

E-ISSN: 2709-0256, P-ISSN: 1991-8690, Vol. 10, No. 2, Dec. 2023

Phytochemical constituents of bark essential oils of *Cinnamomum zeylanicum* Blume and effects on liver tissue of rats

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Received: 2023-11-21, Revised: 2023-12-12, Accepted: 2023-12-24, Published: 2023-12-26

Abstract— Cinnamomum zeylanicum Blume is an evergreen tropical tree belonging to the Lauraceae family; it is a useful plant in traditional and modern medicines. Essential oils of Cinnamomum zeylanicum were prepared and identified using gas chromatography-mass spectrometry (GC-MS). For histological study 80 adult male rats were employed in this study. It is the same weight (30 \pm 5 gm) and 75 days in age. The animals were divided into 4 groups, with ten rats in each group. The results indicated that C. zeylanicum bark contained 27 different phytochemical components. The main components included Cinnamaldehyde (2-Propenal, 3-phenyl-) (46.46%), 9-Methoxybicyclo, [6.1.0] nona-2,4,6-triene (31.31%), alpha-, Muurolene (7.14%) and Copaene (1.63%). Sections of livers were examined for histological abnormalities, such as the breakdown of the liver architecture and increased inflammation, in comparison to control samples, some hepatocytes were swollen with pyknotic, large nuclei and dilated sinusoids. As well as in the rats treated with noticed sever congestion of central vein (CV), degeneration and sluggish of endothelial lining larger, Bile ducts hyperplasia and necrotic hepatocytes. It can be deduced that the main component of cinnamon bark oil is cinnamaldehyde and the liver displayed some tissue changes, including hepatocyte necrosis and the existence of steatosis foci. Histological and cellular changes in liver tissue were dramatically attenuated by C. zeylanicum essential oil.

Keywords: bark, *Cinnamomum zeylanicum*, essential oil, liver, rats.

I. INTRODUCTION

Cinnamomum zeylanicum (common Cinnamon) is an evergreen tropical tree 50 m high belonging to the family Lauraceae. The bark of trees of *C. zeylanicum* is a useful plant in traditional and modern medicines. More than 100 species of the genus *Cinnamomum* are found throughout Asia. Australia, Vietnam, Madagascar, and Mexico. It is widely, cultivated in southern India and Sri Lanka [1,2].

Numerous health benefits of cinnamon have been suggested, including a reduction in triglycerides, LDL cholesterol, total cholesterol, anti-yeast activity [3], antibacterial, antifungal activity, insects [4,5,6,7,8], blood circulation improvement, anti-platelet aggregation, antiinflammatory activity, Enhancing mental function, decreasing colon cancer risk, anti-Parkinson, anti-Alzheimer, anti-angiogenesis, anti-HIV-1 activity, antiinflammatory qualities, antibacterial action, heart disease prevention, and blood sugar regulation. Furthermore, cinnamon's possess an ability to reduce inflammation and its primary ingredients in a different variety of tissues, such as the pancreas, liver, kidney, heart, and brain [9].

Essential oils extracted from plants have long been used to enhance the flavor of food and drink [10,11]. Oil from the bark of the cinnamon plant is typically utilized in food and drink. It is highly valuable in industry [12,13], Because of their positive health effects. Traditional plants are increasingly being used as supplemental medicines, including their ability to prevent diabetic mellitus and cardiovascular disease, as well as their anti-inflammatory and antioxidant properties [14,15]. It was discovered that in healthy adults, *Cinnamon burmannii* tea significantly decreased maximal glucose levels after meals and variance in maximum concentration [16].

There are numerous components.in Cinnamon such as Cinnamaldehyde, trans-cinnamaldehyde (Cin), and cinnamic acid, also known as aromatic aldehyde 3-phenyl-2(E)propenal, are the three main components of cinnamon [17]. As well as eugenol, linalool, cinnamic acid, and *cinnamaldehyde* [18]. It has offered some health benefits include reducing blood glucose and analgesic properties, as well as antioxidant, anti-cholesterolemic, antibacterial, antiinflammatory, anti-yeast, and anti-gastric ulcer properties [19]. Cinnamaldehyde was identified as the primary constituent of *Cinnamonum burmannii* oil (68.3%-82%), cinnamyl acetate (2.5%-16%), cinnamyl alcohol (2.25%-4.6%), and cinnamic acid (3%-8%) [20].

Several studies have reported that C. zeylanicum bark was rich in essential oil, while cinnamaldehyde is the main chemical compound that was isolated from *C. zeylanicum* bark [21,22,23,24]. Research showed that the *C. zeylanicum* contains thirty-eight components [4], Cinnamaldehyde was



the major compound (68.41%), followed by benzaldehyde. [25] main constituents of the C. zeylanicum leaf are eugenol (79.75%), transcinnamaldehyde (16.25%), Linalool (0.14%), cinnamaldehyde (75 %), cinnamyl acetate (5%), and eugenol (1-10 %). [18] identified thirty essential oil compounds, including cinnamic acid, eugenol, linalool, and cinnamaldehyde. [26] investigated the essential oil from the bark of C. zevlanicum and its antibacterial, antioxidant, and antiproliferative properties, and found that the following constituents were present: (E)-cinnamaldehyde (71.50 %), linalool (7.00 %), β-caryophyllene, eucalyptol, and eugenol. Moreover, limonene, a-pinene, b-cadinene, p-cymene, and ahumulene were important components. Other projectreported that the oil of cinnamon contains cinnamaldehyde (55-76%), eugenol and safrole_[27]. The aim of the study identified chemical compounds in C. zeylanicum and assess the preventive properties of it against liver.

II. MATERIALS AND METHODS

A. Plant material

This study was conducted in the Basrah University, Science College, Biology Department, Iraq. The GC-MS Chromatography carried at the Basrah University, Agriculture College of, Iraq..

B. Preparation of samples

The bark samples of *C. zeylanicum* were collected from the market of Basrah governorate. One hundred gram of the bark sample was washed with water, dried and ground in a grinder for 20 seconds. The small pieces were homogenized for 3 min to 40-mesh size. The air-dried sample of cinnamon was pulverized to get the powdered form [18].

C. Essential oils Extraction

A Clevenger apparatus was used to extract essential oils using water distillation for four hours at 100_°C, it was collected in sealed container to prevent any evaporation. Furthermore, Anhydrous sodium sulfate was used to dry the recovered oil, which was then placed in a dark glass container for analysis [28].

D. GC-MS analysis

GC-MS analysis was carried out by utilizing a Shimadzu GC-QP 2010 Ultra gas chromatograph. The temperature of the GC oven was set to rise at a rate of 4.3 °C per minute from 40 °C to 250 °C. The carrier gas was helium. The linear velocity was 48.1 cm/sec and the input pressure was 100.0 kPa. Column flow was 1.78 mL/min, Injector temperature: 250_{\circ} C; injection mode: split. MS scan conditions: source temperature, 200 °C; interface temperature, 250 °C; Detector Gain, 0.70 kV +0.10 kV; Scan speed, 1666 Start 50 m/z, End 800 m/z. The components of the Cinnamon oil were identified by comparing the spectra with those of known compounds stored in the NIST library (2005) (Figure 1-5).

E. The animals

In total, 80 adult male rats were employed in this study. They were in good health, approximately having the same weight $(30 \pm 5 \text{ gm})$ and 75 days in age having cages with three animals in each group. at a temperature of 18-25 °C under a 12_h dark–light cycle. The animals were left for one week before starting the treatments. The animals were

divided into 4 groups, with ten rats in each group. First, distilled water was given orally to the control group. Diethyl ether was used to anesthetize the animals following each treatment, fixed liver was taken for sectioning. Experimental design was approved by College of Science/ Basrah University. Twenty male adults healthy Two groups of rats were created.: Group 1 represented Control rats (n= 10) no treatment and Group 2: The animals were dosed orally with a concentration of 50mg/kg atenolol for thirty days (n=10).

F. Preparation of tissues

Tissue samples were fixed in equivalent formaldehyde solution (10%), then washed with tap water. The samples were then dehydrated by being passed through serious of ethanol from 30% up to 100%. Xylene was then applied for 30-40 minutes to clear the samples from ethanol. The tissues were subjected to paraffin infiltration using melted paraffin wax inside the oven at 60 °C for one hour. and the tissue samples were transferred from the oven inside these molds which were then cooled to room temperature. The tissue sections were stained by using hematoxylin for one minute then in alcoholic eosin 1% [29].

III. RESULTS AND DISCISSION

A. Chemical composition of the essential oil

Essential oils extracted by hydro-distillation from C. zeylanicum bark are light yellow in color and have a pungent odor at room temperature. The essential oil content of the bark extract of C. zeylanicum was determined by GC-MS as summarized in (Table 1). The structures of the major compounds are shown in (Figure 1). The results that the existence of 27 components in C. zeylanicum is demonstrated by GC/MS. phytochemical constituents). The chemical components of C. zeylanicum bark are essential oil which include Cinnamaldehyde (2-Propenal, 3-phenyl-) 46 %, followed by 9-Methoxybicyclo[6.1.0]nona-2,4,6-triene (31.31%), alpha.-Muurolene (7.14%), tau-Muurolol (1.34%) and Copaene (1.63%) (Table 1). Our results associated with some research by found eight compounds Cinnamaldehyde, tau.-Muurolol, Cubenol, Copaene, Naphthalene, (E)-, 1,2,3,5, 6, 8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-, Benzenepropanal, while we associated with [30] cinnamaldehyde (73.345%, Benzaldehyde, Borneol and Copaene. Our studied similar with Gotmare and Tambe (2019) three compounds cinnamaldehyde, Benzenepropanal and Benzaldehyde. Also found Trans-cinnamaldehyde (91.56%), cis-cinnamaldehyde (1.28%), eucalyptol (1.26%), Benzenepropanol (0.76%) , α -Muurolene (0.62%) and α -Cubebene, some researchs indicates that the main constituent of cinnamon bark oil is cinnamonaldehyde, on contrast the primary constituent of cinnamon leaf oil is eugenol [2].

The principal compound of *C. zeylanicum* is called 2-Propenal, 3-phenyl-, cinnamaldehyde, transcinnamaldehyde (Cin), and cinnamic acid, Cinnamal and cinnamic aldehyde [17]. Numerous researchers concluded that cinnamaldehyde is the main chemical component of cinnamon oil [17,31], Our findings support [32] recollection that 49.9% of cinnamon barks are essential oil.

The percentage of Cinnamaldehyde differ between the researchers, [33] showed the percentage of Cinnamaldehyde (91.82%) in India. In Malysia [34] reported trans-

cinnamaldehyde (84.97%) and Borneol (1.03%). While [35] recorded Trans-cinnamaldehyde (87.013%) in Ethiopia. it is found (45.13% and 64.84%) in Egypt [36, 37]. [38] reported that E-cinnamaldehyde has 88.2%. B-phellandréne (1.19%), E-cinnamaldehyde (85.77%), Z-cinnamaldehyde (3.22%), linalool (3.70%), and Eugenol (0.48%) [39].

Researchers have different estimates of how many chemical components are present in cinnamon essential oil. It was 4% of the essential oil, which is predominantly composed of 60-75% cinnamaldehyde [24]. [4] reported that C. zeylanicum consists of 38 components, the majority components (68.41%) was Cinnamaldehyde, followed by benzaldehyde. While [33] discovered that cinnamaldehyde made up about 91.82% of the composition of cinnamon oil extract, with eight compounds having small percentage. Cinnamic aldehyde was discovered to be the primary component in the essential oil extract when GC-MS analysis of C. zevlanicum essential oil was performed [40]. Cinnamaldehyde content in C. zeylanicum extracted was 90% [28]. While [41] identified thirty compounds. Other constituents include cinnamyl acetate, cinnamyl alcohol, eugenol, linalool, cuminaldehyde, and pinene [42].

Climate, soil, harvest season, drying technique, storage conditions, and even the specific portion of the plant tissue are examples of environmental variables. The amount of essential oil in cinnamon species (*Cinnamomum burmannii*) differs between the tree age of plant [20]. Different agricultural dates, origins, vegetable states, plant growth seasons, and market storage conditions can all affect the composition of essential oils [43,44]. Although there were discrepancies between the present study and past studies in the number of essential oils, the analytical results were compatible with earlier publications [45]. This may be brought on by various cultivars and extraction techniques. It could be because the microwave's heating effect caused the essential oil's cinnamic aldehyde to change into 2-methoxycinnamaldehyde [30].



Figure (1) Chromatogram of essential oil of C. zeylanicum bark.



Figure (5): Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1methylethyl)-, (1S-cis)-

Table (1): Compounds Identified By Mass Spectrometry
Gas Chromatography (Gc-Ms) In The Essential Oil Of C.
Zeylanicum Bark.

Peak	Components	Retention	Area %
	F	Time	
1	Benzaldehyde	9.194	0.10
2	Benzaldehyde dimethyl acetal	13.115	0.11
3	Benzenepropanal	14.455	0.04
4	Borneol	14.645	0.02
5	Cinnamaldehyde, (E)-	17.095	0.23
6	2-Propenal, 3-phenyl- or Cinnamal or	20.506	46.46
	cinnamaldehyde		
7	Consene	22 100	1.63
8	1 4-Methano-1H-indene_octabydro-4-	22.155	0.57
0	methyl_8_methylene_7_(1_	23.033	0.57
	methylethyl)- [1S-(1 a]		
9	9-Methoxybicyclo[6.1.0]nona-2.4.6-	26.155	31.31
-	triene		
10	2-Propenoic acid, 3-(2-	27.105	0.29
	hydroxyphenyl)-		
11	2-Propen-1-ol, 3-phenyl-, acetate, (E)-	28.005	0.21
12	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-	28.633	1.18
	octahydro-1,6-dimethyl-4-(1-		
	methylethyl)-, [1R-(1.alp		
13	.alphaMuurolene	31.155	7.14
14	Naphthalene, 1,2,3,5,6,8a-hexahydro-	32.729	5.95
	4,7-dimethyl-1-(1-methylethyl)-, (1S-		
1.5	C1S)-	22.215	0.60
15	Naphthalene, 1,2,3,4,4a,/-hexahydro-	33.315	0.69
16	Panzana 1 mathyl 4 [(1	22 921	1.04
10	methylethylidene)cyclopropyll	55.651	1.04
17	Carvophyllenyl alcohol	35 987	0.15
18	1-Hydroxy-1 7-dimethyl-4-isopropyl-	37.402	0.09
10	2.7-cvclodecadiene	37.102	0.09
19	Illudol	38.155	0.09
20	Epiglobulol	38.954	0.32
21	Naphthalene, 1,2,3,5,6,8a-hexahydro-	39.766	0.27
	4,7-dimethyl-1-(1-methylethyl)-, (1S-		
	cis)-		
22	Cubenol	40.781	0.38
23	1H-Cycloprop[e]azulene, decahydro-	41.205	0.06
	1,1,7-trimethyl-4-methylene-		
24	tauMuurolol	42.893	1.34
25	Cycloheptane, 4-methylene-1-methyl-	44.891	0.22
26	2-(2-methyl-1-propen-1-yl)-1-vinyl-	51.246	0.07
26	2-Butanone, 4-(2,6,6-trimethyl-2-	51.346	0.07
27	cyclonexen-1-ylidene)-	52 011	0.07
21	2,3,3,8a-1 etramethyl-4-methylene-	55.911	0.07
	4a-yl hydropero		
			100.00
		1	100.00

B. Histopathology of the liver

Examinations of liver sections under a light microscope for histopathology found that when compared to the control group, the rat given doses of the essential oil extract showed a number of alterations. In the section in rat liver from group treated with *C. zeylanicum* referred to dilated branch of portal duct, hepatocytes with cytoplasmic vacuolation, some hepatocytes were swollen with pyknotic, large nuclei and dilated sinusoids (Figure 6 A). As well as in the rats treated with noticed sever congestion of central vein (CV) (Figure 6 B), degeneration and sluggish of endothelial lining larger, Bile ducts hyperplasia and necrotic hepatocytes (Figure 6 C). A section of the rat liver from the *C. zeylanicum*-treated group revealed vacuolation in most hepatocytes, congested central vein, dilated sinusoid, complete degeneration of endothelial lining layer of central vein and the parenchyma tissue lost normal structure (Figure 6 D).

The histological sections taken from the livers of animals dosed orally from the volatile oil extract of cinnamon at a concentration of 20 mg/kg showed congestion, inflammations around central vein in treated rat after 30 days and showed congestion around portal triads vein (Figure 6); as well as found necrosis and degenerations liver cells in addition inflammation of cells after 30 days, In addition. One of the mechanisms of plant extracts is the process of inhibiting the synthesis of purine and pyrimidine [46,47]. Under a microscope, liver sections were examined for histological alterations such as When compared to the control, there was greater inflammation and damage of the liver architecture [48].



Figure -6: Photomicrographs of H and E stained liver sections from rat treated with essential oil extracts of *C. zeylanicum* (A): Section in rat liver from group treated with *C. zeylanicum* referred to dilated branch of portal duct (\longrightarrow), hepatocytes with cytoplasmic vacuolation (\longrightarrow), some hepatocytes was swollen with pyknotic, large nuclei (\longrightarrow) and dilated sinusoids (\longrightarrow). B: Section in rat liver from group treated with *C. zeylanicum* showed sever congestion in hepatic portal vein and blood vessels (\longrightarrow), hyperplasia in lining layer of bile duct (\longrightarrow), destruction of most hepatocytes (\leftrightarrow) and necrotic stromal tissue(\longrightarrow). D: Section in rat liver from group treated with *C. zeylanicum* and sluggish of endothelial lining larger (\longrightarrow). Bile ducts hyperplasia (\checkmark) and necrotic hepatocytes (\leftrightarrow). D: Section in rat liver from group treated with Cinamonum showed vacuolation (\longrightarrow) in most hepatocytes, congested central vein (\longrightarrow), dilated sinusoid (\longrightarrow), complete degeneration of endothelial lining layer (*M*) of central vein (*M*) of central vein (*M*) of central vein (*M*) and necrotic hepatocytes (\longrightarrow).

IV. CONCLUSION

From the current investigation, it can be deduced that the main component of cinnamon bark oil is cinnamaldehyde. Consequently, cinnamaldehyde may be thought of as a component used to identify cinnamon bark oil. The liver displayed several tissue changes, including hepatocyte necrosis and the existence of steatosis foci. Histological and cellular changes in liver tissue were dramatically attenuated by *C. zeylanicum* essential oil. From this investigation, we may infer that essential oil of *C. zeylanicum* possesses hepatoprotective properties [39].

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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