Chemical Composition, Antioxidant and Antimicrobial Activity of Turmeric Essential Oil (*Curcuma longa* L.)

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ABSTRACT: *The objective of this work is to identify and examine the antioxidant and antibacterial effects of the phytochemicals found in the crude extract of Eugenia caryophyllus rhizome. The phytochemical screening, GC-MS were determined using standard methods. Antibacterial activities were evaluated by disc diffusion and agar well diffusion methods. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) were determined using standard procedures. The aqueous and methanolic extracts of turmeric (Curcuma longa) rhizome showed the presence of phytochemicals like tannins, flavonoids, alkaloids, reducing sugar and saponin. Mineral composition analysis shows that the plant contains Na, Ca, Mg, K and Fe. Nineteen compounds were identified using GC-MS analysis of turmeric with a R-Turmerone being the most abundant with peak area of 50.05%. The study evaluates the phytochemical screening, Gas chromatography–mass spectrometry (GC-MS) and antibacterial activities of aqueous and methanolic extracts of turmeric (Curcuma longa) rhizome against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus).*

Keywords: Eugenia caryophyllus. Phytochemicals. Antibacterial agent. Antioxidant efficacy. Eugenol

1. INTRODUCTION

Curcuma longa L., often known as turmeric, belongs to the Zingiberaceae family. Turmeric is a golden spice obtained from the rhizome of the Curcuma longa plant [1]. Curcuma longa has served as the primary component in culinary preparations from Nigeria, India, and Bangladesh due to its color, flavor, and taste. In West Africa, it is mostly utilized as a dye to provide a golden yellow hue to items such as cotton fabric, tanned leather, palm fibers, and thread. The use of the yellow hue from turmeric rhizome and other botanical derivatives as dyes is rising, aiming to substitute synthetic additives with natural components [2]. The yellow hue of turmeric results from three primary curcuminoids found in the rhizome. Dry turmeric has 5.2% oils, 6.3% proteins, 69.45% carbs, 3.7% minerals, and other components [3] Approximately 235 compounds, predominantly terpenoids and phenolics, have been identified from various turmeric species, including 22 diarylheptanoids and diarylpentanoids, 8 phenylpropenes alongside other phenolics, 109 sesquiterpenes, 68 monoterpenes, 5 diterpenes, 4 sterols, 3 triterpenoids, 2 alkaloids, and 14 additional compounds [4]. Curcuminoids, namely curcumin, and essential oils, chiefly monoterpenes, are the principal bioactive components exhibiting various bioactivities. Calebin-A, vanillic acid, vanillin, quercetin, and other phenolic chemicals have been previously found in turmeric [2,5]. Research indicates that the aqueous extract of turmeric rhizomes had antibacterial efficacy against Staphylococcus aureus and Escherichia coli which is Gram-negative, rod-shaped bacteria often located in the lower intestine of warm-blooded organisms, induce serious infectious illnesses linked to elevated mortality and morbidity rates [6]. This study assesses the phytochemical screening, gas chromatography-mass spectrometry (GC-MS), and antibacterial properties of aqueous and methanolic extracts of turmeric (Curcuma longa) rhizome against Escherichia coli and Staphylococcus aureus.

Essential oils may be extracted using many processes, including hydro-distillation, steam distillation, water/steam distillation, expression, and supercritical carbon dioxide extraction [7]. Due to their fragrance, scent, and several advantageous effects, they are extensively utilized in fragrances, cosmetics, aromatherapy, and nutrition [8-9]. The

turmeric rhizome has two primary categories of secondary metabolites: phenolic curcuminoids and essential oil. These metabolites predominantly account for the pharmacological actions of turmeric [10]. Curcuminoids provide the yellow hue to turmeric, while its essential oil contributes to its scent and flavor. The principal and most researched curcuminoid in turmeric is curcumin, acknowledged as the primary ingredient responsible for the bulk of the positive benefits exhibited by this remarkable plant. In addition to curcumin, there are two more curcuminoids: demethoxycurcumin and bisdemethoxycurcumin [11]. The essential oil may be extracted from fresh and dried leaves, fresh flowers, dried roots, and both fresh and dried rhizomes of turmeric. Essential oil extraction in the business utilizes dried rhizomes and leaves. Rhizomes, although they possess a greater concentration of active chemicals than other plant parts, have higher oil content than leaves, with 5-6% compared to 1-1.5%, respectively. Essential oils derived from leaves and flowers are predominantly composed of monoterpenes, but those extracted from roots and rhizomes generally consist of sesquiterpenes [12]. The primary volatile constituents of the rhizome oil are α- and β-turmerone, together with ar-turmerone [13].

It demonstrates numerous advantageous effects due to its phytochemical constituents, including: anti-carcinogenic, anti-inflammatory, anti-microbial, anti-fungal, anti-mutagenic, hypocholesteremic, insect repellent, anti-rheumatic, anti-fibrotic, anti-venomous, anti-diabetic, anti-viral, and anti-hepatotoxic properties. Turmeric has been utilized for religious purposes as an amulet within Hindu culture; as a spice and food colorant due to its flavor and golden hue, as well as a food preservative in India. In Ayurveda, it is administered orally as a stomachic and blood purifier, addressing gallbladder and cardiac issues, liver disorders, bloating, menstrual complications, urinary tract disorders, allergies, arthritis, and other chronic ailments. Topically, it is employed in the treatment of various dermatological conditions, and via inhalation, it is used for chronic rhinitis and coryza.

This study aimed to ascertain the chemical composition of the essential oil extracted from turmeric rhizome via Clevenger hydrodistillation employing the GC-MS technique, evaluate its antioxidant activity through the DPPH assay, and assess its antimicrobial efficacy using the disc-diffusion method, thereby enhancing the application of turmeric in the pharmaceutical and food industries in many countries. Despite extensive research on the isolation and characterization of turmeric essential oil, the literature referenced in this paper indicates that the chemical composition and biological activity of the essential oil derived from the commercially sourced spice have yet to be examined.

2. EXPERIMENTAL

Materials and Methods

Empirical Vegetative matter

A high-quality yellow turmeric powder, derived from the dried and ground rhizome of Curcuma longa, was acquired from a local food store. Reagents and chemicals are essential components in various scientific experiments and analyses.Essential oil was extracted using Clevenger hydrodistillation, employing a hydromodulus of 1:5 m/V over duration of 240 minutes. The quantity of essential oil was quantified per 100 g of plant material.The extracted oil was dried using anhydrous sodium sulfate and stored at 4 °C until analysis.

Analysis Method of Gas Chromatography:

Gas Chromatographic Conditions:

Analysis was performed by Agilent Technologies 6890N Network system at Centre for Aromatic Plants (CAP), Dehradun (Uttarakhand) India.

Column	HP-55% Phenyl methyl siloxane capillary column (30mX0.32mm film thickness 0.25mm)
Carrier Gas	Nitrogen gas
Detector temprature	250^0C
Injector temp(Inlet)	210^0C
Injection volume	0.2µl
Column Oven temp	60^0C for 2 min ramp 3^0C/min up to 210^0Cfor 5 min
Detector	FID
Split Ratio	50/1

Analysis Method of Gas Chromatography-Mass Spectrometry:

GC-MS Chromatographic Conditions:

Column	Rtx^R-5 Capillary column (60M x 0.32 mm ID X film thickness 0.25µm) cross bond® 5% diphenyl siloxane.
Carrier Gas	Helium gas flow
Injector temp(Inlet)	210^0C
Injection volume	0.2µl
Column Oven temp	60^0C for 2 min ramp 3^0C/min up to 210^0Cfor 5 min
Mass range	
Solvent delay time	5 min
MS scan time	5.1 min to 55 min
Ionization mode	EI^+

Identification of Components:

The constituents of the oil were identified by comparison of their mass spectra with those of computer library search(NIST/PFLEGER/WILEY) and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature.

The retention indices of the components from the analyzed samples were determined experimentally using a homologous series of n-alkanes ranging from C_8 to C_{20} as standards. Compound identification was conducted by comparing their retention indices with literature values [14-15], in addition to analyzing their mass spectra against those from Willey, NIST, and PFLEGER libraries. The percentage composition of specific components in the essential oil was determined using the automatically integrated peak areas of the GC-MS signal.

3. RESULTS AND DIDCUSSION

DPPH assay

The capacity of the essential oil to scavenge free DPPH radicals was assessed through the DPPH assay. The essential oil was dissolved in ethanol, and a range of different concentrations was prepared. 1 cm^3 of the ethanol solution containing DPPH radical (300 µmol, or 3 x 10^{-4} mol/dm^3) was combined with 2.5 cm^3 of the prepared essential oil solutions. Absorption was measured at 517 nm immediately following the addition of the DPPH radical and after 20, 30, and 45 minutes of incubation with the radical. The absorption at 517 nm was measured for the ethanolic solution of DPPH radical, which was diluted in the specified ratio (1 cm^3 of DPPH radical at the given concentration with 2.5 cm^3 of ethanol added). Ethanol served as the blank in the experiment. The free radical scavenging activity was determined using the formula.

Control ethanolic solution of the DPPH radical

All absorptions were measured using a UV-VIS VARIAN Cary 100 Conc. spectrophotometer. The concentration of essential oil required to neutralize 50% of the initial DPPH radical concentration is referred to as the EC50 value. The value was established through linear regression analysis within the concentration range of 0.008 to 2 mg/cm3 of essential oil incorporated into the reaction mixture.

Analysis of the Mineral Composition of Turmeric

Two grams of turmeric were digested with 10 mL of aqua regia, consisting of trioxonitrate (v) acid and hydrochloric acid in a 1:3 ratios. The resulting mixture was heated in a crucible for several minutes until the brown fumes produced during the process dissipated, leaving only white fumes. The solution was subsequently filtered through filter paper into a universal bottle. The analysed minerals included Ca, Fe, K, Na, Mg, Cu, Zn, and Pb (Table.1).

Initial phytochemical assessment

The qualitative methods employed confirmed the presence of saponins, tannins, alkaloids, flavonoids, anthraquinones, glycosides, and reducing sugars [16]. The qualitative analyses of the extract were conducted based on the intensity of the color change (Table.3).

Testing for antibiotic susceptibility

The organisms' susceptibility to various antibiotics was assessed using the disk diffusion method. Mueller Hinton agar was freshly prepared and standardized according to the methods of Famuyide et al. [17] and the National Committee for Clinical Laboratory Standards (NCCLS), 2000. The following antibiotics were utilized: Rocephin (25µg/disk), chloramphenicol (30µg/disk), streptomycin (30µg/disk), erythromycin (10µg/disk), ciprofloxacin (10µg/disk), and septrin (30µg/disk). /disk). The experiment was conducted in triplicate for each combination of antibiotics and bacterial strains (Table.4 and 5).

Measurement of the diameter of the inhibition zone via the agar well diffusion technique. The agar well-diffusion method was utilized to assess the antimicrobial activity of aqueous and methanolic root extracts of turmeric (Curcuma longa). Eighteen hours of culture from the two microorganisms were suspended in sterile nutrient broth. The

standardization involved the incremental addition of 9% normal saline to achieve turbidity comparable to the McFarland standard of 0.5, corresponding to approximately 1 x 10^8 colony-forming units per mL. Petri dishes were prepared by adding approximately 25 mL of autoclaved nutrient agar to sterile plates, which were then allowed to solidify. The surface of each plate was drilled with a sterile cork borer (6 mm), resulting in three wells being created on each plate. A total of 100 µL of a standardized culture (adjusted to 0.5 McFarland) of the two organisms was added to different agar plates. Subsequently, 100 µL of the aqueous and methanolic root extracts of turmeric were loaded into the wells and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 37ºC for 18 to 24 hours to assess bacterial pathogens. The diameters of the inhibition zones (mm) were quantified. The susceptibility of Staphylococcus aureus and Escherichia coli to aqueous and methanolic extracts of turmeric was assessed using standard methods[6]. The experiment was conducted three times, with readings taken in three distinct fixed directions for each replicate, and the average values were documented. The inhibitory responses were categorized as follows: potent response (++++), with a zone diameter greater than 30 mm; strong response (+++), with a zone diameter between 21 and 30 mm; moderate response (++), with a zone diameter between 16 and 20 mm; weak response (+), with a zone diameter between 10 and 15 mm; and little or no response, with a zone diameter less than 10 mm [18].

The minimum inhibitory concentration (MIC) of aqueous and methanolic root extracts of turmeric (Curcuma longa). The minimum inhibition concentration refers to the lowest concentration of extract that prevents the growth of test organisms, as evidenced by the lack of visible turbidity in the experimental tubes compared to the control tubes. The minimum inhibitory concentration (MIC) of the aqueous and methanolic extracts of turmeric rhizome was determined using a standard method [6]. The minimum inhibitory concentration (MIC) of the aqueous and methanolic root extracts of turmeric was determined using the serial dilution method. A total of 1 mL of Mueller-Hinton broth was distributed into various test tubes and subsequently autoclaved. Subsequently, 1 mL of 100% aqueous and methanolic root extracts of turmeric (2 g/mL) was added to the first separate test tubes to achieve a concentration of 50%. Two-fold serial dilutions were then performed by transferring 1 mL from one tube to another, resulting in the following series: 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%, 0.39%, etc. An overnight culture of the various test organisms was adjusted to the McFarland turbidity standard, and 100 µL of each cell suspension was added to separate tubes. The tubes underwent aerobic incubation at 37°C for duration of 18 hours. A negative control tube was prepared by adding 1 mL of normal saline in place of the aqueous and methanolic root extracts of turmeric. The minimum inhibitory concentration was defined as the lowest dilution concentration at which no bacterial growth occurred(Table.4).

Minimum Bactericidal Concentration (MBC) of aqueous and methanolic extracts from turmeric roots

The minimum inhibitory concentration (MIC) of the aqueous and methanolic root extracts of turmeric was determined using standard methods [6]. In the procedure, 0.1 mL aliquots of test samples from the non-turbid tubes of the minimum inhibition concentration assay were sub-cultured onto nutrient agar plates. The plates were incubated aerobically at 37°C for duration of 24 hours.

The mediums utilized for microbial growth include nutrient agar for bacteria and Sabouraud maltose agar for fungi. Microorganisms originate from the collection of the Microbiology Laboratory. The agar disc-diffusion method was employed to evaluate the antimicrobial activity of turmeric essential oil. The media were sterilized for 15 minutes in an autoclave at 121 °C under 110 kPa. An inoculum of 0.1 cm^3 from an overnight culture was added to 10 cm^3 of the medium and subsequently poured into petri dishes. For

screening, sterilized filter paper disks (12.7 mm diameter, Schleicher & Schuell) were positioned on the surface of inoculated media and infused with 60 µl of the essential oil (1:10 V/V in DMSO). The plates were incubated for 24 hours at 37 °C for bacterial growth and for 48 hours at 25 °C for fungal growth. Following incubation, the diameters of the inhibition zones were measured and reported in millimetres (Table.6). The inhibition zone signifies the efficacy of the tested samples against bacterial or fungal organisms. Standardized discs of Ampicillin (10 µg/disc), Bactrim (25 µg/disc), Cefalexin (30 µg/disc) from Bio Rad, and Nystatin (100 U/disc) from Bio-analyse were utilized as reference standards. DMSO served as the negative control.

Table 1. Mineral composition of *Curcuma longa*

Elements	Conc. in mg/L	%RSD
Na	1.363 ±0.02	Not Available
Mg	0.802±0.01	Not Available
Ca	0.797 ±0.01	1.16
K	0.002 ±0.00	0.55
Fe	1.011 ± 0.02	0.50
Zn	0.048 ± 0.00	30.3
Ag	0.002 ± 0.00	23.8
As	0.0097 ± 0.00	50.1
Cd	0.0059 ±0.01	80.2
Co	0.0086 ± 0.00	140.7
Cu	0.0061 ± 0.00	307.3
Ni	0.016 ±0.00	12.4
Pb	-0.028 ± 0.00	363.1

Values are mean ± standard deviation for triplicate determinates

Table 2. Compounds found in the turmeric analysed using Gas Chromatography–Mass Spectrometry

S.No	R-Turmerone RT	Compounds	Mol.Formula	Mol. Weight	Peak Area%	Ref#	Cas#
1	8.359	Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-	$C_{15}H_{22}$	202.335	1.68	66865	000644-30-4
2	8.879	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-	$C_{15}H_{24}$	204.351	1.89	68734	020307-83-9
		methylene-,[S-R*,S*)]-					
3	9.613	Benzene,1-ethyl-3,5-dimethyl-	$C_{10}H_{14}$	134.218	1.86	15214	000934-74-7
4	9.989	Benzene,1-(1,5-dimethylhexyl)-4-methyl-	$C_{15}H_{24}$	204.351	5.84	68654	001461-02-5
5	10.814	aR-Turmerone	$C_{15}H_{20}O$	216.318	50.06	79922	000532-65-0

6	11.246	2-Methyl-6-(4-methylene cyclohex-2-	$C_{15}H_{22}O$	218.335	20.04	81679	082508-14-3
		en-1-yl)hept-2-en-4-one					
7	11.595	3-Methyl-6-(6-methyl hept5-en-2-yl)- cyclo hex -2 -enone	$C_{15}H_{24}O$	220.350	1.43	83600	066964-98-5
8	11.697	Gamma - Terpinene	$C_{10}H_{16}$	136.234	1.13	16078	000099-85-4
9	11.788	Binapacryl	$C_{15}H_{18}N_2O_6$	322.317	1.27	180130	000485-31-4
10	11.894	Benzonitrile, 3-hydroxy	C_7H_5NO	119.120	1.31	9294	000873-62-1 43
11	11.927	(E)-Atlantone	$C_{15}H_{22}O$	218.334	2.15	81630	108645-54-1
12	12.099	Cumenyl angelate, o	$C_{14}H_{18}O_2$	218.29	0.99	81511	1000383-67-238
13	12.188	3,5-Dimethylanisole	$C_9H_{12}O$	136.191	1.29	16778	000874-63-5
14	12.297	Prop-2-ynyl (E)-2-methyl but-2-enoate	$C_8H_{10}O_2$	138.16	1.98	17804	1000373-72-522
15	13.108	Diglycolic acid, nonyl 3-phenylpropyl ester	$C_{22}H_{34}O_5$	378.5	3.36	241428	1000382-18-035
16	13.279	(S)-3-Methyl-6-((S)-6-methyl-4-oxohept-5-en-2-	$C_{15}H_{22}O_2$	234.33	0.89	96682	949081-10-1
17	13.698	But-2-enamide, N-ethyl -N-(3-methyl phenyl)-3-methyl-	$C_{14}H_{19}NO$	217.31	1.32	80637	1000308-23-638
18	14.197	Cyclohexanecarboxylic acid, 4-nitrophenyl ester	$C_{13}H_{15}NO_4$	249.262	1.28	110342	1000307-70-8
19	15.605	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.445	0.44	140138	000060-33-3

Table. 3. Phytochemistry of aqueous and methanolic extracts of turmeric (*Curcuma longa*)

Phytochemical constituent	Test performed	Water	Methanol
Alkaloids	Mayer`s test	+	+
Protein	Biuret test	+	+
Reducing sugar	Fehling`s solution test	+	+

Simple phenolics	Ferric Chloride test	+	+
Steroid	Liebermann-Burchard's test	-	+
Carbohydrate	Molisch`s test	+	+
Tannins	Ferric chloride test	+	+
Saponins	Froth test	+	+
Flavonoids	Lead Acetate test	+	+

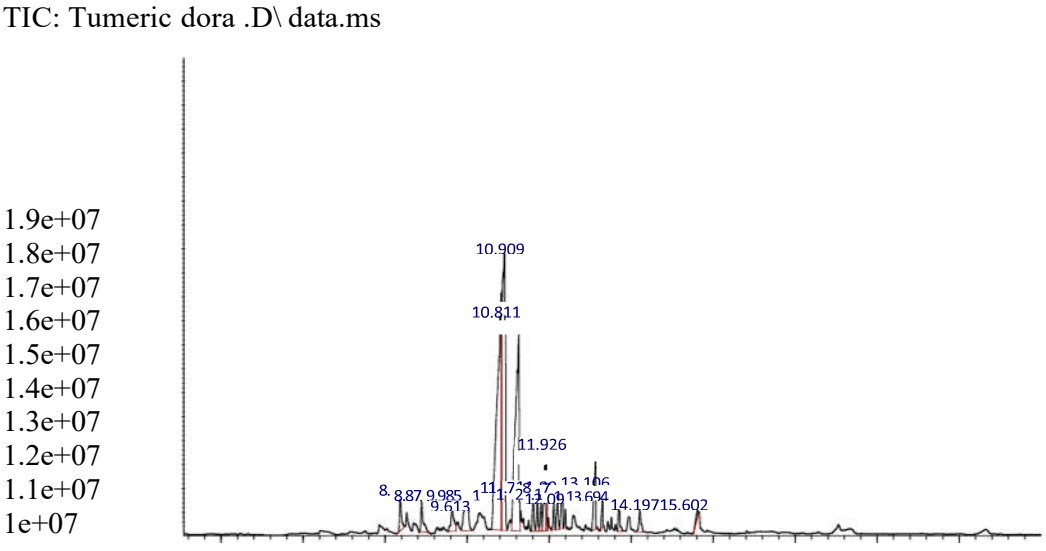

Fig. 1. Gas-Chromatography–Mass Spectrometry chromatogram of turmeric (*Curcuma longa*)

Table. 4. Antimicrobial susceptibility pattern of standard antibiotics agent against *Escherichia coli*

Antibiotic sensitive disc	Concentration (µg)	Diameter of zone of inhibition (mm)	Interpretation
Septrin (SXT)	30	18.07 ± 0.6	++
Erythromycin (E)	15	20.00± 1.6	++
Streptomycin (S)	30	21.37± 1.3	+++
Roceplin (R)	20	23.00± 1.9	+++
Chloramphenicol (CH)	25	14.80 ± 0.4	+
Ciprofloxacin (CPX)	15	19.16± 1.2	++

Table.5.Antimicrobial susceptibility pattern of standard antibiotics agent against *Staphylo coccus aureus*

Antibiotic sensitive disc	Concentration (µg)	Diameter of zone of inhibition (mm)	Interpretation
Septrin (SXT)	30	16.83± 0.3	++
Erythromycin (E)	15	16.64± 0.4	++
Streptomycin (S)	30	18.21 ± 0.2	++
Roceplin (R)	20	18.00 ± 0.6	++
Chloramphenicol (CH)	25	15.13 ± 0.6	+
Ciprofloxacin (CPX)	15	18.55± 0.3	++

The concentration of aqueous and methanolic root extracts at which no colonies of Escherichia coli and Staphylococcus aureus were seen is designated as the minimum bactericidal concentration. The results were compared with those of the control tube utilizing sterilized distilled water. The experiment was conducted in duplicate. The minimum bactericidal concentration (MBC) was defined as the concentration of the aqueous and methanolic root extracts of turmeric that exhibited no growth on a new batch of agar plates. The minimal inhibitory concentration (MIC) that demonstrated no observable growth was considered the minimum bactericidal concentration. The MBC/MIC number was determined to be either bactericidal or bacteriostatic.

Table. 6. Zone of inhibition of *Curcuma longa* aqueous and methanolic extract against *Escherichia coli* and *Staphylococcus aureus*

Test organisms	Aqueous extract of Tumeric concentration	Zone of inhibition for aqueous extract of Tumeric	Methanolic extract of Tumeric concentration	Zone of inhibition of methanolic extract of Tumeric	Concentration of azithromycin solution used	Zone of inhibition of azithromycin
	(mg/ml)	(mm)	(mg/mL)	(mm)	(mg/mL)	solution (mm)
Escherichia coli	250	22.29±2.4	250	21.79±1.1	12.50	21.67±1.09
Staphylococcus aureus	250	22.31±1.6	250	22.96±1.0	12.50	19.35±1.4
Escherichia coli	500	29.56±2.3	500	29.95±1.8	25	32..03±1.2
Staphylococcus aureus	500	28.67±1.5	500	30.13±1.95	25	28.76±0.96

Table. 7.Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *Curcuma longa* extracts against *Escherichia coli* and *Staphylococcus aureus*

ORGANISMS	*Escherichia coli*	*Staphylococcus aureus*
MIC for aqueous extract of Tumeric (mg/mL)	62.6	31.3
MIC for methanolic extract of Tumeric (mg/mL)	31.3	15.6
MBC for aqueous extract of Tumeric (mg/mL)	250.0	62.5
MBC for methanolic extract of Tumeric (mg/mL)	62.6	31.3
MBC/MIC for aqueous extract of tumeric	4.0	2.0
MBC/MIC for aqueous extract of tumeric	2.00	2.00

The findings of this investigation indicate that sodium (1.3637 ± 0.007) was the predominant element in turmeric, succeeded by iron (1.0108 ± 0.003), magnesium (0.8026 ± 0.002), and calcium (0.7974 ± 0.002). Other elements such as K, Zn, Ag, As, Cd, Co, Cu, Ni, and Pb were detected in negligible levels that are not significant

(Table.1). Enemor et al. [19] demonstrate that Curcuma longa rhizomes have elevated concentrations of calcium, magnesium, potassium, and sodium, measured in parts per million (ppm) at 38.69 ± 0.115, 19.76 ± 0.002, 9.21 ± 0.003, and 7.07 ± 0.017, respectively. Their findings parallel those derived from our research. Ogidi et al. [20] demonstrate that salt contributes to the therapy of cardiovascular disorders. Hartwig [21] conducted studies demonstrating that magnesium is essential for genomic integrity and DNA repair mechanisms. Additional research indicates that magnesium stimulates more than 300 distinct enzymes, therefore contributing to several metabolic processes, rendering it a crucial micronutrient, and facilitating electrolyte transport across cellular membranes [22]. A research indicates that magnesium and calcium are essential for the development of robust bones and teeth. Calcium ions facilitate the conversion of prothrombin to thrombin during blood coagulation and are also utilized in milk coagulation. Calcium ions facilitate the activation of several enzymatic activities within the body. Iron is a crucial metal utilized in the synthesis of red blood cells. Fig.1 illustrates the Gas Chromatography-Mass Spectrometry chromatogram of Curcuma longa (turmeric). Nineteen compounds were discovered, comprising two major compounds and seventeen minor compounds (Table.2). The two primary compounds and their percentage abundances are: aR-Turmerone (RT=10.81, peak area=50.5%) and 2-Methyl-6-(4-methylenecyclohex-2-en-1-yl) hept-2-en-4-one (RT=11.24, peak area=20.3%). aR-Turmerone (peak area = 50.05%) is the predominant component, succeeded by 2-Methyl-6-(4-methylenecyclohex-2-en-1-yl) hept-2-en-4-one (peak area = 20.0%). Ar-turmerone, the most volatile constituent in the rhizome, exhibited significant inhibition of α-amylase (IC50 of 24.5 μg) and α-glucosidase (IC50 of 0.28 μg). According to study [23-24], ar-turmerone at concentrations of 2 and 1 mg/disk significantly prevented the growth of C. perfringens and somewhat decreased the development of E. coli, without any detrimental effects on the Proliferation of four lactic acid bacteria (B. adolescentis, B. bifidum, B. longum, and L. casei) at 2 mg per disk. The study by Marliyana et al. [25] demonstrates that ar-turmerone had no activity against Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853, and Klebsiella pneumoniae ATCC 13883 when assessed using the disc diffusion technique. Hucklenbroich et al. [26] demonstrate through experimental findings that both in vitro and in vivo studies of aromatic (ar-) turmerone promote the proliferation of neural stem cells (NSC). Ar-turmerone aids regeneration in neurological disorders. Research indicates that ar-turmerone possesses anticancer effects through the production of apoptosis and the suppression of tumor cell invasion. The work by Park et al. [27] demonstrates that ar-turmerone has anti-inflammatory effects by inhibiting critical signaling pathways in microglia. Microglial activation is a characteristic of neuroinflammation and is linked to several neurological conditions, including neurodegenerative diseases and stroke.

The initial qualitative study of the various secondary metabolites in both extracts of Curcuma longa was conducted. The aqueous and methanolic root extracts of turmeric revealed the presence of alkaloids, flavonoids, tannins, saponins, simple phenolics, proteins, reducing sugars, and carbo- hydrates, but steroids were lacking in the aqueous root extract. Flavonoids have greater solubility in water or polar solvents due to their bonding with hydroxyl groups. Glycosides are substances including sugar and non-sugar moieties. Saponins often exist as glycosides, rendering them polar in nature. Saponins are surfactant chemicals that generate foam when agitated in water. This occurs due to saponins possessing both polar and non-polar groups that facilitate micelle formation. Upon micelle formation, the polar groups orient outward while the non-polar groups aggregate inside, like foam. Tannins, being phenolic chemicals, are soluble in water and exhibit polar characteristics. Terpenoids are lipophilic. Triterpenoids, a class of terpenoids, have antibacterial potential, whereas steroids are lipid groups that fall within the category of triterpenoids.

Staphylococcus aureus and Escherichia coli were chosen for the study and evaluated against certain antibiotics, as well as aqueous and methanolic extracts of turmeric.

Roceplin and streptomycin antibiotics exhibited a robust response, with zone diameters ranging from 21 to 30 mm against Escherichia coli. Ciprofloxacin, septrin, and erythromycin demonstrated a moderate response, with zone diameters between 16 and 20 mm, whereas chloramphenicol displayed a weak response, with zone diameters below 16 mm (Table.4). Ciprofloxacin, streptomycin, rocephin, septrin, and erythromycin had a moderate response to Staphylococcus aureus, with zone diameters ranging from 16 to 20 mm, but chloramphenicol demonstrated a poor response with a zone diameter of less than 16 mm (Table.5). The aqueous and methanolic extracts of turmeric demonstrated significant antibacterial activity against Escherichia coli and Staphylococcus aureus, with zones of inhibition ranging from 21.78±1.06 to 30.14±1.95 at concentrations of 250 and 500 mg/dl, respectively. The investigation indicates that 500 mg/ml aqueous and methanolic rhizome extracts of turmeric exhibited sensitivity to the tested organisms and were considerably ($P< 0.004$) different from the 250 mg/ml of the various extracts utilized in the study. Staphylococcus aureus exhibited greater sensitivity to the two distinct extracts utilized in the investigation (Table.7). Azithromycin solution at 25 mg/ml exhibited a robust reaction against Escherichia coli, with a zone diameter over 30 mm, and a significant response against Staphylococcus aureus, with a zone diameter below 30 mm. At a concentration of 12.50 mg/ml, azithromycin demonstrated a robust efficacy against Escherichia coli and a moderate efficacy against Staphylococcus aureus (Table.6).

Studies by Kim et al. [28]and Chandrana et al. [29] indicated that turmeric extract effectively inhibited Bacillus subtilis, Escherichia coli, and Staphylococcus aureus, likely attributable to the presence of curcuminoids, a class of phenolic compounds. Negi et al. [30]observed that the components curlone and turmerone in turmeric had superior antibacterial action against several microorganisms, including Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Bacillus coagulans, Pseudomonas aeruginosa, and Escherichia coli. The antibacterial properties of turmeric are attributed to curcuminoids, turmerol, curcumins, veleric acid, essential oil, and turmeric oil [31-32]. The antibacterial efficacy of aqueous and methanolic extracts of turmeric against S. aureus and E. coli pathogens was examined for their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. MIC or MBC values represent the minimal concentration of an antimicrobial agent required to limit bacterial growth or eradicate bacteria, respectively [6]. The work by Aderele et al. [6] demonstrates that the MIC test is crucial in laboratories for confirming microbiological resistance to antimicrobial drugs and for monitoring the efficacy of novel antimicrobial agents. The aqueous and methanolic rhizome extracts of turmeric exhibit MIC values of 62.50 and 31.25 mg/ml for E. coli, and 31.25 and 15.62 mg/ml for S. aureus, respectively. The two extracts exhibit MBC values of 250.00 and 62.50 mg/ml for E. coli, and 62.50 and 31.25 mg/ml for S. aureus, respectively (Table.6). The findings of this investigation indicated that the gram-negative bacteria (Escherichia coli) exhibited reduced susceptibility to the two rhizome extracts in comparison to the gram-positive bacterium (Staphylococcus aureus). Research also indicates that curcumin, the active component of turmeric, has inhibitory effects on methicillin-resistant Staphylococcus aureus (MRSA) strains, with minimum inhibitory concentration (MIC) values between 125 and 250 μg/mL. This drug exhibited significant antibacterial action, with MIC values between 5 and 50 μg/mL against 65 clinical isolates of Helicobacter pylori [33].

The methanolic extract of C. longa has inhibitory activities against S. aureus (MIC value of 128 μg/mL) and Bacillus subtilis (MIC value of 16 μg/mL)[33]. In a study conducted by All the aforementioned studies corroborate the findings of our research about the antibacterial efficacy of turmeric against Staphylococcus aureus and Escherichia coli. The study by Aderele et al.[6]shown that a computed MBC/MIC ratio is bactericidal when the ratio is less than or equal to 4, and bacteriostatic when the ratio exceeds 4. The aqueous and methanolic extracts of turmeric rhizome have bactericidal activity against Escherichia coli and Staphylococcus aureus, respectively.

4. CONCLUSION

Curcuma longa contains vital minerals, phytochemicals, and other natural medicinal compounds that have antibacterial properties against Escherichia coli and Staphylococcus aureus, potentially preventing illnesses caused by these pathogens. The essential oil was extracted from the commercially accessible turmeric spice at the local market. Eight chemicals were discovered and quantified using GC-MS analysis (Fig.1): eugenol, (E)-caryophyllene, ar-curcumen, α-zingiberene, β-sesquiphellandrene, ar-turmerone, turmerone, and curlone. The composition of the acquired essential oil closely resembles that derived from the turmeric rhizome cultivated in India. The predominant ingredient in the oil was turmerone (26%). Turmeric essential oil showed a significant capacity for DPPH radical neutralization. The concentration of the essential oil required to neutralize 50% of the initial DPPH radical concentration (EC50 value) was 0.045 mg/cm3 following 45-minute incubation with the radical. A DPPH radical neutralization of 92% was attained with a concentration of 2 mg/cm3 of essential oil. The extracted oil exhibited the most effective antimicrobial (antifungal) action against C. albicans. Considering the acquired results, the utilization of turmeric is not only rational but should also be prioritized in nutrition, as well as in the processing of food and pharmaceutical sectors.

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CONFLICT OF INTERESTS

Authors declare that there is no Conflict of Interests

REFERENCES

[1] M. Z. Stanković, LJ. P. Stanojević, Tehnologija lekovitog i začinskog bilja, Tehnološki fakultet, Leskovac, p. 138(2014).

[2] P. Tongnuanchan and S. Benjakul, Essential oils: extraction, bioactivities, and their uses for food preservation, Journal of Food Science, 79(7) R1231 - R1249 (2014).

[3] S. Burt, Essential oils: their antibacterial properties and potential applications in foods a review, International Journal of Food Microbiology, 94, 223 - 253(2004).

[4] F. Bakkali, S. Averbeck, D.Averbeck and M. Idaomar, Biological effects of essential oils a review, Food and Chemical Toxicology, 46, 446 - 475(2008).

[5] V. K. Raina, S.K. Srivastava and K.V. Syamsundar, Rhizome and leaf oil composition of Curcuma longa from the lower Himalayan region of Northern India, Journal of Essential Oil Research, 17 1 - 4(2005).

[6] S. Li, W. Yuan, G. Deng, P. Wang, P. Yang and B. B. Aggarwal, Chemical composition and product quality control of turmeric (Curcuma longa L.), Pharmaceutical Crops, 2, 28 - 54(2011).

[7] M. Akram, Shahab-Uddin, A. Ahmed, K. Usmanghani,A. Hannan, E. Mohiuddin and M. Asif, Curcuma longa and curcumin: a review article, Romanian Journal of Biology – Plant Biology, 55(2), p. 65 - 70(2010).

[8] R. P. Adams, Identification of essential oil components by gass chromatography/mass spectrometry, 4th Ed. Allured Publishing Corporation, Illinois (2007).

[9] S.-L. Hong, G.-S. Lee, S. N. S. A. Rahman, O. A. A. Hamdi, K. Awang, N. A. Nugroho, S. N. A. Malek, Essential oil content of the rhizome of Curcuma purpurascens Bl

[10] K. Awang, N. A. Nugroho and S. N. A. Malek, Essential oil content of the rhizome of Curcuma purpurascens Bl. (Temu Tis) and its antiproliferative effect on selected human carcinoma cell lines, The Scientific World Journal, Article ID 397430, 1-7 (2014).

[11] I.M. Famuyide, A.O. Aro, F.O. Fasina, J.N. Eloff, L.J.McGaw, Antibacterial activity and mode of action of acetone crude leaf extracts of under investigated Syzygium and Eugenia (Myrtaceae) species on multidrug resistant porcine diarrhoeagenic Escherichia coli. BMC Vete Res., 15,162(2019).

[12] K. Moo-Key, K. Young-Mi and K. Hoi-Seon, Growth-inhibiting Effects of Juniperus virginiana Leaf-Extracted Components towards Human Intestinal Bacteria, Food Sci. Biotechnol, 14(1)164-167(2005).

[13] V.H.A. Enemor, U.C. Ogbodo, O.F. Nworji, O.C. Ezeigwe, C.O. Okpala and G.C.Iheonunekwu Evaluation of the Nutritional Status and Phytomedicinal Properties of Dried Rhizomes of Turmeric (Curcuma longa). J. Biosci.Medicines, 8,163-179(2020). https://doi.org/10. 4236/ jbm.2020.88015.

[14] O.I. Ogidi, N.G. Esie and O.G.Dike, Phytochemical, Proximate and Mineral Compositions of Bryophyllum pinnatum (Never Die) Medicinal Plant, J. Pharmacognosy Phytochem., 8, 629-635(2019).

[15] Hartwig Role of Magnesium in Genomic Stability. Mutation Res., 475 113-121(2001). Available:https://doi.org/10.1016/S0027- 5107(01)00074-4.

[16] K. Pasternak, J. Kocot and A.Horecka, Biochemistry of Magnesium. J. Elementol., 15,601-616(2010).

[17] P.C. Lekshmi, R. Arimboor, P.S. Indulekha, A.N.Menon, Turmeric (Curcuma longa L.) volatile oil inhibits key enzymes linked to type 2 diabetes, Int. J. Food Sci. Nutr., 63(7), 832–834(2012).

[18] L.Hoi-Seon, Antimicrobiai Properties of Turmeric Curcuma longa L.) Rhizome— Derived ar-Turmerone and Curcumin, Food Sci. Biotechnol., (15) 4, 559-563(2006).
[19] S.D. Marliyana, F.R. Wibowo, M.W. Wartono1 and G. Munasah, Evaluation of antibacterial activity of sesquiterpene Ar- Turmerone from Curcuma soloensis Val. Rhizomes. IOP Conf. Series, Materials Science and Engineering, 578(2019): 012060 IOP Publishing DOI:10.1088/1757-899X/578/1/012060.
[20] J. Hucklenbroich, R. Klein, B. Neumaier, R. Graf, G.R. Fink, M. Schroeter and M.A.Rueger, Aromatic-turmerone induces neural stem cell proliferation in vitro and in vivo. Stem Cell Res. Therapy., 5(100), 1-9(2014).
[21] S.Y. Park, M.L. Jin, Y.H. Kim, Y. Kim and S.J.Lee, Anti-inflammatory effects of aromatic- turmerone through blocking of NF-kappaB, JNK, and p38 MAPK signaling pathways in amyloid beta-stimulated microglia, Int. Immunopharmacol., 14, 13–20(2012).
[22] K.J. Kim, H.H. Yu, J.D. Cha, S.J. Seo, N.Y. Choi and Y.O.You, Antibacterial activity of Curcuma longa L. against methicillin resistant Staphylococcus aureus. Phytother Res., 19, 599–604(2005).
[23] H. Chandrana, S. Baluja and S.V.Chanda, Comparison of antibacterial activities of selected species of Zingiberaceae family and some synthetic compounds, Turk J. Biol., 29(29), 83–97(2005).
[24] P.S. Negi, G.K. Jayaprakasha, Rao L. Jagan Mohan and K.K.Sakariah, Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture, J. Agric. Food Chem., 47(10) 4297–4300 (1999).DOI: 10.1021/jf990308d. PMID: 10552805.
[25] S. Cikricki, E. Mozioglu, H.Y.ylmaz, Biological activity of curcuminoids isolated from Curcuma longa, Rec. Nat. Prod., 12, 19–24(2008).
[26] R.K. Basniwal, H.S. Butter, V.K. Jain and N.Jain Curcumin nanoparticles: preparation, characterization, and antimicrobial study. J Agric Food Chem., 59, 2056–2061(2011).
[27] P. De R. Kundu, S. Swarnakar, T. Ramamurthy, A. Chowdhury and G.B. Nair, Antimicrobial activity of curcumin against Helicobacter pylori isolates from India and during infections in mice, Antimicrob. AgentsChemother, 53(4), 1592–1597(2009).
[28] Kim KJ, Yu HH, Cha JD, Seo SJ, Choi NY, You YO. Antibacterial activity of Curcuma longa L. against methicillin resistant Staphylococcus aureus. Phytother. Res.2005; 19:599–604.
[29] Chandrana H, Baluja S, Chanda SV. Comparison of antibacterial activities of selected species of Zingiberaceae family and some synthetic compounds. Turk J. Biol.2005;29(29):83–97
[30] Negi PS, Jayaprakasha GK, Jagan Mohan Rao L, Sakariah KK. Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture. J. Agric. Food Chem. 1999; 47(10):4297–4300.DOI: 10.1021/jf990308d. PMID: 10552805.
[31] Cikricki S, Mozioglu E, Yylmaz H. Biological activity of curcuminoids isolated from Curcuma longa. Rec. Nat. Prod. 2008; 12:19–24.
[32] Basniwal RK, Butter HS, JainVK, JainN(2011) Curcumin nanoparticles: preparation, characterization, and antimicrobial study. J Agric Food Chem.2011; 59:2056–2061.
[33] De R, Kundu P, Swarnakar S, Ramamurthy T, Chowdhury A, Nair GB.Antimicrobial activity of curcumin against Helicobacter pylori isolates from India and during infections in mice. Antimicrob. AgentsChemother.2009; 53(4):1592– 1597.