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Metabolic profile, bioactivities, and variations in chemical constituents of essential oils of twenty mango ginger (*Curcuma amada*) accessions

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ABSTRACT

Curcuma amada Roxb. (Zingiberaceae), commonly called as amba ada or mango ginger, is an important aromatic plant having both medicinal and culinary properties. In the present investigation, an attempt has been made to select high essential oil yielding germplasm among *C. amada* accessions collected from different regions and to evaluate its antioxidant as well as antimicrobial activities. Out of 20 accessions analyzed, Ca17 showed highest oil yield (1.35 ± 0.036%), while Ca10 accession showed lowest yield (0.12 ± 0.015%) in rhizome oil. Gas chromatography and mass spectrometry analysis revealed 56 bioactive compounds identifying myrcene (67.59–72.97%), (Z)-(Z)-Geranyl linalool (4.3–7.79%), (Z)-(E)-Geranyl linalool (4.05–7.23%), β-ocimene (2.9–6.33%), and β-pinene (1.23–4.82%) as the dominant compounds. The antimicrobial activity of the essential oil of *C. amada* was tested against four different bacteria, in which Ca17 was found to have good to moderate antimicrobial activities against all the tested microorganisms. Further, the antioxidant activity of all the accessions were also evaluated, in which Ca17 showed considerable antioxidant property with IC_{50} value 32.05 µg/mL. *C. amada*, being an untapped plant known for its morphological resemblance with ginger and mango-aroma and having antimicrobial and antioxidant properties, could serve as a good source of bioactive compounds having food additive properties. Based on these results, it could be suggested that *C. amada*'s rhizome oil could be used for food and pharmaceutical applications as a bioresource of antioxidants and antimicrobials.

1. INTRODUCTION

Zingiberaceae family is a notable family of medicinal, economic, and aromatic plants recognized for volatile oils and also have been used in the cosmetic industry [1]. In the family Zingiberaceae, constituting the genus *Curcuma* contains over 80 species of paramount importance endowed with widespread adaptation to a variety of environments [2]. Most of the coloring and flavoring agents found in Asian cuisine, traditional medicine, spices, dyes, perfumes, cosmetics, and ornamental plants come from this genus [3].

Curcuma amada Roxb., commonly acknowledged as amba ada or mango ginger [4], is a prime aromatic and medicinal plant grows extensively in the countries of Indian subcontinent and has morphological characteristics similar to ginger (*Zingiber officinale*), but its taste recalls raw mango [5]. There is 43% of amylose in mango ginger starch, which shares the same characteristics as *Curcuma longa* and *Z. officinale* starch [6]. It is being cultivated in many parts of Odisha [7] but has no commercial cultivation. From ancient times, *C. amada* has been used in traditional systems of medicine for a number of uses, including coolant, appetizer, antipyretic, diuretic, expectorant, and laxative. It is also likely to alleviate biliousness, and itching, and cures an array of skin diseases, bronchitis, asthma, and inflammation caused by injury [8,9]. Moreover, enterokinase found in mango-ginger improves digestion, detoxifies the body, and improves skin tone [10]. Among its many pharmaceutical properties, the rhizome essential oil (ROs) of *C. amada* has antimicrobial [11], anti-inflammatory, analgesic, anticancer, antihyperglycemic, and antioxidant activity [7,9,12]. Furthermore, camphor present in the RO reduces inflammation, which helps clear blocked bronchi, larynx, pharynx, and other airway parts of phlegm and mucus [13]. Besides its medicinal properties, rhizomes of this plant are used to flavor various foods such as chutney, dahi vada, pickles, curd water rice, and more in Odisha (India) [4]. In addition, it is a key ingredient in candies, sauces, curries, and salad dressings [14]. Dried ginger powder is used in a variety of foodstuffs, including baked goods and desserts [15].

As a part of many natural biological processes in our bodies, including digestion, breathing, converting fats into energy, and

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metabolizing alcohol and drugs produce harmful substances known as free radicals [16,17]. Moreover, modern lifestyle factors such as unhealthy diets, insufficient exercise, heavy metals, smoking, food additives, pesticides, and environmental pollution can contribute in the occurrence of oxidative stress [18]. Free radicals can increase oxidative stress and can damage the macromolecules, contributing to the pathological processes of various diseases [17]. Antioxidants are found to be effective against diseases related to degenerative disorders such as diabetes, arthritis, immune-related disorders, and many others [19]. Again, allopathy is generally used due to its "quick-fix" nature, but its efficacy can eventually get diminished after years as the bacterial strains evolve to resist the drug made to destroy them; however, medicinal plants destroy the root cause of diseases [20]. The essential oil of *C. amada* extracted from rhizome has efficient antioxidant and antimicrobial characteristics [4,21]. Phytochemicals such as myrcene, β-pinene, ocimene, α-pinene, sabinene, and many others are found in the ROs of *C. amada* evaluated through Gas chromatography-mass spectrometry (GC-MS) [19,21]. A number of epidemiological studies have linked phytochemicals with a series of bioactivities associated with health benefits. The bioactivity of many phytoconstituents is believed to be higher in the form in which they are found in nature [8,21].

There are several factors that influence the yield of essential oil and phytocomposition of mango ginger, including its genetic makeup, growing conditions, origin, chemotypes, and the nutritional value of soil [11]. At present, only a few reports are available on chemical analyses, antioxidant, and antimicrobial studies of *C. amada* [4,7,21]. However, phytochemical characterization of bioactive compounds using GC-MS along with bioactivity screening including antimicrobial and antioxidant of different accessions collected from Odisha has not yet been done to date. Therefore, an attempt has been made to assess the variation in phytochemicals and bioactivities of different accessions of *C. amada*.

2. METHODOLOGY

2.1. Collection of Plant Samples

The plant samples of different accessions of *C. amada* were collected from various geographical locations of Odisha [Table 1 and Figure 1] and were later identified by a taxonomist. The identified samples were planted in the green house for sample maintenance in the Siksha O Anusandhan herbarium for further future use.

2.2. Extraction of Essential Oil

Fresh samples of rhizome (100 g) of *C. amada* were taken for oil extraction through hydro distillation for about 5–6 h with the help of a Clevenger-type apparatus. To remove the moisture content in the extracted oil, it was treated with anhydrous sodium sulfate and was preserved in the refrigerator (4°C) until further analysis. The oil yield percentage was evaluated on the fresh weight basis (v/w).

2.3. Chemical Analysis of Essential Oil

GC-MS analysis was carried out using Clarus 580 Gas Chromatogram (Perkin Elmer, USA) equipped with a MS detector with Helium gas as a carrier gas with flow rate of 1 mL/min. 0.1 µL of rhizome essential oil was injected and the Elite-5 column (30 cm length × 0.25 mm i.d., film thickness 0.25 µm) was used. The oven temperature was equilibrated at 50°C for 1 min, heated at 5°C/min to 230°C with 5 min hold, and finally raised at 15°C/min to 260°C with 1 min hold. At 250°C and 260°C, the temperature of the injector and both the transfer line, and ion source was set, respectively. The total run time was 45 min. The scanning was done over a mass scan range of 50–600 m/z. The

Table 1: Geographical coordinates of collected *Curcuma amada* accessions.

S. No.	Sample code	Place of collection	Voucher specimen number	Altitude (m)	Latitude	Longitude
1.	Ca 1	Udala, Mayurbhanj	2420/CBT	322	22.00313°	86.2574°
2.	Ca 2	Dutiala, Kendrapara	2421/CBT	13	20.5848°	86.6611°
3.	Ca 3	Daspalla, Nayagarh	2422/CBT	110	20.09556°	85.01240°
4.	Ca 4	Patrapur, Kendrapara	2423/CBT	13	20.5848°	86.6611°
5.	Ca 5	Barabati, Jajpur	2424/CBT	331	20.7652°	86.1752°
6.	Ca 6	Fakirpur, Keonjhar	2425/CBT	480	21.6289°	85.5817°
7.	Ca 7	Berhampur, Ganjam	2426/CBT	9	19.5860°	84.6897°
8.	Ca 8	Kandhamal	2427/CBT	915	19.541331°	84.74916°
9.	Ca 9	Raikia, Phulbani	2428/CBT	485	20.4797°	84.2331°
10.	Ca 10	Udayagiri, Gajapati	2429/CBT	1501	19.1912°	84.1857°
11.	Ca 11	Tulasipur, Cuttack	2430/CBT	36	20.4625°	85.8830°
12.	Ca 12	Sahebnagar, Khurda	2431/CBT	75	20.1301°	85.4788°
13.	Ca 13	Jatamundia, Cuttack	2432/CBT	36	20.4625°	85.8830°
14.	Ca 14	Andapur, Keonjhar	2433/CBT	480	21.6289°	85.5817°
15.	Ca 15	Ambiki, Jagatsinghpur	2434/CBT	15	20.1976°	86.3377°
16.	Ca 16	Dumduma, Khurda	2435/CBT	75	20.1301°	85.4788°
17.	Ca 17	Choudwar, Cuttack	2436/CBT	36	20.4625°	85.8830°
18.	Ca 18	Patia, Khurda	2437/CBT	75	20.1301°	85.8830°
19.	Ca 19	Jaraka, Jajpur	2438/CBT	331	20.7652°	86.1752°
20.	Ca 20	Nabarangpur	2439/CBT	59	18.1322°	85.451°

Figure 1: *Curcuma amada* rhizomes collected from different regions of Odisha.

ion chromatogram and mass spectra were acquired using Turbo mass TM software 5.4. The n-alkane series was used for retention index (RI) identification, and compound identification was done through Adams Library [22].

2.4. Antioxidant Activities

The antioxidant activity was evaluated by DPPH radical scavenging assay following the protocol of Sahoo *et al.* with slight modifications [23]. Different concentrations (1, 5, 10, 20, and 30 µg/mL) of methanolic solution of essential oils were mixed with 1 mL of 0.1 mM DPPH. The reaction mixtures were mixed properly and were kept at room temperature for 30 min in dark. At 517 nm, the absorbance of the sample was measured using ultraviolet-visible spectrophotometer (Thermo Scientific, Waltham, MA). Butylated hydroxytoluene and ascorbic acid were taken as the positive control meanwhile methanol and DPPH solution was taken as control and IC_{50} value was measured.

2.5. Bacterial Strains

The antimicrobial activity of essential oil of *C. amada* rhizome was illustrated against two Gram-negative bacteria (*Escherichia coli* and *Acinetobacter baumannii*) and two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). The bacterial strains were collected from the Department of Microbiology, SOADU, Bhubaneswar.

2.6. Antimicrobial Activity

The antimicrobial activity was measured by checking the minimum inhibitory concentrations (MIC) by broth microdilution method as described by the guidelines of Clinical and Laboratory Standards Institute and following the protocol of Dash *et al.* [24]. The experiment was accomplished using Mueller–Hinton Broth (MHB) for all the bacterial strains. The rhizome essential oils (100 µL) with a concentration of 100 µg/mL were prepared by mixing them with dimethyl sulfoxide in sterile Eppendorf tubes. The viable bacterial culture (10^6 CFU/mL of microorganisms) was prepared from overnight suspension. The rhizome volatile oils were analyzed by a 2-fold serial-dilution method with MHB in a 96-microtiter (enzyme-linked immunosorbent assay) plate. Ampicillin was taken as standard. MIC was defined as the concentration that showed no growth visibility or turbidity during the highest dilution of the sample.

Table 2: Yield percentage of *Curcuma amada* rhizome oil collected from different regions.

S. No.	Sample code	% of yield (mean±SD)
1.	Ca1	0.49±0.01
2.	Ca2	0.55±0.015
3.	Ca3	0.19±0.015
4.	Ca4	0.42±0.025
5.	Ca5	0.54±0.01
6.	Ca6	0.17±0.015
7.	Ca7	0.23±0.015
8.	Ca8	0.18±0.005
9.	Ca9	0.13±0.005
10.	Ca10	0.12±0.005
11.	Ca11	0.46±0.025
12.	Ca12	0.13±0.005
13.	Ca13	0.17±0.015
14.	Ca14	0.15±0.01
15.	Ca15	0.50±0.01
16.	Ca16	0.61±0.02
17.	Ca17	1.35±0.029
18.	Ca18	0.76±0.02
19.	Ca19	0.53±0.01
20.	Ca20	0.61±0.02

3. RESULTS AND DISCUSSION

3.1. GC-MS Analysis

Rhizomes of *C.amada* yields a good amount of essential oil (0.12–1.35% v/w) [Table 2] which was a pale yellow liquid having a strong aroma. It was reported that 0.5% (v/w) of rhizome oil yield on fresh weight basis taken from the foothills of Uttarakhand, India [25], while 1.25% (v/w) of rhizome oil yield was reported from Kerala Agriculture University, India [26]. Later, in the present experiment, the RO was subjected to GC-MS analysis which detected 56 peaks. The analysis revealed a total of 84.38–97.37% detectable area percentage comprising all major and minor constituents. Parenthetically, the *C. amada* ROs of all the accessions were composed mainly of monoterpenoids (89%) with 83% hydrocarbons and 6% of oxygenated counterparts [Figure 2]. Alike the present study findings monoterpenoids (97.22%) with a major 96.75% of hydrocarbon fraction and 0.97% of oxygenated ones were found predominantly in *C. amada* RO [25]. The essential oils of the mango ginger (Curcuma amada) accessions were found to contain a total of 56 compounds. The major constituents identified were myrcene (67.59–72.97%), (Z)-(Z)-Geranyl linalool (4.3–7.79%), (Z)-(E)-Geranyl linalool (4.05–7.23%), β-ocimene (2.9–6.33%), and β-pinene (1.23–4.82%). Additionally, α-pinene (0.1–0.96%) and (E)-caryophyllene (0.01–1.95%) were present as minor constituents in the essential oils [Table 3 and Figure 3]. A similar study on *C. amada* RO reported myrcene (40%) and β-pinene (11.78%) as the major constituents [7]. On contrary, a phytochemical screening of RO EOs detected 28 constituents in which ar-curcumene (28.1%), camphor (11.2%), β-cumene (11.2%), curzerenone (7.1%), and eucalyptol (6.0%) were

Table 3: Qualitative phytochemical analysis of rhizome samples of *Curcuma amada*.

S. No.	Compound	Classification	Retention index		Relative area percentage (%)									
			RI[a]	RI[b]	Ca1	Ca2	Ca3	Ca4	Ca5	Ca6	Ca7	Ca8	Ca9	Ca10
1.	α-pinene	Monoterpene Hydrocarbon	935	932	0.6	0.65	0.1	0.4	0.54	0.5	0.4	0.3	0.3	0.46
2.	Camphene	Monoterpene Hydrocarbon	952	946	0.01	0.06	0.02	0.02	0.04	0.02	0.02	0.01	0.01	0.01
3.	Sabinene	Monoterpene Hydrocarbon	975	969	0.02	0.04	0.03	0.01	0.03	0.02	0.03	0.02	0.01	0.04
4.	β-pinene	Monoterpene Hydrocarbon	982	974	2.31	3.11	1.23	3.24	4.13	4.16	3.59	3.11	3.6	3.48
5.	Myrcene	Monoterpene Hydrocarbon	1004	988	69.35	71.56	69.24	67.59	72.81	68.67	70.46	71.42	72.65	68.51
6.	α-Terpinene	Monoterpene Hydrocarbon	1017	1014	0.02	0.03	0.01	0.02	0.02	0.1	0.03	0.2	0.2	0.14
7	p-Cymene	Monoterpene Hydrocarbon	1024	1020	0.03	0.01	0.01	0.01	0.04	0.02	0.01	0.01	0.01	0.01
8.	Limonene	Monoterpene Hydrocarbon	1029	1024	0.02	0.3	0.02	0.01	0.01	0.01	0.02	0.01	0.02	0.02
9.	Eucalyptol	Oxgenated Monoterpene	1033	1026	0.01	0.01	0.02	0.03	0.02	0.02	0.01	0.02	0.01	0.4
10.	(Z)-β-Ocimene	Monoterpene Hydrocarbon	1036	1032	0.4	0.64	0.1	0.5	0.45	0.42	0.5	0.3	0.2	0.02
11.	(E)-β-Ocimene	Monoterpene Hydrocarbon	1049	1044	4.61	6.33	3.65	4.34	5.43	4.52	4.37	3.26	5.02	2.9
12.	Transdecahydronapthalene	Monoterpene Hydrocarbon	1052	1053	-	-	-	0.01	0.12	0.02	0.01	0.01	-	-
13.	γ-Terpinene	Monoterpene Hydrocarbon	1059	1054	-	-	-	-	0.01	-	-	-	0.01	0.04
14.	2-Nonanone	Ketone	1080	1087	-	0.03	0.01	0.01	0.03	0.02	0.02	0.01	-	-
15.	Linalool	Oxygenated Monoterpene	1093	1095	0.02	0.23	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.42
16.	Cis- Thujone	Oxygenated Monoterpene	1101	1101	0.4	0.84	0.4	0.5	0.74	0.01	0.02	0.01	0.2	0.4
17.	Perillene	Oxygenated Monoterpene	1103	1102	0.3	0.2	0.02	0.04	0.1	0.3	0.02	0.02	0.3	0.15
18.	Camphor	Oxygenated Monoterpene	1144	1141	0.01	0.03	0.01	0.01	0.02	0.02	0.01	-	-	0.01
19.	Isoborneol	Oxygenated Monoterpene	1158	1155	0.02	0.03	0.01	0.01	0.01	0.02	0.03	-	-	0.01
20.	Borneol	Oxygenated Monoterpene	1164	1165	0.01	0.05	0.02	0.04	0.04	0.02	0.01	-	-	0.01
21.	Terpien-4-ol	Oxygenated Monoterpene	1182	1174	0.01	0.04	0.02	0.03	0.06	0.01	0.01	0.02	0.01	0.02
23.	α-Terpineol	Oxygenated Monoterpene	1187	1186	0.02	0.02	0.01	0.01	0.03	0.01	0.02	0.03	0.02	0.01
24.	Myrtenol	Oxygenated Monoterpene	1198	1194	0.01	0.01	0.01	0.01	0.01	0.02	0.04	0.02	0.01	0.02
25.	γ-Terpineol	Oxygenated Monoterpene	1208	1199	-	-	-	0.02	0.01	0.03	0.02	0.01	-	-
26.	Linalool formate	Oxygenated Monoterpene	1228	1214	-	0.05	-	0.01	0.03	0.01	-	-	-	-
27.	Nerol	Oxygenated Monoterpene	1230	1227	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.02	-	-
28.	β-Patchoulene	Sesquiterpene Hydrocarbon	1374	1379	0.02	0.03	0.2	0.4	0.1	0.03	0.02	0.01	0.01	0.02

(*Contd*...)

Table 3: (*Continued*)

S. No.	Compound	Classification	Retention index		Relative area percentage (%)									
			RI[a]	RI[b]	Ca1	Ca2	Ca3	Ca4	Ca5	Ca6	Ca7	Ca8	Ca9	Ca10
29.	β-Cubebene	Sesquiterpene Hydrocarbon	1388	1387	0.01	0.04	0.01	0.01	0.01	0.01	0.03	0.02	0.02	0.03
30	(E) - Caryophyllene	Sesquiterpene Hydrocarbon	1418	1417	1.01	1.43	0.01	0.01	0.02	-	-	-	-	-
31	γ–Elemene	Sesquiterpene Hydrocarbon	1428	1434	0.01	0.01	0.02	0.01	0.04	-	0.03	-	-	-
32	(E)-β– Farnescene	Sesquiterpene Hydrocarbon	1453	1454	0.01	0.04	0.01	0.02	0.02	-	-	-	0.01	-
33	Germacrene D	Sesquiterpene Hydrocarbon	1480	1480	-	-	-	-	-	-	0.01	0.01	-	-
34	Curzerene	Furan	1495	1499	0.02	0.04	0.02	0.02	0.03	0.02	0.03	0.01	0.01	0.03
35	γ-Cadinene	Sesquiterpene Hydrocarbon	1519	1522	0.02	0.05	-	0.06	0.04	-	0.01	-	-	-
36	δ–Cadinene	Sesquiterpene Hydrocarbon	1523	1522	0.04	0.04	-	0.01	0.02	-	-	-	-	-
37	Germacrene B	Sesquiterpene Hydrocarbon	1561	1559	0.15	0.17	0.02	0.02	0.2	0.02	-	0.02	0.02	0.02
38	E-Nerolidol	Oxygenated Sesquiterpene	1567	1561	0.1	0.23	-	0.01	0.3	-	-	0.02	-	-
39	Spathulenol	Oxygenated Sesquiterpene	1577	1577	0.01	0.02	0.01	0.01	0.01	0.02	-	0.03	0.02	0.03
40	Caryophyllene-oxide	Oxygenated Sesquiterpene	1585	1582	-	0.02	-	-	0.02	-	-	-	-	-
41	ar-Turmerol	Oxygentaed Sesquiterpene	1580	1582	-	0.04	-	0.01	0.03	0.02	-	-	-	0.02
42	Viridiflorol	Oxygenated Sesquiterpene	1599	1592	-	0.01	-	0.02	0.04	-	-	0.02	-	0.01
43	Ledol	Oxygenated Sesquiterpene	1600	1602	0.01	-	0.01	0.01	-	0.02	0.02	0.02	0.02	0.01
44	Curzerenone	Furan	1607	1605	0.02	0.02	0.02	0.02	-	0.03	0.02	0.03	0.02	0.02
45	Humulene epoxide	Ether	1611	1608	0.03	0.01	-	-	-	0.01	0.01	-	-	-
46	γ-Eudesmol	Oxygenated Sesquiterpene	1626	1630	0.01	0.05	-	0.01	0.01	-	0.01	0.01	-	0.02
47	α-epi-Cadinol	Oxygenated Sesquiterpene	1630	1638	0.01	0.02	-	-	0.02	0.02	0.01	0.01	-	-
48	α-epi-Muurolol	Oxygenated Sesquiterpene	1646	1640	0.01	0.03	0.01	0.03	0.01	0.01	-	0.02	0.02	0.02
49	β-Eudesmol	Oxygenated Sesquiterpene	1663	1649	0.02	-	-	0.01	0.02	0.01	-	0.02	0.01	-
50	ar-Turmerone	Oxygenated Sesquiterpene	1668	1668	0.3	0.4	-	-	0.03	-	-	-	-	-
51	α-Bisabolol	Oxygenated Sesquiterpene	1680	1685	0.01	0.04	0.01	-	0.03	0.01	0.01	0.02	0.02	-
52	Curcuphenol	Oxygenated Sesquiterpene	1712	1717	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.01	-	0.02
53	Zerumbone	Oxygenated Sesquiterpene	1744	1732	0.02	0.03	0.01	0.01	0.02	-	0.02	0.01	0.02	0.03
54	(Z)-(Z)-Geranyl linalool	Oxygenated Sesquiterpene	1948	1960	4.3	7.23	6.42	4.02	4.45	-	5.69	6.24	-	5.63
55	(E)-(Z)-Geranyl linalool	Oxygenated Sesquiterpene	1981	1987	2.1	2.92	2.63	3.01	2.15	3.43	3.12	3.59	3.51	3.27
56	(Z)-(E)-Geranyl linalool	Oxygenated Sesquiterpene	1990	1998	-	-	-	-	-	5.13	-	-	4.59	-

(*Contd*...)

Table 3: (*Continued*)

S. No.	Compound	Classification	Retention index		Relative area percentage (%)									
			RI[a]	RI[b]	Ca1	Ca2	Ca3	Ca4	Ca5	Ca6	Ca7	Ca8	Ca9	Ca10
	Total				86.44	97.22	84.38	84.64	92.382	87.79	88.73	88.94	90.89	86.26
	Monoterpene Hydrocarbon				77.38	82.74	74.43	76.18	83.65	78.48	79.45	78.67	82.04	76.03
	Oxygenated Monoterpene				5.83	8.53	4.3	5.59	7.13	5.48	5.11	3.74	5.79	4.41
	Sesquiterpene Hydrocarbon				1.29	1.85	0.29	0.56	0.48	0.08	0.13	0.07	0.07	0.1
	Oxygenated Sesquiterpene				6.96	11.08	9.13	7.19	7.152	8.73	8.93	10.05	8.23	9.08
	Other groups				0.07	0.1	0.86	0.05	0.06	0.08	0.08	0.05	0.03	0.05
S. No.	**Compound**	**Classification**	**Retention index**		**Relative area percentage (%)**									
			RI[a]	**RI[b]**	**Ca11**	**Ca12**	**Ca13**	**Ca14**	**Ca15**	**Ca16**	**Ca17**	**Ca18**	**Ca19**	**Ca20**
1.	α-pinene	Monoterpene Hydrocarbon	935	932	0.4	0.1	0.61	0.34	0.84	0.76	0.74	0.96	0.75	0.84
2.	Camphene	Monoterpene Hydrocarbon	952	946	0.01	0.02	0.02	0.01	0.04	0.02	0.02	0.04	0.01	0.03
3.	Sabinene	Monoterpene Hydrocarbon	975	969	0.02	0.01	0.01	0.02	0.02	0.03	0.04	0.02	0.04	0.05
4.	β-pinene	Monoterpene Hydrocarbon	982	974	3.01	4.03	4.01	3.49	3.45	4.82	3.66	4.54	3.65	4.12
5.	Myrcene	Monoterpene Hydrocarbon	1004	988	68.47	71.06	69.42	69.43	69.65	70.94	72.93	70.65	69.89	68.45
6.	α-Terpinene	Monoterpene Hydrocarbon	1017	1014	0.1	0.01	0.23	0.02	0.01	0.03	0.02	0.14	0.04	0.02
7.	p-Cymene	Monoterpene Hydrocarbon	1024	1020	0.01	0.02	0.01	0.01	0.03	0.02	0.02	0.03	0.02	0.02
8	Limonene	Monoterpene Hydrocarbon	1029	1024	0.05	0.25	0.02	0.13	0.3	0.04	0.21	0.06	0.01	0.03
9.	Eucalyptol	Oxgenated Monoterpene	1033	1026	0.31	0.2	0.12	0.15	0.21	0.32	0.16	0.22	0.3	0.05
10.	(Z)-β-Ocimene	Monoterpene Hydrocarbon	1036	1032	0.44	0.15	0.35	0.43	0.43	0.51	0.42	0.62	0.6	0.72
11.	(E)-β-Ocimene	Monoterpene Hydrocarbon	1049	1044	5.21	3.28	4.68	5.1	6.23	4.65	4.21	5.44	4.23	5.62
12.	Transdecahydronapthalene	Monoterpene Hydrocarbon	1052	1053	0.01	0.01	0.01	0.02	0.01	-	0.12	-	0.01	0.01
13.	γ-Terpinene	Monoterpene Hydrocarbon	1059	1054	-	0.02	0.02	0.02	0.02	0.03	-	0.02	0.02	-
14.	2-Nonanone	Ketone	1080	1087	0.02	-	0.02	0.01	0.01	0.05	0.06	0.02	0.03	0.03
15.	Linalool	Oxygenated Monoterpene	1093	1095	0.01	-	0.01	0.12	0.1	0.16	0.13	0.08	0.2	0.16
16.	Cis- Thujone	Oxygenated Monoterpene	1101	1101	0.03	0.02	0.1	0.34	0.5	0.75	0.9	0.65	0.4	0.96
17.	Perillene	Oxygenated Monoterpene	1103	1102	0.01	0.26	0.21	0.02	0.03	0.02	0.18	0.16	0.23	0.19
18.	Camphor	Oxygenated Monoterpene	1144	1141	0.01	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.01	0.26
19.	Isoborneol	Oxygenated Monoterpene	1158	1155	0.01	-	0.01	0.02	0.02	0.04	0.04	-	0.01	0.06
20.	Borneol	Oxygenated Monoterpene	1164	1165	0.02	-	0.04	0.03	0.02	0.06	0.03	-	0.01	-
21.	Terpien-4-ol	Oxygenated Monoterpene	1182	1174	0.04	-	0.02	0.04	0.04	0.04	0.05	0.04	0.01	0.05
23.	α-Terpineol	Oxygenated Monoterpene	1187	1186	0.03	0.02	0.03	0.02	0.04	0.03	0.04	0.03	0.02	0.04

(*Contd...*)

Table 3: (*Continued*)

S. No.	Compound	Classification	Retention index		Relative area percentage (%)									
			RI[a]	RI[b]	Ca11	Ca12	Ca13	Ca14	Ca15	Ca16	Ca17	Ca18	Ca19	Ca20
24.	Myrtenol	Oxygenated Monoterpene	1198	1194	0.02	0.03	0.01	0.02	0.02	0.02	0.06	-	0.04	-
25.	γ-Terpineol	Oxygenated Monoterpene	1208	1199	0.02	-	0.02	0.02	0.03	0.02	0.02	-	0.02	-
26.	Linalool formate	Oxygenated Monoterpene	1228	1214	0.04	-	0.01	0.01	0.04	0.06	0.03	-	0.03	0.04
27.	Nerol	Oxygenated Monoterpene	1230	1227	0.01	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.03
28.	β-Patchoulene	Sesquiterpene Hydrocarbon	1374	1379	0.03	-	0.02	0.01	0.01	0.03	0.03	0.02		0.02
29.	β-Cubebene	Sesquiterpene Hydrocarbon	1388	1387	0.02	0.01	0.03	0.02	0.03	0.03	0.04	0.04	0.02	0.04
30.	(E) - Caryophyllene	Sesquiterpene Hydrocarbon	1418	1417	0.89	-	0.01	0.64	0.4	1.95	0.88	1.14	0.93	1.65
31.	γ–Elemene	Sesquiterpene Hydrocarbon	1428	1434	0.01	-	-	0.02	0.02	0.03	0.06	0.06	0.04	0.05
32.	(E)-β– Farnescene	Sesquiterpene Hydrocarbon	1453	1454	-	0.02	-	0.12	0.42	0.46	0.24	0.37	0.32	0.34
33.	Germacrene D	Sesquiterpene Hydrocarbon	1480	1480	-	-	0.03	-	-	0.02	-	0.1	-	-
34.	Curzerene	Furan	1495	1499	0.01	0.03	0.02	0.01	0.03	0.06	0.02	0.09	0.01	0.07
35.	γ-Cadinene	Sesquiterpene Hydrocarbon	1519	1522	0.02	-	-	0.01	0.02	0.03	0.03	0.02	0.02	0.03
36.	δ–Cadinene	Sesquiterpene Hydrocarbon	1523	1522	0.02	-	-	0.02	0.01	0.01	-	0.03	-	0.04
37.	Germacrene B	Sesquiterpene Hydrocarbon	1561	1559	0.01	0.05	0.04	-	0.03	0.05	0.49	0.46	0.32	0.045
38.	E-Nerolidol	Oxygenated Sesquiterpene	1567	1561	0.2	-	0.03	0.01	0.1	-	0.19	-	-	0.21
39.	Spathulenol	Oxygenated Sesquiterpene	1577	1577	0.01	0.04	0.02	-	0.02	0.01	-	0.33	0.3	0.42
40.	Caryophyllene-oxide	Oxygenated Sesquiterpene	1585	1582	0.02	-	-	-	0.01	0.05	0.24	-	-	0.32
41.	ar-Turmerol	Oxygentaed Sesquiterpene	1580	1582	0.01	0.03	-	-	0.02	0.02	-	0.03	0.01	0.04
42.	Viridiflorol	Oxygenated Sesquiterpene	1599	1592	0.01	0.01	-	-	0.03	0.01	-	0.19	0.03	0.19
43.	Ledol	Oxygenated Sesquiterpene	1600	1602	0.01	0.01	0.03	-	0.01	0.01	0.03	-	0.01	0.03
44.	Curzerenone	Furan	1607	1605	0.01	0.04	0.02	0.01	0.01	0.35	0.05	0.25	0.3	0.23
45.	Humulene epoxide	Ether	1611	1608	0.02	-	-	-	0.03	0.02	0.05	-	0.01	0.05
46.	γ-Eudesmol	Oxygenated Sesquiterpene	1626	1630	0.01	0.02	0.02	-	0.01	0.01	0.02	0.02	-	0.01
47.	α-epi-Cadinol	Oxygenated Sesquiterpene	1630	1638	-	0.02	-	0.01	0.03	0.03	0.03	0.03	0.02	0.03
48.	α-epi-Muurolol	Oxygenated Sesquiterpene	1646	1640	-	0.03	0.03	0.02	0.03	0.03	0.02	0.05	0.01	0.02
49.	β-Eudesmol	Oxygenated Sesquiterpene	1663	1649	-	0.02	-	-	-	-	-	0.03	-	-
50.	ar-Turmerone	Oxygenated Sesquiterpene	1668	1668	-	-	-	0.25	0.4	-	-	0.66	0.52	-
51.	α-Bisabolol	Oxygenated Sesquiterpene	1680	1685	-	0.02	0.03	0.02	0.06	0.01	0.05	0.03	0.03	0.05

(*Contd...*)

Table 3: (*Continued*)

S. No.	Compound	Classification	Retention index		Relative area percentage (%)									
			RI[a]	RI[b]	Ca11	Ca12	Ca13	Ca14	Ca15	Ca16	Ca17	Ca18	Ca19	Ca20
52.	Curcuphenol	Oxygenated Sesquiterpene	1712	1717	0.02	0.02	0.02	0.03	0.03	0.02	0.02	-	0.02	0.03
53.	Zerumbone	Oxygenated Sesquiterpene	1744	1732	0.02	0.03	0.02	0.02	0.02	0.05	0.05	-	-	0.01
54.	(Z)-(Z)-Geranyl linalool	Oxygenated Sesquiterpene	1948	1960	-	-	-	4.65	6.21	-	7.79	-	-	5.65
55.	(E)-(Z)-Geranyl linalool	Oxygenated Sesquiterpene	1981	1987	2.31	3.01	0.24	2.56	3.54	3.01	2.91	2.51	3.12	2.65
56.	(Z)-(E)-Geranyl linalool	Oxygenated Sesquiterpene	1990	1998	6.22	4.05	4.69	-	-	6.23	-	6.25	7.23	-
	Total				88.19	86.99	85.33	88.29	93.66	95.99	97.37	96.48	93.86	94.005
	Monoterpene Hydrocarbon				78.04	79.16	79.51	79.17	81.24	82.17	82.55	82.74	79.57	79.96
	Oxygenated Monoterpene				6.24	4.03	5.7	6.41	7.79	6.8	6.49	7.33	6.18	8.22
	Sesquiterpene Hydrocarbon				1.01	0.11	0.15	0.85	0.97	2.67	1.79	2.33	1.66	2.285
	Oxygenated Sesquiterpene				8.87	7.35	5.15	7.58	10.56	9.86	11.45	10.38	11.61	9.94
	Other groups				0.06	0.07	0.06	0.03	0.08	0.48	0.18	0.36	0.35	0.38

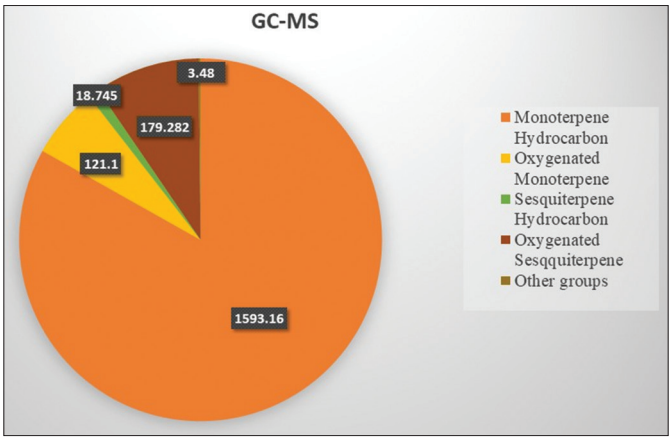

Figure 2: Class distribution of compounds studied by gas chromatography-mass spectrometry in the rhizome essential oil of *Curcuma amada.*

identified as the major constituents which deviate from the present study findings [27]. The analysis result showed a mixture of different compounds in which oxygenated sesquiterpenes (17 compounds) were found in majority followed by oxygenated monoterpene (13 compounds) and monoterpene hydrocarbons (12 compounds). The characteristic aroma of *C. amada* is contributed by the various combinations of compounds, that is, myrcene, ocimene, cis-, and trans-dihydroocimene [28].

3.2. Antioxidant Activity

DPPH free radical-scavenging activity of the *C. amada* ROs of different accessions was investigated. The results were expressed against different concentrations and the IC_{50} values were calculated [Table 4]. As per the calculated IC_{50} values, it was observed that Ca17 has got considerable antioxidant properties with an IC_{50} value of 32.05 µg/mL, whereas Ca10 has shown the lowest with IC_{50} value of 38.2 µg/mL, indicating the influence of phytochemical variation, geographic distribution, and edaphic factors on the measurement of antioxidant properties. The present study results are in agreement with one of the previous reports, which showed IC_{50} value of RO to be

Table 4: DPPH free radical scavenging activity of *C. amada* rhizome essential oil.

Accession	IC_{50} (µg/mL)
Ca1	34
Ca2	33.4
Ca3	36.65
Ca4	34.6
Ca5	33.47
Ca6	36.81
Ca7	34.56
Ca8	35.5
Ca9	37.34
Ca10	38.2
Ca11	34.4
Ca12	37.46
Ca13	36.8
Ca14	37.8
Ca15	36.67
Ca16	35.6
Ca17	32.05
Ca18	33.09
Ca19	34.2
Ca20	34.8
Ascorbic acid	5

34.7 µg/mL [21]. A report has shown appreciable antioxidant property of RO of *C. amada* with IC_{50} of 25 µg/mL [7]. It can be concluded

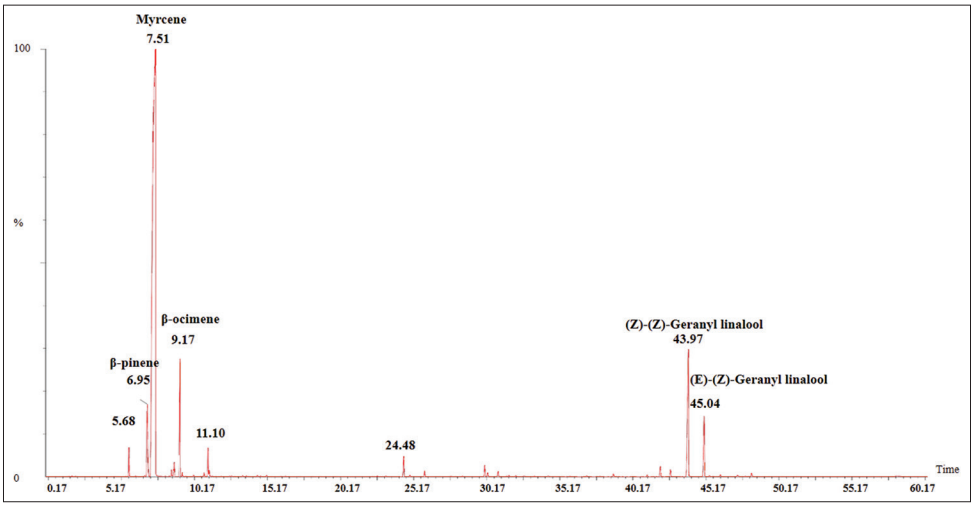

Figure 3: Gas chromatography-mass spectrometry chromatogram of *Curcuma amada* rhizome oil detecting various volatile constituents.

Table 5: Minimum inhibitory concentration (MIC in µg/ml) of *C. amada* essential oils against different strains.

Accession no.	Micro-organisms			
	B. subtilis	*S. aureus*	*A. baumannii*	*E. coli*
Ca1	12.5	6.25	6.25	12.5
Ca2	12.5	6.25	6.25	6.25
Ca3	25	25	12.5	12.5
Ca4	12.5	12.5	12.5	6.25
Ca5	6.25	3.12	6.25	6.25
Ca6	12.5	6.25	12.5	6.25
Ca7	12.5	12.5	25	12.5
Ca8	6.25	6.25	6.25	12.5
Ca9	6.25	3.12	3.12	6.25
Ca10	25	12.5	12.5	25
Ca11	6.25	6.25	12.5	6.25
Ca12	25	12.5	3.12	6.25
Ca13	6.25	12.5	6.25	12.5
Ca14	12.5	6.25	6.25	25
Ca15	6.25	12.5	12.5	12.5
Ca16	6.25	6.25	6.25	6.25
Ca17	3.12	3.12	1.56	6.25
Ca18	25	6.25	12.5	12.5
Ca19	6.25	3.12	6.25	12.5
Ca20	3.12	6.25	3.12	6.25
Ampicillin (standard)	8	4	4	8

that with an increase in the concentration of the RO, an increase in the scavenging activity was observed. Various reports were presented on different extracts of leaf and rhizome [4,9,29], but to date, very scanty reports were presented on the antioxidant activity of RO [4,9]. Therefore, the present report can be used for further analysis of the scavenging property of RO which can be used in baking industry as natural antioxidant.

3.3. Antimicrobial Activity

The antimicrobial activity of rhizome essential oils of *C. amada* accessions was evaluated against Gram-positive and Gram-negative bacterial strains by measuring the MIC values. The ROs of all the accessions demonstrated variable degrees of antibacterial potential against tested microbes. The inhibitory activity of *C. amada* was compared to that of the commercially available antibiotic Ampicillin which was used as the control. The MIC values of rhizome essential oils ranged from 1.56 to 25 µg/mL [Table 5]. It was observed that the rhizome essential oils showed more activity against *A. baumannii* (MIC: 1.56 µg/mL), followed by *S. aureus* (MIC: 3.12 µg/mL), *E. coli,* and *B. subtilis* (MIC: 6.25 µg/mL, respectively). Among all the accessions, Ca17 showed the highest antimicrobial potential against all the strains (3.12 µg/mL against *B. subtilis* and *S. aureus*, 1.56 µg/mL against *A. baumannii* and 6.25 µg/mL against *E. coli*). A similar report was showing potential antimicrobial activity by agar disk-diffusion method against *S. aureus*, *E. coli*, and *B. Subtilis* (18 mm, 16 mm, and 16 mm of inhibition zone, respectively) using ROs [19]. In the current study, it has been shown that the growth of the tested bacteria can be inhibited using *C. amada* ROs and that the bactericidal activity becomes more potent with an increasing concentration of RO. Although, one report exhibited antibacterial activity using ROs against *Ralstonia solanacearum* showing inhibition zone ranging from 3 to 7 mm [30], but no clear-cut data are available on the MIC values of rhizome essential oil of *C. amada* till date. The antimicrobial properties of the essential oil are believed to be attributed to its high levels of monoterpenes, which have been found to exhibit effectiveness against a wide range of susceptible microorganisms [19]. The present study findings from the GC-MS analysis of the rhizome samples constituted a rich amount of monoterpenes; its synergistic effects may contribute to its antimicrobial properties. Moreover, the variation in antimicrobial activity may be possible due to various edaphic factors and different geographical locations. From the above results, it can be concluded that the tested microbes are sensitive toward the ROs of *C. amada*. Therefore, the data can be utilized for making value-added products in the food industry.

4. CONCLUSION

With the advent of modernity, consumers are concerned about synthetic additives in foods, which have forced food processors to seek

alternatives, resulting in the need for "clean label" products in the food industry. In the present study, *C. amada* rhizomes were found to have good antioxidant and antimicrobial potential which may be attributed to the presence of terpenoids. These findings could enhance the use of *C. amada* rhizome oil and could meet the demands of consumers for healthier foods by promoting natural alternatives.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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