Study of Phytochemical Constituents & Antimicrobial Activity of Common Plants of Hadoti region of Rajasthan, India

Kiran Choudhary 1*, Maninder Singh 2, Surender K. Meghwal 1, Banwari Lal Mathuriya 1 and Jitendra Nagar 1

1department of Botany, Govt. College, Kota Rajasthan
2Department of Biotechnology, Shekhwati College, Sikar
*Corresponding Author Email: choudharykiran01@gmail.com

INTRODUCTION
The world is rich with natural and unique medicinal plants. The investigation of plants for bioactive secondary metabolites is an area which most plant scientists have recently focused with the aim of developing new clinically useful; and commercially important plant products.[1] It is estimated that 70-80% people all over the world largely depend on traditional herbal medicine to meet their primary health care needs.[2] The global demand for herbal medicine is growing.[3][4] In India, Ayurvedic system of medicine has existed for over four thousand years. From ancient literature it is evidence that the various parts of the plants were used in Siddha, Ayurveda and Unani medicine for the treatment of disease of human beings.[5] A short survey prior to this study was undertaken between known farmers about their interest in ethnobotany and treatment of their cattle sources. Amongst cattle diseases bovine mastitis is a serious problem which affects the basic income of the farmers destroying their dairy sources. Mastitis is an inflammation of the udder. It adversely affects milk production whereby losses due to subclinical mastitis are more severe than those due to clinical cases. The use of antimicrobials over long periods has triggered the development of multidrug resistant strains, which has resulted in the use of increasing doses of antimicrobials, causing the danger of increasing amounts of drug residues in milk, a potential biohazard.

The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body. Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds and many more. These natural compounds formed the foundations of modern prescription drugs as we know today. Phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds and many more such as flavonoids, tannins and so on. The description of the common plants used for the present study are as follows:

Tridax procumbens (Compositae) is a common weed present along with economically important crops. The exomorphology and histomorphology

ABSTRACT
The present review analyze the ethnobotanical knowledge base for treatment of cuts and wounds which includes a usage of plants, methods employed by tribals and folklore practices prevailing in India. Solanum trilobatum, Mimosa pudica, Tridax procumbens and S.indicus, are chosen to study. They have been reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects.

Key Words- Ethnobotanical, Tribals, Folklore, Antisecretolytic, Antiphlogistic.
of leaf, petiole, internode and root of this plant were studied. The extracts of T. procumbens have been reported to have various pharmacological effects including antimicrobial activity, wound healing property and immunomodulatory activity on the experimental animals. Flavones and glycosides have been isolated from the leaves of the plant.

*Sphранanthus indicus* (Asteraceae) a medicinal plant is wide spread in India and Malaysia. *S. indicus* has long been used in the treatment of skin infection, bronchitis, jaundice and nervous depression. The roots and seeds are considered anthelmintic. The herb is also reported to be useful as a tonic to treat indigestion, asthma, leucoderma and dysentery. A novel isoflavone glycoside from leaves and a new sesquiterpene glycoside and sphaeranthanolide were isolated from the flowers of *S. indicus* and it was found to be an immune stimulant.

*Mimosa pudica* (Fabaceae) is a creeping annual or perennial herb, native to Brazil, but is now a pan tropical weed. The other names given to this plant are Humble plant, Shame plant, Touch me not, Sleeping grass (Tropical Biological Association), Prayer plant, The species epithet “pudica” is a latin equivalent for “Bashful” or “Shrinking”, because of its curious nature and easy procreation.

*Solanum trilobatum* (Solanaceae) is extensively used in Indian traditional and folklore medicines to cure various human ailments. The leaves contain rich amount of calcium, iron, phosphorus, carbohydrates, protein, fat, crude fibre, and minerals. This herbal plant is used as medicine for asthma, vomiting of blood, reducing blood glucose level and bilious matter phlegmatic rheumatism and several kinds of leprosy. It is also antibacterial, antifungal antimitotic, antioxidant and antitumouours.

**MATERIAL AND METHODS**

**Plant collection:**
Fresh plant leaves were collected from the surroundings of Govt. College campus, Kota (Raj.), India. The taxonomic identities of plants were confirmed by Govt. P.G. College, Kota. Fresh plant material were washed with tap water and used for the study.

**Preparation of aqueous extract of plant samples:**

The aqueous extract of each plant sample is prepared by soaking 10 g of powdered samples in 200 ml of distilled water for 12 h. The extracts are then filtered using filter paper or Whatmann filter paper.

**Phytochemical analysis:**
Chemical tests are conducted on the aqueous extract of each plants sample and also of the powdered form of the plant samples using standard methods.

**Qualitative analysis on phytochemical constituents:**

**Test for tannins:**
0.5 g of powdered sample of each plant is boiled in 20 ml of distilled water in a test tube and then filtered. The filtration method used here is the normal method, which includes a conical flask and filterpaper. 0.1% FeCl$_3$ is added to the filtered samples and observed for brownish green or a blue black colouration, which shows the presence of tannins.

**Test for phlobatannins:**
10 ml of aqueous extract of each plant sample is boiled with 1% HCl acid in a test tube or conical flask. If the sample of plant carries phlobatannins, a deposition of a red precipitate will occur and indicates the presence of phlobatannins.

**Test for saponins:**
2 g of powdered samples of each plant is boiled together with 20 ml of distilled water in a water bath and filtered. 10 ml of the filtered sample is mixed with 5 ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing is then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicates the presence of saponins.

**Test for flavonoids:**
A few drops of 1% NH$_3$ solution is added to the aqueous extract of each plant sample in a test tube. A yellow coloration is observed if flavonoid compounds are present.

**Test for terpenoids:**
5 ml of aqueous extract of each plant sample is mixed with 2 ml of CHCl$_3$ in a test tube. 3 ml of concentrated H$_2$SO$_4$ is carefully added to the mixture to form a layer. An interface with a
reddish brown coloration is formed if terpenoids constituent is present.

**Test for cardiac glycosides:** 1 ml of concentrated H\textsubscript{2}SO\textsubscript{4} is prepared in a test tube. 5 ml of aqueous extract from each plant sample is mixed with 2 ml of glacial CH\textsubscript{3}COOH containing 1 drop of FeCl\textsubscript{3}. The above mixture is carefully added to the 1 ml of concentrated H\textsubscript{2}SO\textsubscript{4} so that the concentrated H\textsubscript{2}SO\textsubscript{4} is underneath the mixture. If cardiac glycoside is present in the sample, a brown ring will appear, indicating the presence of the cardiac glycoside constituent.

**Bacterial strains**

Bacterial strains such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* used in this study were obtained from Govt. Medical College, Kota. All the strains were confirmed by cultural and biochemical characteristics and maintained in slants for further use.

Testing of antimicrobial activity- The antimicrobial activity of the selected plants were done by the following methods ie.,

Preparation of discs- The disc were dipped in plant extract and allowed to dry. Then the disc was introduced onto the upper layer of the medium with the bacteria

Kirby Bauer method (disc diffusion or agar diffusion method)- 15ml of Nutrient agar was prepared and autoclaved. Then the medium was poured into a sterile petri-plate under aseptic conditions and allowed to solidify. The bacterial culture was spread on the agar surface using sterile swab. The disc was introduced onto the upper layer of the medium with the bacteria. The plates were incubated at 37 C for 24 hrs. After the formation of zone, the inhibition was measured using a scale.

**RESULTS AND DISCUSSION**

In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization. Although a significant number of studies have been used to obtain purified plant chemical, very few screening programmers have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants.

*T.procumbens* showed the presence of Alkaloids, tannins, Saponins and flavonoids while Phlobatannins and terpenoids were absent. Antimicrobial activity of the above plant showed that the methanol extract of flower exhibited highest activity against *S. aureus*. (Table-1)

*S. xanthocarpus* showed the presence of alkaloids, tannins, saponins, phlobatannins, flavanoids while terpenoids was absent. Antimicrobial activity of the above plant showed that the methanol extract of flower exhibited highest activity against *S.aureus* and *P.aeruginosa*.

*S. indicus* showed the presence of tannins, saponins, phlobatannins, terpenoids and flavanoids were absent. Antimicrobial activity of the above plant showed that the methanol extract of flower exhibited highest activity against *S.aureus* and *P. aeruginosa*.

*Mimosa pudica* showed the presence of alkaloids, tannins, saponins, flavonoids, and terpenoids while phlobatannins was absent. The phytochemical screening revealed the presence of alkaloids, tannin, saponin, steroids, terpenoid and falvonoids (Table 1). Most of the secondary metabolites were identified in the polar (methanol and water) extracts. Alkaloids are one of the characteristic secondary metabolites in leaves of this genus. Flavonoids are known to be synthesized by plants in response to microbial infection. Tannins (commonly referred to as tannic acid) are also known as antimicrobial agents. They are water-soluble polyphenols and precipitated proteins present in many plant foods. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein. The growth of many fungi, yeasts, bacteria, and viruses were inhibited by this compound. They have been reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. (Table-2)

Phyotherapeutically, tannin-containing plants are used to treat nonspecific diarrhoea, inflammations of mouth, throat and slightly injured skins.
CONCLUSIONS
The present study is based on determining the phytochemical constituents of selected wild plants and their antimicrobial activity. As these plants are widely available they can be collected locally and used to cure diseases such as diabetes, hypertension, skin diseases etc. As these are natural remedies they are economical and safe to use.

From the above studies, it is concluded that the traditional plants may represent new sources of anti-microbials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

REFERENCES

TABLE-1 PHYTOCHEMICAL CONSTITUENTS OF DIFFERENT PLANTS

<table>
<thead>
<tr>
<th>S.no.</th>
<th>PHYTOCHEMICALS</th>
<th>\textit{T.procumbens}</th>
<th>\textit{S.xanthocarpus}</th>
<th>\textit{S.indicus}</th>
<th>\textit{Mimosa.pudica}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Phlobatannin</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Terpenoid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Flavanoid</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

TABLE – 2: Antimicrobial Activity of different Plants against \textit{E.coli} (A) , \textit{S.aureus} (B) \textit{P.aeroginosa} ( C). Zone of inhibition in  mm.

<table>
<thead>
<tr>
<th>Name of plant</th>
<th>\textit{E.coli} Aq.extract</th>
<th>Met.extract</th>
<th>\textit{S.aureus} Aq.extract</th>
<th>Met.extract</th>
<th>\textit{P.aeruginosa} Aq.extract</th>
<th>Met.extract</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf Flower Leaf Flower</td>
<td>Leaf Flower Leaf Flower</td>
<td>Leaf Flower Leaf Flower</td>
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</tr>
<tr>
<td>1. \textit{T.procumbens}</td>
<td>- 2.2</td>
<td>1.0 2.2</td>
<td>- 2.0</td>
<td>1.0 2.5</td>
<td>- 1.5</td>
<td>0.5 2.0</td>
<td></td>
</tr>
<tr>
<td>2. \textit{S.indicus}</td>
<td>- -</td>
<td>1.0 1.0</td>
<td>1.5 -</td>
<td>2.0 2.5</td>
<td>- -</td>
<td>2.0 2.5</td>
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</tr>
<tr>
<td>3. \textit{S.trilobatum}</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>2.0 2.5</td>
<td>- -</td>
<td>2.0 2.5</td>
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</tr>
<tr>
<td>4. \textit{Vinca rosea}</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>1.5 0.5</td>
<td>- -</td>
<td>1.0 -</td>
<td></td>
</tr>
<tr>
<td>5. \textit{Mimosa pudica}</td>
<td>5.0</td>
<td>1.0 - -</td>
<td>1.0 1.0</td>
<td>- -</td>
<td>1.5</td>
<td>1.0 -</td>
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