Antimycotic and Antibacterial activities of Gynandropsis pentaphylla DC extracts and its Phytochemical Studies

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Citation

Abstract
Aim: To study the antimycotic, antibacterial and phytochemical properties of inherent in leaves, roots, stems, seeds and seed pods of Gynandropsis pentaphylla DC.

Procedure: Fresh leaves, roots, stems, seeds and seed pods of G. pentaphylla were used to extract with methanol, acetone and water. Fresh extracts of were tested against 2 bacteria and also against 3 fungi using well diffusion method of susceptibility testing on sensitivity testing agar medium. Extracts of roots, stems, leaves, seeds and seed pods were screened phytochemically for the presence of secondary metabolites such as alkaloid, flavones, sugar, phenolic group, essential oil, amino acids and saponin.

Results: Bacterial cultures of Escherichia coli and Staphylococcus aureus were highly susceptible to the acetone extracts of all the five parts of G. pentaphylla. Aspergillus niger, Aspergillus flavus and Metarhizium anisopliae were highly susceptible to the methanol extracts of all the parts of G. pentaphylla. The seed showed highest activity among all the plant parts. Highest activity index was observed for all extracts against the entomopathogenic fungi M. anisopliae. Both M. anisopliae and G. pentaphylla are active pest control agent, since both are incompatible to each other, it is difficult to use the both in integrated pest management (IPM) combainly to manage the pests.

Conclusion: The active antimicrobial ingredients in G. pentaphylla should be identified while its medicinal value to humans properly investigated in this regard.

INTRODUCTION
The cat's whiskers (Gynandropsis pentaphylla DC.) is one vegetable, which grows as a weed in most tropical countries. The leaves and seeds of cat's whiskers are used in indigenous medicine in many countries. In India the common names include kurhur and karaila. Gynandropsis pentaphylla belongs to the plant family Capparidaceae. Gynandropsis pentaphylla DC syn Gynandropsis gynandra L. (Briq.) and Cleome gynandra L. (Briq). It is a herb indigenous to the tropical and sub tropical regions. The herb is edible and grows up to 60cm high.

Kurhur has been used for several years in Indian traditional medical practices. G. pentaphylla leaves with a high percentage of vitamin C is taken as a pot herb in soups, fresh or dried. The leaves are used as disinfectants. Inhalation of the leaves also relieves headaches; leaf juice and oil, for earache and eye wash. Seeds have been reputed to have antihelmintic properties and oil is used as fish poison. Sterols are also found in in these plants; with lupeol, campesterol and epi-lupeol having been isolated from this plant. In previous studies, the anthelmintic and antimicrobial properties of Capparidaceae plants have been reported from different countries and in continuation of these objectives on this plant family, the phytochemical, antibacterial and antifungal properties of Gynandropsis pentaphylla are presented in the current study for the Indian G. pentaphylla.

G. pentaphylla plants have been observed to have insecticidal, antifeedant and repellent characteristics. The leaves have anti-tick properties. They also have repellent and acaricidal properties for larvae, nymphs and adult Rhipicephalus appendiculatus and Amblyomma variegatum ticks. Ticks may not be found for a distance of
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2-5 m from the plant. The ethanol extract is toxic to insect pests, such as the painted bug (Bagrada cruciferarum Kirk) and the diamond back moth (Plutella xylostella L.) of cruciferous vegetables. The volatile oils permanently repel the diamond back moth larvae from treated cabbage leaves. The plant has an anti-feedant action against the tobacco caterpillar (Spodoptera litura F.). The extract from the mature seeds is toxic to brinjal aphid (Aphis gossypii Glov.), and the larvae of Helicoverpa armigera (Hubner). The seeds contain phenolic compounds, which are natural products. Lipids from seeds could be used in soap manufacture. The biocontrol agent, Metarhizium anisopliae (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) is an extensively studied cosmopolitan filamentous fungus, which is a key regulatory organism of insect populations. No reports were available on the activity of G. pentaphylla against this entomopathogenic fungi. With this views in mind the present is designed to carried out the activity of leaves, roots, stems, seeds and seed pods extracts of indigenous G. pentaphylla against two bacterial and three fungal species such as Escherichia coli and Staphylococcus aureus and Aspergillus niger, Aspergillus flavus and M. anisopliae.

MATERIALS AND METHODS

COLLECTION OF PLANT AND PREPARATION OF EXTRACT

Gynandropsis pentaphylla was collected from Rajgurunagar area of Pune, Maharashtra, India in March 2008. About 200g of G. pentaphylla leaves were ground in mortar and pestle with 10 ml of sterile distilled water. Resulting paste collected in a conical flask and allowed to mix properly by placing it in a shaker at 200 rpm for 24 hr. Then the paste was filtered through double layered muslin cloth, the filtrate of leaves in water was used for the further studies. Similar protocol was adapted to prepare leaves extract using other solvents such as methanol and acetone. The same procedure was used to prepare the extract from other plant parts like roots, stems, seeds and seed pods. All the extracts were stored at 4 °C for further analysis.

PHYTOCHEMICAL SCREENING

Qualitative phytochemical evaluation was carried out to test the presence of alkaloids, flavones, sugar, phenolic group, saponin, amino acid and essential oil in the extracts samples using modified method of Brindha et al.

Alkaloids: Test solution (500µl) was taken with 2N HCl (500µl). The aqueous layer was decanted. To the lower layer 2 drops of Mayer’s reagent was added. Development of white turbidity in precipitate represented the presence of alkaloids

Flavones: Test solution (500µl) was mixed with 100µl of alcohol, 0.02g of para-dimethyl amine benzaldehyde and two drops of conc. HCl. Development of red or pink color indicated the presence of flavones

Sugar: Test solution (500µl) was taken in clean test tube. 0.01g of anthrone and 3 drops of conc. H2SO4 were added in the test tube. The solution was heated for 1 to 2 minutes. Change of green to purple color was noted to detect the presence of sugar in the sample.

Phenolic group: Alcoholic plant extract (500µl) was taken in a test tube. Two drops of 1M ferric chloride was added. Appearance of intense color indicated the presence of phenolic groups.

Saponin: Test solution (500µl) with distilled water (2 drops) taken in a test tube. Development of foamy lather indicated the presence of saponin.

Amino acid: Test solution (500µl) and two drops of 1% ninhydrine in alcohol were taken in a test tube. Blue or violet color development indicated the presence of amino acid.

Essential oil: Test solution (500µl) with two drops of 1M alcoholic K2Cr2O7 and 3 drops of phenolphthalein were added in a clean test tube. Soap formation indicated the presence of essential oil.

ANTIMICROBIAL ASSAY

MICROORGANISM USED

Pure culture of two human pathogenic bacteria such as one gram negative strain, Escherichia coli NCIM 2064 and one gram positive strain Staphylococcus aureus NCIM 2120 were used for the present in vitro antibacterial assay. For the antifungal assay, three fungal cultures like Aspergillus niger NCIM 501, Aspergillus flavus NCIM 650 and Metarhizium anisopliae NCIM 1311 were used. All the bacterial and the fungal strains were obtained from the National Collection of Industrial Microorganism (NCIM), National Chemical Laboratory, Pune, India.

MEDIA USED

Luria agar (LA) and Luria broth (LB) and potato dextrose agar (PDA) and potato dextrose broth (PDB) (HiMedia, Mumbai, India) were used for the bacterial and fungal bio
Antimicrobial agents

Cloroamphenicol (10mg/ml) and fluconazole (10mg/ml) (HiMedia, Mumbai, India) were used as standard antibacterial and antifungal agent respectively. They were included in the current study as standard reference drugs.

Determination of antimicrobial activity

Sterile cottons swap was taken and dipped in 24 hours old S. aureus culture. The entire agar surface of LA plate was seeded first in horizontal direction and then in vertical direction to ensure the even distribution of organism over the agar surface using the above swap. The seeded agar surface was allowed to dry for five min. Tip of well cutter was sterilized by heating on the blue flame of Bunsen burner and used for the well preparation in the seeded LA plates. Three wells were prepared in each LA plate. As soon as the wells were prepared all plant part extracts (10µl) were poured in each well separately using sterile micro-tips. The same procedure was followed for E. coli in LA plate. The above protocol was adapted for A niger, A. flavus and M. anisopliae in PDA plates using 48 hours old PDB cultures. Antimicrobial agents [Cloroamphenicol (10mg/ml) and fluconazole (10mg/ml)] were pored as positive control and respective solvents were used as negative control. All LA plates and PDA plates were incubated at 37±2 °C for 24 hrs and 27±2 °C for 72 hrs. After incubation the zone of inhibition was measured using HiAntibiotic Zone Scale (HiMedia, Mumbai, India).

Activity index

The zone of inhibition in extract and the standard antimicrobial agents were used to calculate the activity index.

\[ AI = \frac{\text{Zone of inhibition by extract}}{\text{Zone of inhibition by standard antimicrobial agents}} \]

Proportion index

Number of positive results obtained for water, methanol and acetone extract of one plant part was against all the microbials and total number tests carried out were used to evaluate the proportion index.

Results and discussion

The phytochemical screening of Gynandropsis pentaphylla revealed the secondary metabolites which are of medicinal interest as presented in table 1. G. pentaphylla is rich in sugar, amino acid and phenolic group. However, in G. pentaphylla seeds extract (both water and other solvents) contained alkaloids, flavones, sugar, phenolic group, saponin, amino acid and essential oil in plenty of amounts (table 1). Saponins was comparatively less in this species. The variance in the quantitative composition of the secondary metabolite establishes the fact that different plant parts are not likely to have the same medicinal potential. These secondary metabolites are known to exhibit medicinal activity as well as physiological activity.

Table 1: Phytochemical screening of and yields of extracts

<table>
<thead>
<tr>
<th>S. No</th>
<th>Phytochemical name</th>
<th>Solvent name</th>
<th>Leaf</th>
<th>Root</th>
<th>Stem</th>
<th>Seed</th>
<th>Seed pod</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaldoid</td>
<td>Water</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>flavones</td>
<td>Water</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>sugar</td>
<td>Water</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>4</td>
<td>Phenolic group</td>
<td>Water</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>5</td>
<td>Essential oil</td>
<td>Water</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>6</td>
<td>Amino acid</td>
<td>Water</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>7</td>
<td>Saponin</td>
<td>Water</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

* ++ for excellent, + for good, + for moderately positive, + for doubtful.

The results of the antimicrobial activity presented in table 2 shows that all extracts exhibited appreciable antimicrobial properties, inhibiting the growth of all microorganisms. While stem aqueous extracts not inhibit the growth of
Escherichia coli, Staphylococcus aureus, Aspergillus niger and Aspergillus flavus. This is interesting as water is one of the medium through which traditional healers utilize medicinal plants to their clients. Two clinical strains of human pathogenic fungi like A. niger and A. flavus and one entomopathogenic fungi, Metarhizium anisopliae were used to find out the antymycotic activity of G. pentaphylla. M. anisopliae was the most sensitive, this was closely followed by A. flavus and A. niger was the least sensitive in the assay.

**Figure 4**

Table 2: Antimicrobial activities of extracts

<table>
<thead>
<tr>
<th>No.</th>
<th>Plant Parts</th>
<th>Solvent name</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>A. niger</th>
<th>A. flavus</th>
<th>M. anisopliae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaves</td>
<td>Methanol</td>
<td>10.0±0.0</td>
<td>11.6±0.5</td>
<td>-</td>
<td>10.2±0.0</td>
<td>11.0±0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetone</td>
<td>28.6±0.0</td>
<td>28.0±0.0</td>
<td>11.3±0.6</td>
<td>-</td>
<td>15.1±0.5</td>
</tr>
<tr>
<td>2</td>
<td>Roots</td>
<td>Methanol</td>
<td>14.5±0.4</td>
<td>17.1±0.2</td>
<td>11.3±0.1</td>
<td>12.2±0.3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetone</td>
<td>19.6±0.1</td>
<td>20.0±0.0</td>
<td>11.0±0.0</td>
<td>-</td>
<td>15.6±0.4</td>
</tr>
<tr>
<td>3</td>
<td>Stems</td>
<td>Methanol</td>
<td>13.2±0.2</td>
<td>10.4±0.0</td>
<td>20.2±0.2</td>
<td>14.5±0.2</td>
<td>12.1±0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetone</td>
<td>21.4±0.4</td>
<td>25.2±0.2</td>
<td>11.0±0.0</td>
<td>12.2±0.1</td>
<td>10.3±0.4</td>
</tr>
<tr>
<td>4</td>
<td>Seeds</td>
<td>Methanol</td>
<td>11.0±0.0</td>
<td>13.2±0.5</td>
<td>13.0±0.0</td>
<td>13.6±0.5</td>
<td>13.4±0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetone</td>
<td>22.2±0.1</td>
<td>25.4±0.1</td>
<td>15.4±0.1</td>
<td>15.4±0.4</td>
<td>14.8±0.3</td>
</tr>
<tr>
<td>5</td>
<td>Pods</td>
<td>Methanol</td>
<td>14.0±0.2</td>
<td>10.0±0.5</td>
<td>14.0±0.0</td>
<td>10.7±0.3</td>
<td>12.1±0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetone</td>
<td>15.0±0.3</td>
<td>25.4±0.1</td>
<td>10.0±0.0</td>
<td>-</td>
<td>16.3±0.1</td>
</tr>
<tr>
<td>6</td>
<td>SAA</td>
<td>Methanol</td>
<td>22.0±0.0</td>
<td>22.0±0.0</td>
<td>17.0±1.0</td>
<td>20.0±2.6</td>
<td>15.0±1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>Methanol</td>
<td>12.0±0.0</td>
<td>17.0±0.0</td>
<td>13.1±0.1</td>
<td>12.0±0.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetone</td>
<td>12.1±0.0</td>
<td>10.0±0.0</td>
<td>11.0±0.0</td>
<td>11.0±0.0</td>
<td>-</td>
</tr>
</tbody>
</table>

SAA - Standard antimicrobial agent

Generally, proportion index of antimicrobial activities of G. pentaphylla extracts shows the highest activity in seeds extract as presented in figure 1.

**Figure 5**

Figure 1: Proportion index of antimicrobial activities of extracts

A high correlation coefficient (near 1 or -1) means a good relationship between two variables and its value around zero means no relationship between them at a significant level of p < 0.05. More precisely, it can be said that parameters showing r > 0.7 are considered to be strongly correlated whereas r between 0.5 and 0.7 shows moderate correlation.

In the present study, the resultant matrix reveals strong positive correlation among different bacteria (table 3). The relationship between the antimicrobial activity pattern of different extracts against various microbials used for the assay had been studied. Highest correlation (0.99) was observed in between aqueous extract of root and acetone extract of seeds. Higher correlation (0.97) was observed in between acetone extract of leaves and roots; acetone extract of stems and standard antibiotic. 0.96 correlation was observed in between aqueous extracts of leaves and seed pods.

A data matrix of five organisms and 19 variables (different extracts with one aqueous and two solvents) were used for this cluster analysis, the results are presented in figure 2.

**Table 3: Correlation matrix of sensitivity of different microorganism by extracts**

<table>
<thead>
<tr>
<th></th>
<th>E. coli</th>
<th>S. aureus</th>
<th>A. niger</th>
<th>A. flavus</th>
<th>M. anisopliae</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.77*</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>0.51*</td>
<td>0.22</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. flavus</td>
<td>0.25</td>
<td>0.14</td>
<td>0.67*</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>M. anisopliae</td>
<td>0.58*</td>
<td>0.49</td>
<td>0.48</td>
<td>0.10</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* Marked correlations are significant at p < .050000

The relationship between the sensitivity pattern of different microbials against various extracts used for the antimicrobial assay had been studied using STATISTICA/w 5.0. software (table 3).
Antimycotic and Antibacterial activities of Gynandropsis pentaphylla DC extracts and its Phytochemical Studies

Figure 7
Figure 2: Cluster analysis of sensitivity of different microorganism by extracts

The microbes were separated into two main groups I and II based on their sensitivity: the bacteria, S. aureus with the highest resistant was clustered alone in group II and all the remaining organism with moderate and low resistant were grouped into I (figure 2). In the group I, only one fungi, A. flavus was sub clustered into IB, which is the second resistant organism (figure 2). G. pentaphylla extracts were more active against fungi than that of bacteria as presented in figure 3.

Figure 8
Figure 3: Activity index of extracts

Presence of alkaloids and reducing sugars in leaves and stems were reported by Ajaiyeoba et al. In the present study also we observed the same results in stem and leaves. Apart from both leaves and stems we additionally observed the presence of alkaloids and reducing sugars in seeds, roots and seed pods extracts of G. pentaphylla. This is not surprising for plants of the Capparidaceae family 18, 20, 5.

From the result of the antibacterial studies as shown in Table 2, all the extracts exhibited appreciable antibacterial properties, inhibiting the growth of all the bacteria and fungi. The same results were reported by Ajaiyeoba et al., but the zone of inhibition was comparatively less in methanol extracts of stems and leaves. However, in the present study we noted the highest activity (28.4±0.0) against E. coli in acetone extract of leaves. In most instances, activities were greater than the standard therapeutic agent, chloramphenicol as presented in figure 3. The current study was correlated with the previous report 5. However, in the current investigation we haven't observed any insensitive bacteria to chloramphenicol.

In the antimycotic assay, G. pentaphylla have again displayed high antifungal activities. Methanol extract of G. pentaphylla stem showed the highest activities, inhibiting the growth of A. niger up to 20.2±0.2mm. Ajaiyeoba et al. observed less activity (13.0±0.5) against this fungi in his study. This difference in activity is may be because of the difference in geographical origin 11. Highest activity index was observed generally against the entomopathogenic fungi M. anisopliae. M. anisopliae and G. pentaphylla 11, 24 are active pest control agents, since both are incompatible to each other, it is difficult to use the both pest control agents simultaneously in integrated pest management (IPM).

Conclusively, all extracts have displayed antimicrobial activities from present studies. The leaves and seeds of cat's whiskers are used in indigenous medicine in many countries 11. This has further confirmed the use of this plants in Indian ethnopharmacology for treatment of bronchitis, boils, earache, eye wash, disinfectant and nasal congestion, analgesic, headaches, epileptic fits, facilitate childbirth in pregnant women, stomach-ache, constipation, conjunctivitis, thread-worm infection, chest pains, arthritis, inflammation, neuralgia, rheumatism, localized pains, pus, anaemia, uterine complaints, malaria, pneumonia, head lice and reduce coughing 10, 3, 17, 14, 11, 16, 5. There is need for the development of new antibiotics due to acquired resistance, more importantly from natural sources as this delays resistance. G. pentaphylla used for the study provide good opportunities for drug development in this area. Further studies are needed to find out the exact compound responsible for the antimicrobial activity using thin layer chromatography, column chromatography and HPLC.
Further purification and bioactivity is in progress in our laboratory and will be communicated in future.

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