Phytochemical and Antimicrobial Screening of Cocculus hirsutus L.

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Abstract: Many number of the plant species including Cocculus hirsutus L. is being used as the sources of herbal medicine. Present work was mainly focused with the identification of the therapeutic properties of Cocculus hirsutus L. leaf extracts. The leaf extracts of methanol, aqueous, chloroform and benzene showed solvent dependent phytochemical present and anti-microbial activity. Whereas the leaf extracts of methanol and chloroform showed significant antimicrobial activity than water and benzene extracts. These extracts were further studied and analyzed for different phytochemical compounds. Leaf extracts possesses more flavonoids, steroids, alkaloids, tannins, glycosides and lignins in methanol, chloroform, benzene extracts than water extracts. The antimicrobial activity of different extracts of Cocculus hirsutus were analyzed and discussed.

Key words: Phytochemical screening, Anti-microbial activity, Cocculus hirsutus L.

I. Introduction

Cocculus hirsutus belongs to the family minispermaceae. It is a tropical, invasive creeper, native to India, Pakistan and tropical Africa with the common name broom creeper, dussaratheega (in telugu). Cocculus hirsutus having wide medicinal properties including skin infection, dhierea, anticancer, eczema, rheumatism, gonorrhea etc. A decoction is applied against fever, rheumatism and server weight loss. Other than medicinal properties Cocculus hirsutus having some house hold uses like making of baskets, brooms and carry bags by using shoots.

Traditional medicine has a long history of serving people all over the world. The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main source of drugs [1-2]. There is an evidence of herbs being used in the treatment of diseases and for revitalizing body systems in almost all ancient civilizations. Since time-immemorial plants are being used in all cultures as a source of medicine. Apart from primitive and ancient civilizations, the present contemporary cultures all over the world are relying on herbs to reap the benefits that Mother Nature has extended to mankind [3-4]. It is estimated that, 7,500 plants are used in local health traditions mostly in rural and tribal villages of India. Out of these, the real medicinal value of over 4000 plants is either little or not known to the main stream of population [5].

Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin. Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine. Many food crops have medicinal effects, for example garlic. Medicinal plants are resources of new drugs. It is estimated that there are more than 2, 50,000 flowering plant species. A phytopharmaceutical preparation (or) herbal medicine is any manufactured medicine obtained exclusively from plants, either in the crude state (or) as pharmaceutical formulation [6]. The main objective of present study is to analyze and evaluation of wild relatives of Cocculus hirsutus for their phytochemical, antimicrobial and anti fungal potential properties, which might be used to formulate effective herbal medicine.

II. Materials and methods

2.1. Collection of plant material:

The plant samples of Cocculus hirsutus were collected from the surrounding field areas of Rayalaseema University campus, Kurnool district, Andhra Pradesh. The leaves were washed thoroughly with tap water followed with sterilized distilled water for the removal of dust and sand particles, then shade dried at room temperature. Samples were homogenized to fine powder by a mechanical grinder and used for experimental analysis.

2.2. Preparation of plant extracts

5g of leaf sample powder were sequentially extracted with solvents namely methanol, chloroform, benzene and also with water by soxhelt apparatus for 48 hours. Then it was filtered through whatman No.1 filter.

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paper. The crude extracts were obtained by dissolving a known amount of dry extract in different solvents such as methanol, chloroform, benzene and aqueous to obtain a stock solution of 1000 μg/ml. The stock solutions were serially diluted with the respective solvents to obtain lower dilutions (50, 25 and 10 μg/ml). Crude extracts were analyzed for phytochemical and antimicrobial studies.

2.3. Preliminary phytochemical analysis

The individual extracts were subjected to qualitative chemical investigation for the identification of different phytochemical compounds and plant secondary metabolites by using standard protocols followed by mayer’s and wagner’s procedures.

2.4. Antibacterial and antifungal activity

The method called agar well plate method was adapted for screening of antimicrobial study. The hot sterile medium was poured into the sterile petri plates to form a 2-3mm thick. The plates were lawn cultured with bacterial broth and fungal spore suspension. Then make a hole with a diameter of 6-8 mm is punched aseptically with a sterile tip, and then poured a volume 150 μl of crude extract at desired concentrations (50, 25, 10 μg) were introduced into the each well. Then agar plates were incubated under ambient conditions in an incubator at 37°C for 24 hours depending upon the test organisms. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested, then evaluated the inhibition zones.

2.5. Preparation of Standard Solutions

Ciprofloxacin was the positive reference (control) standard with the dilutions of 50, 25, 10 μg/mL.

2.6. Test Organisms (Bacterial and Fungal stains)

The microorganisms used for the present study include bacteria like Pseudomonas, Klebsiella pneumonia, bacillus subtilis. Fungal species includes Candida albicans, Aspergillus fumigates. All bacterial and fungal species were procured from biotechnology lab, S.K.University, Anantapur. The bacterial and fungal stock cultures were maintained on different nutrient media which were stored at 4°C.

III. Results and discussion

3.1. Preliminary Qualitative and quantitative Phytochemical Studies

We analyzed qualitative and quantitative analysis of different phytochemical compounds in leaf extracts of Cocculus hirsutus by using the soxhlet apparatus with different solvents (methanol, chloroform, benzene and water). It was observed that, methanol, chloroform and benzene extracts of leaf crude extract was shown more phytochemical presence such as flavonoids, steroids, alkaloids, tannins, glycosides and lignins in methanol, chloroform, benzene extracts than water extracts respectively, results were represented in Table 1. Similar results were reported by [7, 8, and 9] and their reports stated that methanol, chloroform and ethanol extracts shows more phytochemical than water extracts.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compounds</th>
<th>Methanol</th>
<th>Aqueous</th>
<th>Chloroform</th>
<th>Benzene</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>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Phenols</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Glycosides</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Tannins</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Steroids</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Quinones</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Saponins</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Lignin’s</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Fixed oils</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

(+)= Presence of phytochemical compound, (-) = absence of phytochemical compound

3.2. Anti microbial activity

We measured antimicrobial activity of Cocculus hirsutus leaf extracts in different solvent extracts on common bacterial and fungal species. The disc diffusion method was used to determine the zone inhibition range. Efficiency of crude drug on different micro organisms was calculated based on the inhibition zone in diameter (mm), results were represented in Figure 1. Aqueous, methanol, ethanol and benzens extracts were showed significant activity against Pseudomonas, Klebsiella pneumonia, in gram negative and Bacillus subtilis in gram positive at the concentration of 50 μg. Whereas aqueous, methanol, ethanol extracts shows maximum inhibition activity and benzene extract showed less antimicrobial activity. Our results correlated with earlier reports [10] and the ethanol extracts of Tectona grandis shows maximum bacterial inhibition.

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3.3. Anti fungal activity

Anti fungal activity of different leaf extracts of *Cocculus hirsutus* was studied and measured by using different fungal species. The leaf extracts of aqueous, methanol and ethanol showed significant activity against *Candida albicans*, *Aspergillus fumigates* at 50 µg/ml concentration compared with fluconazole which is used as a positive control. Whereas benzene extracts shows less inhibition activity compared to other extracts, results were depicted in Figure 2. Similar results were reported by [11].

IV. Conclusions

Present study has been concluded that *Cocculus hirsutus* extracts using ethanol, methanol and aqueous solvents were most effective against *Pseudomonas*, *Klebsiella pneumonia*, and *Bacillus subtilis*. Out of all three extracts from *Cocculus hirsutus* maximum significant antimicrobial activity showed by aqueous and methanol leaf extracts against bacterial as well as fungal species, *Candida albicans*, *Aperigillus fumigates*. Results of present study have justified the traditional indirect use of herbal medicine in curing diseases. Some of these observations have helped in developing drugs for therapeutic use in different diseases. Our future aim from this basic study is to characterize the structural compounds from this plant crud extract.

References

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