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Estimation of antimicrobial efficacy of phenolics characterized from Iraqi *Cinnamomum cassia* cortex

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Abstract
The greatest importance of different medical plants can be explained through presence of a wide spectrum of chemical compounds as potent metabolites. *Cinnamomum cassia* is a healthy plants is abundant in various countries. The current research was established to isolate. Characterize the phenolics compounds belonging to this plant cortexes. The results of gas-chromatography – mass spectroscopy analysis showed existence of seven phenolics in *Cinnamomum cassia.* The medicinal efficacy of synergistic interaction belonging the mixture of these active compounds recorded inhibition diameters equal to 3, 25, 25, 26 and 27 mm against *Pseudomonas aeruginosa* bacteria at concentrations 50, 100, 150, 200, 250 µg/ml represented by 0, 10, 25, 27 and 30 mm against growth of pathogenic bacteria represented by *Enterobacter cloacae*. Therefore the seven phenols have ability to kill the great number of micro-organism pathogens then *Cinnamomum* phenolics can be carried out as natural remedies against most diseases resulting from infection by these pathogenic bacteria.

Keywords: *phenolics, Cinnamomum cassia,* synergistic interaction, GC-MS, zearalenone

1. Introduction
Medicinally there are many plants possess therapeutic features for various dangerous diseases resulting from the pathogenic action of multi micro-organism especially bacteria. The significance value for any medicinal plant originate from abundance of the numerous active metabolic compounds like phenolics, anthocyanin, tannins, alkaloids, essential oils and terpenes which represent the main compounds of chemical system of all parts of plants [1, 2]. Various biochemical and medical studies were performed successfully in many countries concerning the phytochemical constituents existing in all parts of different sorts of medical plants. Also the biochemical efficacy was established completely for these potent metabolites against biological system belonging to multi-microorganisms especially fungi and bacteria. This medicinal potential was compared with many antibiotics at the same time [3, 4] *Cinnamomum cassia* a (Cinnamon) is a benefit healthy medicinal was used for multi – purposes such as a traditional refreshing drink around the world and also this plant was carried out against growth of various pathological microorganisms by estimation and evaluation the photochemical compounds which are available in all parts. Some pre-researches indicated and showed the chemical significance of many active compounds isolated and characterized from *Cinnamomum cassia* plant such as essential oils, alkaloids, phenolics, anthocyanins, lipids, antioxidants, vitamins and terpenes.
The phytochemical constituents were carried out successfully as antifungal, anti-tumor and antibacterial against. At the same time these various potent chemical compounds had chemical compounds had chemical ability as anti-hyperglycemic materials induced by alloxane [5, 6, 7]. The antioxidant capability of some active natural metabolic compounds from *Cinnamomum* was gave an excellent results to capture the chemical action of some free radicals represented by reactive oxygen, therefore the various compounds belonging to cinnamon can reduce or finish the biochemical functions of multi-free radicals [8].
Natural phenols are active constituents are spreading in different plants. Chemically they have aromatic ring attached with one or more hydroxyl group. The most of phenolics compounds are water-soluble compounds since they contain the polar groups represented by hydroxyl [5]. Also phenols can be found as glycosides when they like with mono or disaccharide [9]. Some pre-studies showed the high and unique feature of phenolics compounds isolated and identified from many medicinal plants as antibacterial constituents [10, 11].

Hence aim of the present research is to estimate and evaluate the antimicrobial and antioxidant potential of the phenols characterized from *Cinnamomum cassia* cortexes.

2. Materials and methods

2.1 Research plant collection

The cortex belonging to *Cinnamomum cassia* were bought from Al-Ashar Market in Basrah Governorate – Iraq, and taxonomic of plant was performed by a botany scientist at – college of education for pure sciences in Basrah University – Iraq. *Cinnamomum* cortex were cleaned by using distilled water to isolated impurities dust, then the cortexes were put in dark place to dry at room temperature. Then *Cinnamomum* was milled completely and the powder was put in dark containers in the sake of various procedures.

2.2 Phytochemical isolation of active phenolics

The chemical phenolics belonging to cinnamon cortexes were isolated by taking twenty five gm of plant then was treated with 500 ml of 2% hydrochloric acid (HCl). The mixture was placed in water bath at 80 °C for nine hours then it was filtrated gently by using Buchner funnel for elimination of precipitate. The filtrate was mixed with 15 ml of diet hylether then the solution was put other time in water bath at 40 °C for four minutes. After that the mixture was underwent to rotary evaporation in order to concentrate the phenolics which they finally collected and kept in plastic container [12].

2.3 Pathological bacterial strains

Tow pathological bacteria used in the current work are *Pseudomonas aeruginosa* and *Enterobacter cloacae* were isolated clinically from different patients and they were characterized by a microbiologist in the laboratory of Basrah University - Iraq.

2.4 Phytochemical test of *Cinnamomum phenolic*s

The phenolics compounds belonging to *Cinnamomum cassia* were underwent several qualitative reagents for ensurance of presence of phenolics in the extract and determination of their purity. The reagents were ferric chloride (1%w⁄v), lead acetate (1%w⁄v), ethanolic potassium hydroxide (5N), Dragendroff solution, ninhydrin (1%w⁄v) and Liberman – Burchard [13, 9].

2.5 Characterization test of *Cinnamomum phenolic*s

To certainty of existence of active phenolics and their fine structures therefore it was depended on technique of separation and identification represented by gas chromatography – mass spectroscopy (GC-MS) to determine he phenolics compounds number with their mass spectra.This phytochemical analysis of GC-MS was performed by using instrument in sort shimadzu GC-QP –zolo ultra system containing type CTC as an aromatic sampler supplied with comi pal robotic arm. Also this characterization was achieved in GC-MS laboratory in department of chemistry in Education College of pure sciences at university of Basrah – Iraq.

2.6 Estimation and Evaluation of medicinal efficacy of *Cinnamomum phenolic*s

To estimate and evaluate the medicinal efficacy of phenolics compounds mixture, so several concentrations were depended represented by 50, 100, 150, 200, 250 mg⁄ml to inhibit. The biological system of *Pseudomonas aeruginosa* and *Enterobacter cloacae* bacteria. Disc diffusion method was carried out by using several Petri dishes have PDA and SDA as culture media digs containing both bacteria for six days at 25 °C and all concentrations were placed autoclave. After that the inhibition was noticed for all bacteria at the different concentrations then diameters of inhibition zones were recoded [14].

3. Results

Currently more various studies were established concerning the phyto-chemistry for several medical plants and investigation of the potent chemical compounds existing in many parts of these plants. *Cinnamomum cassia* is a healthy, useful and economic plant therefore the natural phenolics as secondary metabolites were chemically isolated with amount equal to 3.2 gm thus the percentage of extraction was calculated with value equal to 12.8%. After that the phenols were tested for emphasis its abundance alone in phenolics crude so different detectors were carried out for the sake of guarantee purity of these active metabolic compounds as indicated in table (1).

Table 1: Phytochemical qualitative tests of phenols belonging to *Cinnamomum cassia* cortex

Chemical reagent	Detection	Practical results	Inference
$FeCl_3$ (1%w⁄v)	+	Bluish-green colour	Existence of phenols
C_2H_5OH-KOH (5N)	+	Yellow precipitate	Existence of flavonoids
Lead acatae (1%w⁄v)	-	White-brown precipitate	Absence of tannins
Wagner	-	No brown precipitate	Absence of alkaloids
Benedict	-	No red precipitate	Absence of glycosides
Mercuric chloride (1%w⁄v)	-	No yellow precipitate	Absence of saponin
Ninhydrin	-	No violet colour	Absence of amino acids

From the results gotten from the table (1), this means that the phenolics extract has only compounds including flavonoids while the other active metabolites were not detected. This experimental case insures the separation and purity the phenolics alone because the reagent represented by ferric chloride (1%w⁄v) and ethanolic potassium hydroxide (5N) proved formation of Bulsh-green colour and yellow precipitate respectively.

Cinnamon phenolics compounds which were isolated and purified, they were spectrophotometrically characterized by gas chromatography – mass spectrum technique. It was found the isolated phenolics were seven compounds represented by [1, 5-Diacetyl-2, 6-naphthalenediol, 1-Acetyl-2, 6-naphthalenediol, 2-Benzofuranbutanoic acid, 7-acetyl-4,6-dihydroxy-α-(1-hydroxyethylidene)-3, 5-dimethyl-β-oxo-, ethyl ester, Phenol, 2-(1, 1-dimethylethyl)-4-(1, 1, 3, 3-tetramethylbutyl), 11, 13-Dihydroxy-4-methyl-4, 5, 6, 7, 8, 9-hexahydro-1H-benzo[d]oxacyclododecine-2,10-dione,

Phenol, 2, 6-bis(1, 1-dimethylethyl)-4-(Methoxymethyl), Zearalenone] and these compounds have different retentions times. Also the mass spectra belonging to *Cinnamomum cassia* phenolics were recorded as shown in figures (1, 2, 3, 4, 5, 6 and 7) which indicated the mass spectrum of each phenolics compound and its fine chemical structure.

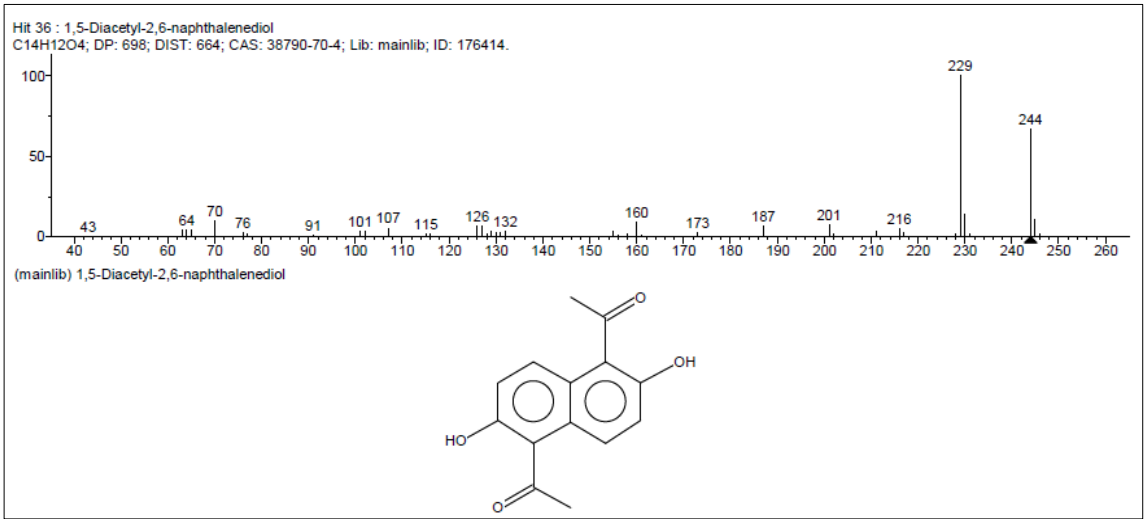

Fig 1: Mass spectrum of 1, 5-Diacetyl-2, 6-naphthalenediol characterized from *Cinnamomum cassia* cortex

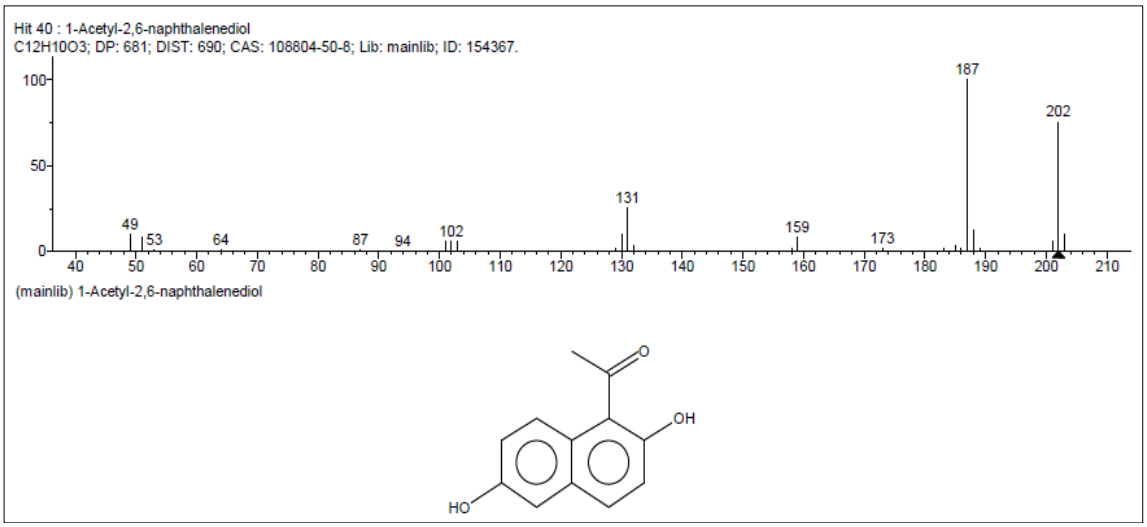

Fig 2: Mass spectrum of 1-Acetyl-2, 6-naphthalenediol characterized from *Cinnamomum cassia* cortex

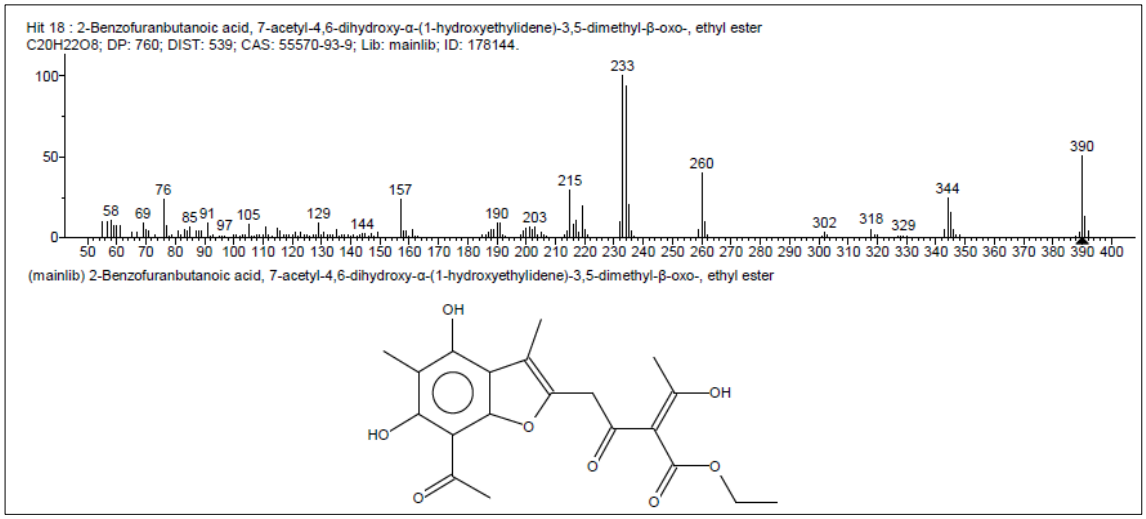

Fig 3: Mass spectrum of 2-Benzofuranbutanoic acid, 7-acetyl-4, 6-dihydroxy-α-(1-hydroxyethylidene)-3,5-dimethyl-β-oxo-, ethyl ester characterized from *Cinnamomum cassia* cortex

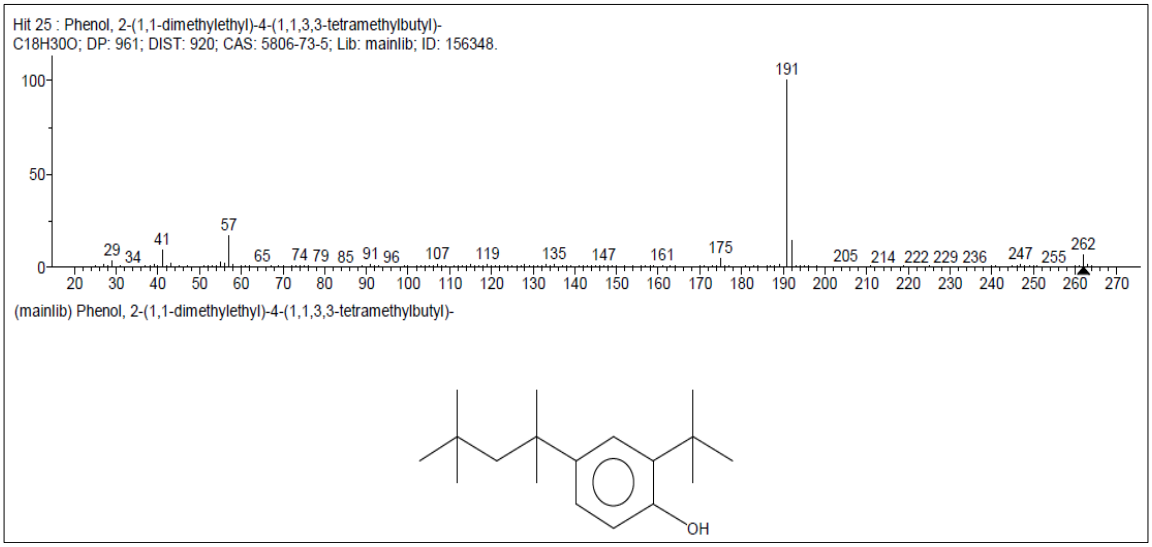

Fig 4: Mass spectrum of Phenol, 2-(1, 1-dimethylethyl)-4-(1, 1, 3, 3-tetramethylbutyl) characterized from *Cinnamomum cassia* cortex

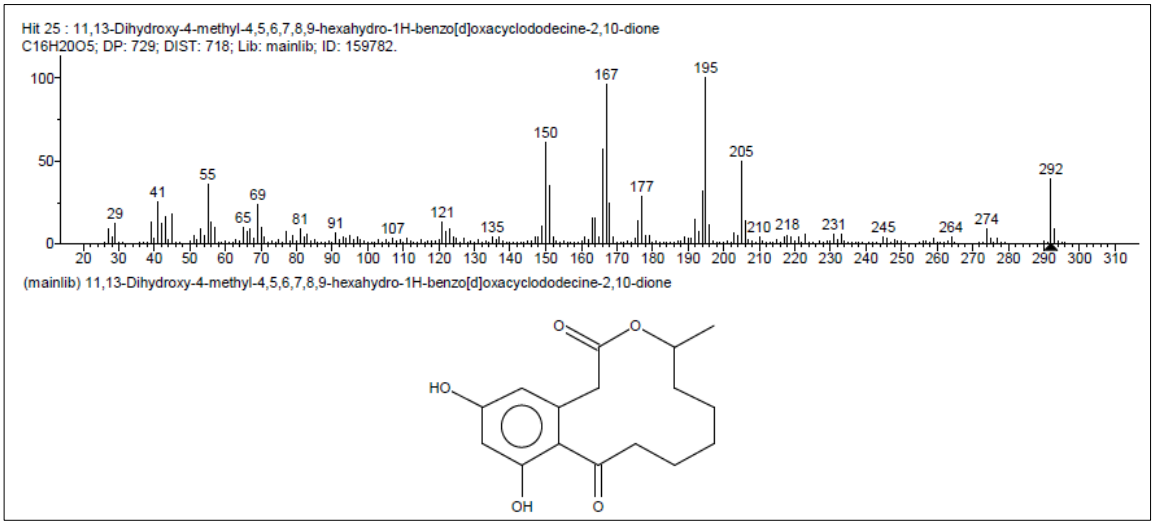

Fig 5: Mass spectrum of 11, 13-Dihydroxy-4-methyl-4, 5, 6, 7, 8, 9-hexahydro-1H-benzo[d]oxacyclododecine-2, 10-dione characterized from *Cinnamomum cassia* cortex

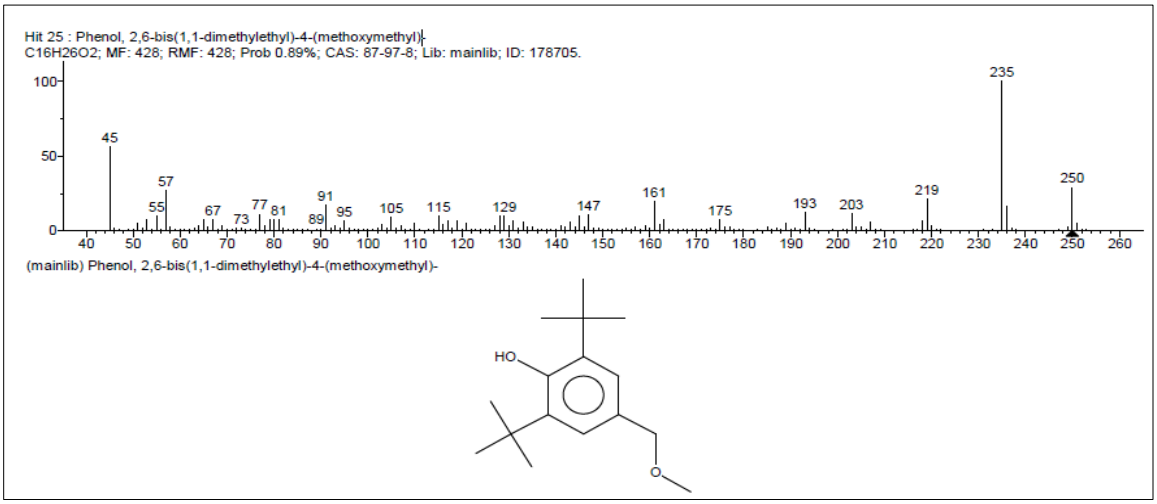

Fig 6: Mass spectrum of Phenol, 2, 6-bis (1, 1-dimethylethyl)-4-(Methoxymethyl) characterized from *Cinnamomum cassia* cortex

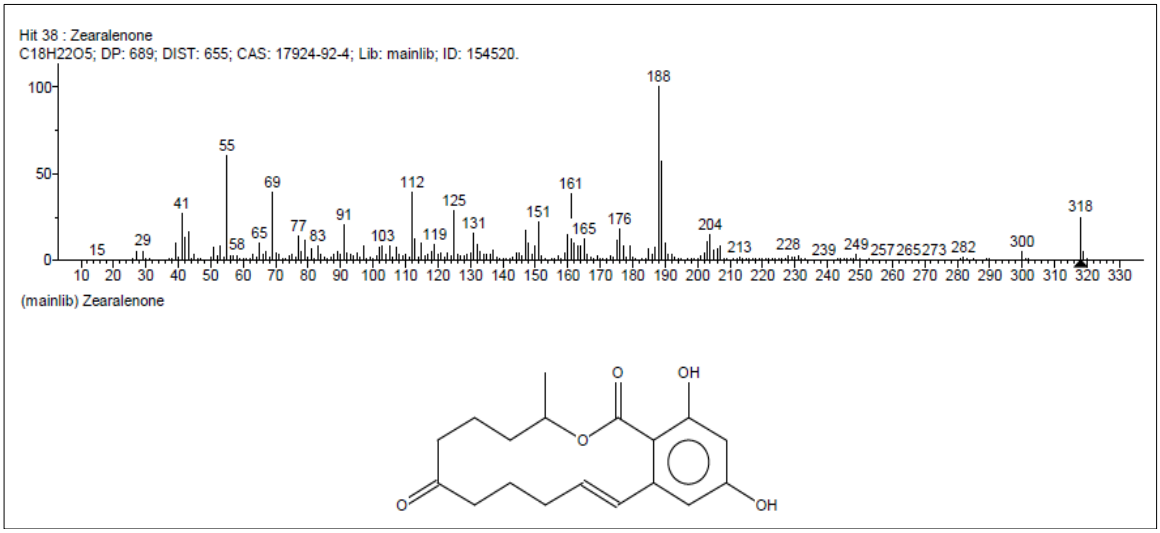

Fig 7: Mass spectrum of Zearalenone characterized from *Cinnamomum cassia* cortex

Given the significance of natural phenols belonging to *Cinnamomum cassia* cortex, so the medicinal efficacy was measured for the seven phenolics as synergistic interaction principle. Table (2) illustrates the values of diameters of inhibition zones recorded against and bacteria.

Table 2: Measurements of medical efficacy of the mixture of seven phenolics of *Cinnamomum cassia* cortex

Concentrations of phenolics mixture (mg⁄ml)	Diameters of inhibition zone (mm)	
	Pseudomonas aeruginosa	***Enterobacter cloacae***
50	3	0
100	25	10
150	25	25
200	26	27
250	27	30

The inhibition diameters of phenolics mixture were calculated with values equal to 3, 25, 25, 26 and 27 mm at concentrations represented 50, 100, 150, 200, 250 mg⁄ml against *Pseudomonas aeruginosa* bacteria. While the same concentrations showed various inhibition diameters equal to 0, 10, 25, 27 and 30 mm against growth of *Enterobacter cloacae* bacteria. Also it was found the concentration of 250 mg⁄ml had the highest value of inhibition concerning *Pseudomonas aeruginosa* bacteria, also the same concentration had the greatest value to inhibit *Enterobacter cloacae* bacteria.

4. Discussion

Given the importance of active chemical metabolic compounds belonging to different medicinal plant therefore the biochemical activities of these natural metabolites were carried out and estimated successfully against the biological and chemical systems belonging to various micro-organisms especially pathogenic bacteria. In the current work, seven phenolics compounds were isolated and characterized from cortex of *Cinnamomum cassia* by using GC-MS analysis which insured the existence of these phenolics compounds. Phytochemical screening of phenolics extract proved presence of only phenols and this represents the purity of phenolics. Multi-studies confirmed the medicinal significance of natural phenolics characterized from several plants because of these potent metabolites contain many functional groups especially hydroxyl [16].

The seven phenolics were characterized by GC-MS analysis which asserted successfully presence of these active chemical compounds with their various retention times. Also use of the phenolics as mixture by principle of synergistic interaction increased the medicinal efficacy against the biochemical system belonging to pathological bacteria. The increase of concentration of phenolics strengthened the biochemical activity of the seven compounds and covalent in inhibition of action of biological system belonging to pathogenic bacteria [17, 18]. Phenolics have different functional and structural groups like hydroxyl, aromatic ring, phenolics ring, carbonyl and carbonyl, so these chemical groups have multi-chemical roles for destruction of chemical systems represented by enzymes, hormones, carbohydrates, amino acids, proteins and lipids belonging to pathogenic bacteria. One of mechanism of activity of phenolics is destroy of wall and membrane of living cell of the micro-organism then dissociation of the various proteins [19, 20]. Also natural metabolic phenols have chemical ability to bind with amine and carboxyl groups belonging to different enzymes y hydrogen bonding. *Cinnamomum cassia* phenolics have a high capability to inhibit the biochemical roles of nucleic acids (DNA and RNA) belonging to the two pathogenic bacteria leading to decrease or finish the chemical functions of nucleotides [21, 22, 23].

5. Conclusions

The synergstic medicinal efficacy of the seven phenolics compounds isolated from *Cinnamomum cassia* cortex showed the high ability of these active metabolites for inhibition the action of chemical systems belonging to *Pseudomonas aeruginosa* and *Enterobacter cloacae* bacteria. Also the *Cinnamon* phenolics recoded the great capability as antioxidant agents and consequently they can maintain reducing status in the human body. As an inference, *Cinnamon* phenolics can be used as natural therapies for treatment of several diseases resulting from infection by pathological bacteria instead of some antibiotics.

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Conflicts of interest

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the

ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

Life science reporting

No life science threat was practiced in this research.

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