European Journal of Advanced Chemistry Research
Vol 5 | Issue 3 | August 2024
ISSN 2684-4478

RESEARCH ARTICLE

Phytochemical Identification of Alkaloidic Compounds from *Cinnamomum cassia* Cortex and Estimation of their Antifungal Efficacy

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ABSTRACT

Cinnamomum caresia **is defined as a medical and traditional plant grows in diverse countries in the world. It has therapeutic effects for treating various diseases resulting from infection by different microorganisms. In the current study, six alkaloids compounds were isolated and characterized from cinamom cortexes by using the GC-MS technique. The synergistic interaction was applied for the alkaloidic mixture against growth of two isolates of pathogenic fungi represented by** ***Urocystis agropyri*** **and** ***Candida albicans*** **by using the concentrations (25, 50, 75, 100, and 125 mg/ml) which recorded diverse diameters of inhibition equal to 58, 66, 72, 74, and 78 mm and 68, 52, 70, 72, and 74 mm, respectively. As a result, these alkaloidic compounds are recommended for use as natural therapy for diseases caused by the current pathogenic fungi.**

Keywords: *Candida albicans*, *Cinnamomun cassia*, Medical efficacy, Natural therapy.

Submitted: July 15, 2024

Published: August 31, 2024

10.24018/ejchem.2024.5.3.155

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1. Introduction

The medicinal significance and the therapeutic benefit of various medicinal plants result from the existence of diverse, active chemical components that have healthy actions leading to treating different infections and diseases. cinamom (*Cinnamomum cassia*) is considered one of the traditional and medical plants which are available in diverse countries of the world. In many societies, this plant is traditionally used as a drink in social occasions [1], [2]. Medicinally and chemically, cinamom has the therapeutic features as a natural drug because it contains various active metabolites compounds represented by phenolics, alkaloids, flavonoids, terpenes, tannins, coumarins, xanthenes, glycosides, and essential oils [3], [4].

Therefore, *Cinnamomum cassia* cortexes were carried out in biochemistry laboratories, and different chemical extracts were prepared. Then, the phytochemical metabolites were detected qualitatively and quantitatively against the growth of various micro-pathogens, especially fungi, parasites, and bacteria. Pre-studies were achieved concerning the phytochemical screening of natural active compounds present in all parts of cinamom. Also, multiple studies indicated and ensured the biochemical role of diverse, active metabolites as natural therapies for the treatment of various diseases caused by different pathogenic microorganisms [5]–[7]. Most studies about cinamom ensured the great significance and highest value of the presence of various chemical metabolites in this medical plant. The existence of alkaloids, terpenes, summaries, xanthenes, flavonoids, and phenolic acids in cinamom ensures the medicinal importance of these chemical compounds as natural therapies against the diverse diseases caused by different living pathogens. Chemically alkaloids are heterocyclic compounds that contain one or more nitrogen atoms having the chemical ability to bind with different compounds of the chemical system of pathogenic micro-organisms [8], [9]. Pre-studies indicated isolating and characterizing the alkaloid compounds from various medicinal plants, including (*Cinnamomum cassia*) in different countries of the world [10], [11]. So in the current research, alkaloids were isolated and

characterized from the cinnamon cortex, and the antifungal efficacy was measured for these chemical compounds.

2. Materials and Methods

2.1. Collection of Plant

Cortex of *Cinnamomum cassia* was bought from the Abu Al-Khasseb local market in the Iraqi Basrah Governorate. A professor of botany from the University of Basrah's Department of Biology, College of Education for Pure Sciences, classified the plant. After that, distilled water was used to wash the cinamom cortexes in order to remove any dust. Cortexes were then dried, ground into a powder, and stored in a dark container so that the necessary analysis could be carried out.

2.2. Preparation of Cold Aqueous Extract

In a conical flask, 500 ml of distilled water were used as a polar solvent, and 50 g of powdered cinnamon was added. All of the components were mixed thoroughly. After agitating the mixture for six hours at room temperature using a magnetic stirrer, the contents were filtered using a Buchner funnel. After extracting the filterate and removing the precipitate, the filterate's constituent parts were gently dried. Ultimately, 6.22 g of solid crude were collected [12].

2.3. Investigation of Qualitative Analysis of Alkaloids

Alkalodic compounds were detected qualitatively in a cold aqueous extract of cinnamon using Dragendroff reagent to know the presence of various alkaloids. One hundred mg was treated with 1 ml of distilled water in a test tube then 2.5 ml of Dragendroff reagent was mixed with aqueous solution. Finally orange precipitate was formed [13].

2.4. Isolation of Alkaloids from Cinnmomum cassia

In a conical flask, 500 ml of 10% w/v ethanolic acetic acid was added to 50 gm of cinamom cortex powder, and the mixture was thoroughly stirred. After ten hours of vigorous stirring with a magnetic stirrer, the plant and solvent mixture was filtered through a Buchner funnel, removing any precipitate and condensing the filtrate to its volume quarter. After the filterate and 5 ml of concentrated sulfuric acid were combined, the mixture was well shaken, and ammonium hydroxide was added to bring the pH to 9. After adding 25 ml of chloroform to the mixture and giving it a good shake, the ingredients were put into a separating funnel [14]. The weight of alkaloidic extract was equal to 2.34 gm.

2.5. Characterization of Alkaloids by Gas Chromatography-Mass Spectroscopy

Gas chromatography-mass spectroscopy (GC-MS) was the method used to chemically characterize the alkaloidic extract from *Cinnamomum cassia* cortex in the chemistry department of University of Basrah, Iraq's College of Education for Pure Sciences. The device is a Shimadzu GC-MS-QP-2101 Ultra type. Following characterization, many alkalodic compounds were obtained.

2.6. Pathogenic Fungi Isolates

Pathogenenic fungi stanines were gotten and identified by microbiologist in the microbiology laboratory at the college of science in Basrah University, Iraq. The pathogens are represented by *Urocystis agropyri* and *Candida albicans*.

2.7. Culture Medium of Pathogenic Fungi

The culture media represented by dextrose agar (PDA) and sabourad dextrose agar (SDA) were prepared according to the instructions of manufacturing company [15].

2.8. Estimation and Evaluation of Antifungal Activity

To estimate and evaluate the antifungal efficacy of alkaloids isolated from *cinnamomum cassia*, various concentrations represented by 25, 50, 75,100, and 125 mg/ml were prepared. Three ml of Mollur Hinton Agar as culture medium was put in each petridish and this medium was treated with the fungal suspension with volume equal to 0.2 ml and the optical density was 0.1 with wavelength equal to 450 nm by using a glass sterilized spreader. After that, all dishes were left to stable for 30 min, then some wells were made, and the mixture of alkaloids was added with the five diverse concentrations. The petridishes were incubated at 37 °C for one day by using incubator then different values of inhibition zone diameters were calculated [16].

TABLE I: Extraction Percentages Belonging to Cold Aqueous Extract and Alkaloids of *Cinnamomum cassia*

No.	Extract	Plant weight (gm)	Extract yeild	Extratction percentage (%)
1-	Cold aqueous	50	6.22	12.44
2-	Alkaloids	50	2.34	4.68

TABLE II: Preliminary Qualitative Detections for Alkaloids Isolated from *Cinnamomum cassia* Cortex

Reagent kind	Test result	Test indication	Conclusion
Dragendroff	+	Orange precipitate	Presence of alkaloids
Ferric chloride (1%)	–	No bluish-green colour	No phenols
Benedict	–	No red precipitate	No glycosides
$Pb(Ac)_2$ (1%)	–	No white- light brown	No tannins
Ethanolic KOH (5 N)	–	No yellow precipitate	No flavonoids
Ninhydrin (1%w/v)	–	No violet colour	No amino acids

3. Results and Discussion

The medicinal significance of the various medicinal plants was proved through the presence of diverse, active metabolic compounds in their various parts. Alkaloids are one of the chemical compounds existing naturally in plants, including *Cinnamomum cassia*, and they were carried out as potent therapies against different diseases [17]. Aqueous extract belonging to cinamom was prepared with weight equal to 6.22 gm then alkaloids were isolated with yield equal to 2.34 gm. The extraction percentages of aqueous extract and alkaloids were 12.44% and 4.68%, respectively, as indicated in Table I.

It is noticed that the extraction percentages for both cold aqueous and alkaloid extracts were very good. The explanation of the great value of extraction of cold aqueous belongs to an abundance of inactive and active compounds, including alkaloids. For insurance of the existence of alkaloids alone in the alkaloidic extract, various qualitative reagents were used to prove the absence of other active chemical compounds, as shown in Table II.

It was noticed from Table II that alkaloidic extract contains only alkaloids because of formation of orange precipitate which insure existence of alkalodic compounds while the other active chemical compounds were absent in a alkaloidic extract. Therefore this is considered as a great and clear evidence for purification of alkalods. Multi pre-studies were established about the presence of alkaloids as phytochemical compounds in diverse medicinal plants, including *Cinnamomum cassia* [18], [19].

To separate and characterize the alkaloidic compounds existing in the alkaloidic extract, gas chromatography- mass spectroscopy technique was carried out successfully to determine of number of alkaloidic compounds and identification their fine chemical structures. It was found the alkaloidic compounds are 2-(4,5-dihydro-3-methyl-5-oxo-1-phenyl-4-pyrazolyl)-5-nitro benzoic acid, ethanone, 1-(3-amino-4-methoxy methyl-6-methylthieno[2,3-b] pyrid-2-yl), 1H-1,2,3-triazole, 1-ethyl-4-[2-(1-ethyl-1H-1,2,3-triazole-4-yl)diozenyl]-,N-oxide, methaqualone, propanoic acid, 3-(perhydro-2,5-dioxo-imidazol[4,5-d] imidazol-1-yl) and benzimidazole-5-carboxylic acid, 2-methyl-1-phenyl. Mass spectra for these alkaloidic compounds were recorded as shown in Figs. 1–6.

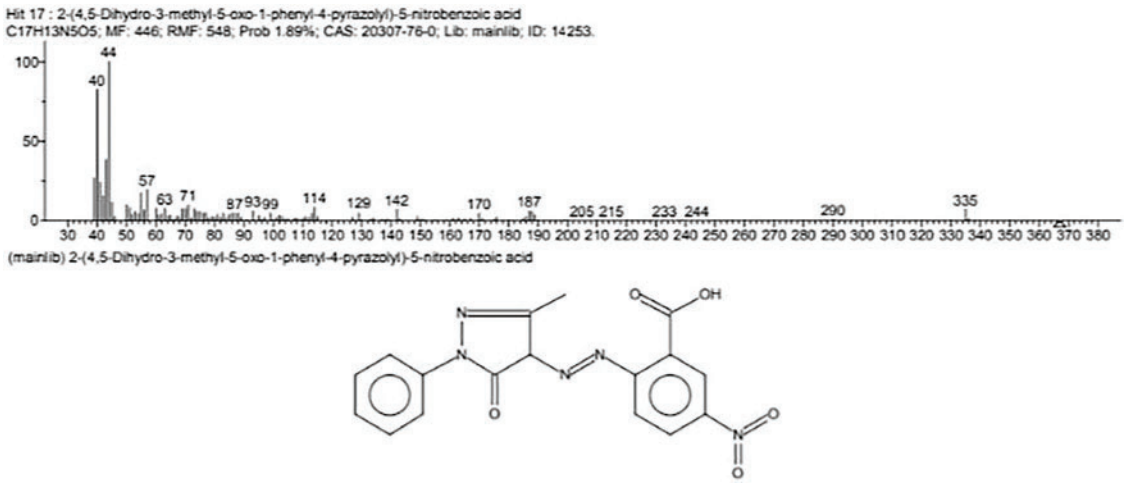

Fig. 1. Mass spectrum of 2-(4,5-Dihydro-3-methyl-5-oxo-1-phenyl-4-pyrazolyl)-5-nitrobenzoic acid.

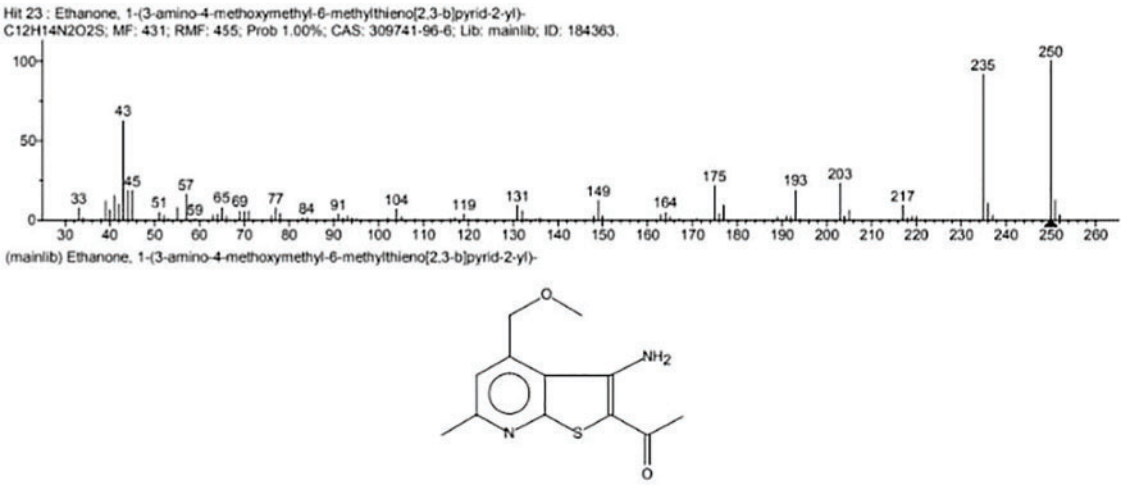

Fig. 2. Mass spectrum of ethanone, 1-(3-amino-4-methoxymethyl-6-methylthieno[2,3-b] pyrid-2-yl).

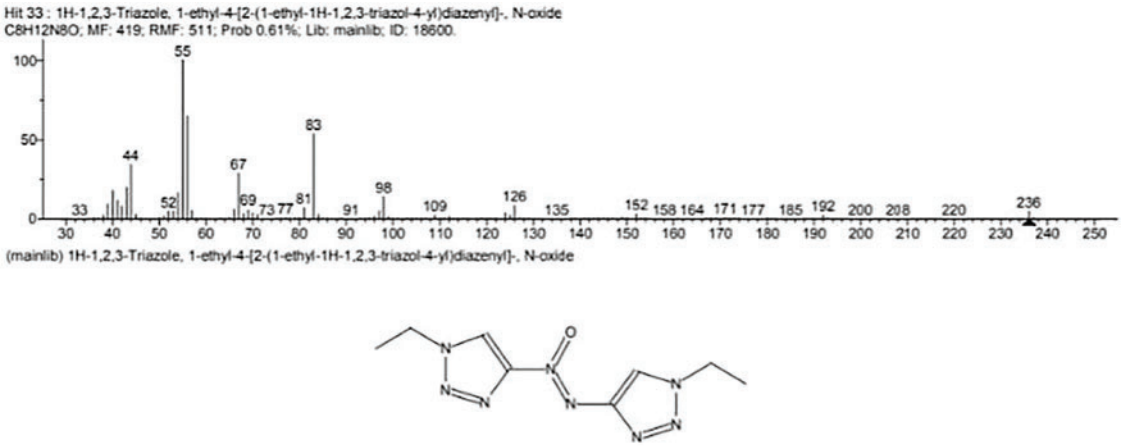

Fig. 3. Mass spectrum of 1H-1,2,3-Triazole, 1-ethyl-4-[2-(1-ethyl-1H-1,2,3-triazol-4yl)diazenyl]-N-oxide.

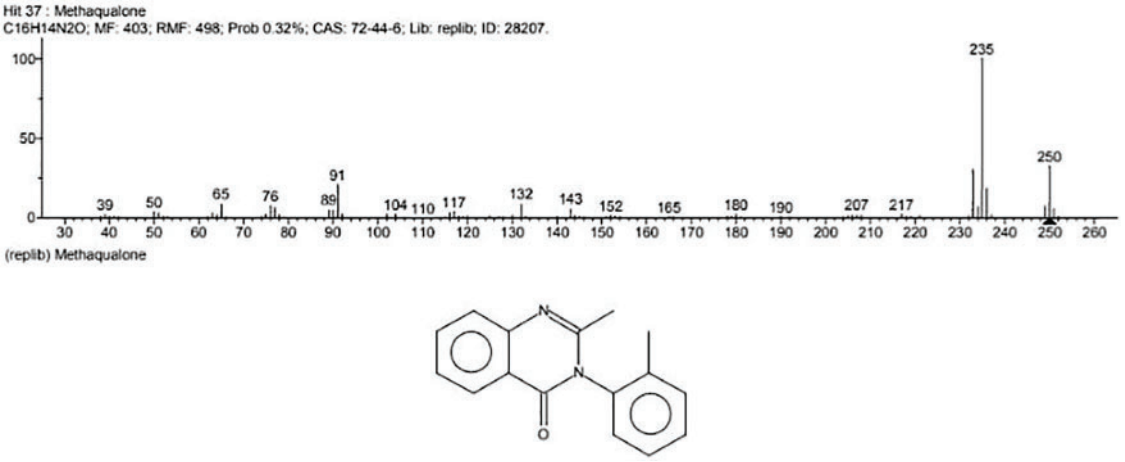

Fig. 4. Mass spectrum of methaqualone.

The six alkaloidic compounds contain many active nitrogenous groups that are responsible for antimicrobial efficacy, especially against pathogenic fungi [20]. The concentrations of alkaloidic compounds mixture represented by 25, 50, 75, 100, and 125 mg/ml recorded different values of inhibition zone diameter equal to 58, 66, 72, 74, and 78 mm against growth of *Urocystis agropyri* furgus whereas the same concentrations showed inhibition values equal to 68, 52, 70, 72, and 74 mm against *Candida albicans* fungus as shown in Table III.

It was found that the greatest value of inhibition diameter was 78 mm, which was recorded against Urocystis agropyri fungus, while the maximal value of inhibition diameter was equal to 74, which was calculated against *Candida albicans* fungus. Also, it was found that synergistic interaction of all

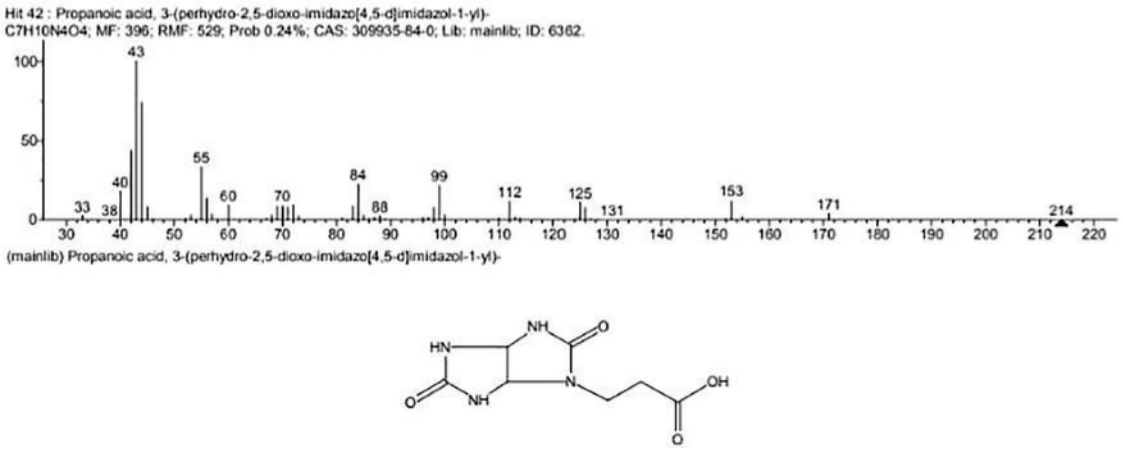

Fig. 5. Mass spectrum of propanoic acid, 3-(perhydro-2,5-dioxo-imidazo[4,5-d]imidazol-1-yl).

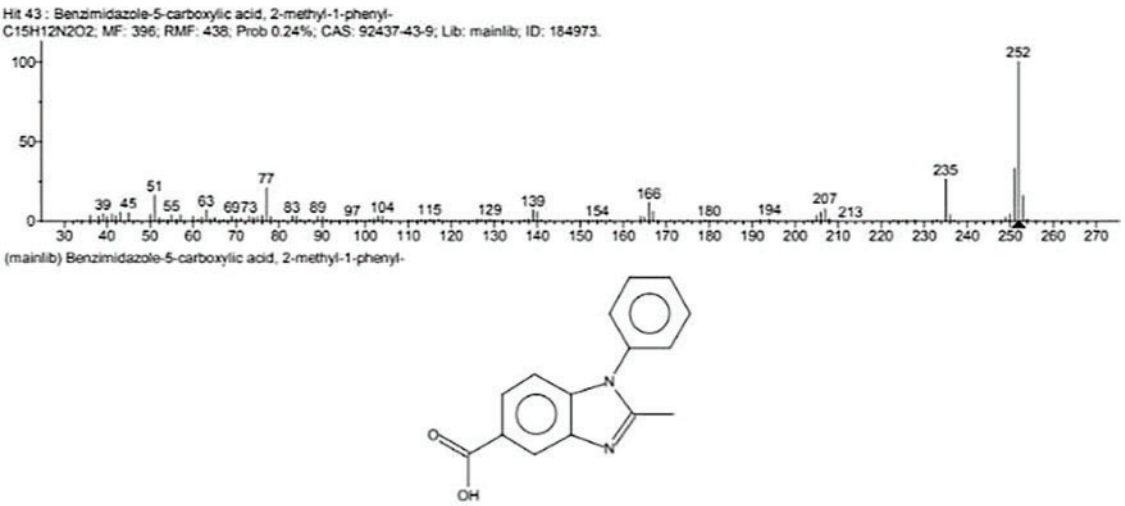

Fig. 6. Mass spectrum of benzimidazole-5-carboxylic acid, 2-methyl-1-phenyl.

TABLE III: Inhibition Diameter Values of Alkaloidic Compounds Mixture Isolated from *Cinnamomum cassia* agaist Pathogenic Fungi

Active compounds	Concentration (mg/ml)	Diametars values of inhibition zone (mm)	
		Urocystis agropyri	*Candida albicans*
Mixture of six alkaloidic compounds	25	58	68
	50	66	52
	75	72	70
	100	74	72
	125	78	74

alkaloidic compounds has the highest ability to inhibit or kill the most pathogenic fungi. The medicinal and biochemical mechanisms of the chemical action of active alkaloids belong to the capability of these chemical compounds to link with chemical system of living cell of pathogenic fungi by hydrogen bonding with nucleic acids represented by deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) leading to destruct the natural chemical structures of these acids [21], [22].

Diverse studies ensured the biochemical action of alkaloids isolated from various medicinal plants to inhibit the metabolism of lipids, amino acids, proteins, and carbohydrates belonging to the chemical system of micro-organisms, especially pathogenic fungi, by bonding between the nitrogen of alkaloid and hydrogen of these metabolites in the living cell. Alkaloidic compounds can also interact with the hydrogens of different enzymes present in the chemical system of pathogenic fungi leading to reduce or minimize the catalytic effect of these enzymes [23]–[25].

4. Conclusions

The synergistic interaction of the mixture of six alkaloidic compounds isolated and characterized from *Cinnamomum cassia* cortex proved its high medicinal efficacy against the growth of the pathogenic fungi represented by *Candida albicans* and *Urocystic agropyri*. Also, it was found the maximal inhibition concentrations of active alkaloids showed the greatest inhibition values, leading to the killing of most pathogenic fungi. Therefore, these alkaloidic compounds can be used as excellent natural therapy to treat various diseases caused by pathogenic fungi of current research, depending on the synergistic interaction principle.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

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