

Original Article

PHYTOCHEMICAL, ANTIOXIDANT AND ANTIBACTERIAL STUDIES ON THE LEAF EXTRACTS OF *CURCUMA AMADA* ROXB

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ABSTRACT

Objective: This study investigates the chemical composition, *in vitro* antioxidant activity and antibacterial activity of leaf extract of *Curcuma amada*

Methods: GC-MS analysis of these extracts was performed using a Perkin-Elmer GC Clarus 500 system. The antioxidant activity was done by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, total antioxidant assay, Ferric reducing power assay, Hydrogen peroxide scavenging activity, and nitric oxide scavenging assay. Evaluation of *in vitro* antibacterial activity was carried out by the disc diffusion method. Thirteen bacterial strains were used in this study.

Results: The GC-MS analysis of the ethanolic extracts has shown a profile of 15 compounds. The antioxidant study proves that the leaf extracts could serve as an important bio-resource of antioxidants for the pharmaceutical industry. The extracts of leaf remarkably inhibited the growth of 13 bacterial strains

Conclusion: This study revealed that phytochemicals compound present in ethanolic extract, antioxidant properties of ethanol and acetone extract and antimicrobial activity.

Keywords: *Curcuma amada* Roxb., Leaf extracts, GC-MS Analysis, Antibacterial, Antioxidant

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INTRODUCTION

The use of herbs to treat illness has its roots in an ancient holistic healing tradition that originated in Asia more than 3000 y ago. Plants rich in a wide variety of secondary metabolites like tannins, terpenoids, alkaloids, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial and antioxidant properties. Some of the 20th-century practitioners of western treatment, including herbal therapies in healing practices, such as traditional Chinese medicine, Japanese kampo and Indian ayurveda, which are rapidly gaining acceptance in the west. The development of the science of phytopharmaceuticals and the hopes of the remedies in chronic diseases generated enthusiasm in the researchers to develop herbal medicines [1]. The driving factor for the renewed interest in plant antimicrobials in the past 20 y has been the rapid rate of species of extinction [2].

The genus *Curcuma* belongs to the family Zingiberaceae comprising more than 80 species of rhizomatous herbs. They have originated in the Indo-Malayan region and are distributed widely in the tropics from Asia to Africa and Australia [3]. Mango ginger (*Curcuma amada* Roxb.) is a perennial herb and its rhizomes are morphologically similar to ginger but impart a mango flavour.

In an aerobic environment, all animals and plants want oxygen and hence reactive oxygen species (ROS) are ubiquitous. Recently fruits and vegetables have play a significant part in the chemoprevention of diseases and aging and are recognized as natural antioxidants

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 the oxidative damage. Furthermore, studies about antioxidant substances in foods and natural medicinal 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 [4-9]. Taking into consideration of the medicinal importance of the plant, the ethanol extract of *C. amada* was analyzed for the GC-MS. This work will help to identify the compounds of therapeutic value. GC-MS is one of the technique to identify the bioactive constituents of long chain branched chain hydrocarbons, alcohols, acids, esters, The objectives of the present study were to identify chemical composition as well as assess the antioxidant and antibacterial properties of the leaf extracts of *C. amada* using gas chromatography combined with mass spectrometry (GC-MS) and flame ionization detector.

MATERIALS AND METHODS

A collection of Plant Sample *C. 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.

Solvent extraction

Thoroughly washed leaves of *C. amada* were dried in shade for five days and then powdered with the help of Waring blender. 25 g of shade-dried powder was filled in the thimble and extracted successively with methanol, ethanol, chloroform, petroleum ether and acetone solvent in Sechelt extractor for 48h. The solvent extracts were concentrated under reduced pressure and preserved at 5 °C in the airtight bottle until further use.

GC-MS analysis

GC-MS analysis of these extracts was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30 mm X 0.25 mm 1D X 1 µMdf, composed of

100% Dimethyl polysiloxane). For GCMS detection, an electron ionization system with the ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 2 μ L was employed (split ratio of 10: 1); Injector temperature 250 °C; Ion source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min.), with an increase of 10 °C/min, to 200 °C, then 5 °C/min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative % amount of each component was calculated by comparing its average peak area to the total areas; software adapted to handle mass spectra, and chromatograms was a Turbo mass. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight, and structure of the components of the test materials were ascertained.

Antioxidant activity

DPPH radical scavenging activity

Radical scavenging activity was measured by using DPPH scavenging method of [10]. A solution of DPPH in methanol (24 μ g/ml) was prepared, and 2 ml of this solution was added to ethanol and acetone extract at different concentrations (10-50 μ g/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.

Percent inhibition of DPPH Radical Scavenging assay = $\frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$

Where, Abs control was the absorbance of the control (without extract) at 517 nm; Abs sample was the absorbance in the presence of the extract at 517 nm. The experiment was repeated in triplicate.

Total antioxidant capacity assay

The total antioxidant capacity assay was determined as described by Prieto *et al.* [11]. Different concentrations of the ethanol and acetone extracts (10-50 μ g/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 recapped 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.

Percent inhibition of DPPH Radical Scavenging assay = $\frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$

Where, Abs control was the absorbance of the control (without extract) at 695 nm; Abs sample was the absorbance in the presence of the extract at 695 nm. The experiment was repeated in triplicate

Reducing power assay

The reducing power of extract was determined by the method of Yen and Duh [12]. Different concentrations of ethanol and acetone extracts (10-50 μ g/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 [13]. The ethanol and acetone extracts were added

to different test-tubes in varying concentrations (10-40 μ g/ml). Sodium nitroprusside (5 mM) in phosphate buffer was added to each test tube to make volumes up to 1.5 ml. Solutions were incubated at 25 °C for 30 min. Thereafter, 1.5 ml of Griess reagent (1% Sulphanilamide, 0.1% Naphthyl ethylenediamine 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.

Percent inhibition of Nitric oxide scavenging assay = $\frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$

Where, Abs control was the absorbance of the control (without extract) at 546 nm; Abs sample was the absorbance in the presence of the extract at 546 nm. The experiment was repeated in triplicate

Hydrogen peroxide scavenging activity

The Hydrogen peroxide scavenging activity of the extract was determined by the method of Ruch *et al.* [14]. The ethanol and acetone extracts were dissolved in 3.4 ml of 0.1M phosphate buffer (pH7.4) and mixed with 600 μ L of 43 mM solution of hydrogen peroxide. The absorbance value (at 230 nm) of the reaction mixture was recorded at 10 min intervals between zero and 40 min. for each concentration, a separate blank sample was used for background subtraction.

Percent inhibition of H₂O₂ assay = $\frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$ where Abs control was the absorbance of the control (without extract) at 230 nm; Abs sample was the absorbance in the presence of the extract at 230 nm. The experiment was repeated in triplicate.

Calculation of IC₅₀

Various concentrations (10-50 μ g/ml) of ethanol and acetone extracts of *C. amada leaf* were taken for the study and IC₅₀ values which show 50 % inhibition.

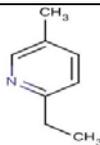
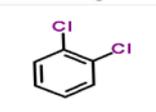
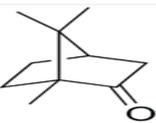
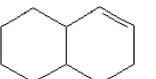
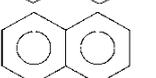
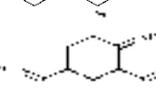
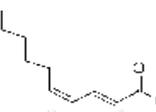
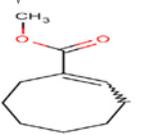
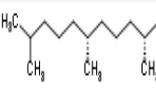
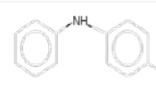
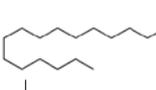
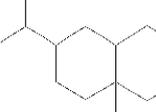
Antimicrobial studies

Thirteen bacterial strains were used in this study: *Escherichia coli*, (MTCC # 119) *Pseudomonas aeruginosa* (MTCC #2474), *Salmonella paratyphoid* (MTCC # 734), *Vibrio cholera* (ATCC # 14104), *Streptococcus pneumonia* (ATCC # 7066), *Bacillus subtilis* (MTCC # 441), *Bacillus cereus* (ATCC #4342), *Proteus vulgaris* (MTCC # 1771), *Proteus mirabilis* (MTCC # 1429), *Serratia marcescens* (MTCC # 2645), *Klebsiella pneumonia* (MTCC # 3040), *Staphylococcus aureus* (MTCC#3163) and *Enterobacter aerogenes* (MTCC#2990). Evaluation of *in vitro* antibacterial activity was carried out by the disc diffusion procedure as described by Perez *et al.* (15). The five extracts 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 37 °C.

RESULTS AND DISCUSSION

Plants are known to have beneficial therapeutic effects documented in Traditional Indian System of Medicine. The secondary metabolites in plants are produced in abundance against self-protection and other functions. GC-MS analysis provides the broad idea about the chemical structure, and molecular formula and idea about the functional groups present in the compound [16]. The GC-MS study of *C. amada* has shown many photochemical which contributes to the medicinal activity. The *C. amada* leaf contains about 15 photochemical compounds such as Pyridine, 2-ethyl-5-methyl, (+)-2-Bornanone, n-Hexadecanoic acid, etc. These 15 compounds are responsible for antimicrobial, antifungal, sedative, antitumor, antioxidant and insecticidal in this plant. (+)-2-Bornanone is used anaesthetic and antimicrobial; n-Hexadecanoic acid used as antioxidant and anti-inflammatory agent; Hexadecanoic acid, 2-methyl-, methyl ester is used as antioxidant, wound healing, arthritis, antiemetics; 4,9-Decadienoic acid, 2-nitro-, ethyl ester is used as insecticidal activity (table 1).

Table 1: GC-MS analysis of Ethanolic extract of *Curcuma amada* leaf

S. No.	Name of compound	Molecular formula	Molecular mass	Structure	Rt	% area	Uses
1	Pyridine, 2-ethyl-5-methyl	C ₈ H ₁₁ N	121.18		3.304	50.55	Flavour Ingredient
2	Benzene, 1,2-dichloro	C ₆ H ₄ Cl ₂	147.002		3.522	2.08	Herbicide and pesticide
3	(+)-2-Bornanone	C ₁₀ H ₁₆ O	152.2334		5.33	6.33	local anesthetic and antimicrobial
4	1,2,3,4,4a,5,6,8a-Octahydro-naphthalene	C ₁₀ H ₁₆	136.23404		5.547	1.72	anti-filarial, perfume
5	Naphthalene	C ₁₀ H ₈	128.1705		6.154	2.45	effective pesticide, make dyes, resins, lubricants
6	Cyclohexane, 1,5-diethenyl-3-methyl-2-methylene-, (1.alpha.,3.alpha.5.alpha.)-	C ₁₂ H ₁₈	162.27132		11.641	2.52	to treat dysentery
7	4,9-Decadienoic acid, 2-nitro-, ethyl ester	C ₁₂ H ₁₉ NO ₄	241.28356		15.526	1.52	Insecticidal
8	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E,E)-	C ₁₅ H ₂₂ O	218.3346		18.033	1.33	inhibited cell proliferation, increased lactate dehydrogenase (LDH) release, anti-ulcer agents
9	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.4241		23.320	7.62	Anti-inflammatory property, antioxidant
10	Hexadecanoic acid, 2-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	270.4507				Antioxidant, wound healing, arthritis, antiemetics
11	1-Cyclooctene-1-carboxylic acid, methyl ester	C ₁₀ H ₁₆ O ₂	168.23284		24.390	2.17	Antioxidant, Flavor
12	Phytol	C ₂₀ H ₄₀ O	296.531		26.146	11.50	Antioxidant
13	1,4-Benzenediamine, N-(1-methylethyl)-(N'-phenyl)	C ₁₅ H ₁₈ N ₂	226.3168		26.518	1.32	Antimicrobial, Antioxidant
14	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.48		26.993	5.46	Soaps, cosmetics, detergents
15	2(1H)-Naphthalenone, octahydro-4a-methyl-7-methylethyl)-(4a.alpha.,7.beta.,8a.beta.)-	C ₁₄ H ₂₄ O	208.33976		28.006	2.06	improving the immunity activities

Antioxidant assays

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* leaf

was evaluated using DPPH radical scavenging method by comparing with the activity of the ascorbic acid as a known antioxidant. The antioxidant capacity of ethanolic extract showed higher activity than acetone extract of the leaf. IC₅₀ value of ethanol and acetone extracts was respectively 21 and 25 µg/ml. (fig. 1).

The total antioxidant capacities of ethanol and acetone extracts were determined by phosphor molybdenum with using Ascorbic acid as standard. In phosphor molybdenum assay, the concentrations range from 10-50 μ g/ml; ethanol extract showed a higher dose-dependent reducing activity than acetone extract. IC₅₀ Value of ethanol and acetone extracts was respectively 26 and 30 mg/ml (fig. 2).

Ethanol extract exhibited a significant dose-dependent inhibition of reducing power assay activity compared to acetone extract. Reductive capabilities of ethanol and acetone extracts of leaf compared to ascorbic acid shown in [fig. 3]. The plant extract could reduce the most Fe³⁺ions, which had a lesser reductive activity than the standard of ascorbic acid.

The scavenging of nitric oxide by ethanol and acetone were increased in a dose-dependent manner as illustrated in [Fig.3]. At a concentration of 24 μ g/ml and 30 μ g/ml of extracts, 50% of nitric oxide generated by incubation was scavenged.

The composition of hydrogen peroxide into water may occur according to the antioxidant compounds as the antioxidant components present in the extract are good electron donors, they may accelerate the conversion of H₂O₂ to H₂O. The H₂O₂ inhibition activities of ethanol and acetone extracts are given in (fig. 4). The ethanol extract showed the highest H₂O₂ inhibition values (28.6 \pm 1.67-62.1 \pm 2.41%) while Acetone extract had the less than H₂O₂ inhibition values at (26.7 \pm 1.7-60.1 \pm 3.4%). IC₅₀ Value of ethanol and acetone extracts was respectively 35 and 38 μ g/ml.

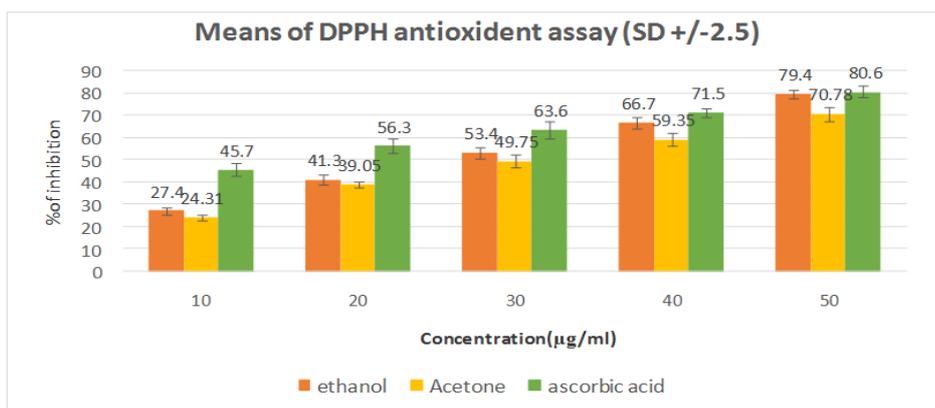


Fig. 1: DPPH Scavenging assay of ethanol and acetone extracts of *C. amada* leaf, compared to ascorbic acid
IC₅₀ value of ethanol extract=21 μ g/ml, IC₅₀ value of acetone extract=25 μ g/ml

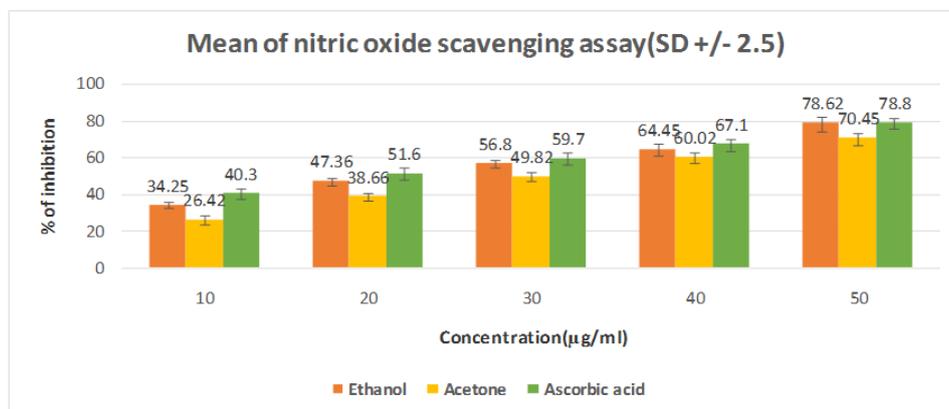


Fig. 2: Nitric oxide scavenging assay of ethanol and acetone extracts of *C. amada* leaf, compared to ascorbic acid
IC₅₀ Value of ethanol extract=24 μ g/ml, IC₅₀ Value of acetone extract=30 μ g/ml

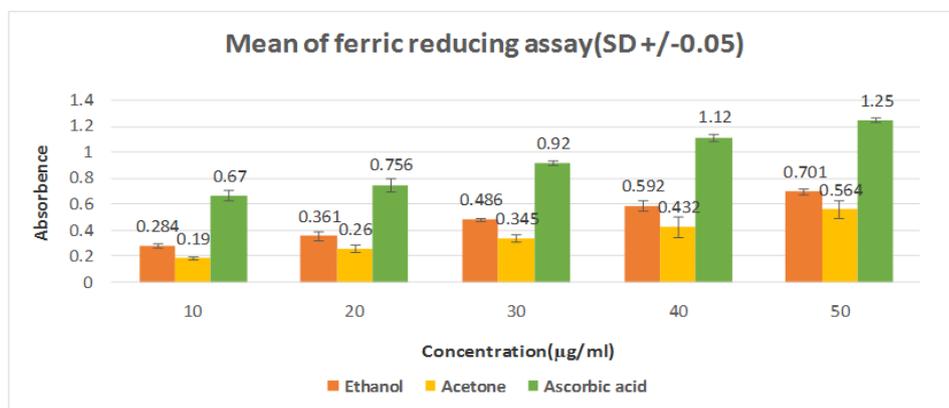


Fig. 3: Ferric reducing assay of ethanol and acetone extracts of *C. amada* leaf, compared to ascorbic acid

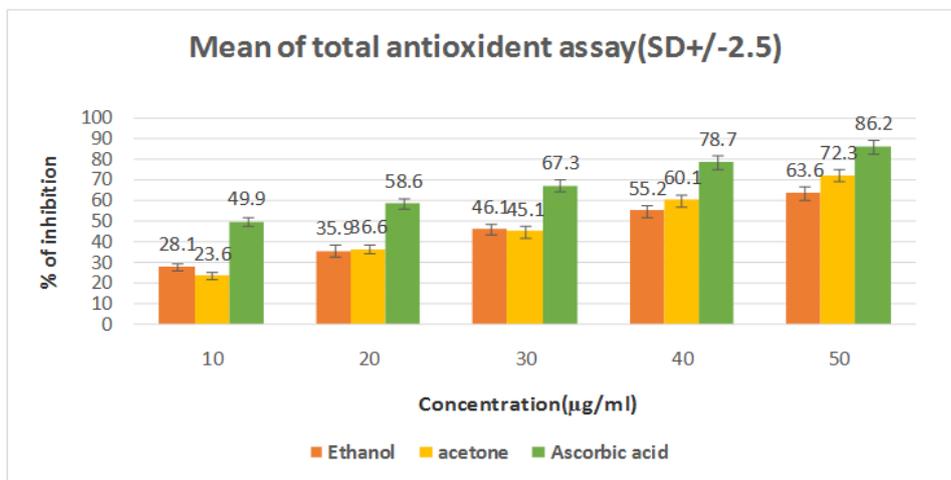


Fig. 4: Total antioxidant assay of ethanol and acetone extracts of *C. amada* leaf, compared to ascorbic acid
 IC₅₀ Value of ethanol extract=26µg/ml, IC₅₀ Value of acetone extract=30µg/ml

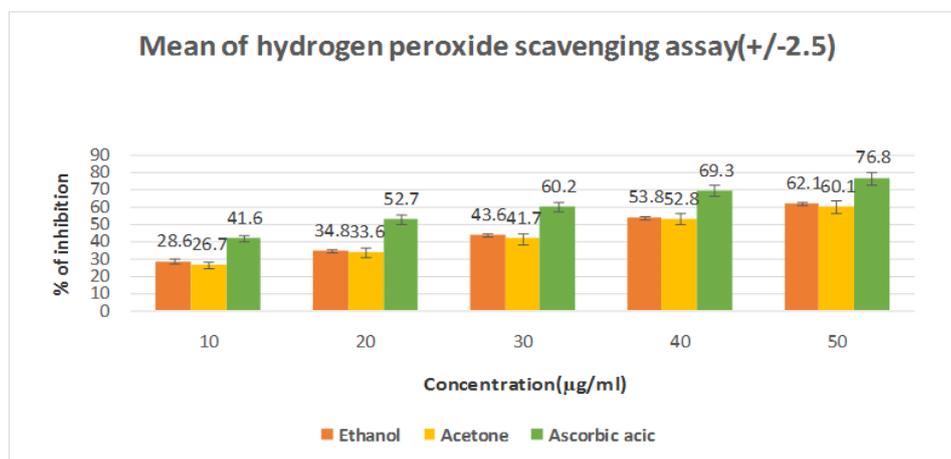


Fig. 5: Hydrogen peroxide scavenging of ethanol and acetone extracts of *C. amada* leaf, compared to ascorbic acid
 IC₅₀ Value of ethanol extract=35µg/ml, IC₅₀ Value of acetone extract=38µg/ml

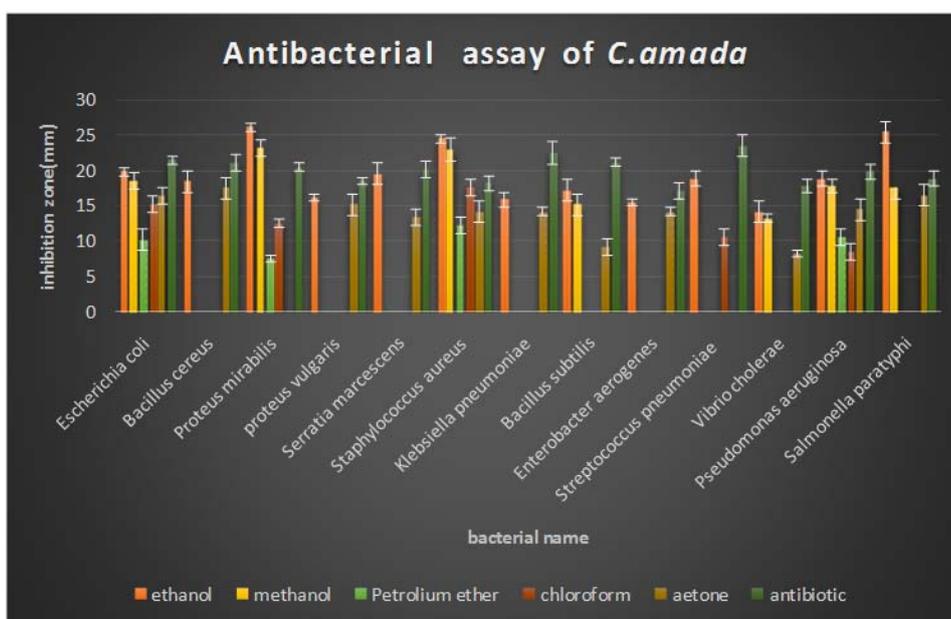
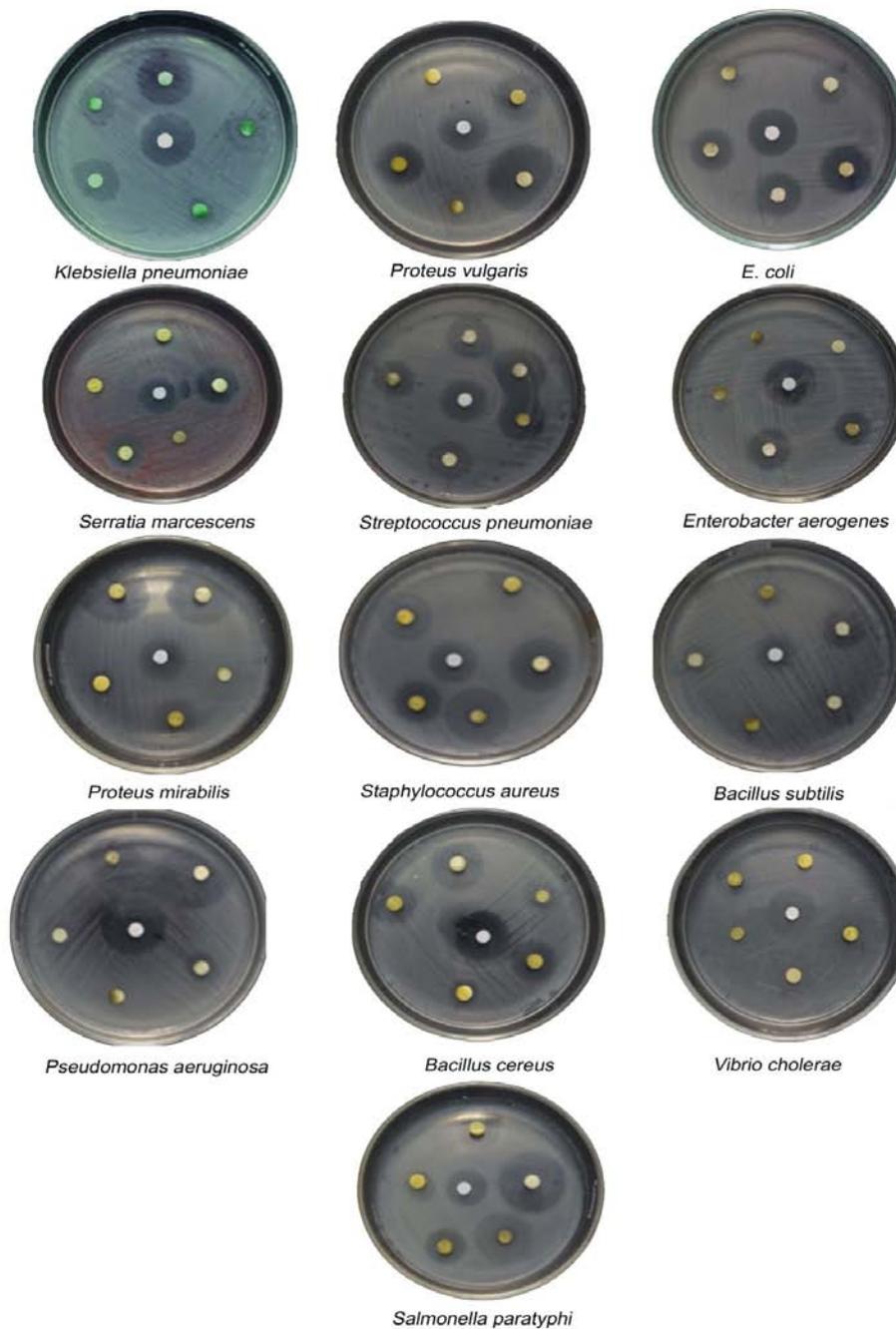


Fig. 6: Antibacterial activity (inhibition zone) of the leaf extracts of *C. amada*

Table 2: Antibacterial activity (inhibition zone) of the leaf extracts of *C. amada*

S. No.	Name of organisms	Solvent name					
		Ethanol	Methanol	Acetone	Chloroform	Pet. ether	antibiotic
1	<i>Staphylococcus aureus</i>	24±1.73	23±1.52	12.33±1.15	17±1.15	14.33±1.52	18±1
2	<i>Bacillus cereus</i>	18.66±1.52		17.66±1.2			21.3±0.57
3	<i>Proteus mirabilis</i>	26.3±0.57	23.33±1.15		12.66±0.57	7±0.58	20.6±1.15
4	<i>proteus vulgaris</i>	16.33±0.57		15.66±1.52			18.66±0.57
5	<i>Serratiamarcescens</i>	19.66±1.52	13±1.52				20.33±0.57
6	<i>Escherichia coli</i>	20±0.52	18±1.15	16.33±1.54	15±1.15	10.66±1.15	21.6±1.15
7	<i>Klebsiellapneumoniae</i>	16±1		14.33±0.57			22.66±1.15
8	<i>Bacillus subtilis</i>	17.33±1.52	15.66±1.72			9.33±1.15	21.3±1.54
9	<i>Enterobacteraerogenes</i>	15.66±0.52		14.33±0.57			17.33±1.15
10	<i>Pseudomonas aeruginosa</i>	19±1			10.66±1.52		23.66±1.52
11	<i>Vibrio cholerae</i>	14.33±1.52	13.33±0.52	8.33±0.52			18±1
12	<i>Streptococcus pneumoniae</i>	19±1	18±1	14.66±1.52	10±1	8.66±1.15	20±1
13	<i>Salmonella paratyphi</i>	25.66±1.52	17.33±1.52	16.66±0.57			19±1

Plate 1: Antibacterial activities of *Curcuma amada* leaf extracts

Antibacterial assay

The antibacterial property of the essential oil and extracts has led to the basis of many applications. Curcuma, is gaining importance worldwide as a potential source of new drugs to combat a variety of ailments as the species contains molecules credited with anti-inflammatory, hypocholesteremic, choleric, antimicrobial, insect repellent, ant-rheumatic, antifibrotic, antivenomous, antiviral, anti diabetic, ant hepatotoxic as well as anti cancerous properties (17). The antibacterial activity of the essential oil of *C. caesia*, *C. amada* and antifungal activity of essential oil of *C. aromatica* was earlier reported (18,19,20). In the present study methanol, ethanol, chloroform, petroleum ether and acetone extract of leaves of *C. amada* were tested against selected Gram-positive and Gram negative bacterial species and inhibition zone also noticed. (table 2). This study demonstrated that the five solvent of leaf extract displayed antimicrobial activity on Gram negative and Gram positive bacteria. The strong antimicrobial activity of the ethanol extract against almost all the susceptible microorganisms can be attributed to the presence of high concentration of secondary metabolites. The ethanolic and acetone extract remarkably inhibited the growth of tested Gram positive and Gram negative bacteria (fig. 6) (Plate 1).

CONCLUSION

Quantitative analyzes of the chemical composition of the investigated ethanolic extract of *Curcuma amada* were tested. Gas chromatography/mass spectrometry (GC-MS) analysis revealed the presence of 15 major chemicals in all three of the oils. Chemical identification of the Ethanolic extract 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 Ethanolic and acetone were subjected to screening for their possible antioxidant activity by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, total antioxidant assay, Ferric reducing antioxidant power, nitric oxide scavenging assay, and hydrogen peroxide assay. Results showed that the ethanol and acetone extract of leaves possessed a strong degree of antioxidant activity. The ethanol, methanol, chloroform, pet. ether and acetone extracts remarkably inhibited the growth of tested Gram positive and Gram negative bacteria.

CONFLICT OF INTERESTS

Declare none

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