Preliminary phytochemical screening and antibacterial activity of *Pueraria tuberosa* (Roxb. Ex Willd)DC. – A High Value Veterinary Significant Plant

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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-Positive and Gram-negative bacteria *Yeast candida*, *Aspergillus niger*, *Staphylococcus aureus*, *Eschericha coli*, *Salmonella typhi*, *Bacillus subtilis*, *Pseudomonas fluorescence*, *Klebsiella pneumonia* and *Streptococcus 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.

Keywords: Phytochemicals, *Pueraria tuberosa* (Roxb. Ex Willd)DC., Antibacterial activity and Pathogens.

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 as antispasmodics, emetics, anti-cancer, antimicrobials etc (Tiwari et al., 2011). Preliminary Phytochemical Screening and Evaluation of Anti-Inflammatory Activity of Methanolic Extract of *Barleria cristata* Linn. Roots in Experimental Animals (Banu et al., 2011). Antimicrobial activity and phytochemical analysis 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 *Dennettiatripetala* 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 presence 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 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 Etawa forest of Betul district. The Medicinal Plants *Parthenocissus quiquefolia* (L) Planch. was collected from follow land in and around Etawa 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\textsuperscript{15}, Trease and Evans\textsuperscript{16} 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* growth inhibitory activity against different microbes *E. coli*, *Pseudomonas fluroscence*, *Salmonella typhi*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus* Yeast candida, *Aspergillus niger*. using disc-diffusion method. The bacteria rejuvenated in Nutrient broth (Hi-media – laboratories, Mumbai, India) at 37\textdegree c for 18 hrs. and then stored at 40\textdegree c on Nutrient agar subcultures were prepared from the stock for bioassay.
Table 1: Phytochemical activity of root extracts of *Parthenocissus quiquefolia* (L) Planch.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Test / Reagents Used</th>
<th>Ethanol Extract E</th>
<th>Benzen e Extract B</th>
<th>Chloroform Extract C</th>
<th>Acetone Extract A</th>
<th>Petroleum Ether P</th>
<th>Distil Water extrac t W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>Alkaloids (Hager’s Test)</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Glycosides (Libermann’s Test)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Phenols</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Saponins (Foam Test)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Tannins (Braymer’s Test)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Flavonoids</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Terpenoids</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Steroids (Salkowski Test)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Phobatannins (Precipitate Test)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Coumarins</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Proteins (Xanthoproteic Test)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Emodins</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Carbohydrates (Molisch Test)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Present -- *+ve* Absent -- *-ve*
Table 2: Antimicrobial activity of root extracts of Parthenocissus quiquefolia by Disc Diffusion Method (Zone of Inhibition in mm at 100 µg/disc)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Microorganism</th>
<th>Ethanol m</th>
<th>Benzene</th>
<th>Chloroform m</th>
<th>Acetone</th>
<th>Petroleum ether</th>
<th>Distil water</th>
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<tr>
<td>1</td>
<td>YC</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>2</td>
<td>AN</td>
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<tr>
<td>3</td>
<td>SA</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>4</td>
<td>EC</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>ST</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>BS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>PF</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>8</td>
<td>KP</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>SP</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Data represented in mean of three replicates.

YC = Yeast candida, AN = Aspergillus niger, SA = Staphylococcus aureus, EC = Escherichia coli, ST = Salmonella typhi, BS = Bacillus subtilis, PF = Pseudomonas fluorescence, KP = Klebsiella pneumoniae, SP = Streptococcus pyogenes

Fig. 1: Analysis of antimicrobial sensitivity of root extracts of Parthenocissus quiquefolia (L)
Fig. 2: Antimicrobial activity of root extracts of *Parthenocissus quinquefolia* (L) Planch.

**Phytochemical screening:**

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

**Alkaloids**

It was found that concentration of alkaloids have been extracted in Ethanol and Acetone extracts. This is evident from positive test with Hager’s reagent. Benzene, Chloroform, Petroleum ether and Distil water have shown negative test for Alkaloids.

**Glycosides**

All extracts have shown negative test for Glycosides with Libermann’s reagent.

**Phenols**

All extracts have shown negative test for Phenols.

**Saponins**

All extracts have shown positive test for Saponins.

**Tannins**

All extracts have shown negative test for Tannins with Braymer’s reagent.

**Flavonoids**

It is found that concentration of Flavonoids have been extracted in Ethanol and Acetone extract. This is evident from the positive test. Benzene, Chloroform, Petroleum ether and Distil water have shown negative test for Flavonoids.

**Terpenoids**

All extracts have shown negative test for Terpenoids.
Steroids
All extracts have shown positive test for Steroids with Salkowski reagent.

Phlobatannins
All extracts have shown negative test for Phlobatannins.

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

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

Emodins
All extracts have shown negative test for Emodins.

Carbohydrates
All extracts have shown negative test for Carbohydrates with Molisch reagent.

Antimicrobial activity :-
Ethanol extracts showed very promising results against three pathogens like *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. The maximum zone of inhibition of 18 mm was observed in ethanol extract against pathogen *Escherichia coli*. Ethanol extract was found non reactive to other test organisms. Acetone extracts also showed positive results against *Escherichia coli*. The maximum zone of inhibition of 16 mm was found in acetone extracts against pathogen *Escherichia coli*. The acetone extract was found non reactive to other test organisms. The aqueous extracts also showed microbial zone of inhibition against Salmonella typhi. The maximum zone of inhibition of 8 mm was observed in aqueous extracts. The aqueous extract was found non reactive to other test organisms.

REFERENCES


