World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Phytochemical analysis and antifungal effect of extracted cinnamaldehyde against some fungal species

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World Journal of Biology Pharmacy and Health Sciences, 2024, 20(03), 193–202

Publication history: Received on 29 September 2024; revised on 07 December 2024; accepted on 09 December 2024

Article DOI: https://doi.org/10.30574/wjbphs.2024.20.3.1007

Abstract

This study focused on the extraction of cinnamaldehyde, a key bioactive compound, from cinnamon bark using the Soxhlet extraction method, followed by steam distillation and a three-stage solvent extraction process. The Soxhlet extraction method efficiently extracted various phytochemicals from the cinnamon bark, including aldehydes, alkaloids, steroids, flavonoids, tannins, saponins, resins, and phenols. The isolation of cinnamaldehyde was achieved through steam distillation, a technique known for its ability to selectively extract essential oils. The identification of cinnamaldehyde was confirmed through Schiff's test, which showed a positive reaction for aldehydes, and UV spectrophotometry, where cinnamaldehyde exhibited a maximum absorption at 286 nm. The quantitative analysis demonstrated a high degree of precision, with a coefficient of determination (r^2) of 0.9999, indicating the reliability and reproducibility of the measurement. The extracted cinnamaldehyde was further evaluated for its antifungal activity using the agar well diffusion method against two common fungal strains, *Candida albicans* and *Aspergillus niger*. The results showed substantial antifungal activity, with inhibition zones of 4.01 mm and 3.12 mm, respectively, which were comparable to the standard fluconazole. These findings suggest that cinnamaldehyde possesses significant antifungal properties, particularly against *Candida albicans* and *Aspergillus niger.*

Keywords: Cinnamaldehyde; Cinnamon Bark; *Candida albicans; Aspergillus niger*; Phytochemical Analysis; Antifungal Activity

1. Introduction

Cinnamaldehyde, a naturally occurring aromatic compound, is a key phytochemical found predominantly in the bark of cinnamon trees (*Cinnamomum spp*.). Structurally, it is an aldehyde with a phenyl group attached to an α,β-unsaturated carbonyl system, making it both chemically reactive and biologically active. It is primarily extracted through steam distillation of cinnamon bark oil [1]. Cinnamaldehyde is widely used in flavoring, fragrances, and traditional medicine. Its antimicrobial, anti-inflammatory, and antioxidant properties have made it a subject of interest in pharmaceutical and agricultural applications [2].

Phytochemicals, such as cinnamaldehyde, offer a natural alternative to synthetic antifungal drugs. These bioactive compounds exhibit a range of therapeutic activities, including disrupting fungal cell membranes, inhibiting spore germination, and reducing biofilm formation [3]. Unlike synthetic antifungals, phytochemicals often have fewer side effects and lower risk of resistance development. This makes them promising candidates for combating the growing challenge of antifungal resistance [4].

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Fungal infections, particularly those caused by *Candida,* and *Aspergillus* species, are becoming increasingly prevalent due to immunosuppression from diseases such as HIV/AIDS and diabetes. These infections pose a significant public health challenge, compounded by the emergence of antifungal resistance to drugs like azoles and echinocandins [5]. The limited pipeline of novel antifungal drugs underscores the urgency to explore natural compounds like cinnamaldehyde as alternative treatments [6].

Current antifungal treatments are often limited by issues such as high costs, toxicity, and the emergence of drug-resistant fungal strains. Consequently, there is a critical need for safe, effective, and affordable antifungal agents derived from natural sources. Phytochemicals like cinnamaldehyde, with their proven bioactivity and low resistance potential, represent a viable solution to address this challenge [7].

1.1. Research Objectives

The primary objectives of this research are to explore the potential of cinnamaldehyde as a natural antifungal agent. Firstly, the study aims to extract cinnamaldehyde from its natural sources, specifically from *Cinnamomum spp.,* using efficient extraction techniques to obtain it in pure form. Secondly, it seeks to analyze the phytochemical composition of the extracted cinnamaldehyde, characterizing its chemical structure and verifying its purity through advanced analytical methods such as Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC). Finally, the research focuses on evaluating the antifungal efficacy of cinnamaldehyde against selected fungal species, including *Candida albicans,* and *Aspergillus niger*, by assessing its ability to inhibit fungal growth and elucidating its mechanism of action.

1.2. Significance of the Study

The significance of this study lies in the diverse applications of cinnamaldehyde as a natural antifungal agent. In medicine, it offers the potential to serve as an alternative or adjunct to conventional antifungal therapies, helping to reduce the reliance on synthetic drugs that often have limitations such as resistance and toxicity [8]. In agriculture, cinnamaldehyde could be utilized as a biopesticide to protect crops from fungal infections without leaving harmful chemical residues, promoting sustainable farming practices [9]. Additionally, its antifungal properties have implications for preservation, as it can extend the shelf life of food and cosmetic products by preventing fungal contamination, thereby contributing to improved product safety and quality [10].

2. Literature Review

2.1. Overview of Cinnamaldehyde-Extraction and Chemical Properties

Cinnamaldehyde, the primary active compound in cinnamon bark oil, is an aromatic aldehyde with the chemical formula C_9H_8O. It is characterized by a phenyl group attached to an α,β-unsaturated aldehyde. The compound is predominantly extracted from *Cinnamomum spp.* through steam distillation, a process that efficiently isolates volatile oils. Purification methods such as chromatography enhance the yield and quality of cinnamaldehyde [11]. Its chemical structure is responsible for its broad biological activities, including antimicrobial and antifungal properties [12].

2.2. Previous Studies on Cinnamaldehyde's Antimicrobial/Antifungal Effects

Numerous studies have demonstrated the antimicrobial potential of cinnamaldehyde. Research indicates that cinnamaldehyde exhibits significant antifungal activity against pathogens such as *Candida albicans, Aspergillus* and *niger*. For instance, one study found that cinnamaldehyde inhibited the growth of *Candida albicans* by disrupting cell membrane integrity and mitochondrial function [13]. Another study reported its efficacy in reducing fungal biofilm formation, a common resistance mechanism [14]. Additionally, cinnamaldehyde has been tested in combination with conventional antifungal drugs, showing synergistic effects that enhance antifungal activity while reducing drug dosage [15].

2.3. Mechanisms of Action of Phytochemicals against Fungi

Phytochemicals like cinnamaldehyde act through various mechanisms to inhibit fungal growth. These include disrupting fungal cell membranes by targeting ergosterol, impairing cell wall synthesis, and inducing oxidative stress through the generation of reactive oxygen species (ROS) [16]. Cinnamaldehyde has been found to penetrate fungal membranes, causing leakage of intracellular contents and eventual cell death [17]. It may also interfere with fungal signaling pathways, such as quorum sensing, thereby inhibiting spore germination and biofilm formation [18].

2.4. Challenges in Current Antifungal Treatments and Potential Role of Plant-Based Alternatives

Conventional antifungal treatments face challenges such as limited drug options, high toxicity, and the emergence of drug-resistant fungal strains. Resistance to azoles, echinocandins, and polyenes has been reported, posing a significant public health concern [19]. Moreover, the development of new antifungal drugs is slow and resource-intensive. Plant-based alternatives, like cinnamaldehyde, offer a promising solution due to their natural origin, broad-spectrum activity, and lower propensity for resistance development [20]. Phytochemicals are also more environmentally sustainable and can be integrated into both medical and agricultural practices, providing dual benefits in combating fungal infections.

3. Materials and Methods

3.1. Materials

3.1.1. Plant Material

Cinnamon bark (*Cinnamomum spp.)* will be used as the primary source of cinnamaldehyde. Cinnamon bark was procured from the local market in Satana, Nashik (Maharashtra), dried, and powdered for extraction.

3.1.2. Chemicals, Reagents and Instruments

All chemicals and reagents used in this study were of analytical grade. Solvents such as water, ethanol, and dichloromethane were employed in the extraction process. Instrumentation included a Soxhlet extractor, an incubator, and a UV-visible spectrophotometer (Model: Shimadzu UV-1800).

3.1.3. Fungal Species

The fungal strains *Candida albicans* and *Aspergillus niger* were sourced from the Microbiology Department at Sanjivani College of Pharmaceutical Education & Research, Kopargaon. These fungi were cultivated on nutrient agar medium under controlled conditions at 28°C for three days and subsequently stored at 4°C until needed for susceptibility testing.

3.2. Methods

3.2.1. Extraction of Cinnamon Bark by Soxhlet Extraction Method

One hundred grams of dried and powdered cinnamon bark were placed in a robust filter paper thimble within the Soxhlet extractor's primary chamber. Ethanol (150 mL) was used as the extraction solvent in a hot continuous percolation method. The extraction was carried out for 4–5 hours. The resultant extract was filtered through Whatman filter paper and stored for subsequent phytochemical analysis [21].

3.2.2. Qualitative Phytochemical Screening for Bioactive Constituents

The following qualitative tests were conducted to identify bioactive constituents, including aldehydes, alkaloids, glycosides, steroids, flavonoids, tannins, saponins, resins, proteins, phenols, and carbohydrates. The procedures are detailed below:

3.2.3. Test for Aldehydes

Tollens Test: A solution of silver nitrate in ammonia, when heated with the extract, formed a reflective silver coating, indicating aldehyde presence.

Fehling's Test: Heating Fehling's solution A and B with the extract resulted in a red precipitate of cuprous oxide, confirming aldehydes [22].

2. Test for Alkaloids: The extract was treated with dilute hydrochloric acid, filtered, and reacted with Hager's reagent. The appearance of a yellow precipitate confirmed the presence of alkaloids.

3. Test for Glycosides: A mixture of the extract, glacial acetic acid, ferric chloride, and sulfuric acid produced a green-blue coloration, indicating glycosides.

4. Test for Steroids (Salkowski Test): The extract was combined with chloroform and concentrated sulfuric acid. A crimson coloration in the chloroform layer indicated the presence of steroids.

5. Test for Flavonoids: The extract turned yellow upon treatment with sodium hydroxide. The yellow colour changed to colourless with the addition of dilute hydrochloric acid, confirming flavonoids.

6. Test for Tannins: Mixing the extract with ferric chloride resulted in a grey-green or dark blue coloration, confirming the presence of tannins.

7. Test for Saponins: Vigorous mixing of the extract with distilled water produced a stable foamy layer, indicating saponins.

8. Test for Resins: The extract was treated with hydrochloric acid, which produced a pink coloration, confirming the presence of resins.

9. Test for Proteins (Biuret Test): The extract, treated with sodium hydroxide and copper sulfate, developed a violet coloration, indicating proteins.

10. Test for Phenols: The extract reacted with ferric chloride to form a dark or violet complex, confirming the presence of phenols [23].

11. Test for Carbohydrates (Molisch's Test): Addition of Molisch's reagent to the extract followed by sulfuric acid produced a purple coloration, confirming carbohydrates [24].

3.2.4. Extraction of Cinnamaldehyde from Cinnamon Bark

Following stepwise procedure effectively extracts cinnamaldehyde from cinnamon bark, utilizing solvent extraction, steam distillation, and careful separation techniques to ensure maximum yield and purity of the compound.

Preparation of Cinnamon Bark: A total of 25.5 grams of cinnamon bark was carefully disintegrated using a mortar and pestle. The aim was to avoid turning the cinnamon into a fine powder, as leaving it coarse helps minimize excessive foaming during the distillation process.

Solvent Addition: Dichloromethane (DCM) was chosen as the solvent for the extraction process. A volume of 300 mL of DCM was added to the cinnamon bark in a round-bottom flask.

Distillation Setup: The mixture was subjected to steam distillation in a three-necked distillation flask with a volume of 250 mL. Approximately 200 mL of distilled water was added to the flask. To ensure continuous water supply, another 100 mL of distilled water was added through a separate funnel attached to one of the necks of the distillation flask. The system was heated using a heater to induce the distillation process.

Collection of Distillate: The distillate was collected in a graduated cylinder with a volume capacity of 100 mL. Initially, a turbid liquid, indicative of cinnamaldehyde, began to accumulate. About 100 mL of this turbid distillate was collected and transferred to an Erlenmeyer flask. The presence of an insoluble cinnamaldehyde suspension in the distillate confirmed the cinnamaldehyde content.

Secondary Distillation: To maximize cinnamaldehyde extraction, an additional 100 mL of water was added to the distillation flask, and the distillation process was repeated. This second distillate was clearer, indicating a reduced cinnamaldehyde content. Both the turbid and clear distillates were combined in a separatory funnel for further processing.

Triple-phase Extraction: The combined distillate was subjected to a triple-phase extraction process. In each cycle, 60 mL of DCM was added to the Erlenmeyer flask, followed by thorough mixing to ensure optimal separation of phases. The separatory funnel was sealed and vigorously agitated to separate the dichloromethane (DCM) from the aqueous phase. Due to its higher density, the DCM layer settled at the bottom of the funnel. The DCM extract was carefully transferred to a separate flask. This extraction step was repeated three times, using fresh DCM (60 mL for each cycle).

Aqueous Phase Clarification: After the third extraction cycle, the upper aqueous layer became clear, indicating that most of the cinnamaldehyde had been successfully extracted into the DCM phase. The remaining aqueous phase was discarded.

Solvent Recovery: The combined DCM extract from all three extraction cycles was treated with sodium chloride solution to remove residual water. It was then transferred into a flask containing calcium chloride ($CaCl_2$) to further dry the DCM extract. The mixture was vacuum filtered to remove the calcium chloride, and the clear DCM solution was transferred into a round-bottom flask (RB). Any residual calcium chloride was rinsed with a small amount of DCM to recover any trapped cinnamaldehyde.

Distillation of DCM: The DCM solvent was removed from the extract using distillation under reduced pressure. After complete removal of the DCM, the remaining yellowish cinnamaldehyde oil was collected and transferred to a dram vial for storage or further analysis [25].

3.2.5. Tests for Extracted Cinnamaldehyde

Schiff's, tollens and solubility tests were carried out for identification of cinnamaldehyde.

1. Schiff's Test: Schiff's reagent, a solution of fuchsin hydrochloride dissolved in sulfur dioxide (SO_2), is used to detect aldehydes. In this test, the aldehyde group in cinnamaldehyde reacts with the sulfur dioxide component of Schiff's reagent. The reaction restores the bright reddish-purple color of the dye, providing a clear visual indication of the presence of aldehydes in the sample.

2. Tollen's Test: Tollens' reagent, consisting of ammonia and silver nitrate, is employed to test for the presence of aldehydes. When an aldehyde is gently heated with Tollens' reagent, silver ions are reduced to elemental silver, forming a reflective silver mirror on the inner surface of the test tube. This reaction, known as the "silver mirror test," is a qualitative indicator of the presence of aldehyde functional groups in cinnamaldehyde.

3. Solubility Test: Cinnamaldehyde is known for its limited solubility in water but is soluble in oils and alcohols. In this test, cinnamaldehyde is added to three solvents: water, chloroform, and ethanol. The solubility in each solvent is observed and recorded, with cinnamaldehyde being expected to dissolve in chloroform and ethanol but remain insoluble in water [26].

3.2.6. Quantitative Analysis of Cinnamaldehyde

For the quantitative analysis, five dilutions are prepared from a standard stock solution of cinnamaldehyde (100 µg/mL) in 10 mL volumetric flasks. The concentrations of the prepared solutions are 0.5 µg/mL, 1 µg/mL, 1.5 µg/mL, 2 µg/mL, and 2.5 µg/mL. The absorbance of these solutions is measured at 286 nm. The results are plotted to create a calibration curve, which illustrates the relationship between the concentration of cinnamaldehyde and its absorbance [27].

3.2.7. Antifungal Activity of Extracted Cinnamaldehyde

The antifungal activity of extracted cinnamaldehyde was assessed using the agar well diffusion method. Mueller-Hinton agar plates were prepared and inoculated with fungal suspensions of Aspergillus niger and Candida albicans. The fungal strains were cultured in nutrient broth, and their concentrations were adjusted to 1×10^6 CFU/mL. Wells were made in the agar plates using a sterile cork borer, and 100 µL of cinnamaldehyde extract, dissolved in DMSO at a concentration of 1–2 mg/mL, was placed into each well. Fluconazole, used as a positive control, was also tested at a concentration of 1 mg/mL. DMSO was used as the negative control. The plates were incubated at 28°C for 24–48 hours, and the zone of inhibition surrounding each well was measured [28].

4. Result and Discussion

4.1. Phytochemical Screening of Successive Extract of Cinnamon Bark

Phytochemical screening of Cinnamon bark was conducted to identify natural compounds, including tannins, aldehydes, glycosides, alkaloids, steroids, and flavonoids, which may possess medicinal benefits. These phytochemicals are known for their diverse biological actions, including antifungal and antibacterial properties, contributing to human health. A quantitative analysis of the ethanolic extract of cinnamon bark revealed the presence of various phytochemicals such as alkaloids, flavonoids, tannins, saponins, steroids, aldehydes, and phenols. The extract from Cinnamon bark exhibits a rich and diverse composition, as detailed in the comprehensive findings of the sequential phytochemical screening illustrated in Table 1.

Table 1 Qualitative Phytochemical Screening of the Cinnamon Plant's Ethanolic Bark Extract

Sr. No.	Plant constituents	Test performed	Result
1	Aldehyde	Tollen's Test Fehling's Test	+Ve +Ve
2	Alkaloids	Hager's Test	+Ve
3	Glycosides	Killer Killiani Test	-Ve
4	Steroids	Salkowski Test	+Ve
5	Flavonoids	Alkaline Reagent Test	+Ve
6	Tannis	Ferric Chloride Test	+Ve
7	Saponin	Test For Saponins	+Ve
8	Resins	Hcl Test	+Ve
9	Protein	Biuret	-Ve
10	Phenol	Ferric Chloride Test	+Ve
11	Carbohydrate	Molisch's Test	-Ve
+Ve = Present, -Ve = Absent			

4.2. Tests for Extracted Cinnamaldehyde

4.2.1. Schiff's Test

The Schiff's test, a chemical assay commonly utilized for the identification of aldehydes, was employed to analyze the extracted product. Positive outcomes from the Schiff's test substantiated the presence of Cinnamaldehyde in the substance obtained through the extraction process. The specific results of the Schiff's test are presented in Table 2.

Table 2 Results for Schiff's Test

Cinnamaldehyde	When the sample and Schiff's reagent were mixed, a deep magenta (purple) solution was created.
Appearance of Distillate	The distillate had an oily appearance.

4.3. Solubility Test

Cinnamaldehyde exhibits solubility in alcohols and oils while being insoluble in water. A solubility test was systematically conducted on cinnamaldehyde, and the results of this assessment are detailed in Table 3.

Table 3 Results for Solubility Test

Sr. No.	Solvents	Result
1	Water	Insoluble
2	Chloroform	Soluble
3	Ethanol	Soluble

4.4. Qualitative Analysis of Cinnamaldehyde

The spectrophotometric methodology leverages the inherent absorbance characteristics of Cinnamaldehyde at a precise wavelength, specifically 286 nm. The selection of this wavelength is attributed to its significance in capturing the distinctive electronic structure of Cinnamaldehyde molecules, ensuring precise and sensitive detection. A standard

solution was subjected to a spectral scan ranging from 200 nm to 400 nm. The wavelength of maximum absorption for Cinnamaldehyde was determined through this analysis, revealing that Cinnamaldehyde exhibited maximal absorbance at 286nm, as depicted in Figure 1. The correlation coefficient, denoted as 0.9999 and presented in Table 4, signifies exceptional linearity within the concentration range of 0.5-2.5 µg/mL. The dataset yields a regression equation expressed as y = 0.1514x - 0.0006, as demonstrated in Figure 2.

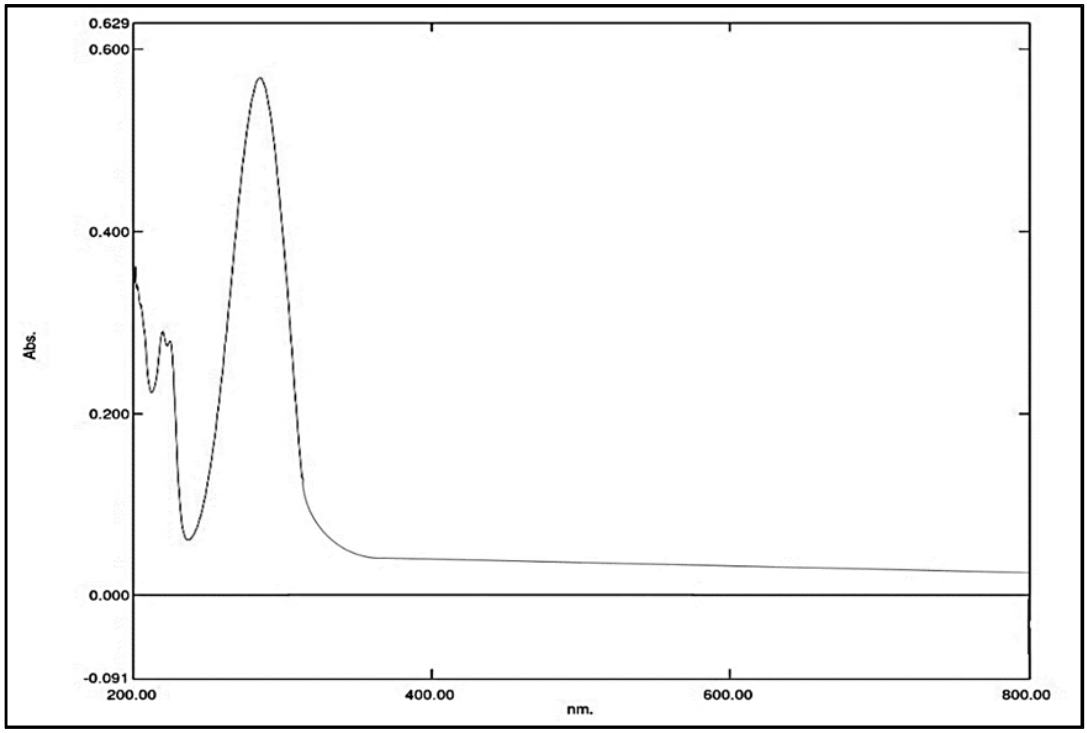

Figure 1 UV Spectrum of Cinnamaldehyde

Table 4 Evaluation Data of Linearity

Sr. No.	**Concentration**	**Absorbance**
1	0.5 µg/mL	0.153
2	1 µg/mL	0.302
3	1.5 µg/mL	0.449
4	2 µg/mL	0.606
5	2.5 µg/mL	0.758
Regression (r^2)		0.9999
Slope of Regression Line		0.1514
Y- intercept		0.0006

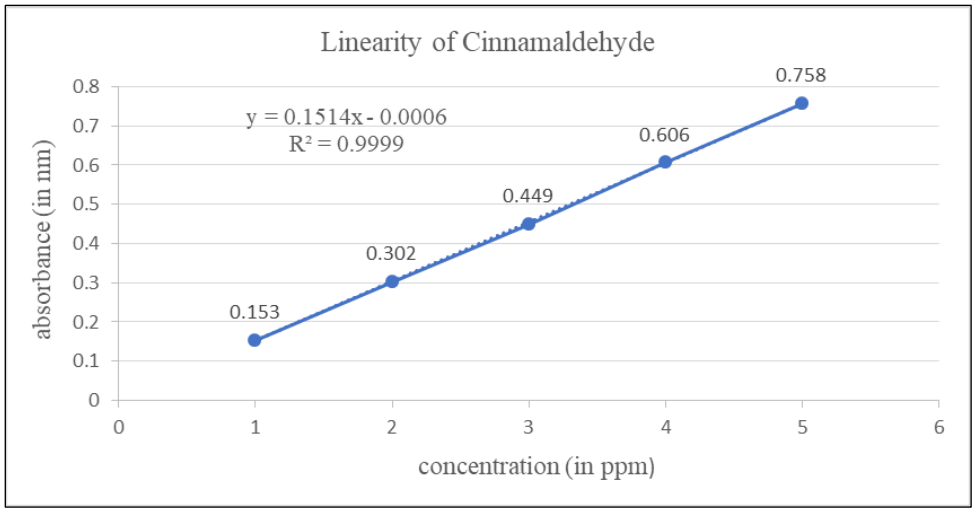

Figure 2 Calibration Curve of Cinnamaldehyde

4.5. Antifungal Activity of Extracted Cinnamaldehyde

The agar well diffusion technique was employed to assess the antifungal property of sample against two common fungal strains, *Aspergillus Niger* and *Candida albicans,* in contrast to the fluconazole standard.

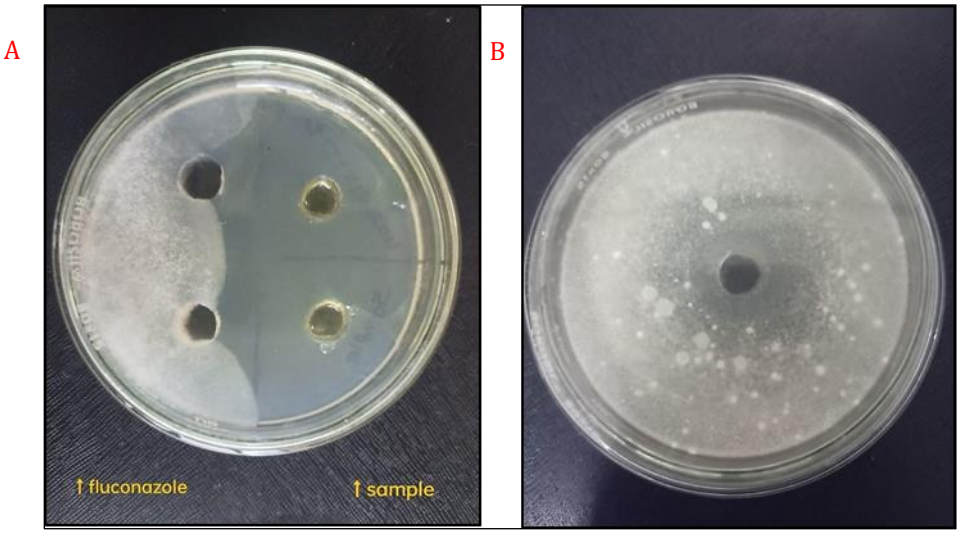

Figure 3 Zone of Inhibition of Cinnamaldehyde against a) *Candida albicans* and b) *Aspergillus niger*

Table 5 Results for Antifungal Activity

Sr. No.	Antifungal Agent (Sample)	Fungal Species	Zone of Inhibition (mm)
1	Cinnamaldehyde	*Candida albicans*	4.01 mm
2	Cinnamaldehyde	*Aspergillus niger*	3.12 mm

The sample demonstrated remarkably potent antifungal activity against *Candida albicans,* surpassing the zone of inhibition observed for the standard fluconazole. This suggests a higher efficacy in inhibiting the growth of *Candida albicans.* Similarly, the antifungal activity of the sample against *Aspergillus Niger* was notably more pronounced than that of the standard fluconazole, as the sample's zone of inhibition is larger than the standards, as shown in Figure 3. These results imply that the tested sample possesses potent antifungal properties, particularly against *Candida albicans*

and *Aspergillus Niger,* outperforming the standard fluconazole in both cases. The antifungal efficacy of cinnamaldehyde to *Aspergillus Niger* and *Candida albicans* is shown in Table 5, along with the greatest zone of inhibition for each species.

5. Conclusion

Extraction of cinnamon bark utilizing the Soxhlet method facilitated a comprehensive phytochemical analysis, revealing the presence of aldehydes, alkaloids, steroids, flavonoids, tannins, saponins, resins, and phenols. The isolation of cinnamaldehyde, a key constituent, was achieved through steam distillation and a three-stage extraction process. The confirmation of cinnamaldehyde was established through Schiff's test and UV Spectrophotometry at 286 nm. The quantitative analysis demonstrated high precision, with a coefficient of determination (r^2) of 0.9999. Evaluation of the antifungal activity against *Candida albicans* and *Aspergillus Niger* exhibited substantial inhibition zones (4.01 mm and 3.12 mm, respectively), comparable to the standard fluconazole.

Compliance with ethical standards

Acknowledgments

We thank to Department of Microbiology, Sanjivani College of Pharmacy, Kopargaon (Maharashtra) for providing lab space to conduct anti-fungal activity.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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