Studies on GC/MS Spectroscopic Analysis of some Bioactive Antimicrobial Compounds from *Cinnamomum zeylanicum*

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Abstract

Background: Infectious diarrhoea is an emergent problem in both developing and developed countries for high rates of mortality in infants. In recent years, drug resistance to microbial pathogens has been commonly reported from all over the world. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries especially in India and are a source of many potent and powerful drugs. This study was aimed to explore the bioactive antimicrobial compounds present in *Cinnamon zeylanicum*, which is used as a spice in Indian foods.

Objective: The aim of this study was to carry out Gas chromatography and Mass spectroscopy studies and to determine the antimicrobial activity of *Cinnamon zeylanicum* extract against some common microbial pathogens isolated from diarrhoeal patients.

Methods: The extract was prepared by the method of maceration using methanol as extraction solvent. The antimicrobial activity was performed by disc diffusion method at the concentration 200, 100, 50, 25 and 10 mg/ml against *E.coli, Salmonella typhi, Salmonella typhimurium, Shigella dysenterae, Shigella flexneri, Pseudomonas aeruginosa* and *Klebsiella pneumonia* and the fungi *Candida albicans, Candida tropicalis, Candida krusei* which are isolated from the paediatric diarrhoeal samples. Gas chromatography and Mass spectroscopy studies were performed to identify the bioactive compounds.

Results: The methanol extract was found very sensitive against all the isolated organisms. The preliminary phytochemical screening shows the presence of carbohydrate, glycosides, phenolic compounds, tannins, terpenes and proteins. The GC/MS study on the active principles shows the presence of 38 components.

Conclusion: This study shows that the *Cinnamon zeylanicum* extract exhibit high antifungal activity as compared with antibacterial activity. The most identified compound by GC/MS to have antimicrobial property were monoterpenes, sesquiterpenes, aromatic aldehydes and ketones. Cinnamaldehyde was the major compound responsible for the antimicrobial activity.

Keywords: **Antimicrobial activity, *Cinnamon zeylanicum*, Gas chromatography/Mass Spectroscopic Studies**

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Introduction

In developing countries like India, the majority of people living in rural areas were almost exclusively using traditional medicines in treating all sorts of disease including diarrhea [1]. Infectious diarrhea is one of the principal causes of death in the infants in developing countries particularly in malnourished and unhygienic conditions. It thus becomes important to identify and evaluate commonly available medicinal plant to treat infectious diarrhea as alternative to currently used antimicrobial drugs. Several studies have evaluated the antimicrobial properties in some traditional medicines in treating infectious diarrhea [2]. *Cinnamon zeylanicum* Blume (Family. Lauraceae) is a small evergreen tree native to tropical southern India and Sri Lanka., growing from sea level to nine hundred meters. *Cinnamon* barks are widely used as a spice. It has also been used to treat diarrhea and other problems of the digestive system and as a component of compounds used in Indian Ayurvedic medicine [3]. The *Cinnamon* bark tea infusion was used for complaints such as a feeling of distension, flatulence, and mild cramp-like gastrointestinal disorders due to reduced production of gastric juice. *Cinnamon* bark is also traditionally used to treat symptoms of digestive disorders, functional asthenias, and also to facilitate weight gain [4]. Many publications have documented the antimicrobial activity of *Cinnamon* against different microbial spices [5-8]. The *Cinnamon* bark contains volatile oils (14%) of cinnamaldehyde (60%), eugenol (up to 10%) and *trans*-cinnamic acid (51%); phenolic compounds (41%), condensed tannins, catechins, and proanthocyanidins; monoterpenes and sesquiterpenes, (pinene); calcium-monoterpenes oxalate; gum; mucilage; resin, starch, sugars, and traces of coumarin [9]. The previous GC/MS studies on *Cinnamon* bark have shown that the major constituents were cinnamaldehyde and cinnamic acids [10]. The objective of the present study was to determine the antimicrobial activity of *Cinnamon* bark against various diarrheal pathogens isolated from patients and to Analysis the composition of alcoholic extract of dried fruits of *Cinnamon zeylanicum* by GC/MS. Although a lot of work has been reported in the literature by different workers on the chemical composition *Cinnamon zeylanicum* conventional methods and by GC/MS, yet it is the first time that the characterization has been carried out in *Cinnamon zeylanicum* by GC/MS in our region.

Material and Methods

Plant Materials

Cinnamon zeylanicum dried barks were collected from Kodaikanal area in Tamilnadu, India, and were identified and confirmed the by Department of Botany, Annamalai University, Annamalainagar.

Extraction of plant materials

The barks were shade dried, powered and were extracted with methanol using a maceration process and with occasional shaking for 3 days. The extract was then filtered, dried at 50 to 60 ^{o} and the residue was weighed and percentage yield was calculated and subjected to preliminary phytochemical analysis. The residue was dried and stored in air tight container.

Antibiotic testing of plant extract

The Micro-organism used were *E. coli*, *Salmonella typhi*, *Salmonella typhimurium*,

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Shigella dysenterae, *Shigella flexneri*, *Pseudomonas aureginosa* and *Klesiella pneumonia* and the fungi *Candida albicans, Candida tropicalis, Candida krusei* which are isolated from the diarrhoeal samples from the paediatric patients. The dried extracts were reconstituted with 5% aqueous dimethyl sulphoxide (DMSO) at concentration of 500mg/ml. plant extracts were then diluted at a concentration of 200, 100, 50, 25 and 10 mg/ml in 5% aqueous DMSO. The antimicrobial assay was performed by disc diffusion method. Ciprofloxacin (10 mcg/disc) and Fluconazole (10 mcg/disc) were used as positive control for bacteria and *Candida* respectively and 5% DMSO impregnated disc was used as negative control. The plates were incubated at 37^{o}C for 24 to 48 hours. The results were recorded by measuring the zones of inhibition. The experiment was performed in triplicate and the mean values of results are given in Table 1.

GC/MS analysis of plant materials

The methanol extract was filtered with sodium sulphate [2g] and concentrated the extract to 1ml by bubbling nitrogen into the solution. The extracted material was taken for GC MS analysis. The Gas chromatography–Mass spectroscopy (Agilant 6890/Hewlett-packard 5975) was fitted with electron impact (EI) mode. The Helium was used as the carrier gas at a flow rate of 1mL/min. The temperature was programmed at 80^{o}C for 5 min then increased to 300^{o}C at the rate of 15^{o}C/min. The temperature of injector and EI detector (70eV) were 280^{o}C and 300^{o}C, respectively. Each plant extract of 29µL was injected with a Hamilton syringe to the GC/MS manually.

Table 1- Antimicrobial activity of *Cinnamon zeylanicum*

Microorganisms	Diameter of zones of inhibition in mm						
	Methanol extracts in mg/ml conc.					Ciprofloxacin	Fluconazole
	200	100	50	25	10	(5µg/disc)	(10µg/disc)
K. pneumonia	3+	2+	1+	-	-	26	-
Ps. aureginosa	3+	2+	1+	-	-	26	-
E. coli	4+	3+	2+	1+	-	24	-
S. typhimurium	4+	3+	2+	1+	-	24	-
S. enteritidis	4+	3+	2+	1+	-	22	-
S. typhi	4+	3+	2+	1+	-	30	-
Sh. dysenteriae	4+	3+	2+	1+	-	28	-
Sh. flexneri	4+	3+	2+	1+	-	24	-
C. albicans	3+	2+	1+	-		-	20
C. tropicalis	3+	2+	1+	-		-	20
C. krusei	3+	2+	1+	-		-	20

1+ indicates zone of inhibition in average of 7 to 10 mm; **2+** indicates zone of inhibition in average of 11 to 14 mm; **3+** indicates zone of inhibition in average of 15 to 18 mm; **4+** indicates zone of inhibition in average of 19 to 22 mm; **-** No activity

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Results and Discussion

The methanol extract exhibits a good antimicrobial activity against all the micro-organisms tested, was shown in Table 1. The zone of inhibition exhibited by the alcohol extract was comparable with standard antibiotics. This study shows that the *Cinnamon zeylanicum* extract exhibit high antifungal activity when compare with antibacterial activity. However the concentration of extract required for activity against *Candida, Klebsiella* and *Pseudomonas spp*. were very higher than the other pathogens tested.

Many studies support the above findings [5-8]. The percentage yield obtained was 15.4%. The preliminary phytochemical screening shows the presence of carbohydrate, glycosides, phenolic compounds, tannins, flavonoids and proteins. The studies on the active principles content in *Cinnamon zeylanicum* by GC/MS clearly shows the presence of 38 components (Table 2). The most identified compound to have antimicrobial property were monoterpenes, sesquiterpenes, aromatic aldehydes and ketones. Cinnamaldehyde was the major compound (68.41%), followed by benzaldehyde. The fragrance of plants was mainly due to presence of essential or volatile oil fractions. *Cinnamon* bark was rich in essential oil which possesses activity against fungi and bacteria due to the presences of antimicrobial compounds. These oils are secondary metabolites that are highly enriched in compounds like cinnamaldehyde, cinnamic acid, benzaldehyde, eugenol, benzoic acid, monoterpenes, triterpenes, and sesquiterpenes [11]. The inhibitory activity by *Cinnamon* extract could be due to the presence of cinnamaldehyde, benzaldehyde and terpenes. It was reported that 60% of essential oil derivatives examined were inhibitory to fungi while 30% inhibited bacteria [11]. The fungicidal effect of *Cinnamon* extract is due to the presence of natural products that protects the plant from various diseases and pests. The mechanisms involved are cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular enzymes. It has been reported that lytic enzymes act on the fungal cell wall, causing breakge of β-1,3glycan, β-1,6glycan and chitin polymers [12].

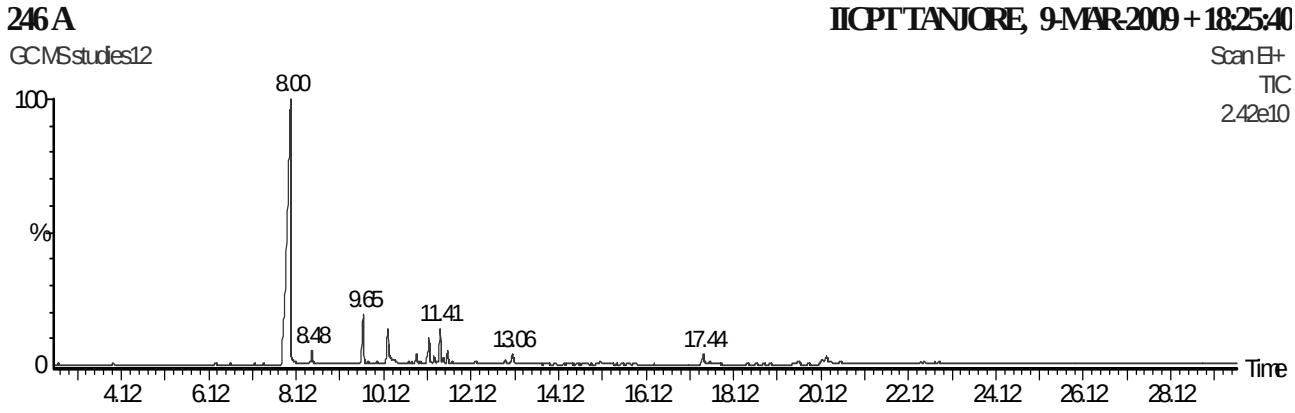

Figure 1- GC/MS spectrogram for the *Cinnamon zeylanicum* methanol extract

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Table 2- Activity of Phytocomponents identified in the plant *cinnamon zeylanicum* (GC-MS Study)

No	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature
1	6.62	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	$C_{10}H_{18}O$	154	0.09	Monoterpene alcohol
2	6.89	Naphthalene	$C_{10}H_8$	128	0.04	Aromatic compound
3	7.36	Benzenepropanol	$C_9H_{12}O$	136	0.11	Aromatic alcohol
4	8.00	Cinnamaldehyde, (E)-	C_9H_8O	132	68.41	Aldehyde
5	8.48	4-(1-Hydroxyethyl)benzaldehyde	$C_9H_{10}O_2$	150	1.54	Aromatic Aldehyde
6	9.65	Copaene	$C_{15}H_{24}$	204	5.27	Sesquiterpene
7	9.76	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]-	$C_{15}H_{24}$	204	0.17	Sesquiterpene
8	9.98	1,4-Methano-1H-indene, octahydro-4-methyl-8-methylene-7-(1-methylethyl)-, [1S-(1à,3aá,4à,7à,7aá)]-	$C_{15}H_{24}$	204	0.25	Sesquiterpene
9	10.22	2H-1-Benzopyran-2-one	$C_9H_6O_2$	146	5.27	Ketone compound
10	10.70	à-Caryophyllene	$C_{15}H_{24}$	204	0.22	Sesquiterpene
11	11.16	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1à,4aà,8aà)-	$C_{15}H_{24}$	204	2.99	Sesquiterpene
12	11.27	2-Propenal, 3-(2-methoxyphenyl)-	$C_{10}H_{10}O_2$	162	0.56	Aldehyde
13	11.41	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	$C_{15}H_{24}$	204	3.22	Sesquiterpene
14	11.48	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1à,4aá,8aà)-(ñ)-	$C_{15}H_{24}$	204	0.38	Sesquiterpene
15	11.58	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	$C_{15}H_{24}$	204	1.14	Sesquiterpene
16	11.69	à-Calacorene	$C_{15}H_{20}$	200	0.31	Hydrocarbon
17	12.24	4aH-Cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1aà,4á,4aá,7à,7aá,7bà)]- (Palustrol)	$C_{15}H_{26}O$	222	0.37	Sesquiterpene alcohol
18	12.38	Caryophyllene oxide	$C_{15}H_{24}O$	220	0.11	Sesquiterpene oxide
19	12.59	3,7-Cycloundecadien-1-ol, 1,5,5,8-tetramethyl-	$C_{15}H_{26}O$	222	0.17	Sesquiterpene alcohol
20	12.90	Cubenol	$C_{15}H_{26}O$	222	0.51	-do-
21	13.06	tau.-Muurolol	$C_{15}H_{26}O$	222	1.35	-do-
22	13.23	à-Cadinol	$C_{15}H_{26}O$	222	0.10	-do-
23	13.50	à-Bisabolol	$C_{15}H_{26}O$	222	0.09	-do-
24	14.37	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.07	Fatty acid
25	14.69	Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane], 1-methyl-4-isopropyl-7,8-dihydroxy-, (8S)-	$C_{15}H_{24}O_3$	252	0.11	Dihydroxy compound
26	14.77	cis-9-Hexadecenal	$C_{16}H_{30}O$	238	0.07	Aldehyde

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Continue Table 2- Activity of Phytocomponents identified in the plant *cinnamon zeylanicum* (GC-MS Study)

No	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature
27	14.87	Isolongifolene, 7,8-dehydro-8a-hydroxy-	$C_{15}H_{24}O$	220	0.02	Hydroxy compound
28	15.09	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	0.72	Unsaturated fatty acid ester
29	15.85	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.09	Saturated fatty acid
30	17.44	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.63	Palmitic acid
31	17.58	Palmitic acid vinyl ester	$C_{18}H_{34}O_2$	282	0.42	Ester compound
32	17.75	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.17	Fatty acid ester
33	19.62	E-2-Octadecadecen-1-ol	$C_{18}H_{36}O$	268	0.56	Alkane compound
34	20.15	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.80	Linoleic acid
35	20.25	Oleic Acid	$C_{18}H_{34}O_2$	282	1.84	Oleic acid
36	20.57	Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.33	Stearic acid
37	20.74	Stearic acid hydrazide	$C_{18}H_{38}N_2O$	298	0.14	Nitrogen compound
38	22.81	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356	0.36	Ester compound

A study shows that the Cinnamaldehyde was completely inhibiting both sensitive and resistant strain of *Helicobacter pylori* [13]. Terpenes are phenolic compounds that exhibit the antimicrobial activity and mostly mono and sesquiterpenes are active against bacteria, fungi, virus and protozoa [14]. The terpenes observed in this study, consist of one monoterpene (Bicyclo [2.2.1] heptan-2-ol, 1, 7, 7-trimethyl-, (1S-endo)-), and 11 sesquiterpenes. Some of them were Copaene, Cyclohexane,1- ethenyl -1- methyl - 2, 4- bis (1-methyl ethenyl)-, [1S-(1à,2á,4á)]-, and Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-, and that exhibit the antimicrobial activity. Terpenes are found in latex and resins of some plants and physiological function of these compounds is generally believed to be a chemical in defense against certain pathogens causing human and animal disease [15]. Their activity is a function of the lipophilic properties of the constituents terpenes, the potency of their functional groups and their aqueous solubility [16].

Summarizing these results, we conclude that the antimicrobial activity of the *Cinnamon zeylanicum* was mainly due to presence of cinnamaldehyde, benzaldehyde, and phenolic compounds monoterpenes, triterpenes and sesquiterpenes. These compounds can used as an antidiarrhoeal agent against infective diarrhoeal after successful invivo analysis.

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