PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY ANALYSIS OF SWERTIA CHIRAYITA AND ARTEMISIA ABSINTHIUM PLANT EXTRACTS

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Abstract: The Swertia chirayita and Artemisia absinthium (Afsanteen) plants extracts were evaluated for phytochemicals and their antimicrobial activity against 3 bacteria and 2 fungal strains by using the disc diffusion technique. Phytochemical analysis showed the presence of tannins, flavonoids, saponins, and terpenoids in Swertia chirayita while in Artemisia absinthium only saponins, terpenoids were present. To a very small extent tannins were present in the ethanolic extract of Afsanteen. But both plant extracts gave negative results for the presence of cardiac glycosides. Antibacterial activity of Swertia chirayita and Afsanteen plant extracts were screened against 3 bacterial strains (E. coli, Bacillus subtilis, Pseudomonas syringola) and 2 fungal strains (Aspergillus niger and Fusarium Solani). Ampicillin was used as a standard drug for antibacterial and antifungal activity. Results showed that Afsanteen and chirayita extracts showed activity against bacterial strains except chirayita n-hexane and ethanol extract for Bacillus subtilis and Chirayita n-hexane extract for Pseudomonas syringola. A maximum zone of inhibition was noticed for chirayita ethanol extract against Pseudomonas syringola. But both plant extracts showed zero activity against fungal strains except Chirayita acetone extract against Fusarium solani while chirayita ethanol extract against Aspergillus niger. Both extracts of plants gave concentration-dependent activity. It was concluded that the presence of antimicrobial activity for both plant extracts indicated that is due to the presence of phytochemical compounds.

Keywords: antimicrobial, Swertia chirayita, Artemisia absinthium, ethanol, n-hexane, acetone, plant extract

Introduction

Swertia chirayita is a very famous herb which grows under temperate climatic condition of Himalaya. Family to which chirayita belongs is Gentianaceae. chrirayita is also called Indian gentian (Joshi et al., 2005; Aleem et al., 2018). Gentianaceae is a flowering family which contains a range of floral patterns and colors. Swertia chirayita is annual or biennial herb of seasonal growth. The size of chirayita stems range from 60cm to 150cm. Stems are cylindrical at the base and upwardly quadrangular (Khanal et al., 2014). Color of stems is greenish brown at the young age of plant and this color changes from light brown to violet when plant at its maturity stage. Its leaves are 10cm long that are in opposite in pair pointed at tips without stalks (Keshebo et al., 2016; Ahirwal et al., 2010). Cross pollination in S. chirayita is promoted due to the presence of nectaries and multi colored corolla (Kumar and Sharma, 2015). S. Chirayita flavours as well as thrives in woodland gardens having partial shade sunny edge as well as in marshy lands.

early summer to early autumn (Hashimi et al., 2019). This plant is native to temperate regions of Northern United States, Northern Africa, Kashmir, Nepal, Afghanistan, US, Canada, westward to the Atlantic, the Midwest, the Great Plains and Eurasia. This plant is aromatically tonic and enjoyed a higher reputation in debility of digestive organs. This herb also regarded as Anthelmintics or antihelmintics (Ashraf et al., 2019). Afsanteen grows in dry waste places in Europe like roadsides (Beigh and Ganai, 2017). *Artemisia absinthium* contains many compounds which are responsible for its bioactivity like huyjl alcohol esters, α-cadinene, guaiazulene-epoxyocimene, sabinyl acetate, (Z) - chrysantenyl acetate. Other important compounds of Afsanteen are bitter sesquiterpenoid lactones, absinthin. Bitter compounds that are obtained from this herb are artamardin, artamarin, artamarinidin, artamarin. This herb also has many flavonoids (Goud and Swamy, 2015; Szopa et al., 2020). The present investigation was undertaken to study the antimicrobial activity of crude extracts of *Swertia chirayita* and *Artemisia absinthium* whole plants in different solvents against bacterial and fungal strains.

**Materials and Methods**

**Plant material**

*A. absinthium* (Afsanteen) plant and *Swertia Chirayita* plant samples were collected from local market of Lahore. The samples were air dried separately after light washing in water (to clean dust and other particles) and after about 5-7 days when these herbs dried properly they were grinded to obtain their powdered form.

**Extracts of whole plant materials**

Ethanol, n-hexane and acetone extracts of *chirayita* and Afsanteen plants were prepared by soaking 15g of each of the dry powdered plant material separately in 150ml of ethanol-hexane and acetone at room temperature for 2days. After 2 days these extracts were filtered through filter paper. The extracts were concentrated by using rotary evaporator with water bath at 40°C or also evaporated by opening the lid of reagent bottles for 1-2 days in which filtered plant solution are present. Then these extracts were stored separately in 1.5ml micro centrifuge tubes by proper labeling of each plant name and solution names (n-hexane, acetone, and ethanol).

**Phytochemical screening**

Qualitative chemical tests were performed for each extract of Afsanteen and *chirayita* plants by using standard procedures.

**Test for terpenoids**

0.5g ethanolic extract of Afsanteen was added to 2ml of chloroform and then 3ml sulphuric acid in concentrated form was added carefully to form a layer. Reddish brown color at interface shows the presence of terpenoid. Perform this test for each extracted sample material in same way.

**Test for flavonoids**

5ml of dilute ammonia was added to a portion of filtered extract of each of plant material separately, and then 1ml of concentrated sulphuric acid was added. Yellow color was disappeared while standing indicated the presence of flavonoids.

**Test for tannins**

0.5g of each extract was boiled separately in 10ml of distilled water in a test tube and then each solution was filtered. Few drops of ferric chloride (0.1%) was added to the filtrate separately. Blue-black or brownish green color shows the presence of tannins.

**Test for saponins**

0.5g of each extract was added to 5ml of distilled water in test tube. Vigorously the solution was shaken and stable persistent froth formation was observed. Then 3 drops of olive oil were mixed to the froth in each test tube and then each test tube was observed for an emulsion formation.

**Keller-Killiani test (cardiac glycosides test)**

5ml of distilled water was added to 0.5g of extract that was in test tubes separately. Then 2ml of glacial acetic acid and 1 drop of ferric chloride solution. 1ml of sulphuric acid was added Afsanteen acetone. At the interface Brown ring shows the presence of deoxy-sugar characteristic of cardenolides. Below brown ring violet ring appears and in acetic acid layer green ring forms above brown ring and slowly spread throughout the layer.

**Antibacterial Activity**

**Media preparation**

12g of nutrient agar powder was added in 500ml of water and after dissolving completely it was autoclaved at 121°C for 15min. After autoclaving media was poured in 18 petri plates for checking antibacterial activity of plant extracts.

**Antibacterial assay**

After complete solidification of media isolated bacterial samples (*E. coli, Pseudomonas syringola*, and *Bacillus subtilis*) were streaked separately on each plate properly. Then ampicillin disc placed on plate and 3 simple discs were also placed on same plate, each plate is labeled properly where the control disc (antibiotic disc) on plate is and where are simple plates. 5µl,10µl and 15µl of each plant extracts were poured separately on simple discs such as 5µl ethanol extract of *chirayita* on one simple disc and 10µl ethanol extract on 2nd simple disc and 15µl ethanol extract of chiriata on 3rd disc. Same concentrations of plant extracts were used for each plate. Then plates were incubated overnight at 37°C. After incubation period plates were observed for clear zone formation.
around the simple discs which corresponds to antimicrobial activity of tested compounds. At last zone of inhibition was measured in mm for each plate.

**Antifungal activity**

**Media preparation**

17g of LB agar was dissolved completely in 500ml water and then autoclaved at 121°C for 15min. Then autoclaved media was poured in 12 petri plates and placed for solidification of media.

**Antifungal assay**

*Fusarium solani* and *A. Niger* isolated fungal samples were streaked properly. First fungal sample was streaked in solidified 6 petri plates and similarly 2nd fungal sample was also streaked in remaining 6 plates sterilized loop. Then ampicillin disc placed on plate and 3 simple discs were also placed on same plate, each plate is labeled properly where the control disc (antibiotic disc) on plate is and where are simple plates. 5µl,10µl and 15µl of each plant extracts were poured separately on simple discs such as 5µl ethanol extract of *chirayita* on one simple disc and 10µl ethanol extract on 2nd simple disc and 15µl ethanol extract of *chirayita* on 3rd disc. Same concentrations of plant extracts were used for each plate. Then plates were incubated for 2-3 days at 37°C. After incubation period plates were observed for clear zone formation around the simple discs which corresponds to antimicrobial activity of tested compounds. At last zone of inhibition was measured in mm for each plate. All of above described procedure was performed in biosafety cabinet.

**Results and discussions**

In this study phytochemical compounds of *chirayita* and Afsanteen plant and their antimicrobial activity were recorded. Results of the study are given below:

**Terpenoid Test**

In this test it was found that in *chirayita* (ethanol extract-hexane and acetone extract) and Afsanteen (acetone, ethanol and n-hexane extract) terpenoid are present because reddish brown coloration at the interface was observed (Table 1). The presence of terpenoids revealed the potential of extracts as antioxidants and pharmacological uses. The plants *chirayita* and Afsanteen may be used as potential medicinal plants (Aleem and Kabir, 2018; Parmar et al., 2012).

**Flavonoids Test**

It was found that in *chirayita* (ethanol, n-hexane and acetone extracts) flavonoid is present which was confirmed experimentally because a yellow color was appeared and that color disappears on standing which indicates the presence of flavonoids. But in Afsanteen flavonoids were not present because no change in color was observed when this test was performed (Table 1). The presence of flavonoids revealed the potential of extracts as antioxidants and pharmacological uses. The plants *chirayita* and Afsanteen may be used as potential medicinal plants (Aleem and Kabir, 2018; Bhargava et al., 2009; Kaloo and Bhat, 2020; Parmar et al., 2012; Rafe, 2017).

**Tannins Test**

If brownish green or blue black color appears it means tannins are present. Here it was observed that ethanolic *chirayita* extract have tannins and in remaining extracts except Afsanteen (n-hexane and acetone extracts) tannins may be present to some extent (Table 1). The presence of tannins revealed the potential of extracts as antioxidants, anticancer and pharmacological uses. The plants *chirayita* and Afsanteen may be used as potential medicinal plants (Hashimi et al., 2019; Ashraf et al., 2019; Szopa et al., 2020).

**Saponins Test**

Emulsion formation indicates their presence. So it was found that saponins are present in *chirayita* (ethanol extract, acetone extract) Afsanteen (n-hexane extract and acetone extract) because they form an emulsion but in *chirayita* n-hexane and in Afsanteen ethanolic saponins are not present because no emulsion was observed (Table 1). The presence of saponins revealed the potential of extracts as antioxidants, anticancer, anti-inflammatory and pharmacological uses (Ansari et al., 2019; Ashraf et al., 2019; Das et al., 2012; Szopa et al., 2020).

**Keller-Killiani test results**

This test showed negative results in Afsanteen and *chirayita* extracts.

**Antibacterial assay results**

Anti-microbial activity evaluation of selected plant extracts was determined initially by disc diffusion method against different bacterial strains. The study revealed that all plant extracts used in research work have a varying degree of antimicrobial activity against used bacterial strains that were used that is explained in given table 2. It was observed that *chirayita* is most effective against *E. coli* as compared to Afsanteen. In case of *P. subtilis* Afsanteen was found to be effective as compared to *chirayita*. In case of *P. syringola* Afsanteen and *chirayita* showed antibacterial property except *chirayita* n-hexane extract. The presence of antibacterial activities revealed the potential of extracts as antibiotics. The plants *chirayita* and Afsanteen may be used as potential medicinal plants (Kumar and Van Staden, 2016; Ahirwal et al., 2010; Ashraf et al., 2019; Medda et al., 1999).

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Antifungal assay results

Anti-microbial activity evaluation of different plant extracts was determined initially by disc diffusion method against different fungal strains (Aspergillus niger, Fusarium solani). It was found that only *chirayita* acetone extract showed antifungal activity to some extent and remaining all the extracts there were used showed that they have no antifungal activity (Table 3).

**Table 1.** Shows results of Phytochemical screening test

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Terpenoids Test</th>
<th>Flavonoids Test</th>
<th>Tannins Test</th>
<th>Saponins Test</th>
<th>Keller-Kiliani test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chirayita</em> Ethanol extract</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td><em>Chirayita</em> n-hexane extract</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve to some extent</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td><em>Chirayita</em> Acetone extract</td>
<td>+ve to some extent</td>
<td>+ve</td>
<td>+ve to some extent</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Afsanteen ethanol extract</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve to some extent</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Afsanteen n-hexane extract</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Afsanteen acetone extract</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

Antibacterial assay results

**Table 2.** Diameter of zones of inhibition (mm) of plant extracts against *E. coli*, *B. subtilis* and *P. syringola* at 5µL, 10µL and 15µL concentrations with control antibiotic disc of Ampicillin

<table>
<thead>
<tr>
<th>Bacterial Names</th>
<th>Plant extracts</th>
<th>Concentration of extract (5µL)</th>
<th>Concentration of extract (10µL)</th>
<th>Concentration of extract (15µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>Afsanteen(ethanol)</td>
<td>1.65</td>
<td>1.57</td>
<td>1.55</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Afsanteen(acetone)</td>
<td>1</td>
<td>0.8</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Afsanteen(n-hexane)</td>
<td>0.6</td>
<td>0.8</td>
<td>0.75</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td><em>Chirayita</em> (ethanol)</td>
<td>1</td>
<td>1.25</td>
<td>1.2</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td><em>Chirayita</em> (n-hexane)</td>
<td>1.25</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td><em>Chirayita</em> (acetone)</td>
<td>1.95</td>
<td>1.75</td>
<td>1.9</td>
</tr>
<tr>
<td><em>B. Subtilis</em></td>
<td>Afsanteen(acetone)</td>
<td>1</td>
<td>1.5</td>
<td>1.1</td>
</tr>
<tr>
<td><em>B. Subtilis</em></td>
<td>Afsanteen(n-hexane)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>B. Subtilis</em></td>
<td>Afsanteen(ethanol)</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td><em>B. Subtilis</em></td>
<td><em>Chirayita</em> (n-hexane)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>B. Subtilis</em></td>
<td><em>Chirayita</em> (ethanol)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>B. Subtilis</em></td>
<td><em>Chirayita</em> (acetone)</td>
<td>0.9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>P. Syringola</em></td>
<td>Afsanteen(acetone)</td>
<td>1.8</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td><em>P. Syringola</em></td>
<td>Afsanteen(n-hexane)</td>
<td>0.9</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td><em>P. Syringola</em></td>
<td>Afsanteen(ethanol)</td>
<td>1.1</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td><em>P. Syringola</em></td>
<td><em>Chirayita</em> (n-hexane)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. Syringola</em></td>
<td><em>Chirayita</em> (ethanol)</td>
<td>2</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td><em>P. Syringola</em></td>
<td><em>Chirayita</em> (acetone)</td>
<td>1.5</td>
<td>1</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Antifungal assay results

**Table 3.** Diameter of zones of inhibition (mm) of plant extracts against *Aspergillus Niger*, *Fusarium Solani* at 5µL, 10µL and 15µL concentrations

<table>
<thead>
<tr>
<th>Fungal strains</th>
<th>Plant extracts</th>
<th>Concentration of extract (5µL)</th>
<th>Concentration of extract (10µL)</th>
<th>Concentration of extract (15µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. Niger</em></td>
<td>Afsanteen (ethanol)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>A. Niger</em></td>
<td>Afsanteen (acetone)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>A. Niger</em></td>
<td>Afsanteen (n-hexane)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>A. Niger</em></td>
<td><em>Chirayita</em> (ethanol)</td>
<td>1mm</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>A. Niger</em></td>
<td><em>Chirayita</em> (n-hexane)</td>
<td>No growth occurs in whole plate</td>
<td>No growth occurs in whole plate</td>
<td>No growth occurs in whole plate</td>
</tr>
<tr>
<td><em>A. Niger</em></td>
<td><em>Chirayita</em> (acetone)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>F. Solani</em></td>
<td>Afsanteen (ethanol)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Conclusion
Based on the results obtained in this study, it may be concluded that plant extracts of *chirayita* and Afsanteen have a stronger and broader spectrum of antimicrobial activity against number of bacteria’s and plant extracts are used for discovering bioactive natural products that behave as a basic source for developing new antimicrobial compounds for overcoming the problem of increasing resistance to antibiotics that are available traditionally. Antibacterial activities could be increased if bioactive compounds are purified and proper dosage is determined. So, further studies can be performed for exploring *Chirayita* and Afsanteen potential for developing a number of drugs which will be effective with no side effects. But antifungal activity tests are not too much satisfied. So, detailed clinical research of Afsanteen and *chirayita* plant can be performed for its antifungal activity and exploring full therapeutic potential of these plants for the establishment of standard drug.

Conflict of interest
The authors declared absence of conflict of interest.

References


