



Isolation and characterization active alkaloidic compounds from *Cinnamomum verum* cortexes and proving of their medicinal efficacy against pathogenic fungus *Alternaria alternata* causing upper respiratory tract inflammatory

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Abstract

Six alkaloid compounds were isolated (the discovery of a natural drug, which is a combination of the six alkaloid compounds). They are:

- 1) 1,3,5-Triazine-2-carboxylic acid, 4-amino-6-methyl
- 2) 1,2,4-Triazino[5,6-E][1,2,4]-triazine-3,6-dione, hexahydro
- 3) 2-(4,5-Dihydro-3-methyl-5-oxo-1-phenyl-4-pyrazol)-5-nitrobenzoic acid
- 4) 4-methyl-3,4-dihydro-[1,2,3] triazolo [4,5-d] pyrimidine-5,7-dione
- 5) Propanoic acid-3-(perhydro-2,5-dioxo-imidazol [4,5-d] imidazole-1-yl)
- 6) 1,2,3-Triazolo[4,5-f] benzotriazole- 4,8(4H,8H)-dione

were isolated from *Cinnamomum verum* cortexes by using ethanolic acetic acid, sulphuric acid and ammonia solvents, and they were characterized by using of preliminary qualitative tests, gas chromatography-mass spectrum GC-MASS. The medicinal efficacy was studied for these six alkaloidic compounds theoretically by using the Autodock program and the results showed presence of high efficacy of the compounds. Then the medicinal efficacy was measured biologically for the six compounds mixture in vitro depending on synergistic interaction principle by using various concentrations (5, 10, 15, 20 and 25 mg / ml) by using of agar diffusion technique. The results showed that six alkaloidic compounds mixture is biologically active against *Alternaria alternata* fungus, where the inhibition zone diameters were recorded equal to 27, 33, 41, 45 and 49 mm respectively towards the pathogenic fungus. Also, cytotoxicity tests for that different concentration of six active alkaloidic compounds mixture proved there is no toxic effect belonging them towards red blood cells, and no hemolysis effect was appeared in blood cells. Therefore, six alkaloidic compounds isolated from *Cinnamomum verum* cortexes can be used as active drug for treatment of various diseases especially caused by pathogenic fungus represented by *Alternaria alternata*, without any side effects.

Keywords: *Fungus, cinnamomum burmannii, Bacteria.*

Introduction

The biological effectiveness of the extract of the cinnamon plant was studied due to the great medical importance of this plant. In 2019, researcher Nita Parisal and his group studied the effectiveness of the extract of the *cinnamomumburmannii* plant as an antibacterial against *Staphylococcus aureus* and *Escherichia coli*, and the study proved the effectiveness of the extract as an antibacterial [1]. The antioxidant and antimicrobial ability of the raw extract from the leaves of the sage plant was also studied by researcher LilianeBezerra de Lima and his group in 2022 [2]. A patent was also registered with the Shanghai Institute of Nutrition and Health of CAS under Application No. CN2006101175121A events, as the invention relates to the process of preparing the extract of scholars.

The extract is used in pharmacy or health care product preparation. The prepared cinnamon extract contains cinnamic alcohol, cinnamic acid, cinnamic aldehyde, and eugenol. The study extract of the invention can be used to prepare medicines or health care products that have the dual functions of lowering blood sugar, reducing blood lipids, and can protect liver functions [3]. In this patent application, six alkaloid compounds (which had not been previously extracted) were extracted from the peels of the *Alternaria* plant and their medicinal effectiveness was proven against the pathogenic fungus *Alternaria alternata*, which causes upper respiratory tract infections (this effect has not been previously studied) at a low cost. Medicinal plants

are one of the natural sources for treating many different diseases because they contain many effective metabolic compounds such as phenols, alkaloids, tannins, flavonoids, glycosides, terpenes, steroids, essential oils and saponins, which are characterized by their high pharmacological effectiveness in reducing various pathological conditions caused by pathogenic microorganisms known for their ability to cause Inflammations and injuries inside the human body [4,5].

The active natural chemical compounds isolated and identified from medicinal plants are biochemically synthesized in the plant through secondary metabolism through many and diverse biochemical metabolic pathways depending on the class of the active compound. Many effective natural compounds, such as phenols and alkaloids, have been isolated from various parts of many medicinal plants, and these chemical compounds have been used as therapeutic drugs for a number of diseases such as diabetes, high blood pressure, cancer, heart disease, and others.

It has led to amazing successes in this field [6-8]. The principle that distinguishes active metabolic compounds is what is called synergistic interaction, which is the contribution of all the active compounds to each other in the effective drug effect within the living cell of the pathogenic microorganism, as well as the absence of any effects. side effects of these compounds, which makes their use safe. The other thing that distinguishes the effective chemical compounds

extracted from medicinal plants is that they are obtained from natural sources and are available at a very low price compared to the cost of industrially produced pharmaceutical compounds [9-11].

The alkaloids isolated from medicinal plants are heterocyclic organic nitrogenous compounds containing one or more nitrogen atoms within this ring structure, and the pharmacological effectiveness of these effective compounds is due to the nitrogen atom or atoms present and their derivatives, especially the imine group, which has the ability to hydrogen bond with various chemical systems. Found in the living cell of pathogenic microorganisms such as bacteria, fungi, and viruses[12]. Many previous studies have proven that alkaloid compounds isolated from medicinal plants have medical and physiological effects as anti-fungal, anti-parasitic, anti-inflammatory, anti-tumor, and anti-cancer compounds. anti-cancer[15-13].

Materials and methods

1. Prepare the sample before extraction in detail

The students' peels were purchased from the local market in the city of Basra, Iraq, and the plant was classified in the Department of Life Sciences - College of Education for Pure Sciences - University of Basra.

The students' peels were cleaned well with distilled water, then dried and ground using an electric grinder and turned into a powder that was stored in opaque plastic containers at room temperature until the day of use.

2. Preparing the cold aqueous extract of the peels of the scholars in detail 25 grams of *Cinnamomum verum* peel powder was weighed, then

mixed with 500 milliliters of distilled water in a conical flask and the mixture was shaken well. Then the contents of the mixture were placed on a rotating magnetic mixer for 12 hours. After that, the aqueous mixture was filtered using a Buechner funnel. The precipitate was discarded, the filtrate was collected and evaporated, and a solid weighing 6.22 grams was obtained.

The initial qualitative detection of alkaloid compounds in the cold aqueous extract of the peels of the scholars was carried out using Druckendorff's reagent. The detection result was positive with the appearance of an orange precipitate.

The chemical and spectroscopic diagnosis of the cold aqueous extract of the aquatic peels was carried out using thin layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS) to identify the phenolic compounds present in the aqueous extract.

The diagnosis was made in the Gas Chromatography-Mass Spectrometry Laboratory in the Department of Chemistry - College of Education for Pure Sciences - University of Basra using the GC-MS Agilent Techn device. HP-5MS, 7890A System.

3. Isolation and diagnosis of alkaloids from the peels of scholars in detail After the presence of alkaloids in the students' peels was confirmed qualitatively, the alkaloids were isolated quantitatively by weighing 25 grams of the students' peels powder, then mixing them with 250 milliliters of aqueous acetic acid (10% V/V), with continuous shaking using a magnetic stirrer for four hours. . After that, the mixture was filtered using a Buechner funnel with Whatman type filter paper

(Whatman No.1), then the sediment was discarded and the filtrate was concentrated to a quarterSize it using a rotary evaporator at a temperature of 50 degrees Celsius. After that, 5 ml of sulfuric acid (2% v/v) was added to the filtrate, and the acid value was adjusted to pH=9 by adding drops of concentrated ammonia, then the basic solution was filtered and the sediment was discarded. Place the filtrate in a separating funnel, then add 20 milliliters of chloroform to it. The solution was then shaken well and left to settle for three minutes, and the organic layer was then isolatedThe separation process was repeated three times, and the sizes of the isolated organic layers were collected each time and dried at room temperature. The weight of the alkaloid was 2.10 grams and was brown in color.

Spectrophotometric diagnosis of the isolated alkaloid extract was carried out using gas chromatography-mass spectrometry (GC-MS) technology to determine the number of alkaloid compounds present, their mass spectra, as well as their precise chemical structures. The diagnosis was made in the Gas Chromatography-Mass Spectrometry Laboratory in the Department of Chemistry - College of Education for Pure Sciences - University of Basra using the GC-MS Agilent Techn device. HP-5MS, 7890A System.

4. Method for estimating the pharmacological efficacy of alkaloids isolated ex vivo in detail

The biochemical aspect included assessing the antifungal activity of the pathogen *Alternaria alternata*, as follows:

1-Prepare the SabouroudDextroseAgar SDA culture medium used for fungi by dissolving 50 grams of the medium in 1000 milliliters of distilled water. It

was poured into special petri dishes and then used to reactivate pathogenic fungal isolates. They were activated by growing them on the culture medium and then incubated at a temperature of 30°C for 1-3 days to isolate the fungus at a temperature of 30°C.

2. After the end of the incubation period, prepare a fungal suspension for each of the isolates to collect the pathogenic isolates by adding a small amount of the fungal colony to a test tube containing 2 ml of distilled water, then shaking the solution using a Vortex electric shaking device until a homogeneous fungal suspension was obtained and its density was measured using Measure the milligrams as a concentration of 1×10^8 cells/ml was obtained.

3. Testing drug effectiveness outside the living bodyEfficacy was measured using the agar diffusion assay. Place 0.2 ml of the fungal suspension on the surface of the SDA culture medium, then spread it evenly over the medium using an L-shaped glass rod. The dishes were then left for a period of 10 minutes for the suspension to be absorbed by the medium. All the dishes with the names of the isolates and the concentrations of the prepared chemical compounds, thenPuncture the center of each dish using a cork drill to create a circular hole with a diameter of 0.9 mm. 5 ml/100 microliters of each concentration of the chemical compound was added to the culture center hole for all pathogenic fungal isolates, in two replicates for each isolate.

5. Method for measuring cytotoxicity in detail

The Xian-guoamdUrsola method was adopted to measure the toxicity of the studied alkaloid compounds using red blood cells, as follows:

A standard stock solution was prepared with a

concentration of 200 mg/ml, then it was diluted and a series of concentrations were prepared (0.2, 0.3 and 0.4 mg/ml). 0.8 ml of each concentration was placed in Eppendorff tubes, then 0.2 ml of red blood cells were added to each tube. At the same time, I prepared two Ependorf tubes and put 0.8 ml in the first of the physiological Ringer's solution (representing the negative control) and the second tube, liquefaction water was placed in it (representing the positive control), then 0.2 ml of red blood cells were placed in each tube. Finally, the results were recorded after incubating these tubes in a special incubator for half an hour at 37 degrees Celsius.

Results and Discussion

Table (1) indicates the extraction percentages for both the cold aqueous extract and the alkaloid extract. The weight of the cold aqueous extract was 7.2 g, while the weight of the alkaloid extract was 2.1 g. Accordingly, the extraction percentage was 28.80% and 8.40%, respectively.

It was found in Table (1) that the extraction percentage of the cold water extract was greater than the extract of alkaloid compounds. The reason for this is that the cold water extract possesses many effective medicinal compounds in addition to the alkaloid compounds. The percentage of isolated alkaloids was very good compared to the compoundsOther active substances found in the peels of the larch plant, such as phenols [16], thiolates [17], and essential oils [18].

Table (1): Extraction percentages of the cold aqueous extract and alkaloids isolated from Iraqi basil leaf powder.

Extraction percentage	Weight of the resulting extract	Weight of ground (plant (g	Type of active compounds
28.80%	7.2	25	Cold aqueous extract
8.40%	2.1	25	Alkaloids

Specific detection of alkaloids

A qualitative detection of the alkaloids present in the cold aqueous extract, as well as in the extract of the isolated alkaloids, was carried out using Druckendorff's reagent. The result was the formation of an orange precipitate in both extracts, and this proves the presence of alkaloids qualitatively in the peels of the students, as shown in Table (2).

Table (2): Qualitative chemical detection of alkaloids in cinnamon plant peel powder

Conclusions	Notes	Detection result	Reveal	Extract type
Presence of alkaloids	Form an orange precipitate	+++	Drakendorff	Cold watery
Presence of alkaloids	Form an orange precipitate	+++++	Drakendorff	Alkaloids

Separation and diagnosis of alkaloid compounds by gas chromatography-mass spectrometry technique
The active alkaloid compounds present in the alkaloid extract were separated by gas chromatography-mass spectrometry technique. It was found that there are six alkaloid compounds with different retention times, as in Table (3).

Table (3) Active alkaloid chemical compounds found in cinnamon bark, separated by gas

chromatography-mass spectrometry.

Compound no.	Alkaloidic compound Name	Retention time(min)
1	1,3,5-Triazine-2-carboxylic acid, 4-amino-6-methyl	0.34
2	1,2,3-Triazolo[4,5-f] benzotriazole- 4,8(4H,8H)-dione	0.75
3	[1,2,3,4]Tetrazolo [1,5-b][1,2,4] triazine, 5,6,7,8-tetrahydro	0.93
4	4-metyl-3,4-dihydro-[1,2,3] trizolo [4,5-d] pyrimidine-5,7-dione	1.17
5	Propanoic acid-3-(perhydro-2,5-dioxo-imidazol [4,5-d] imidazole-1-yl)	2.73
6	2-(4,5-Dihydro-3-methyl-5-oxo-1-phenyl-4-pyrazol)-5-nitrobenzoic acid	3.28

The mass spectra of the alkaloid compounds isolated from the peels of the Darcin plant were recorded by mass spectrometry, and several peaks of different heights appeared for each isolated alkaloid compound.

Gas chromatography-mass spectrometry (GC-MS) technology has proven successful in separating and diagnosing the six alkaloid compounds with high accuracy. This confirms beyond doubt that this technology reveals the chemical information related to the isolation, separation, and diagnosis of secondary metabolic compounds, including alkaloids. As a result, it gives distinctive and wonderful characteristics for obtaining the exact chemical composition of the metabolically active natural compounds that are abundant in medicinal plants [19].

Results of evaluating the pharmacological effectiveness of a mixture of alkaloid compounds isolated from the peels of scholars

The results demonstrated that the mixture of the six

alkaloid compounds isolated from the peels of the scholars and prepared at concentrations of (5, 10, 15, 20, and 25) mg/ml, recorded diameters of inhibition values equal to 27, 33, 41, 45, and 49 mm, respectively, against the growth of the pathogenic fungus. Alternaria alternate, as in Table (4).

Table (4): Pharmacological efficacy of the mixture of alkaloid compounds isolated from cinnamon peels against the pathogenic fungus Alternariaalternata, which causes upper respiratory tract infections.

Diameter of the inhibition (zone (ml	concentration(mg\ml)	Active compounds
65	25	The mixture of six alkaloid compounds
70	50	
71	75	
71	100	
73	125	

It is clear from Table (4) that the mixture of the six alkaloid compounds recorded different diameters of the inhibition zone against the fungus . It was also found that increasing the concentration led to an increase in the diameter of the inhibition zone, which means killing a large number of this pathogenic microorganism, Alternaria alternate, and inhibiting its growth, thus increasing the drug effectiveness of the mixture.The six alkaloid compounds isolated against this pathogenic fungus. This proves that the mixture of the six alkaloid compounds worked according to the principle of synergistic interaction, which led to increased effectiveness. This indicates that these compounds are medicinally effective, and therefore the mixture of the six alkaloid compounds can be used as an

effective and successful drug to treat various diseases. Caused by the pathogenic fungus *Alternaria alternata*.

The explanation of the pharmacological effectiveness is that the six isolated alkaloid compounds possess the chemical ability to bond with the nucleic acids (DNA and RNA) present in the living cells of the pathogenic fungus, leading to the destruction or inhibition of the biological and chemical roles of these acids. Studies have also proven that alkaloids are effective medicinal compounds. It has the chemical ability to inhibit the metabolism of carbohydrates, amino acids, fats, and proteins through hydrogen bonding between the nitrogen atoms present in the chemical structure of alkaloids with the hydrogen atoms present in these compounds in the living cell of pathogenic fungi [20-22]. Also, isolated natural alkaloids. Medicinal plants have proven their ability to inhibit the growth

of pathogenic fungi through hydrogen bonding between the alkaloid nitrogen and the hydrogen atoms present in the chemical structure of microscopic organism enzymes, thus inhibiting the action of enzymes as cofactors and inhibiting their effectiveness, thus stopping their biochemical work [24,23].

Conclusion

- 1- Finding a new natural pharmaceutical alternative that does not have any side effects instead of the antibiotics used that have side effects.
- 2- The isolated compounds are characterized by their inhibitory effect on the fungus *Alternaria alternata*.
- 3- The mixture of isolated compounds is characterized by the possibility of using it as a safe medicine because it is extracted and isolated from a medicinal plant.

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