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GC-MS analysis and *in vitro* antifungal assessment of *Cinnamomum camphora* (L.) J. Presl. against *Fusarium oxysporum* f. sp. vasinfectum

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Abstract

Fusarium wilt caused by *Fusarium oxysporum* f. sp. vasinfectum is a major field disease of several major plants such as tomato, cotton, onion, etc., severely reducing crop yield and quality. It causes the plant to turn yellow, wilt, stop growing, and eventually die. Its conventional management is compelled due to residue establishment, environmental risk and resistance development. In the present study, aqueous and ethanolic extracts of *Cinnamomum camphora* (L.) J. Presl. leaves were evaluated for their potential to inhibit the mycelial growth of plant pathogen *F. oxysporum*. *C. camphora* ethanolic extract showed 64% mycelial growth inhibition (MGI) at 200 ppm, while aqueous extract exhibited 72.5% MGI at 400 ppm concentration. The constituents of *C. camphora* were also identified using GC-MS. Among ten compounds detected, major constituents identified are bicyclo [2.2.1] heptan-2-one-1.7.7-trimethyl-(1S), 16-hentriacontanone and O-methyl-beta-tocopherol. These results highlight the strong antifungal potential of *C. camphora* extracts and may be used as eco-friendly plant-based management of *F. oxysporum*.

1. Introduction

Phytopathogens are infectious organisms that cause plant diseases, resulting in significant economic losses in agriculture. Over 20% of the output of important crops was lost due to pathogenic fungus (Todorova *et al.*, 2021). *Fusarium* species like *F. graminearum*, *F. moniliforme*, *F. oxysporum* and *F. verticillioides* infect cereals, fruits, and vegetables (Ain *et al.*, 2022). *Fusarium* species affects crops directly and indirectly by producing dangerous compounds known as mycotoxins during storage (Seepe *et al.*, 2021). *F. oxysporum* is a widespread soil-borne fungal species complex recognized for inducing severe plant diseases, including *Fusarium* wilt, basal rot, and root rot (Isaac *et al.*, 2014). This fungus affects many crops such as grains, corn, vegetables, bananas, lilies, and trees. Their ability to overcome host resistance emphasize the critical need for novel crop protection approaches (Borrego *et al.*, 2021).

Many chemical based fungicides have existed as the major class of treating fungal diseases. However, the widespread and arbitrary use of synthetic chemicals has harmed the environment, raised the risk to human health, and resulted in the evolution of resistant fungus strains. *Fusarium* species, especially *F. oxysporum* is increasingly resistant to major fungicide classes such as triazoles and benzimidazoles, making traditional prevention techniques unsustainable. In response to these issues, there has been a greater emphasis on researching the plant-based antifungal solutions. Alternative methods

for disease control have been studied, with emphasis on compounds derived from plant sources, such as essential oils and botanical extracts, which can usually have higher safety for consumers and the environment (Diego *et al.*, 2021).

Cinnamomum is one of the largest genus of Lauraceae family and has been used as spices, food, traditional medicine and food additives by the society. More than 200 species of *Cinnamomum* genus are naturalized in Asia, South and Central America, China, and Australia in which 15 *Cinnamomum* species are distributed in different parts of Indian sub-continent. *C. camphora* is an evergreen tree with wide branching. This species is useful in cough, cold, diarrhea, dysentery, skin infections and vomiting traditionally. Some *Cinnamomum* species has reported with antimicrobial properties (Singh *et al.*, 2023). Plant based compounds isolated from *Cinnamomum iners* showed antibacterial and antioxidant characteristics, including flavonoids, alkaloids, tannins and terpenoids (Pedzil *et al.*, 2025). The present research work aims to explore the antifungal efficacy of *C. camphora* leaves extracts against *F. oxysporum* along with phytochemical identification using GC-MS experiment.

2. Materials and Methods

2.1 Collection and extraction of plant material

C. camphora leaves were collected in April month from botanical garden of SKAU, and was identified by taxonomist of Dravyaguna Department of Shri Krishna AYUSH University, Kurukshetra. Leaves Specimen (Herbarium) has been in record (CHEM/0012) for future reference. The *C. camphora* leaves were shade-dried and pulverized into a fine powder. The leaf powder (50 g) was extracted with absolute ethanol using Buchi Universal Extractor. After 6 h extraction, the ethanolic filtrate was concentrated under vacuum using a rotary

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evaporator at 40°C to produce the semisolid ethanolic crude extract (CCEE) (7.2 g). Similarly, the leave powder (50 g) was extracted with distilled water using Buchi Universal Extractor to get aqueous filtrate which was lyophilized at -55°C to get hygroscopic off-white crude extract (CCAЕ) (3.9 g). Both ethanolic and aqueous extracts were kept at 4°C until further use.

2.2 Fungal strains, culture media, and conditions

The plant pathogenic fungus *F. oxysporum* was obtained from the Department of Plant Pathology, College of Agriculture, CCS HAU, Hisar. *F. oxysporum* was subculture on potato dextrose agar (PDA) medium, which consists of 200 g/l potato, 20 g/l dextrose, and 15 g/l agar in distilled water. Subculturing was done in a sterilized Petri dish at 28°C for approximately 8 days in an incubator.

2.3 In vitro antifungal assay

2.3.1 Sample preparation

The CCEE and CCAE stock solutions (20 mg/ml) were prepared in DMSO using ultrasonicator. Different concentrations were prepared for the experiments under laboratory conditions.

2.3.2 Mycelial growth inhibition

In this assay, different concentration of CCEE and CCAE were combined with potato dextrose agar (PDA) medium. Then, *F. oxysporum* mycelium was added, and the colony of fungus growth was measured. Untreated media considered as negative control and Carbendazim was used as positive control. The percentage of mycelial growth inhibition (MGI) was calculated using a formula:

$$MGI = \frac{[(C - d) - (T - d)]}{(C - d)} \times 100$$

where, C represents the diameter of the fungal colony in the control, T represents the diameter of the fungal colonies in the treated sample, and d denotes the inoculum plug diameter (usually 5 mm).

2.3.3 GC-MS analysis

In this study, ethanolic extract of *C. camphora* was analysed on GC-MS/MS (Shimadzu TQ8040) equipped with a Rtx-5MS capillary column (30m x 0.25 mm, 0.25 µm film) for volatile compound identification. The CCEE sample was injected into a split injector system in the GC-MS. The MS ran in EI mode at 70 eV. The carrier gas was helium, which flowed at a rate of 1 ml/min. The injector and detector were set at 250°C and 300°C, respectively. The temperature program was: 80°C (2 min hold), ramped at 20°C/min to 180°C, then 5°C/min to 300°C. The MS scanned in full mode between 20 and 610 AMU.

2.3.4 Experimental design and statistical analysis

The experiment was conducted with three replications. Data were subjected to analysis of variance (ANOVA), and treatment means were compared at a 5% level of significance.

3. Results

3.1 In vitro antifungal activity

The aqueous and ethanolic extract of *C. camphora* were tested for their effect on *F. oxysporum* at four different concentrations: 100, 200, 300, 400 ppm and 50, 100, 150 and 200 ppm, respectively, in petri dishes. The data presented in Table 1 indicate that both extracts reduced the mycelial growth of *F. oxysporum*. The effect was obviously increased by increasing the concentrations of plant extracts from 100 to 400 ppm for CCAE and 50 to 200 ppm for CCEE. The obtained data showed that the CCEE was the most effective (65.6% MGI) in decreasing mycelial growth at concentration of 200 ppm (Figures 1 and 2) while CCAE was most effective at 400 ppm (72.5% MGI) (Figures 3 and 4).

Table 1: In vitro antifungal efficacy of aqueous and ethanolic extracts of *C. camphora* leaves against *F. oxysporum*

Treatment	Mycelial growth inhibition (%)			
	100 ppm	200 ppm	300 ppm	400 ppm
<i>C. camphora</i> aqueous extract	20	27.5	60	72.5
<i>C. camphora</i> ethanolic extract	50 ppm	100 ppm	150 ppm	200 ppm
	33.2	44.7	48.2	65.2

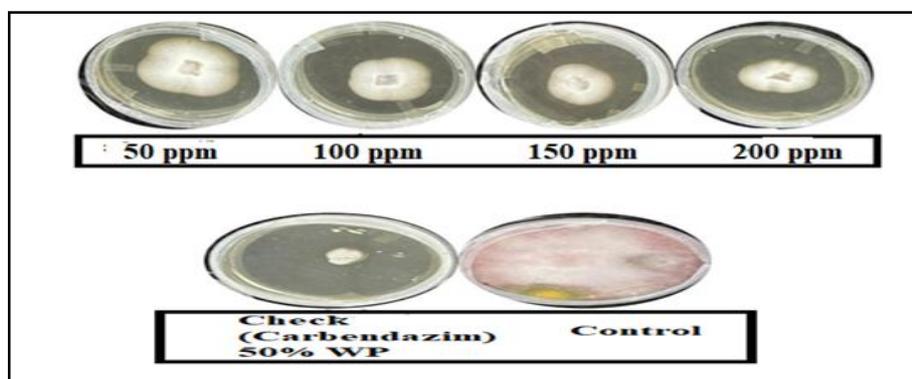


Figure 1: Mycelial growth inhibition of *F. oxysporum* with ethanolic extract of *C. camphora*.

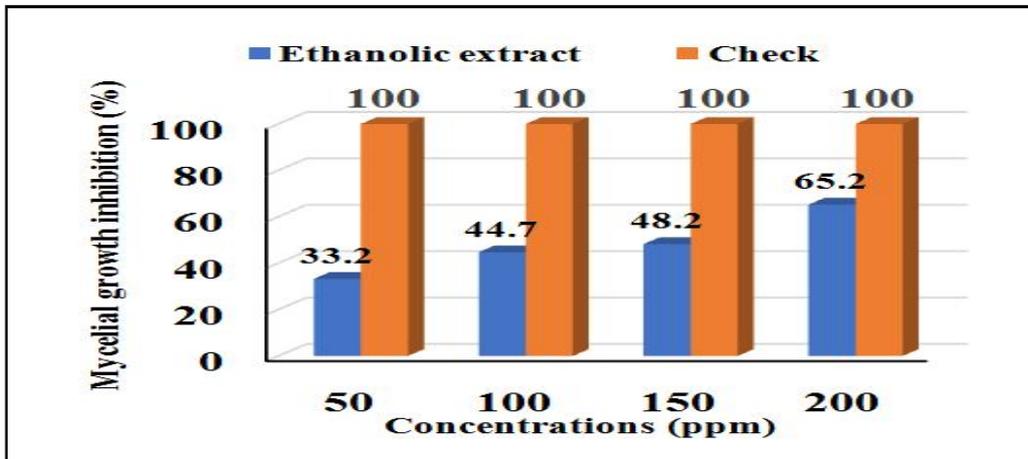


Figure 2: Effect of ethanolic extract of *C. camphora* on mycelial growth of *F. oxysporum* at different concentrations.

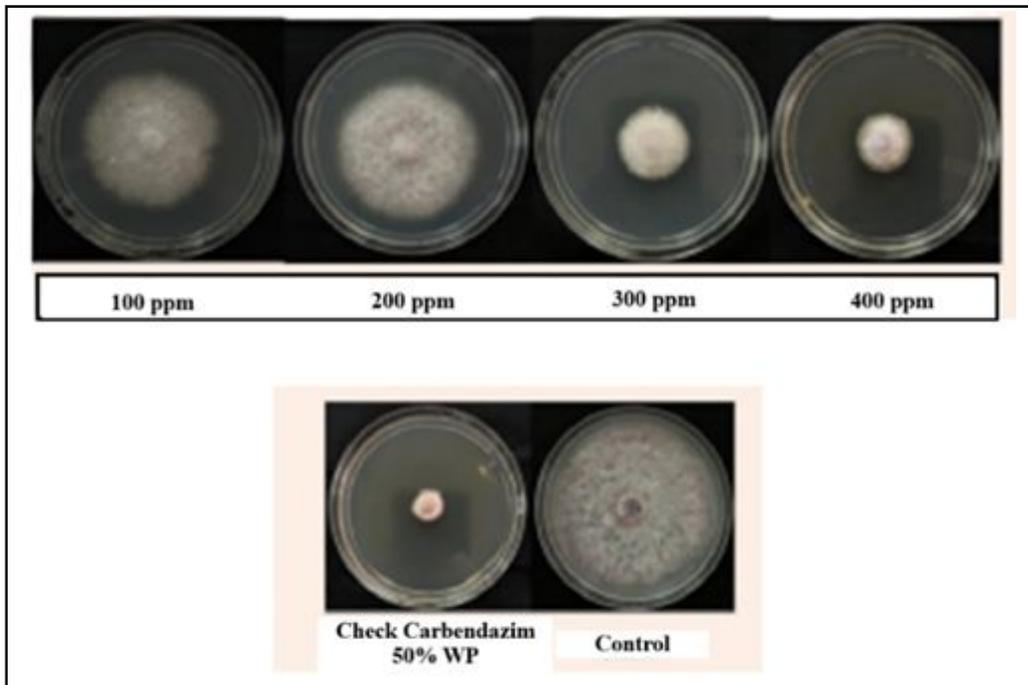


Figure 3: Mycelial growth inhibition of *F. oxysporum* with aqueous extract of *C. camphora*.

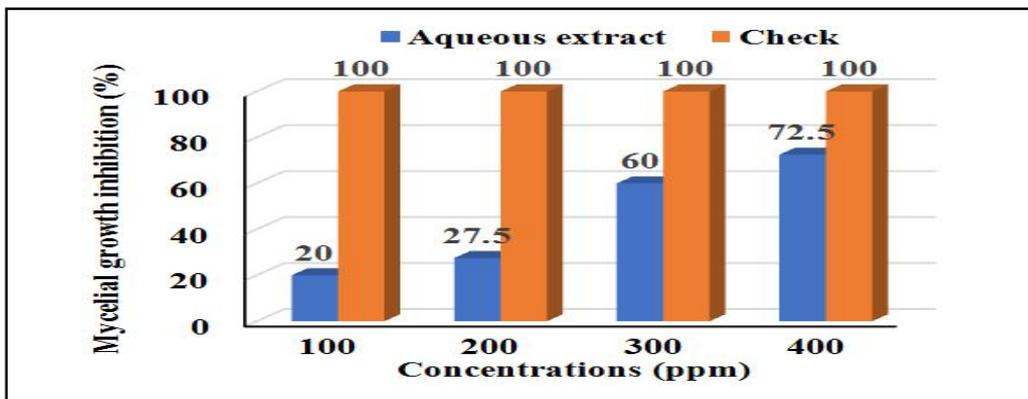


Figure 4: Effect of aqueous extract of *C. camphora* on mycelial growth of *F. oxysporum* at different concentrations.

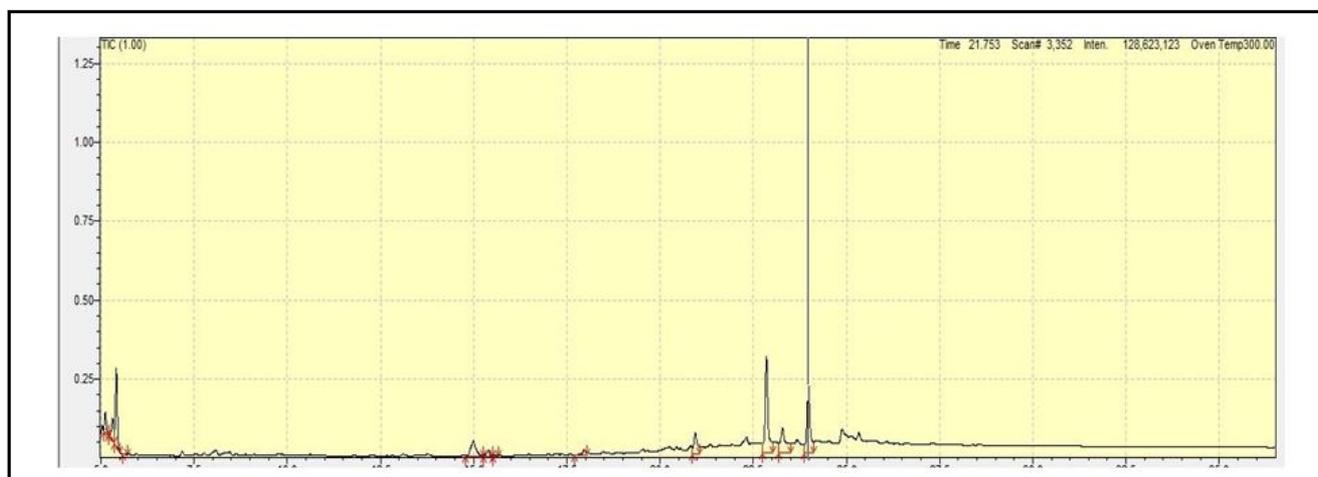


Figure 5: GC-MS chromatogram of ethanolic extract of *C. camphora*.

Table 2: The chemical composition of ethanolic extract of *C. camphora* leaves using GC-MS

S. No.	Compounds	Structure	Retention time	Per cent
1.	2-Bornanone		5.31	7.63
2.	Bicyclo[2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1S)		5.41	20.64
3.	9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)		15.39	1.26
4.	Heneicosane		15.60	0.29
5.	9-Octadecenoic acid (Z)-, oxiranylmethyl ester		17.96	1.03
6.	2-Methylhexacosane		20.94	4.18
7.	O-methyl-beta-Tocopherol		22.85	44.9
8.	Sesamin		23.28	4.44
9.	16-Hentriacontanone		23.98	12.35
10.	Heneicosanoic acid, methyl ester		14.98	2.67

3.2 GC-MS analysis

Through, using a GC-MS/MS system a total of 10 compounds with varying extents were identified in ethanolic extract of *C. camphora* as shown in Table 2. The major chemicals constituents include; Bicyclo [2.2.1] heptan-2-one-1.7.7-trimethyl-(1S), 16-hentriacontanone and O-methyl-beta-tocopherol. While, the other constituents including 2-bornanone, 2-methylhexacosane, sesamin and heneicosanoic acid, methyl ester existed in intermediate levels. The rest constituents were found at minor percentages.

4. Discussion

This study evaluated the aqueous and ethanolic extracts of *C. camphora* at four distinct concentrations: 100, 200, 300, and 400 ppm, and 50, 100, 150, and 200 ppm, respectively. The findings indicate that mycelial growth inhibition increases with increasing extract concentrations. At 100 ppm, the aqueous extract inhibited 20% of mycelial growth, which increased to 27.5% at 200 ppm, 60% at 300 ppm, and 72.5% at 400 ppm. At a concentration of 100 ppm, the ethanolic extract inhibited 33.2% mycelial growth. This concentration-dependent inhibitory pattern is prevalent in natural product bioassays, showed the presence of antifungal components present in the *C. camphora* extract. The secondary metabolites present in *C. camphora* leave ethanolic extract were detected using GC-MS/MS. Ten compounds were identified based on their area percentage and spectral matching. The major chemicals constituents identified as bicyclo [2.2.1] heptan-2-one-1.7.7-trimethyl-(1S), 16-hentriacontanone and O-methyl-beta-tocopherol.

5. Conclusion

The assessment of the antifungal activity of *C. camphora* extracts against *F. oxysporum* f. sp. *vasinfectum*, conducted through *in vitro* mycelial growth inhibition assay, underscores the potential of this traditional medicinal plant as a source of bioactive antifungal compounds. The aqueous extract achieved a 72.5% inhibition of mycelial growth at a concentration of 400 ppm, exhibiting a clear dose-response relationship across all tested concentrations. The ethanolic extract demonstrated even greater efficacy, with a 64% inhibition at 200 ppm, highlighting the significance of solvent selection in the preparation of plant extracts. While antifungal compounds derived from *C. camphora* showed promise for agricultural applications, further investigation is required to characterise the specific bioactive components, optimise extraction procedures, and evaluate field-level efficacy. This research contributes to the expanding

body of evidence supporting natural products as viable alternatives to synthetic fungicides in sustainable agricultural management practices.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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