

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

AFZAL A^{1*}, AFTAB B¹, SIDDIQUE J¹, BABAR S¹, SOHAIL A¹, YASIR M¹, HANIF S²

¹Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore, Pakistan

²Emergency Department, Bahria Town International Hospital Lahore-Pakistan

*Corresponding author email: aminaafzal069@gmail.com

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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 for *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. *chirayita* 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* flourishes as well as thrives in woodland gardens having partial shade sunny edge as well as in marshy lands.

Chirayita plant is very famous because of its therapeutic uses (Kaloo and Bhat, 2020), due to the presence of different chemical compounds like Triterpenoids, Mangiferin, Swerchirin, flavonoids, terpenoids, saponins, Lignans, Pentacyclic Triterpenoids *etc* (Khalid *et al.*, 2011).

Afsanteen is a perennial plant belongs to family Asteraceae or Compositae. Its scientific name is *Artemisia absinthium* and called Afsanteen in Unani system of medicine. Dried leaves, herb and flowering tops are used in Unani system of medicine. This herb also used in preparation of decoction, sharbat and arq (Ansari *et al.*, 2019). *Artemisia absinthium* have fibrous roots and it is woody based perennial herbaceous plant. The leaves of Afsanteen plant are spirally arranged, greenish gray above and below portion are white, covered with silky silvery- white trichomes, bipinnate and tripinnate with long petioles (Erdigrul, 2002; Hashimi 2019). The flowers of this plant are pale yellow, tubular, have ornamental value, clustered in spherical capitula. Flowering occurs from

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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 antihelminthics (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 hujyl 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 artamaridin, artamarin, artamaridin, 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

Artemisia 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 2 days. 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. 5 μ l, 10 μ l and 15 μ l of each plant extracts were poured separately on simple discs such as 5 μ l ethanol extract of *chirayita* on one simple disc and 10 μ l ethanol extract on 2nd simple disc and 15 μ l ethanol extract of *chirayita* 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 ethanol 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 *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).

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

Plant extracts	Terpenoid Test	Flavonoids Test	Tannins Test	Saponins Test	Keller-Killiani test
<i>Chirayita</i> Ethanol extract	+ve	+ve	+ve	+ve	-ve
<i>Chirayita</i> n-hexane extract	+ve	+ve	+ve to some extent	-ve	-ve
<i>Chirayita</i> Acetone extract	+ve to some extent	+ve	+ve to some extent	+ve	-ve
Afsanteen ethanol extract	+ve	-ve	+ve to some extent	-ve	-ve
Afsanteen n-hexane extract	+ve	-ve	-ve	+ve	-ve
Afsanteen acetone extract	+ve	-ve	-ve	+ve	-ve

Antibacterial assay resultTable 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

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

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

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

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<i>F. Solani</i>	Afsanteen (acetone)	0	0	0
<i>F. Solani</i>	Afsanteen (n-hexane)	0	0	0
<i>F. Solani</i>	<i>Chirayita</i> (ethanol)	0	0	0
<i>F. Solani</i>	<i>Chirayita</i> (n-hexane)	0	0	0
<i>F. Solani</i>	<i>Chirayita</i> (acetone)	1	1	1

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.

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