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Physicochemical and Antibacterial Variability of Ginger (*Zingiber officinale* L.) Essential Oil grown at Various Geographical Areas in India

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Abstract: The essential oils derived from diverse plant species have received global attention for their benefits. Ginger (*Zingiber officinale*), an annual herbaceous plant, is well-known for its varied flavor and medicinal values. This study seeks to analyze and evaluate the essential physical (oil yield, specific gravity, refractive index, optical rotation), chemical (acid value, ester value, and percentage (%) of major/ minor chemical molecules in the ginger oil from three different agro-climatic zones in Northern India i.e. Prayagraj (Uttar Pradesh), New Delhi, and Sasaram (Bihar). It has been noticed that ginger from Sasaram (Bihar) produced the oil yield most (1.55%) while from Delhi (1.23%) and Prayagraj (Uttar Pradesh) (1.16%). A significant difference in the number and percentage of chemical constituents was identified by GC-MS analysis in the three different ginger oils. Camphene, zingiberene, myrcene, ar-curcumene, β-sesquiphellandrene, borneol, neral, farnesene, and geranial were among the principal constituents. Zingiberene, 4-thujanol, and camphene were detected in significant amounts in the oil from Delhi, while 4-thujanol, geranial, and camphene were the predominant components in the Prayagraj region. In contrast, the main components of Bihar's oil were zingiberene, camphene, 4-thujanol, and geranial. These differences were attributed to regional environment, drying techniques, distillation techniques, and temperature. The *in-vitro* antibacterial efficacy of ginger oil was analyzed using gram-negative and gram-positive bacterial species. For the tested bacteriae, the ginger essential oil exhibited good to moderate growth inhibition.

Keywords: *Zingiber officinale* oil, composition, oil yield, zingiberene, antibacterial

Introduction

Essential oils are extracted from aromatic plants of different species and are extremely beneficial. Chemically, they are a combination of hydrocarbons and their oxygenated derivatives[1]. Essential oils are secondary metabolites derived from various medicinal, edible, and herbal plants. They are fragrant, oily liquids that are volatile, seldom colored, and their density is lower than water. Essential oils are mainly composed of sesquiterpenes, monoterpenes, and their derivatives like aldehydes, alcohols, ketones, esters, etc. [2]. These oils are

found in various spices including sage, rosemary, thyme, oregano, cloves, cinnamon, basil, nutmeg, lemongrass, coriander, and garlic. The phenolic and aliphatic components of the oils provide strong flavor and confer antimicrobial, antifungal, and antioxidant properties. Because essential oils have no possible harmful effects on humans, and some essential oils have property to inhibit food-borne pathogens, are generally recognized as safe (GRAS), enabling their usage as food additives and preservatives in food industries[3].

Ginger (*Zingiber officinale*) is a perennial herbaceous plant from the Zingiberaceae family and considered as the world's most important spices. Locally referred to as 'adarak' and it has been used for a variety of applications since ancient times. The genus has over 85 species and is cultivated in warm tropical areas such as Southeast Asia, also grown considerably in India, Hawaii, Mexico, Africa, Jamaica, and China[4]. The Indian variety of ginger plant is an upright perennial that can reach a height of 1 to 3 feet. They are thick-lobed, irregularly branched, yellowish-brown, flattened, and rough on the surface. Most cultivars are sterile and produced for their edible rhizomes; flowers rarely appear[5]. It is frequently utilized in food manufacturing, including chutneys, pickles, drinks, jams, and baked goods, and in other industrial areas. Its rhizome is often used in traditional medicine, either raw or processed, and has been used as neuronal cell protecting[6], antibacterial[7], antiemetic[8], analgesic[9], anti-inflammatory[10], anti-tumor[11], antifungal[12], antidiabetic [13], antiobesity[14], and antioxidant[15]. As an alternative medication, it is used to treat conditions including nausea, vomiting, palpitations, and asthma. It has been utilized as a flavoring agent [2].

Ginger rhizome is used for extraction of the ginger essential oil, which is valued for its distinctive aroma and biological effects[16]. Researchers from various fields have extensively studied ginger rhizome, which contains approximately 1 to 2% of the essential oil responsible for its characteristic flavour [5, 17]. The major volatile components found in ginger oil are a mixture of constituents such as monoterpenes, namely limonene, phellandrene, borneol, camphene, geraniol, linalool, citral, citronellol, cineole and sesquiterpenes, namely α-zingiberene, ar-curcumene, β-bisabolene, β-sesquiphellandrene, zingiberol and zingiberenol along with some aliphatic aldehydes and alcohols[17-19]. Gingerols and shogaols are the two major phenolic components present in ginger oils. Gingerols are responsible for the characteristic pungent odour of ginger [20]. β-eudesmol, trans-β-sesquiphellandrol, zingiberene, geranial, bornyl acetate, and neral are significant known components of ginger's odour. The lemon flavour of ginger is attributed to neral and geranial [21]. Since zingiberene is the major component and its concentration has a substantial impact on the quality of ginger oil, traditional distillation methods that use high temperatures may result in low zingiberene concentrations [22,23]. Studies on ginger oil revealed that the composition of main components in ginger oil can differ significantly due to several factors such as drying procedures, geographical conditions, distillation methods, and temperature [15, 24-27].

In addition to identifying possible production zones in North India, the current study aimed to establish the yield

and chemical composition of *Z. officinale* rhizome as an indication of essential oil quality. This study will help farmers and business owners choose where to plant and grow superior-quality ginger. It will also help identify potential suppliers of high-quality *Z. officinale* rhizome essential oil for commercialization.

MATERIALS AND METHOD

Plant and chemical materials

The plant materials used were the rhizomes of domestic cultivated gingers. The freshly matured ginger rhizomes were harvested from three diverse agro climatic zones of Northern India, namely, Delhi and Sasaram in Bihar, which are noted for their subtropical temperatures and Prayagraj, Uttar Pradesh which is distinguished by a humid subtropical climate. After the harvest, the identification of Zingiber officinale rhizomes was carried out by Mr. Kamlesh Kumar, Scientific Officer, Agro and Biotech Division, Fragrance and Flavor Development Centre, Ministry of MSME, Govt. of India. The chemicals used in the experiment were phenolphthalein (0.1N), sodium hydroxide (2.5N), potassium hydroxide (0.5N), sodium sulfate (anhydrous) and distilled water.

Methods

Physical and chemical analysis of ginger essential oil

Physicochemical properties, including the yield, color, refractive index, specific gravity/ relative density, optical rotation, acid vlue and saponification value of ginger essential oil, were determined by the Beauro Indian standard method (IS:761-1988) [28]**.** The Clevenger apparatus hydrodistillation method was carried out the extraction of ginger essential oil by followed the grounded ginger (300 g) added into distilled water (3000 mL) in a round bottom flask. This flask was then kept in a heating mantle with an attached Clevenger. The mixture was allowed to boil at 100°C, and then the temperature was reduced to 70°C and kept for 6 h. The ginger essential oil was collected in a separation flask. Finally, the separated essential oil was dehydrated (de-moistured) by anhydrous sodium sulphate for further studies.

The distillation yield of the obtained essential oil was calculated using following equation: (1);

$$\text{Yield\% of Ginger Essential oil} = \frac{\text{Weight of Ginger E.ssential Oil}}{\text{Weight of Ginger}} \times 100$$

ABBE refractometer, pyknometer or specific gravity bottle, Sodium Lamp Laboratory Research Polarimeter and titration methods were apply to determine the refractive index, relative density, optical rotation and acid value, saponification values of ginger essential oil as per Beauro Indian standard method (IS:761-1988) [28].

GC-MS analysis of essential oil

The composition of ginger oil was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) using a gas chromatograph (Agilent 7890) connected with a mass spectrometer (Accu TOF GCv, Jeol) and an HP-5MS capillary column (30m length, 0.25µm film thickness, 0.25mm internal diameter). Helium was employed as the carrier gas, with a flow rate of 1.0 mL/ min, and n-hexane as the solvent. The sample was injected with a volume of 0.2 µl. The GC-MS interface temperature

was 250ºC. The temperature of the oven was initially set to 60ºC, then raised to 200ºC at a rate of 6ºC per minute, 200ºC to 275ºC at a rate of 8ºC per minute, and lastly 275ºC to 280ºC at a rate of 5ºC per minute. With a mass scan range of 35 to 800 atomic mass units, ionization took place at 70 electron volts and the ionisation mode was EI+.

The result was verified through comparing it to retention indices on the exact same column. Using a homologous sequence of n-alkanes (C_8-C_{20}, Sigma-Aldrich), each unique chemical was identified based on retention duration and retention index by comparing its mass spectra to the NIST Mass Spectral Library, co-injection with genuine samples, and literature[29]. Compound percentages in the oil were computed by dividing the proportion of each compounds peak area by the by the overall peak area calculated according to the gas chromatogram and mass spectrometry findings.

The antibacterial efficacy of the ginger oil was evaluated with *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) using the agar well diffusion method described by Verma et al.[30] with some modifications. Briefly, the pre-autoclaved Muller Hilton agar (MHA) medium was poured into 90 mm diameter petri plates and allowed to solidify for 15-20 minutes. Under aseptic conditions, 100 µl of freshly grown bacterial cultures were spread over the separate Petri plates containing MHA medium through swab. A 6-mm well was made in each petri plate using a cork borer, and then 0.1 ml of ginger oil was added to the wells. Petri dishes containing bacteria and ginger oil were incubated in an upright position at 37ºC ± 2°C for 24 hr. After incubation, the diameter of the inhibition zone (in mm) was measured. The assay was repeated twice with three replications.

Statistical Analysis

The data are expressed as the mean ± standard deviation (SD). The statistical difference and significance among various groups was calculated through t-test by using Graph Pad PRISM version 5.01 software. The values $p < 0.05$ considered as statistically significant.

RESULT AND DISCUSSION

Physicochemical properties of *Z. officinale* oil

The physical properties of ginger oil, such as oil yield, and colour, were identified using traditional methods. The quantity of oil produced is largely determined by the location of the plant, plant species, extraction method, moisture level during harvest, and plant age. In addition, the odour, colour, and fragrance of the oil are significantly affected by the place where it was grown [18]. The oil colour from Uttar Pradesh (Allahabad) region was amber-yellow, while the oil from Bihar (Sasaram) and Delhi area was light yellow. The ginger oil from three distinct regions of North India showed some variation in their yield (Table 1). Bihar state produced ginger recorded the highest oil yield at 1.55% v/w, followed by ginger from New Delhi (1.23% v/w), and Uttar Pradesh state produced ginger had the lowest oil yield (1.16% v/w). Table 1 presents the physico-chemical parameters of the ginger essential oil. Physico-chemical assessment (i.e. odour, colour, refractive index, specific gravity, optical rotation, acid value and

saponification value) reveals that except yield percentage, there are no noticeable significant variations in ginger essential oil due to the fact that it is grown at different geographical locations.

Table 1. Physicochemical characteristics of *Zingiber officinale*, essential oils

Parameters	Sasaram (Bihar)	Delhi	Prayagraj (U.P.)
Yield (%)	1.55±0.01	1.23±0.01	1.16±0.01
Appearance & colour	Clear mobile liquid, light yellow	Clear mobile liquid, light yellow	Clear mobile liquid, light amber-yellow
Odour	Sharp lemony spicy odour	Sharp lemony spicy odour	Sharp lemony spicy odour
Refractive index (27°C)	1.4889±0.01	1.4898±0.01	1.4910±0.01
Specific gravity (27°C)	0.8786±0.01	0.8759±0.01	0.8790±0.01
Optical Rotation (°)	-36°±0.01	-35°±0.01	-36°±0.01
Acid value	8.0±0.0	8.1±0.01	7.8±0.0
Saponification value	20.25±0.02	21.0±0.01	20.1±0.02

Values are represented as mean ± standard error of mean of triplicate values

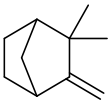
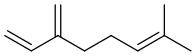
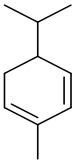
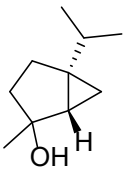
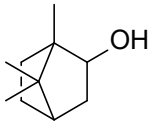
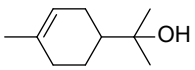
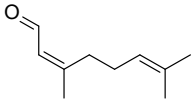
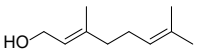
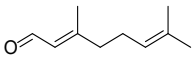
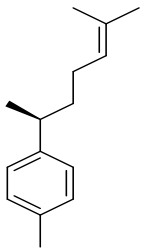
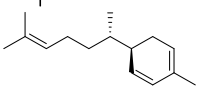
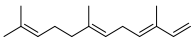
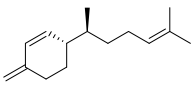
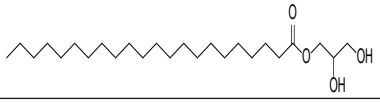
Chemical Composition of *Z. officinale* oil

The volatile oils extracted from ginger grown in three different regions were analysed using gas chromatography-mass spectrometry (GC-MS) to determine their chemical composition. Table-2 shows the primary volatile components discovered by GC-MS evaluation of ginger oil from three different sites.

The GC-MS analysis revealed that total 20 components (99.99% of the total oil) was present in the ginger oil acquired from the Uttar Pradesh and Delhi regions, and 19 components (97.33% of the total oil) in the oil from Bihar state. GC-MS analysis showed that the essential oil is mainly composed of monoterpenes (citral, camphene, geraniol, myrcene, linalool) and sesquiterpenes (β-sesquiphellandrene, farnesene, curcumene, zingiberene). The principal chemical components include camphene, zingiberene, myrcene, ar-curcumene, β-sesquiphellandrene, borneol, neral, farnesene, and geranial. The chemical makeup of essential oil varies greatly because the yield of essential oil extracted differs in all three locations. Some variations have been observed, which might be attributable to local climatic conditions.

The chemical composition of ginger oil from three different regions examined in this work exhibited significant variation. In the Prayagraj region (Uttar Pradesh), the major components were camphene (17.83%), 4-thujanol (16.92%), geranial (12.19%), zingiberene (10.45%), neral (8.69%), α-farnesene (7.62%), β-sesquiphellandrene (5.57%), borneol (4.71%), ar-curcumene (4.16%), and myrcene (3.65%). Conversely, ginger oil from the Delhi region contained higher levels of zingiberene (16.82%), 4-thujanol (15.49%), camphene (13.50%), α-farnesene (11.44%), geranial (7.88%), ar-curcumene (7.75%), neral (5.83%), α-fellandrene (2.67%), myrcene (2.40%), and borneol (2.21%). In Bihar oil, zingiberene (18.43%), camphene (13.61%), 4-thujanol (12.61%), geranial (11.36%), α-farnesene (10.14%),

Table 2: Major chemical components of *Z. officinale* essential oil from three

S. N.	Compounds	Chemical Structure	RI[a]	RI[b]	Area %		
					Sasaram	Delhi	Prayagraj
1	Camphene		951	946	13.61	13.50	17.83
2	β-Myrcene		992	988	2.61	2.40	3.65
3	α-Phellandrene		1007	1002	-	2.67	-
4	trans-4-Thujanol		1075	1065	12.61	15.49	16.92
5	Borneol		1166	1165	-	2.21	4.71
6	α-Terpineol		1190	1186	-	-	2.05
7	Neral		1239	1235	7.66	5.83	8.69
8	Geraniol		1256	1249	1.06	-	-
9	α-Citral (Geranial)		1268	1264	11.36	7.88	12.19
10	Ar-Curcumene		1480	1479	4.48	7.75	4.16
11	Zingiberene		1489	1493	18.43	16.82	10.45
12	α-Farnesene		15.7	1505	10.14	11.44	7.62
13	β-Sesquiphe-llandrene		1523	1521	8.22	9.17	5.57
14	Glyceryl behenate		2564	2560		0.49	

different regions analyzed by GC-MS **RI[a]:** Experimental Retention Index; **RI[b]:** Literature Retention Index

β-sesquiphellandrene (8.22%), neral (7.66%), ar-curcumene (4.48%), myrcene (2.61%), and geraniol (1.06%) were identified as the major constituents. Various studies have revealed variations in the chemical composition of ginger oil. In the Northeast part of India, camphene emerged as the primary component, followed by neral, geranial, and zingiberene [31]. Conversely, in another study from the same region, geraniol was identified as the major constituent, followed by z-citral, eucalyptol, and camphene [32]. Srinivasan [33] and El-Baroty et al. [34] noted β-sesquiphellandrene as a typical component, whereas Singh et al. [4] found geranial (25.9%) to be the primary component, followed by α-zingiberene (9.5%), neral (7.6%), (E, E)-α-farnesene (7.6%), and ar-curcumene (6.6%). Additionally, research conducted in Uttarakhand, India, indicated that ginger essential oil yields ranged from 0.20% at higher altitudes to 0.26% at lower altitudes, with variations in chemical composition linked to altitude. Higher altitudes tended to contain more oxygenated monoterpenes like geraniol, while lower altitudes exhibited higher concentrations of monoterpene hydrocarbons like phellandrene [35]. Furthermore, a literature survey revealed that predominant components of the ginger essential oils from the Sub Himalayan zone of India were zingiberene in the case of the gorubathane and thingria cultivars, and geranial and neral in the case of the shingboi cultivar [36].

Antibacterial activity

The *in-vitro* antimicrobial activity of ginger oil was investigated using well-diffusion method against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) bacteria. The inhibition zones of 13 mm were recorded with *Escherichia coli* and *Staphylococcus aureus* recorded the inhibition zone of 10 mm (Fig 1 A, B). In this study, the ginger oil found more effective to inhibit the growth of gram-negative bacteria i.e. *E. coli.* Previous studies revealed that the bacteriostatic–bactericidal activity of the essential oils depend on their chemotypes and concentrations, as well as type of the bacteria [37]. Our results are in accordance with Singh et al. [4], who reported that Indian ginger essential oil was more effective than ginger oleoresins regarding antioxidant and antimicrobial activities. However, in another study, It is has been reported that Chinese ginger oleoresin exerted significantly higher antimicrobial activity as compared to the ginger essential oil and concluded that origin of plant material is more important when dealing with phytochemical properties[38]. Moreover, the marked antibacterial, antifungal and anti-yeast mold properties of ginger oil is believed to be due to the presence of chemical components mainly, eudesmol, γ-terpinene, a-curcumene, alloaromadendrene and zingiberene, which are responsible for the observed antibacterial activity [39, 40].

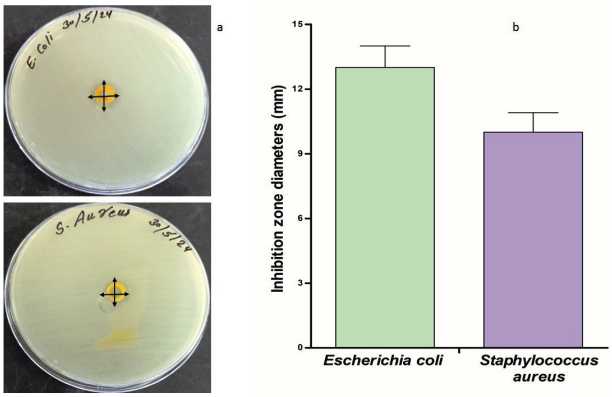


Fig 1. (a) *In-vitro* antimicrobial activity of ginger oil against *E. coli* and *S. aureus* using agar well diffusion assay, (b) Graphical representation of inhibition zone (mm) against the bacterial strains

Conclusion

To provide insight on variations in chemical composition and yield, this study examined the physical characteristics, oil yield, and chemical profile of ginger oil from three distinct northern Indian regions: Prayagraj (Uttar Pradesh), Delhi, and Sasaram (Bihar). Significant differences were seen in the oil output among the three regions, underscoring the significance of geographical considerations in the essential oil production. Ginger oil was subjected to GC-MS analysis, which identified sesquiterpenes such as α-zingiberene, ar-curcumene, β-bisabolene, β-sesquiphellandrene, zingiberol, and zingiberenol, and monoterpenes such as phellandrene, camphene, cineole, linalool, limonene, citral, geraniol, citronellol, and borneol. These chemical compounds give ginger oil its distinct flavor and scent as well as some of its possible therapeutic benefits. The antibacterial efficacy of ginger essential oil was accessed using gram-negative (*E. coli*) and gram-positive (*S. aureus)* bacterial species. The results revealed that the ginger essential oil exhibited good to moderate growth inhibition activity. Since, the essential oil showed moderate to considerable antibacterial activities, it can be utilized in the treatment of many bacterial infections and naturally food additives and preservative in food industries. According to the bioactivity study, ginger oil might be a better option for medicine and food preservatives than chemicals. Further research should look at other factors that influence essential oil content, as well as refinement of cultivation and extraction processes to improve the quality of ginger oil. The study also underlines the importance of improving distillation procedures in order to preserve important compounds like zingiberene and ensure the consistent quality of ginger oil.

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Disclosure statement

The authors report there are no competing interests to declare.

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Data Availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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