ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 07, pp.18098-18104, July, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

PHYTOCHEMICAL, ANTIOXIDANT AND ANTIBACTERIAL STUDIES ON THE ESSENTIAL OIL OF THE RHIZOME OF *CURCUMA AMADA* ROXB.

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ARTICLE INFO

Article History:
Received 20th April, 2015
Received in revised form
18th May, 2015
Accepted 09th June, 2015
Published online 31st July, 2015

Key words:

Curcuma amada Roxb., Essential oil, GC-MS Analysis, Antibacterial, Antioxidant.

ABSTRACT

This study investigates the chemical composition, *in vitro* antioxidant activity and antibacterial activity of essential oil of *Curcuma amada* Roxb. The GC- MS analysis of the oil has shown a profile of 17 compounds. β-Myrcene (69.60%) and β-Pinene (15.15%) are the two major components. The antioxidant activity was done by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, total antioxidant assay, Ferric reducing antioxidant power and nitric oxide scavenging assay. This study proves that the essential oil could serve as an important bio-resource of antioxidants for using in food and pharmaceutical industry. Besides, the essential of *C.amada* remarkably inhibited the growth of 12 bacterial strains. Results indicated that essential oil of *C.amada* included rather higher proportions of mono-terpenoid compounds with good antioxidant and antibacterial properties.

Citation: Mariat George, S. John Britto, M. Thamacin Arulappan, R. R. Marandi, Ignace Kindo and Dessy, V. J. 2015. "Phytochemical, antioxidant and antibacterial studies on the essential oil of the rhizome of *Curcuma amada* roxb.", *International Journal of Current Research*, 7, (7), 18098-18104.

INTRODUCTION

Essential oils are valuable natural products used as raw materials in many fields such as perfumes, cosmetics, aromatherapy, spices and nutrition (Baron and Finegold, 1990). Essential oils could be extracted from foliage, stems, flowers, roots, herbs, brushes, and trees right through distillation. They have been used for therapeutic and curative purposes for numerous years all over the world. Importance of essential oils has amplified in current decades with the reputation of aromatherapy, which claims that essential oils and additional aromatic compounds have useful effects (Alizadeh, 2013; Al-Qudah *et al*., 2014; Topçu *et al*., 2013; Usano-Alemany *et al*., 2014). Zingiberaceae or the ginger family constitutes a vital group of rhizomatous medicinal and aromatic plants (Sabu, 2006) characterized by the presence of volatile oils and oleoresins of export value. The usefulness of curcuma has been studied for decades for its chemical and biological properties. It is extensively used as an aromatic medicinal cosmetic in India, besides its use as medicine for various diseases related to skin, cardiovascular and respiratory system.

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Species of genus *Curcuma* namely *C. longa and C. zedoaria* whose essential oils were found to contain ar-turmerone, turmerone, turmerol and zingiberene as the major constituents, possessed antioxidant, antimicrobial, anti-inflammatory and cytotoxic properties

(Mishra and Gupta, 1997; Singh *et al*., 2002; Mau *et al*., 2003; Lai *et al*., 2004; Saccheti *et al*., 2005; Naz *et al*., 2010). Most of the other tuberising *Curcuma* species produce aromatic rhizomes which are rich in essential oils varying in chemical constituents but which remain unexplored for their pharmacological properties. Studies on their biological activity would be beneficial in medicinal applications. Mango ginger (*Curcuma amada* Roxb.) is a perennial herb, which morphologically resembles the ginger (*Zingiber officinale*) but, it imparts mango (*Magnifera indica*) flavour. The mango ginger starch constitutes 43% of amylose and resembles the characteristic of both *Curcuma longa* and *Zingiber officinale* starch (Policegoudra and Aradhya, 2007). Essential oil from *Curcuma amada* Roxb. could serve as an important bio - resource of antioxidants for using in food and pharmaceutical industry (Policegoudra *et al*., 2007). Antioxidants have great importance because they can reduce oxidative stress which could cause damage to biological molecules. Antioxidant compounds play a crucial role in the treatment of various

diseases related to degenerative disorders, namely, cardiovascular and brain diseases, arthritis, diabetes, cancer and immune system decline, by acting as free radical scavengers, and thus decreasing the extent of oxidative damage. Furthermore, studies about antioxidant substances in foods and medicinal natural sources have attracted increased interest in the recent decades. In addition, the use of plant materials in lipids and lipid-containing foods is important because the plant potentials of decreasing rancidity, delaying the formation of toxic oxidation products, maintaining nutritional quality and increasing the shelf life of food products. Hence, evaluation of radical scavenging properties and antioxidant activity are of commercial interest to the pharmaceutical and food industries as a source of natural antioxidants (Valifard *et al*., 2014; Al-Tawaha *et al*., 2013; El Abdouni Khiyari *et al*., 2013; Salehi *et al*., 2013; Kivrak *et al*., 2009; Tel *et al*., 2010). Microbial actions of essential oils are also one of the most extensively studied features of botanical medicine and various aromatic plant species were being investigated for their pharmacological properties (Bouajaj *et al*., 2013; Ulukanli *et al*., 2013; Kotan *et al*., 2008; Hayet *et al*., 2007; Bouajaj *et al*., 2013). The objectives of the present study were to identify chemical composition as well as assess the antioxidant and antibacterial properties of the essential oil of the rhizome of *Curcuma amada* using gas chromatography combined with mass spectrometry (GC-MS) and flame ionization detector.

MATERIALS AND METHODS

Collection of Plant Sample

Curcuma amada was collected from Kottayam and Poonjar (Kerala, India). They were identified and authenticated by Dr. S. John Britto, the Director and Head, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (*Autonomous*), Tiruchirappalli, Tamilnadu, India. The voucher specimen (RHT 65181) was deposited at Rapinat Herbarium.

Extraction of Essential oil

The fresh rhizomes of plants were subjected to hydrodistillation for 3 hrs using a Clevenger type apparatus. The obtained essential oil was dried over anhydrous sodium sulphate (Na_2SO_4) and preserved in a sealed vial at 4°C until further analysis.

GC-MS analysis

The analysis of the essential oil was performed using a Hewlett Packard 5890 II GC equipped with a FID detector and HP-5 ms capillary column (30m´ 0.25m, film thickness 0.25µm). For GC-MS detection, an electron ionization system was used with ionization energy of 70 eV. Helium was the carrier gas, at a flow rate of 1ml/min. Injector and MS transfer line temperature were set at 220 and 290°C respectively. Column temperature was initially at 50°C, and then gradually increased to 150°C at a 3°C/min rate, held for 10 min and finally increased to 250Vc at 10Vc/min. Diluted samples (1/100 in petroleum ether) of 1.0µl were injected manually and split less. The components were identified based on the comparison of their relative retention time and mass spectra with those of Wiley 7N Library data and standards of the main components.

Antioxidant activity

DPPH Radical Scavenging activity

Radical scavenging activity was measured by using DPPH scavenging method of (Blois, 1958). A solution of DPPH in methanol (24µg/ml) was prepared and 2ml of this solution was added to oil at different concentrations (10- 40µg/ml). Absorbance at 517 nm was determined after 30 min at room temperature and the scavenging activity were calculated as a percentage of the radical reduction. Each experiment was performed in triplicate. Ascorbic acid was used as reference compound.

Total antioxidant capacity assay

The total antioxidant capacity assay was determined as described by Prieto *et al*. (1999) Different concentrations of the essential oil (10-40µg/ml) were taken and added 1.0 ml of the reagent solution (0.6 M Sulphuric acid, 28 mM Sodium phosphate and 4 mM Ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid.

Reducing power assay

The reducing power of extract was determined by the method of Yen and Duh. (1993) Different concentrations of essential oil (10-40µg/ml) were mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1 % Potassium ferricyanide. The mixtures were incubated at 50°C for 20 min. After incubation, 2.5 ml of 10% Trichloroacetic acid were added to the mixtures, followed by centrifugation for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1 % Ferric chloride and the absorbance of the resultant solution were measured at 700 nm.

Nitric oxide scavenging assay

Nitric oxide scavenging activity was measured spectrophotometrically (Govindarajan *et al*., 2003). The essential oil was added to different test-tubes in varying concentrations (10-40 µg /ml). Sodium nitroprusside (5mM) in phosphate buffer was added to each test tube to make volume up to 1.5ml. Solutions were incubated at 25ºC for 30 minutes. Thereafter, 1.5ml of Griess reagent (1% Sulphanilamide, 0.1% Naphthylethylenediamine dichloride and 3% Phosphoric acid) was added to each test tube. The absorbance was measured immediately at 546 nm and the percentage of scavenging activity was measured with reference to ascorbic acid.

Antimicrobial studies

Bacterial isolates and Bioassay

Thirteen bacterial strains were used in this study: *Escherichia coli*, (MTCC # 119) *Pseudomonas aeruginosa* (MTCC #

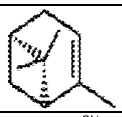
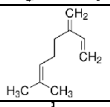
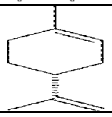
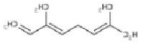
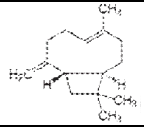
2474), *Salmonella paratyphi* (MTCC # 734), *Vibrio cholerae* (ATCC # 14104), *Streptococcus pneumoniae* (ATCC # 7066), *Bacillus subtilis* (MTCC # 441), *Bacillus cereus* (ATCC # 4342), *Proteus vulgaris* (MTCC # 1771), *Proteus mirabilis* MTCC # 1429), *Serratia marcescens* (MTCC # 2645), *Klebsiella pneumoniae* (MTCC # 3040), *Staphylococcus aureus (MTCC#3163)* and *Enterobacter aerogenes* (MTCC#2990). Evaluation of in vitro antibacterial activity was carried out by the plate diffusion procedure as described by Perez *et al*. (1995). The essential oils were diluted with Dimethyl sulphoxide (DMSO) and aliquots were loaded on a 6 mm diameter disc, air dried and placed on sterile medium in a petri dish. Plates were incubated at 37ºC.

RESULTS AND DISCUSSION

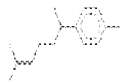
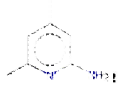
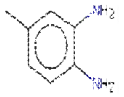
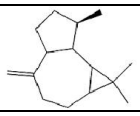
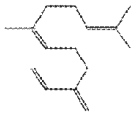
The GC-MS study of *C. amada* has shown many phytochemicals which contributes to the medicinal activity. The *C. amada* rhizome contains about 17 phytochemical compounds such as Caryophyllene, Alloaromadendrene, 1-Heptatriacotanol, cis-β-Farnesene, cis-β-Farnesene, alpha-Pinene, alpha-Pineneand other compounds. These 17 compounds are responsible for antimicrobial, antifungal, sedative, antitumor, antioxidant and insecticidal in this plant. Camphene is used as stimulant; D-Limoneneis used as antioxidant; β-Pinene is used as antimicrobial; Alloaromadendrene is used as antihelmetheic activity; Caryophyllene is used as antifugal; cis-β-Farnesene is used as inflammation and1-Heptatriacotanolis used as antispasmodic (Table 1).

Plants with radical scavenging property and antioxidant capacity are useful for medicinal applications and as pharmaceutical industries. So, in the present study, the antioxidant capacity of *C. amada* was evaluated using DPPH radical scavenging method by comparing with the activity of the ascorbic acid as a known antioxidant. The antioxidant capacity of essential oil of *C. amada* was higher than that of the used synthetic antioxidant (Fig.1).

Table 1. Chemical composition of Essential Oil from the Rhizome of *C.amada*

No.	Name of compound	Chemical formula	Molecular mass	Structure	Rt	% area	Uses
1.	2,6,6-Trimethylbicyclohept-2-ene	$C_{10}H_{16}$	136.24		4.512	4.98	Insecticide, Cosmetics
2.	Camphene	$C_{10}H_{16}$	136.24		4.803	0.42	Stimulant, tonic, antiseptic and antispasmodic
3	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	$C_{10}H_{16}$	136.2340		5.387	15.1	Anti-Infective Agents, Anti-inflammatory, Flavoring agents, Insecticides
4	β-Pinene	$C_{10}H_{16}$	136.23		5.719	69.6	Natural insecticide, antimicrobial activities
5	β-Myrcene	$C_{10}H_{16}$	136.23		5.719	69.6	Analgesic
6	D-Limonene	$C_{10}H_{16}$	136.23		6.491	0.30	Antioxidant and Anti-inflammatory
7	α-Pinene	$C_{10}H_{16}$	136.23		6.652	0.35	Anticancer
8	β-ocimene	$C_{10}H_{16}$	136.23		6.652	0.35	Antioxidant, Anti-inflammatory
9	1,3,6-Octatriene, 3,7-dimethyl-, (Z)	$C_{10}H_{16}$	136.23		6.652	0.35	Antimicrobial
10	Caryophyllene	$C_{15}H_{24}$	204.36		16.60	1.33	Antifungal, Anti-inflammatory effect

Continue……..

11	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	$C_{15}H_{22}$	202.3352		18.10	1.01	Antioxidant Activity
12	2-Pyridinamine, 4,6-dimethyl-	$C_7H_{10}N_2$	122.1677		18.10	1.01	Antioxidant, Antiulcer
13	1,2-Benzenediamine, 4-methyl	$C_7H_{10}N_2$	122.1677		21.03	0.83	Antimicrobial
14	Alloaromadendrene	$C_{15}H_{24}$	204.35106		28.223	1.51	Anthelmintic
15	1-Heptatriacotanol	$C_{37}H_{76}O$	536.998740		28.223	1.51	Anthelmintic, Purgative, Antispasmodic
16	cis-β-Farnesene	$C_{15}H_{24}$	204.3511		28.881	0.55	Perfume
17	α-Farnesene	$C_{15}H_{24}$	204.3511		28.881	0.55	Perfume

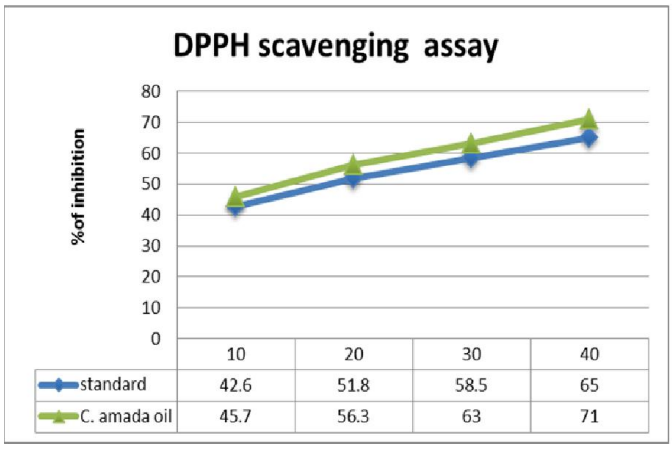


Fig. 1. DPPH Scavenging of *C. amada* essential oil, compared to that of Ascorbic acid

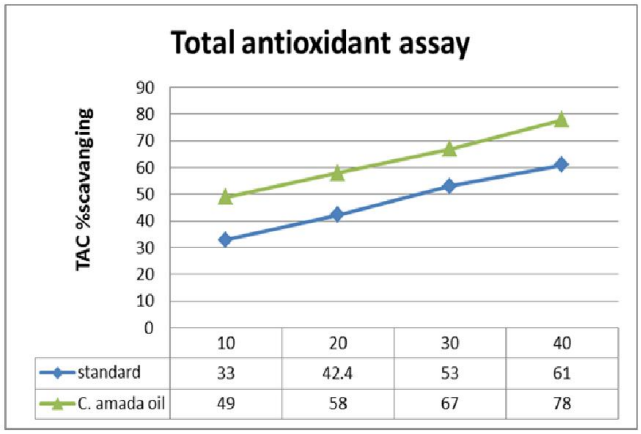


Fig. 2. Total antioxidant assay of *C. amada* essential oil, compared to that of Ascorbic acid

The total antioxidant capacity of the essential oil was determined by phosphormolybdenum with using Ascorbic acid as standard. In phosphormolybdenum assay, the concentrations range from 10-40µg/mL, essential oil showed higher dose dependent reducing activity than ascorbic acid (Fig. 2). The result obtained was confirmed by the high potency of essential oil towards the transition metal ions. The reducing power assay was found to be 0.25 at 40µg/mL in essential oil. This result showed that ascorbic acid exhibited excellent reducing power activity than *C. amada* essential oil (Fig. 3).

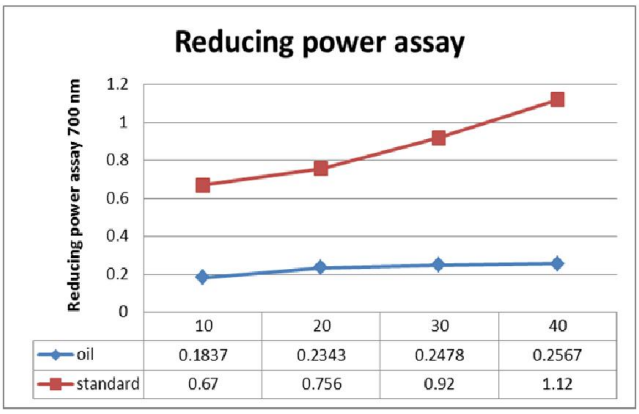


Fig. 3. Reducing Power Assay of *C. amada* essential oil, compared to that of Ascorbic acid

Nitric Oxide (NO) scavenging assay is based on the scavenging ability of essential oil, as well as ascorbic acid, which is used as standard. The scavenging of NO was found to increase in dose dependent manner. Maximum inhibition of NO was observed in the extracts of highest concentration (40µg/ml) for both the samples. At this maximum concentration, inhibition was found to be 67% for ascorbic acid, which serves as the standard. For *C. amada* essential oil inhibition was found to be higher 63 % (Fig. 4).

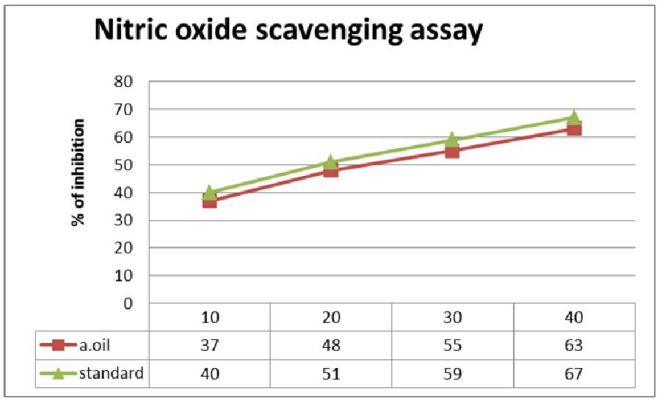


Fig. 4. Nitric oxide scavenging of *C. amada* essential oil, compared to that of Ascorbic acid

Table 2. Antibacterial activity (inhibition zone) of the essential oil of *C. amada*

no	Name of Bacteria	Zone in mm	
		C.amada oil	Antibiotic (Streptomycin)
1	*Staphylococcus aureus*	18mm	17mm
2	*Escherichia coli*	16mm	19mm
3	*Klebsiella pneumoniae*	17mm	20mm
4	*Pseudomonas aeruginosa*	10mm	18mm
5.	*Salmonella paratyphi*	15mm	18mm
6	*Vibrio cholerae*	15mm	20mm
7	*Enterobacter aerogenes*	17mm	19mm
8	*Streptococcus pneumoniae*	19mm	17mm
9	*Bacillus subtilis*	16mm	21mm
10	*Bacillus cereus*	11mm	16mm
11	*Proteus mirabilis*	15mm	18mm
12	*Proteus vulgaris*	14mm	19mm
13	*Serratia marcescens*	-	14mm

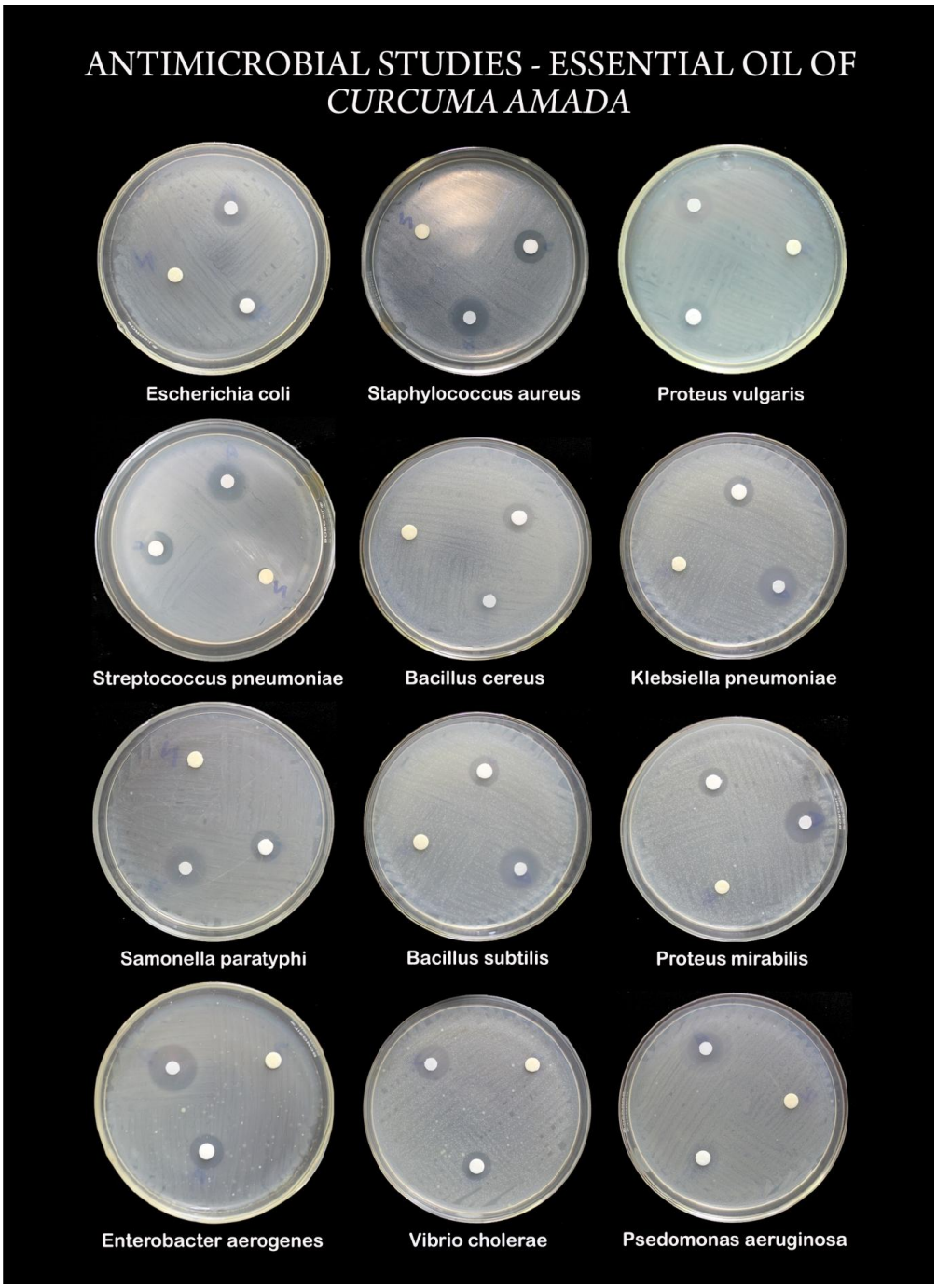


Plate 1. Antimicrobial studies – essential oil of *Curcuma amada*

Therefore, the antioxidant properties of essential oil could play a valuable role in the food conservation and also in the prevention of oxidative damage related to the path physiology of many diseases, including significant and prevalent neurodege. The antibacterial property of the essential oil and extracts has led to the basis of many applications. Curcuma, is gaining importance world wide as a potential source of new drugs to combat a variety of ailments as the species contains molecules credited with anti-inflammatory, hypocholestremic, choleratic, antimicrobial, insect repellent, ant-rheumatic, anti-fibrotic, antivenomous, antiviral, antidiabetic, antihepatotoxic as well as ant cancerous properties (Sukari Mohd *et al*., 2010). The antibacterial activity of the essential oil of *C. ceasia, C.amada* and antifungal activity of essential oil of *C. aromatica* were earlier reported by Banerjee and Nigam (1976) Angel *et al.* (2012) Rao (1976) respectively. This study also demonstrated that the essential oil displayed antimicrobial activity on Gram negative and Gram positive bacteria. The strong antimicrobial activity of the essential oil against almost all the susceptible microorganisms can be attributed to the presence of high concentration of monoterpenes. The essential oil remarkably inhibited the growth of tested Gram positive and Gram negative bacteria except *Serratia marcescens* (Table 2). The extract showed significant antimicrobial activity against (19mm), and *Staphylococcus aureus* (18 mm) (Plate 1).

Primary studies were conducted in advance especially in practical applications of the essential oils in fragrance and flavor industries, as well as in the chemical andpharmaceutical industries. Gas chromatography–mass spectrometry (GC–MS) is certainly a useful and powerful tool in the essential oil analysis. It is noteworthy that the composition of the essential oils from a particular species of a plant can differ between harvesting seasons, extraction methods, and geographical sources, and that those from a different parts of the same plant can also differ widely (Yoshioka *et al*., 2004). Maturation stages constitute an important factor influencing essential oil composition in some plants (Telci *et al*., 2009). The synergistic role of various constituents present in the oil might also features to the antioxidant nature of essential oil. However, it was also considered that minor components, might also have likely interactions between the major components which might also affect the antioxidant activities.

In that sense, for biological determination, it is more enlightening to study the entire oil rather than its components. Many plants species are currently used as a source of nutritional additives because of their antioxidant properties that increase immunity to diseases. The essential oil of *Curcuma amada* showed high amount of in vitro antioxidant activity. This study also demonstrated that the *C. amada* essential oil displayed antimicrobial activity on Gram negative and Gram positive bacteria. The tested microorganisms are pathogens or opportunists for man, animal and plants, and they cause contamination and deterioration in food, water and air. This in vitro experimental study clearly shown the efficient ant bactericidal action of *C. amada* essential oil and support the freely use of this natural, pleasant and eco-friendly product as a preservative in food and water which are susceptible for generating pleasant odors.

Conclusion

Quantitative analyses of the chemical composition of the investigated essential oils of *Curcuma amada* were tested. Gas chromatography/mass spectrometry (GC-MS) analysis revealed the presence of 17 major chemicals in all three of the oils. Chemical identification of the oil constituents was conducted based on their retention time (tR), retention indices (KI) and mass spectral data, as well as by computer search of mass spectral databases. The chemical structures and medicinal properties also identified. The sample was subjected to screening for their possible antioxidant activity by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, total antioxidant assay, Ferric reducing antioxidant power and nitric oxide scavenging assay. Results showed that the essential oil possessed a strong degree of antioxidant activity. The essential oil remarkably inhibited the growth of tested Gram positive and Gram negative bacteria.

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