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# Screening for *in vitro* Antifungal activity and qualitative phytochemical analysis of the fruit extract of *Capparis zeylanica*

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**ABSTRACT**

*Capparia zeylanica is native to Sri Lanka and it was reported to possess antioxidant, antipyretic, anti-inflammatory, antimicrobial and immunostimulant activity. Present study was carried out to evaluate the in vitro antifungal activity of sequentially extracted different solvent extracts of fruits of Capparis zeylanica against plant pathogenic fungi such as Aspergillus spp., Alternaria spp., Trichoderma spp., Penicillium spp. and Fusarium spp. by the standard agar well diffusion method. Synthetic fungicide Dithane M-45(mancozeb) and solvents used for extraction as standard and controls respectively. Qualitative phytochemical analysis of crude extracts was also carried out for the presence of biomolecules using standard procedures. Results indicated that the C.zeylanica fruit extracts exhibited antifugal activity against all tested fungi at least in one solvent. Three solvent extracts of C.zeylanica fruit showed antifungal activity against all fungi. But the hexane fruit extract failed to inhibit the growth of Penicillium spp. only. Effect of ethylacetate fruit extract against, Aspergillus spp.(25,16 mm), Penicillium spp.(20,16 mm) was significant than compared to the effect of hexane , ethylacetate extracts as well as mancozeb after 48 and 72 hours of incubation period. Growth of Trichoderma spp.(16,13 mm) and Alternaria spp.(16,14 mm) was significantly inhibited by methanol fruit extract than compared to other fruit extracts. This effect was also significant when compared to the standard mancozeb after 48 and 72 hours of incubation periods. But the Fusarium spp.(16,15 mm) was significantly inhibited by the hexane fruit extract, though the standard exhibited the highest inhibition against Fusarium spp. Phytochemical analysis revealed the presence of different types of phytochemicals in sequentially extracted hexane, ethylacetate and methanol extracts. Tannins, saponins, phlobatanins and steroids were present in all three solvents extracts. But flavanoids were present only in hexane extract. Trepenoids, alkaloids and cardiac glycosides were absent in all three extracts. This study put a flat form for further developmental studies on isolation and characterization of bio active compounds which exhibit antifungal activity.*

**Key words**: *C.zeylanica* fruit, sequential extraction, antifungal activity, agar well diffusion method, phytochemistry

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## INTROUDUCTION

Phyotpathogenic fungi cause severe losses in plants and crop production. Therefore it is necessary to develop control measures that are cheap, ecologically friendly and environmentally safe. Herbal medicine refers to using a plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes. Medicinal plants can be used as biological control agents against micro organisms due to the presence of phytochemical compounds [1]. *Capparia zeylanica* is native to Sri Lanka and it was reported to possess antioxidant, antipyretic, anti-inflammatory, antimicrobial and immunostimulant activity [2]. Biomolecules with antifungal activity have been found in plants [3]. The plant *C. zeylanica* was found to have variety of chemical constituents. Preliminary phyto-chemical screening of the leaf extracts showed the presence of alkaloids, flavonoids, saponins, terpenoids, tannins, proteins and carbohydrates. The roots of *C.zeylanica* contain alkaloid, phytosterol,acids and mucilage. A new fatty acid E-Octadec-7-en-5-yonic acid

has been isolated from the roots of chloroform extract of *C. zeylanica*. Carotene has also been isolated from the petroleum ether extract of leaves. P-Amyrin, N- Triacontane, Fixed oil and Thioglucoside glucocapparin were identified from seeds and leaves. [4]. So the present study was carried out to evaluate the *in vitro* antifungal activity of sequentially extracted different solvent extracts of fruits of *Capparis zeylanica* against plant pathogenic fungi such as *Aspergillus* spp., *Alternaria* spp., *Trichoderma* spp., *Penicillium* spp. and *Fusarium* spp. and qualitatively elucidate the phytochemicals in the test extracts.

## MATERIALS AND METHODS

### Collection of plant materials

Fruits of *C.zeylanica* were collected from the Chulipuram region of the Jaffna peninsula and identified taxonomically based on herbarium records in the Department of botany, University of Jaffna, Sri Lanka.

### Preparation of plant extract

The fresh fruits were air dried and ground into fine powder using an electric blender. 100 g of powder was soaked in 200 ml of hexane with intermittent shaking for 3 days. The supernatant was filtered in volumetric flask under sterile condition. This procedure was repeated twice to ensure the complete separation of all constituents. The solvent was evaporated under reduced pressure by using rotatory evaporator. The remaining samples were allowed to air dry. Finally the sample of hexane crude was weighed. The sequential extraction was followed by using ethyl acetate and methanol as solvents.

### Test Fungi

Plant fungal pathogens, *Aspergillus* spp., *Alternaria* spp., *Trichoderma* spp., *Penicillium* spp. and *Fusarium* spp. were obtained from the culture collections of the Department of Botany, University of Jaffna, Sri Lanka. These fungal cultures were sub cultured on potato dextrose agar medium and were stored in the refrigerator as slants at 10ºC for the future use.

### Antifungal assay

### Preparation of fungal spore suspension

0.85 g NaCl was weighed and it was dissolved in 100 ml of distilled water in a volumetric flask. Then 9.0 ml of the saline water was transferred into Mac Cartney bottles and those bottles were sterilized by an autoclave. A loopful of spores was taken by a sterile loop and suspended into sterile saline water under aseptic condition. Spore concentration was determined by the Haemocytometer. Then the suspension was stirred well and serially diluted to $10^5$ number of spores/ml.

### Preparation of standard and control solution

The synthetic antifungal agent Mancozeb (Dithane M-45) was prepared in 0.3 mg/150 µl concentration as standard. The solvent used to prepare the crude was used as control.

### Agar well diffusion method

0.1 ml of each fungal spore suspension was spread uniformly on the surface of PDA plate by using sterile glass spreader. 8 mm diameter wells were made by using a sterile cork borer. 100 µl of each test extracts were administered into each well separately. Mancozeb and ethyl acetate were also used as standard and control respectively. Plates were incubated at room temperature for 3-5 days and the zone of inhibition around the well was measured at various time intervals (24, 48, 72 and 96 hours). Each experiment was repeated thrice and the mean value was taken [5,6].

### Qualitative Phytochemical analysis

Phytochemical analysis of crude extracts were carried out by using standard procedures to identify the following components[7,8,9,10]

*Test for Tannins*

About 0.01 g of the crude extract was boiled in 20 ml of water in a boiling tube. Few drops of 0.1% of $FeCl_3$ were added. Formation of brownish green or a blue black colouration indicated the presence of tannins.

*Test for Saponins*

About 0.01g of the crude extract was boiled in 20 ml of distilled water in a water bath. Then it was mixed with 5 ml of distilled water and it was shacked well. Stable persistent forth indicated the presence of saponins.

*Test for Phlobatanins*
About 0.01 g of the crude extract was boiled with 1% aqueous hydrocholoric acid. A deposition of a red precipitate indicated the presence of phlobatanins.

*Test for Flavanoids*
About 0.01 g of the crude extract was dissolved in 2 ml of ethanol solvent. Con. HCl and Mg turnings were added. Formation of yellow colour indicated the presence of flavanoids.

*Test for Steroids*
About 0.01g of the crude extract was dissolved in 2 ml of ethanol solvent. 2 ml of acetic anhydride and 2ml of con.$H_2SO_4$ were added. A colour change from violet to blue or green indicated the presence of steroids.

*Test for Cardiac glycosides*
0.01g of the crude extract was dissolved in 2 ml of ethanol and then 2 ml of glacial aceticacid containing one drop of $FeCl_3$ solution was added. This was underplayed with 1ml of con $H_2SO_4$. A brownish ring of the interface indicated the presence of cardiac glycosides.

*Test for Alkaloides*
About 0.01 g of the crude extract was dissolved in ethanol and it was divided into two parts. Few drops of Wagner's reagent along the wall of the test tube were added to one part. Brownish red precipitate indicated the presence of alkaloids.

Few drops of Mayer's reagent were added to the other part. A creamy white precipitate in indicated the presence of alkaloids.

*Test for Terpenoids*
5ml of the crude extract was treated with 2ml of $CHCl_3$ and 3ml of con. $H_2SO_4$ was added carefully to from a layer. A reddish brown coloration of interface indicated the presence of terpenoids.

## RESULTS AND DISSCUSION

**Extraction of Fruit**
Powered material was sequentially extracted with hexane, ethylacetate and methanol. Bioactive compounds were extracted from the material according to their polarity.

**Table 1: Antifungal activity of *Capparis zeylanica* fruit extracts.**

| Fungus | Incubation Time (Hours) | Mean diameter of clear zone (mm). | | | |
|---|---|---|---|---|---|
| | | Fruit extracts of *Capparis zeylanica* | | | Mancozeb (Standard) |
| | | Hexane | Ethylacetate | Methanol | |
| *Aspergillus* spp. | 24 | - | - | - | 12 |
| | 48 | 12 | 25 | 15 | |
| | 72 | 11 | 16 | 12 | |
| | 96 | - | 11 | 10 | |
| *Penicillium* spp. | 24 | - | - | - | 14 |
| | 48 | - | 20 | 12 | |
| | 72 | - | 16 | 10 | |
| | 96 | - | 13 | 10 | |
| *Trichoderma* spp. | 24 | - | - | - | 11 |
| | 48 | 13 | - | 16 | |
| | 72 | 11 | 12 | 13 | |
| | 96 | 09 | - | 11 | |
| *Fusarium* spp. | 24 | - | - | - | 22 |
| | 48 | 16 | - | 13 | |
| | 72 | 15 | 14 | 12 | |
| | 96 | 14 | 13 | 11 | |
| *Alternaria* spp. | 24 | - | - | - | 13 |
| | 48 | 13 | 10 | 16 | |
| | 72 | 11 | 9 | 14 | |
| | 96 | 9 | - | 14 | |

*No clear zone was observed in controls*

Results indicated that the *C.zeylanica* fruit extracts exhibited antifugal activity against all tested fungi atleast in one solvent. Mean diameter of the clear zone decreased with increasing incubation period. The standard mancozeb showed antifungal activity against all fungi where as control solvents did not exhibit antifungal activity against fungi

tested. Three solvent extracts of *C.zeylanica* fruit showed antifungal activity against all fungi. But the hexane fruit extract failed to inhibit the growth of *Penicillium* spp. only. None of the above extracts showed any inhibitory effect on the growth of fungi after 24 Hrs of incubation.

Effect of ethylacetate fruit extract against, *Aspergillus* spp., *Penicillium* spp. was significant than compared to the effect of hexane , ethylacetate extracts as well as mancozeb after 48 and 72 hours of incubation period. Growth of *Trichoderma* spp. and *Alternaria* spp. was significantly inhibited by methanol fruit extract than compared to other fruit extracts. This effect was also significant when compared to the standard mancozeb after 48 and 72 hours of incubation periods. But the *Fusarium* spp. was significantly inhibited by the hexane fruit extract, though the standard exhibited the highest inhibition against *Fusarium* spp.

Previous study on the antimicrobial activity of *C.zeylanica* leaf stated that petroleum ether, chloroform, ethanol and water extracts exhibited *in vitro* antibacterial activity against *Bacillus subtilis*, *E.coli* and *Pseudomonas fluorescens*. But none of the extracts showed antifungal activity [11].Study on antimicrobial assay showed that chloroform, ethanol and water extracts of *C.zeylanica* root exhibited *in vitro* antibacterial activity against gram positive and gram negative bacteria, where as petroleum ether exhibited antibacterial activity against selected bacterial species as *S.aureus*, *B.subtilis*, *K.pneumoniae* and *P.*vulgaris[12]. But in this study hexane , ethylacetate and methanol fruit extracts showed antifungal activity against plant fungal pathogens, *Aspergillus* spp., *Alternaria* spp., *Trichoderma* spp., *Penicillium* spp. and *Fusarium* spp.

**Table 2 Qualitative phytochemical analysis**

| **Test** | **Fruit extracts of *C.zeylanica*** | | |
|---|---|---|---|
| | Hexane | Ethylacetate | Methanol |
| Tannins | + | + | + |
| Saponins | + | + | + |
| Phlobatanins | + | + | + |
| Steroids | + | + | + |
| Flavanoids | + | - | - |
| Terpenoids | - | - | - |
| Alkaloids | - | - | - |
| Cardiac glycosides | - | - | - |

*Note: + present ; - absent*

This Phytochemical analysis revealed the presence of different types of phytochemicals in sequentially extracted hexane, ethylacetate and methanol extracts. Tannins, saponins, phlobatanins and steroids were present in all three solvents extracts. But flavanoids were present only in hexane extract. Trepenoids, alkaloids and cardiac glycosides were absent in all three extracts. Previous study on phytochemical screening of *C.zeylanica* leaf extract showed the presence of alkaloids, flavanoids, saponins, terpenoids, tannins, protein and carbohydrate[12,13]. Leaves and seeds showed the presence of thioglycoside, glycocapparin, n-tricortane, α-amyrin and fixed oil, where as root bark had alkaloids, phytosterol, water soluable acids[4,14]. Flavanoids of *C.zeylanica* have been known to possess anti-neoplastic, anti-ulcer activities and anti-allergic activity was found in fruits and roots of this plant was also reported [12]. In a particular plant different plant parts had different compounds. The variation in the result of these compounds was also determined by the mode of solvent extraction [3].

## CONCLUSION

The study revealed that *C.zeylanica* fruit extracts exhibited antifugal activity against all tested plant pathogenic fungi at least in one solvent. Different solvent extracts exhibited different degree of antifungal activity among tested fungi. Effect of ethylacetate fruit extract against, *Aspergillus* spp., *Penicillium* spp. was significant than compared to hexane and ethylacetate extracts. Growth of *Trichoderma* spp. and *Alttrenaria* spp. was significantly inhibited by methanol fruit extract and *Fusarium* spp. was significantly inhibited by the hexane fruit extract.

Phytochemical analysis showed the presence of different types of phytochemicals in sequentially extracted hexane, ethylacetate and methanol fruit extracts of *C.zeylanica* . This study put a flat form for further developmental studies on bio active compounds which exhibit antifungal activity.

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