ANTIBACTERIAL STUDIES AND PHYTOCHEMICAL SCREENING OF THE LEAVES OF *STEPHANIA JAPONICA* (THUNB). MIERS AND *COCCULUS HIRSUTUS* (L.) DIELS (MENISPERMACEAE)

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ABSTRACT

*Stephania japonica* (Thunb.) Miers and *Cocculus hirsutus* (L.) Diels (Menispermaceae) are the most commonly used medicinal plants to cure various human ailments. Antimicrobial and qualitative phytochemical studies have been carried out in them using chloroform, ethanol and aqueous extracts. Studies showed that leaf ethanolic extracts of *Stephania japonica* has high potent against *E.coli*, and *Pseudomonas aeruginosa* and while chloroform extract of the same plant has potent against *Vibrio cholera* and *Streptococcus pneumoniae*. Ethanolic extracts of *Cocculus hirsutus*(leaf) showed high potency against *E.coli*, and *Pseudomonas aeruginosa* also. Phytochemical studies reveal the presence of tannins, alkaloids and, proteins and other biocompounds.

KEYWORDS: Antibacterial, phytochemical, biocompounds. *Stephania japonica, Cocculus hirsutus*.

INTRODUCTION

Medicinal uses of plants and their products have a long history especially with folk medicine and through the years they have been incorporated into traditional and allopathic medicine.[1] Since antiquity, many plant species are reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids,
steroids, tannins, alkaloids, terpenes which therefore, should be utilized to combat the disease causing pathogens.\cite{2, 3, 4} Researchers of Pharmacology have recently paid great attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs.\cite{5, 6}

Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, season climate and particular growth phase. Leaf is one of the highest accumulated plant parts of such compounds and people generally preferred it for therapeutic purposes. Some of the active compounds inhibit the growth of disease causing microbes either singly or in combination.\cite{7} Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants and out of the 4, 00,000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities.\cite{8} The plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment.\cite{9}

The present study aims at the study of the preliminary phytochemical and antimicrobial analysis of *Stephania japonica* (Thunb.) Miers and *Cocculus hirsutus* (L.) Diels (Menispermaceae).

**MATERIALS AND METHOD**

**Collection of Plant materials**

The mature plant leaves of *Stephania japonica* (Thunb.) Miers (Fig.1) and *Cocculus hirsutus* (L.) Diels (Fig.2) (Menispermaceae) were collected from the Palni hills (566m and 1825m above sea level), Tamilnadu, India. Taxonomic identification of these plants was carried out by Dr. S John Britto, Director and Head at the Rapinat Herbarium, St. Joseph’s College, Tiruchirappalli. Voucher specimens (*Stephania japonica* RHT 56223, *Cocculus hirsutus* RHT 56229) have been deposited at the Rapinat Herbarium.

**Ethnobotany of the study plants and their uses**

*Stephania japonica* has been claimed to possess various medicinal properties. A juice of the whole plant is employed in treatment of convulsions, skin diseases, cough, asthma like symptoms and kidney disorders.\cite{10-14} In Japan and Taiwan, decoction of the plant is used as
a drink to treat malaria. In Indonesia, the roots are used to provide relief in stomach aches and convulsions.\cite{15, 16} In India, it is used for treatment of fever, diarrhea, dyspepsia and urinary diseases.

*Cocculus hirsutus* is a straggling shrub, with softly villous young parts and resembles the plant path. The plant flowers from November to April and fruits in April-May. According to Ayurveda, *C. hirsutus* is known as Patalagarudi in Sanskrit. Root smell is sweetish and pungent, lessen bile and burning sensation, enrich blood. It is used in diseases of urinary system. According to Unani system of medicine, it is antipyretic, tonic, lessens thirst, good for fractures, and useful in tubercular glands related problems. It is well known herb used as first aid remedy in minor injuries. It alleviates kapha and vata doshas. It is used as deepanee, pachanee and raktdoshagni. It possesses light, oily and slimy attributes. It has a special potency as a detoxifier. It is an aphrodisiac and tonic in properties.\cite{17-19}

**Preparation of extracts**

Leaves of two plants of *Stephania japonica* and *Cocculus hirsutus* were shade dried and then powdered with the help of waring blender. 25g of shade-dried powder was filled in the thimble and extracted successively with ethanol, chloroform, and aqueous in Soxhlet extractor for 48 h. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in air tight bottle until further use.

**Phytochemical screenings**

Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids and terpenoids were carried out for all the extracts by the method described by Mukherjee.\cite{20}

**Test for Phenol (Ferric chloride test)**

To 1ml of the leaf extract and 1ml of tuber extract 2ml of distilled water was added followed by a few drops of 10% ferric chloride. Formation of blue or black colour indicates the presence of phenols.

**Test for Sterol (Liebermann-Burchard test)**

To the test solution, 3-4 drops of acetic anhydride was added, the solution was boiled cooled and conc. Sulphuric acid (3 drops) was added. A brown ring appears at the junction of the two layers. The upper layer turns green showing the presence of steroids.
Test for Tannins

(a) Gelatin test
To 2ml test solution, 1% Gelatin solution containing 10% sodium chloride was added to obtain a white precipitate.

Test for Flavanoids

(a) Zinc chloride reduction test
To 2ml test solution, a mixture of zinc dust (Merck, India) and conc. HCl (Qualigens, India) was added. A red colour was obtained after few minutes.

(b) Alkaline reagent test
To 2ml test solution, sodium hydroxide (Qualigens, India) solution was added to give a yellow or red colour.

Test for Alkaloids

(a) Mayer's test
To 2ml test solution, 2N HCl was added. The aqueous layer formed was decanted and Mayer's reagent (Qualigens, India) was added to it. A cream coloured precipitate indicated the presence of alkaloids.

Test for fats and fixed oils

(a) Stain test
Small amount of the extract was pressed between two filter papers; the stain on the filter paper indicates the presence of fixed oils.

(b) Saponification test
Few drops of 0.5N alcoholic potassium hydroxide was added in small quantity to the extract solution with a drop of phenolphthalein and heated on a water bath for 1-2h. The formation of soap or partial neutralization for the alkali indicated the presence of fats and fixed oils.

Test for Glycosides
To 2ml test solution, equal quantity of Fehling’s solution A and B was added and solution was heated. A brick red precipitate indicated the presence of glycosides.
Test for proteins and amino acids

(a) Millon's test
To 2ml test solution, Millon's reagent was added which gave a white precipitate, which on heating changed to red.

(b) Ninhydrin test
To 2ml test solution, Ninhydrin solution was added and the solution was boiled. Amino acids and proteins when boiled with 0.2% Ninhydrin reagent showed violet colour.

Antimicrobial studies

Bacterial isolates and Bioassay
The extracts of chloroform, ethanol and aqueous from the selected plant tissues were screened against 10 bacterials strains of the test organisms, Escherichia coli (MTCC # 119), Pseudomonas aeruginosa (MTCC # 2474), Salmonella paratyphi (MTCC # 734), Vibrio cholerae (ATCC # 14104), Streptococcus pneumoniae (ATCC # 7066), Bacillus subtilis (MTCC # 441), Bacillus cereus (ATCC # 4342), Proteus vulgaris (MTCC # 1771), Proteus mirabilis (MTCC # 1429), Serratia marcescens (MTCC # 2645), Klebsiella pneumoniae (MTCC # 3040).

Preparation of inoculums
Stock cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to the test tubes of Mueller Hinton broth (MHB) for bacteria and were incubated without agitation for 24h at 37°C.

RESULTS AND DISCUSSION

Phytochemical Studies
The triphytochemical screening (aqueous, ethnanolic and chloroform) of the extracts of leaves in Cocculus hirsutus (leaf) revealed that amino acids and proteins were present in all extracts. Glycosides were observed in ethnolic and chloroform extracts and it was absent in the aqueous extract. Tannins, phenol, flavanoids and alkaloids were found to be present in aqueous and ethnolic extracts and while chloroform showed absence of them. Phytoconstituent saponin was observed from aqueous extract only. The former was present in ethnolic extract and the latter was observed in aqueous extract (Table 1).
The triphytochemical screening was conducted on the leaf extracts of *Stephania japonica*. Proteins and amino acids were observed in all the extracts (aqueous, ethnanolic and chloroform). Glycosides were observed in ethnolic and chloroform extracts and it was absent in the aqueous extract. Ethnolic and aqueous extracts showed the presence phytochemical constituents like phenol, flavonoids and alkaloids. Saponin was observed from aqueous extract only. Tannin was completely absent from all the extracts (Table 2).

**Antibacterial studies**

The ethanolic extracts of both *Stephania japonica* and *Cocculus hirsutus* showed microbial activities in microorganisms like, *E.coli* (21mm & 20mm) and *Pseduomonas aeruginosa* (25mm & 20mm) and while *Vibrio choleare* showed the microbial activities against chloroform extracts of both plants (17mm and 15mm).

The chloroform extract of *Stephania japonica* showed the microbial activities in *Vibrio cholerae* (17mm), *E.coli* (7mm), *Streptococcus pneumoniae* (13mm) and Proteus mirabilis (15mm). *Klebsiella pneumoniae* (14mm) in aqueous and *Bacillus cereus* (7mm) in ethanolic extracts were observed only in *Stephania japonica* (Table 3 and Plate 1).

### Table 1: Phytochemical test in leaves of aqueous, ethanolic and chloroform extracts of *Cocculus hirsutus*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytoconstituents</th>
<th>A</th>
<th>E</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phenol</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>3</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
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<td>-</td>
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<tr>
<td>5</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Proteins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Aminoacids</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

A= Aqueous, E= Ethnolic, C= Chloroform; (+) Present, (-) Absent.

### Table 2: Phytochemical test in leaves of aqueous, ethanolic and chloroform extracts of *Stephania japonica*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytoconstituents</th>
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<th>E</th>
<th>C</th>
</tr>
</thead>
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<tr>
<td>3</td>
<td>Tannins</td>
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<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>S. No.</td>
<td>Micro Organisms</td>
<td>Zone of inhibition (mm)</td>
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<td></td>
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<td></td>
<td>Stephania japonica</td>
<td>Cocculus hirsutus</td>
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<tr>
<td></td>
<td></td>
<td>Aqueous</td>
<td>Ethanol</td>
<td>Chloroform</td>
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<tr>
<td>1</td>
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<td>Vibrio cholerae</td>
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<td>Streptococcus pneumoniae</td>
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<td>Bacillus cereus</td>
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<tr>
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<td>Proteus vulgaris</td>
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<td>-</td>
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<tr>
<td>10</td>
<td>Proteus mirabilis</td>
<td>-</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>11</td>
<td>Serratia marcescens</td>
<td>-</td>
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</tbody>
</table>

Standard Disk = 6 mm

Table 3: Antibacterial activity of *Stephania japonica* and *Cocculus hirsutus* Leaf extract (25/µl) and control Antibiotic (15/µl)

A= Aqueous, E= Ethnolic, C= Chloroform; (+) Present, (-) Absent.

Fig.1 *Stephania japonica*  
Fig.2 *Cocculus hirsutus*
Plate 1: Antibacterial activity of *Stephania japonica* and *Cocculus hirsutus* Leaf extract (25/µl) and control Antibiotic (15/µl)

CONCLUSION

This study supports that *Stephania japonica* and *Cocculus hirsutus* showed has potential antimicrobial activity against different bacterial strains. This antibacterial activity was associated with the variety of phytochemicals found in these plants. The above species has potential to be harnessed for further study in drug discovery.

REFERENCE


