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RESEARCH ARTICLE

Anti-bacterial Effect of Essential Oils Extracted from Selected Spices of *Zingiberaceae*

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Abstract: ***Background*:** Plants are rich source of therapeutic phytochemicals used for treating various ailments. In specific, spices are used in Indian and Chinese system of medicine. Although spices are traditionally used as food preservatives besides food color and flavor, their medicinal values remain unrecognized. This paper discusses the antibacterial activities of essential oil samples extracted from selected spices (cardamom, ginger and turmeric) belonging to *Zingiberaceae*, the ginger family.

***Methods*:** The essential oils from rhizomes of ginger (*Zingiber officinale* R.) and turmeric (*Curcuma longa* L.) as well as capsules of cardamom (*Elettaria cardamom* M.) were extracted by hydrodistillation. The oil samples were analyzed by gas chromatography/mass spectrometry (GC/MS). The chemical composition was identified based on the retention index, and also by co-injection with authentic standards. The oil samples were also evaluated for their antibacterial activities.

***Results*:** The major composition of cardamom is 1,8-cineole (40.11%) and α-terpinyl acetate (39.24%). In the case of ginger, it is dominated by zingiberene (32.01%) followed by β–sesquiphellandrene (16.25%) and farnesene (12.52%). In turmeric, the major composition is found to be turmerone (32.55%) followed by ar-turmerone (23.15%) and curlone (23%). Besides other compounds such as 1,8-cineole, α-curcumene, α-terpinene, α-thujene, β-myrcene, β-sesquiphellandrene, γ-terpinene, limonene, linalool, terpinolene, (Z)-citral and zingiberene were present in varying levels.

***Conclusion*:** The spice oil samples were more effective against Gram positive than Gram negative bacteria. The constituents of cardamom oil are highly antibacterial than ginger and turmeric oils. It was found to inhibit *Lactobacillus sp*., a probiotic Gram positive bacterium. The probiotic inhibitory potential of essential oils is in the following order: ginger > cardamom > turmeric. Although essential oils are generally recognized as safe, it may potentially inhibit probiotic bacteria. Hence, more research is required in this direction.

Keywords: Essential oil, Zingiberaceae, anti-bacterial, cardamom, ginger, turmeric.

1. INTRODUCTION

In Indian and Chinese system of medicine, spices are used as a constituent of several therapeutic formulations. Many spices used in medicine have multiple bioactive principles. The curative properties of natural products are due to the presence of complex chemical substances. Secondary metabolites form the major component of "essential oil" extracted from different parts of the plants [1].

The spice essential oils from the family *Zingiberaceae* demonstrated effective antimicrobial properties [2-4]. They are used for centuries to extend the shelf life of foods and also as seasonings [5-8] and are used to prevent food spoilage due to antimicrobial and antioxidant activities [9, 10]. Due to emerging antibiotic resistance among microorganisms, it is of interest to search for new inhibitory compounds preferably from plant origin. There are numerous *in vitro* studies on the use of plant extracts in combination with antibiotics that act against resistant strains [11].

The medicinal effects of the essential oils are often less well studied than polyphenols [12]. It was demonstrated that the individual oil components (mainly with phenolic compounds) exhibited wide spectrum of antibacterial activity [13]. Besides polyphenols, there are alkaloids, phenolics, and terpenoids, among others. Terpenoids are of interest, they occur as monoterpenes, diterpenes, triterpenes, and tetraterpenes (C10, C20, C30, and C40, respectively), as well as hemiterpenes (C5) and sesquiterpenes (C15). They are termed as 'terpenoids' if it contains an additional element usually oxygen. The mechanism of action of terpenes is not fully understood, it is predicted that due to their lipophilicity it may act by disrupting the bacterial membrane [14].

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There are reports to state that the effect of essential oils is due to combined effect of compounds contained in them rather than the individual components [15, 16]. It is also stated that the individual components are not as active as the essential oils [16]. Moreover, to assess individual components from herbal extracts is complex because many contain mixtures of compounds and exist in varied forms [17]. Hence, it is of research interest to verify whether single compound has any antibacterial effect? For instance, in food industry essential oil is used as food preservatives to inhibit foodborne pathogens but high concentration may lead to undesirable organoleptic effect [18], this can easily be overcome by isolated compounds. If such isolated compound has an important pharmacological activity, it would be of great interest to pharmaceutical and food industry.

The present study is intended to understand the chemical composition of the essential oils of major spices of medicinal interest (cardamom, ginger, and turmeric belonging to *Zingiberaceae*) as well as to evaluate their antibacterial activities against selected Gram-positive and negative bacteria. An attempt was also made to answer whether a single compound has any antibacterial effect? The results are discussed.

2. MATERIALS AND METHOD

2.1. Plant Material

Fresh rhizomes of ginger and turmeric, as well as capsules of cardamom were collected during May 2008 from the Indian Institute of Spices Research - Peruvanamulzhi and Appangala farms.

2.2. Chemicals & Reagents

All chemicals and reagents used were of analytical grade, procured from Merck Chemicals (Trichur, Kerala, India). Sterile distilled water was used, whenever required for the experiment.

2.3. Oil Extraction

The collected fresh rhizomes and the capsules were shade-dried. Then the samples were powdered using pestle and mortar. The powdered samples were weighed. From the weighed samples, 30 g of turmeric powder, 25 g of ginger powder and 20 g of cardamom powder were separately hydro-distilled using a Clevenger-type apparatus [19] for 3.5 hours. At the end of each distillation, the oil samples were collected and dried over anhydrous sodium sulfate and stored in refrigerator at low temperature until analyzed.

2.4. Chemical Identification

The oil samples were analyzed by Shimadzu GC-2010 gas chromatograph equipped with QP 2010 mass spectrometer. RTX–wax (cross bond-PEG) column (30 m x 0.25 mm, film thickness 0.25 μm) was used. Helium was used as the carrier gas at a flow rate of 1.00 mL/min. The injection port was maintained at 250^{o}C; the detector temperature was 220^{o}C. The oven was programmed as follows: 60^{o}C for 5 minutes, up to 110^{o}C @ 5^{o}C/min, up to 200^{o}C @ 3^{o}C/min and up to 220^{o}C@ 5^{o}C/min, at which the column was maintained for 5 minutes. The split ratio was 1:40 and ionization energy 70eV. The chemical composition was identified based on the retention index, library MS search (NIST 05 and Wiley 139), and also by co-injection with authentic standards. The relative amounts of individual components were calculated based on FID response in GC without using correction factors.

2.5. Bacterial Culture

To screen for antibacterial activity, Gram-negative organisms such as *Escherichia coli* (MTCC 1687), *Pseudomonas aeruginosa* (MTCC 1688), *Serratia marcescens* (MTCC 97) and *Salmonella typhimurium* (MTCC 3224) along with Gram-positive organisms such as *Staphylococcus epidermidis* (MTCC 435) and *Lactobacillus* sp. (MTCC 4183) were procured from the Institute of Microbial Technology (IMTECH), Chandigarh.

2.6. Disk Diffusion Method

Kirby-Bauer method was used [20]. In this test, a number of small, sterile Whatman's No. 1 filter paper disks of uniform size (~5 mm) were placed on the surface of agar plates previously inoculated with 10 μL of bacteria to be tested. The filter paper discs have been impregnated with the essential oil samples of cardamom, ginger, and turmeric, respectively. The positive control (antibiotic) used was chloramphenicol (30μg disk) and distilled water as negative control. The diameter of the zone was measured (in millimeters). If there is no inhibition, the organism is reported as resistant (R) to the antibacterial agent in that disc. If a zone of inhibition surrounds the disk, the organism is susceptible (S) to the antibacterial agent being tested.

2.7. Serial Dilution for Minimum Inhibitory Concentrations

Minimum inhibitory concentration (MIC) of nerolidol was performed by serially diluting with 70% ethanol, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 1.95, 0.975 and 0.48 μg/mL. The susceptibility of the test bacterium was screened along with negative controls, water, and ethanol. Paper discs were placed on the agar surface and carefully soaked the discs with different concentrations of samples. After 30 minutes the culture plates were carefully transferred and incubated at 37^{o}C for 24h. The lowest concentration of the sample showed no visible growth, this was determined as MIC.

3. RESULTS AND DISCUSSIONS

The yield and composition of the essential oils were analyzed from turmeric, ginger, and cardamom. Cardamom yielded highest concentration of essential oil (6 % v/w) followed by ginger (4 % v/w) and turmeric (1.6 % v/w). The antibacterial activities of essential oils were investigated by challenging against selected Gram-positive and Gram-negative bacteria. Only cardamom oil exhibited wide spectrum of inhibition against the selected set of bacteria (Table **1**), others were effective only against the selected Gram-positive bacteria and not against the chosen Gram-negative bacteria.

Table 1. Agar disk diffusion assay; '+' indicates < 3mm ; '++' indicates >3mm; '+++' indicates >13mm.

Medicinal Plants	Vol	Antibacterial Activity					
		Gram-Negative				Gram-Positive	
		Serratia marcescens	*Pseudomonas aeruginosa*	*Escherichia coli*	*Salmonella typhimurium*	*Staphylococcus epidermidis*	*Lactobacillus sp.*
Cardamom	10 µL	+	+	++	++	++	++
Ginger	10 µL	-	-	-	-	++	+++
Turmeric	10 µL	-	-	-	-	+	+

3.1. Chemical Profiling

To correlate the antibacterial activities of essential oils with their chemical components, chemical profiling was done using GC/MS. The essential oil components of cardamom, ginger, and turmeric were confirmed over 90% similarities and are listed in Table **2**.

Eighteen components were identified from cardamom, the major compounds were 1,8-cineole (40.11%) followed by α-terpinyl acetate (39.24%) fractions. This is responsible for the characteristic aroma of cardamom [21-24]. In ginger, nineteen components were identified; zingiberene (32.01%), β-sesquiphellandrene (16.25%) and farnesene (12.52%) were prevalent in agreement with Natta *et al.* [25]. Whereas in the case of turmeric, thirteen components were identified, turmerone (32.55%), ar-turmerone (23.15%) and curlone (23%) were abundant and are in congruence with Leela *et al.* [26].

From Table **2**, it is clear that the compounds such as α-curcumene, β-sesquiphellandrene, limonene, and zingiberene, are in common to turmeric and ginger. Whereas, compounds such as α-pinene, α-terpinene, β-myrcene, nerolidol, linalool and (*Z*)-citral are commonly present in ginger and cardamom. Whereas, compounds like α-thujene, 1,8- cineole, terpinene, and terpinolene are in common to turmeric and cardamom. The convergence of major compounds in cardamom, ginger, and turmeric is graphically depicted in Fig. (**1**).

As a result of this comparative investigation of the essential oils of cardamom, ginger, and turmeric, it was found that more similarity in these oil samples as they belong to the same family. In this study, the ginger and turmeric rhizome oil is typically characterized by monoterpenoids and sesquiterpenoids with high content especially zingiberene and β-sesquiphellandrene. This is in agreement with Onyenekwe and Hashimoto [27]. While many of the identified compounds in the capsules of cardamom such as alcohols, esters, and aldehydes, which are commonly found in other spice oils too. Although there is a convergence of compounds in the essential oil samples, there are quantitative differences in the composition of 1,8-cineole, α-curcumene, α-terpinene, α-thujene, β-myrcene, β-sesquiphellandrene, γ-terpinene, limonene, linalool, terpinolene, (*Z*)-citral and zingiberene of the essential oils from the selected spices.

3.2. Antibacterial Activity

The major components of these essential oils were monoterpenoids (C10) and sesquiterpenoids (C15). The former includes 1, 8-cineole, α-phellandrene, α-pinene, α-terpineol, 4-terpineol, β-citronellol, β-myrcene, β-pinene, camphene, limonene, linalool, borneol, terpinene, nerol, cymene, sabinene, terpinolene, and α-terpinene, whereas the latter includes α-curcumene, ar-turmerone, β-bisabolene, citral, curlone, farnesene, germacrene D, nerolidol, turmerone, and zingiberene. Due to the presence of terpenes, the essential oils may act on the cytoplasmic membrane of bacteria, disrupts the cell contents as well as the proton motive force [28]. It was observed that Gram-negative bacteria showed resistance [no inhibition zone] to the oil samples than Gram-positive bacteria, in agreement with Zaika [29]. This is due to the compositional differences in the cell structure between Gram-positive and Gram-negative bacteria. In addition to the cell wall and cell membrane, Gram negative bacteria have an outer lipid bilayer that acts as a protective barrier against these active compounds. In contrast, Gram-positive bacteria lack the outer protection and therefore they are more susceptible to these compounds [30]. Our results were congruent with Bosnic *et al.* [15] where they found *P. aerugionsa* was most resistant to essential oils. This trend is reflected for ginger and turmeric oils, but not in cardamom oil due to its wide spectrum of inhibition. Neither aqueous extract nor essential oils of ginger and turmeric is effective against *E.coli*, in agreement with [25, 31]. This is similar to the case of *S. typhimurium.* This may be due to antimutagenicity of turmeric oil as evaluated by the Ames test using *S. typhimurium* TA100 [32]. The antimutagenic activity of turmeric both *in vitro* and *in vivo* is well known [33] and may be attributed to the major compounds like aromatic (ar)-turmerone in the oil.

3.3. Probiotic Inhibitory Potential

Often very high inhibitory activities are attributed to essential oils due to combined effect of compounds rather than individual components. It is also stated that the individual components are not as active as the essential oils [16]. Hence, it is of interest to verify whether a single compound has any antibacterial effect? We made an attempt to isolate active components by preparative TLC. That was not successful due to instability of each component. Therefore, nerolidol was chosen for further antibacterial studies as it is

Table 2. GC-MS chemical profile of cardamom, ginger, and turmeric oil samples. The retention time, the area percentage and the chemical components are also shown.

Sl. No	CAS Numbers	Components	Cardamom		Ginger		Turmeric	
			R Time	Area %	R Time	Area %	R Time	Area %
1	470-82-6	1,8-cineole	11.23	40.11	--	--	7.373	1.93
2	543-49-7	2-heptanol	--	--	10.834	0.14	--	--
3	562-74-3	4-terpineol	16.152	2.25	--	--	--	--
4	110-93-0	6-methyl-5-heptene-2-one	--	--	11.253	0.43	--	--
5	644-30-4	α-curcumene	--	--	24.95	2.22	24.807	1.11
6	502-61-4	α-farnesene	--	--	24.318	12.52	--	--
7	80-56-8	α-pinene	7.48	1.87	3.264	1.19	--	--
8	4221-98-1	α-phellandrene	--	--	--	--	6.194	4.47
9	99-86-5	α-terpinene	10.473	0.37	9.605	0.16	--	--
10	98-55-5	α-terpineol	16.663	3.44	--	--	--	--
11	8007-35-0	α-terpinyl acetate	22.813	39.24	--	--	--	--
12	3917-48-4	α-thujene	7.224	0.19	--	--	3.273	0.12
13	495-61-4	β-bisabolene	--	--	23.498	5.87	--	--
14	106-22-9	β-citronellol	--	--	24.628	0.96	--	--
15	123-35-3	β-myrcene	9.519	2.08	6.15	1.22	--	--
16	20307-83-9	β-sesquiphellandrene	--	--	24.896	16.25	24.689	1.76
17	127-91-3	β-pinene	9.008	0.28	--	--	--	--
18	99-85-4	γ-terpinene	11.981	0.84	--	--	8.503	0.11
19	532-65-0	ar-turmerone	--	--	--	--	40.338	23.15
20	507-70-0	borneol	--	--	22.222	1.49	--	--
21	79-92-5	camphene	--	--	3.918	4.8	--	--
22	5392-40-5	(Z)-citral	19.433	0.37	21.526	2.87	--	--
23	82508-14-3	curlone	--	--	--	--	39.884	23
24	105-87-3	geranyl acetate	23.697	1.13	--	--	--	--
25	23986-74-5	germacrene D	--	--	22.806	1.5	--	--
26	138-86-3	limonene	--	--	7.084	1.05	7.094	0.2
27	78-70-6	linalool	13.426	1.23	17.551	1.07	--	--
28	144-39-8	linalyl propionate	--	--	22.119	0.38	--	--
29	106-25-2	nerol	18.841	1.84	--	--	--	--
30	7212-44-4	(Z)-nerolidol	30.665	0.46	33.691	1.25	--	--
31	99-87-6	p-cymene	--	--	--	--	9.225	0.69
32	3387-41-5	sabinene	8.907	3.14	--	--	--	--
33	586-62-9	terpinolene	13.005	0.37	--	--	9.633	0.21
34	82508-15-4	turmerone	--	--	--	--	38.062	32.55
35	495-60-3	zingiberene	--	--	23.292	32.01	23.115	1.83

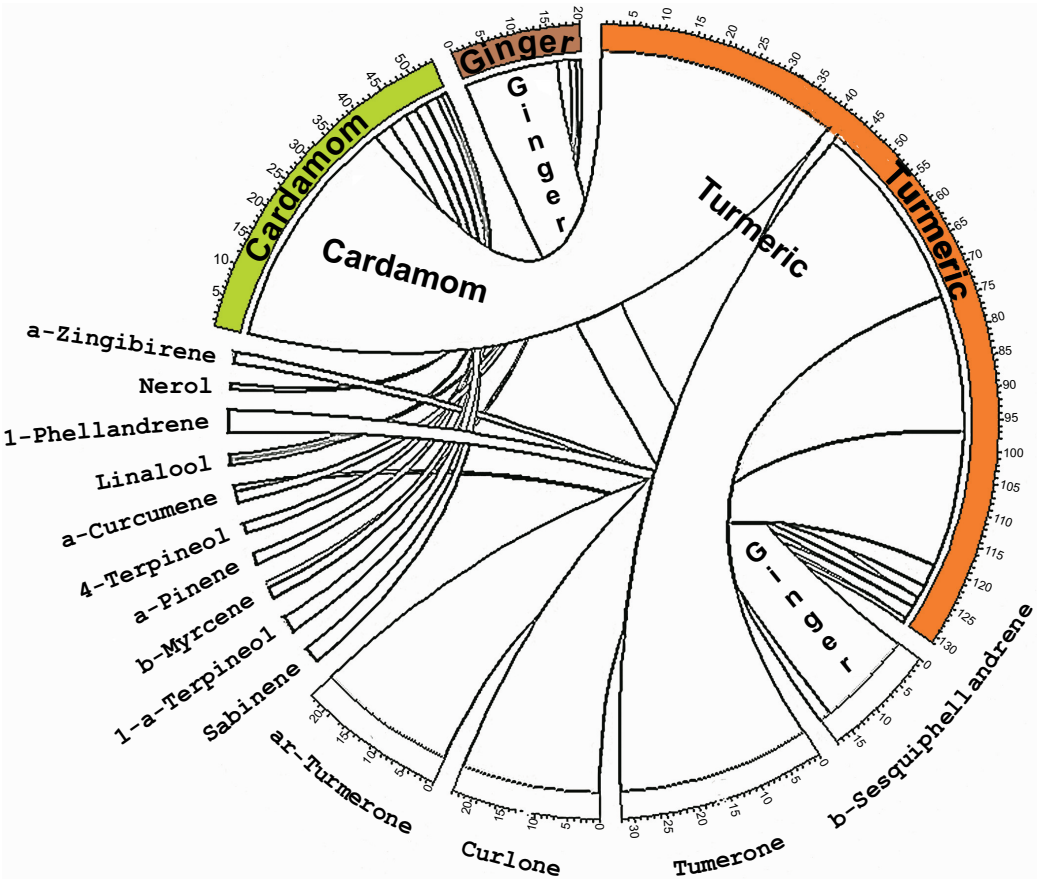

Fig. (1). The convergence of phytochemicals from cardamom, ginger, and turmeric are visualized using Circos (http://circos.ca/). Data is scaled to emphasize major contribution between segments (represented by thick ribbons). The relative composition is showed in percentages.

present in ginger as well in cardamom, and also due to its commercial availability. The results of (*Z*)-nerolidol and the essential oils against *Lactobacillus* sp. (a probiotic Gram-positive bacterium) are tabulated in Table **3.**

Table 3. Inhibitory potential of essential oil against *Lactobacillus* sp.

Sl. No.	Sample	Zone of Inhibition
1	Turmeric oil (T)	10±1mm
2	Ginger oil (G)	34±1mm
3	Cardamom oil (E)	28±1mm
4	Nerolidol (N)	20±1mm

The MIC of nerolidol was found to be <0.5 µg/mL. This may be due to its longer hydrocarbon tail, which may interact with the interior of the lipid bilayer as proposed by Cornwell and Barry [34] and disrupt the cell membrane by inducing leakage of K+ ions from bacterial cells [35]. The susceptibility of *Lactobacillus* sp. is in agreement with the report of Moritz *et al.* [36] on inhibitory effects of essential oils against *L. rhamnosus* used as a starter culture in fermented milk. In particular, clove and mint essential oil caused sub-lethal stress to *L. rhamnosus*. However, this action is undesirable for probiotic foods. Ehsani and Mahmoudi [37] recommended using lower concentration of essential oil when it is combined with probiotics during the manufacturing, ripening, and storage of Iranian white-brined cheese.

The results presented here may slightly vary due to the variations in the chemical profile of essential oils, which in turn influence their biological activities. Even otherwise, due to strain level differences in the bacterial species may further influence the variability in their resistance/ susceptibility of the Gram-negative or positive bacteria. The variation in the essential oil content could be due to plant chemotypes, ecological, climatic and geographical conditions, harvesting time, nutritional status and age of the plant [38]. It could also be due to differences in the extraction methods of essential oils, analytical methods as well as storage conditions [39]. For instance, a couple of compounds from cardamom, α-terpineol, and 1,8-cineol had more variations in their compositions. In the present study, it was found that 40.11% of 1,8–cineole and only 3.44% of α-terpineol, 39.24% exist as α-terpinyl acetate. Another variety of cardamom from South India had 56.87% α -terpinyl acetate and 15.13% of 1,8-cineole [40]. Whereas, cardamom from Turkey had 40.7% α-terpinyl acetate and 25.6% 1,8-cineole [41]. Cardamom from Guatemala had 36.8% terpinyl acetate and 29.2% 1,8-cineole [42]. Whereas, cardamom from Gorakhpur had 44.3% α-terpinyl acetate and 10.7% 1,8-cineole [33].

CONCLUSION

The essential oil samples were more effective against Gram-positive than the Gram-negative bacteria. The degree of antibacterial property of the tested spice oils can be arranged as follows based on their wide spectrum of inhibition: cardamom > ginger > turmeric and based on their probiotic inhibitory potential: ginger > cardamom > turmeric. Since the oil samples are not effective against Gram-negative bacteria, further studies are required to explore their synergistic effects along with membrane-active antibiotics. Since, the essential oil is extensively used in industry as a flavoring agents, preservatives, biocides, and sanitizers, it is of current challenge to identify individual compounds from essential oil that does not inhibit the growth of probiotics. Although nerolidol and certain other sesquiterpenoids are generally recognized as safe, these compounds may potentially inhibit probiotics. Hence, more studies should focus on these aspects and that is our next line of research.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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