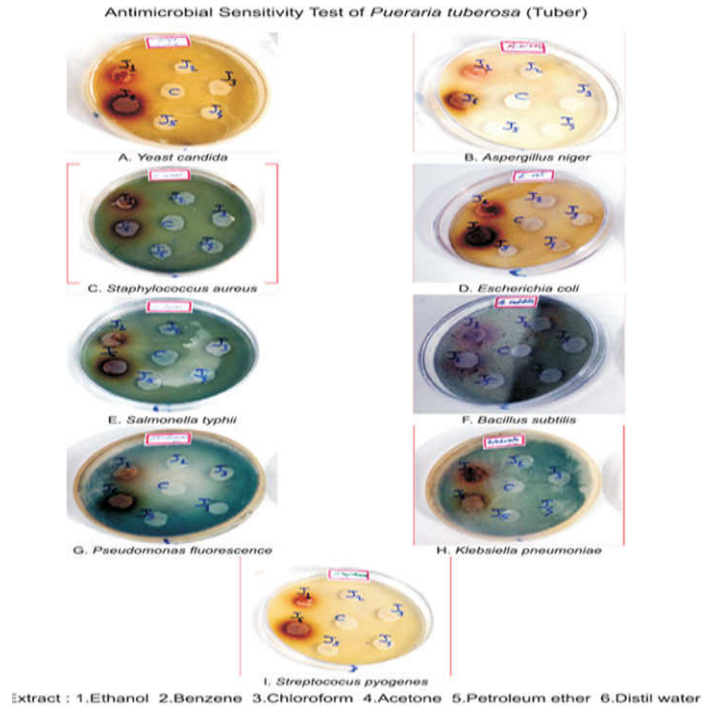

Fig. 2. Antimicrobial activity of root extracts of *Pueraria tuberosa* (Roxb. Ex Willd)DC

Petroleum ether extracts was found non reactive to other test organisms (Fig.2). Ethanol, benzene, chloroform, acetone and aqueous extracts showed no any response to the all test organisms and reactions were nullified (Fig.2). The poor activity of the extracts mentioned as above against tested organisms could be due to the insolubility of the active compounds or could have caused denaturation of the active compounds.

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