ORIGINAL ARTICLE

Comparative phytochemical and anti-bacterial studies of two indigenous medicinal plants *Curcuma caesia* Roxb. and *Curcuma aeruginosa* Roxb

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Background: Traditional medicinal plants could serve as a good supply of new dependable, biodegradable, renewable drugs and can be utilised for its anti-bacterial activity directly or indirectly. **Aims:** To evaluate the phytochemical and anti-bacterial properties of two morphologically similar indigenous medicinal plants *Curcuma caesia* and *Curcuma aeruginosa* belonging to the family Zingiberaceae. **Materials and Methods:** Evaluation of rhizome extracts using methanol was performed for the presence of active principles. Qualitative analysis was carried out for diverse phytoconstituents. Different concentrations (1.25, 2.5, 5.0 mg/ml) of hexane, chloroform, ethyl acetate, acetone, methanol and water serial extracts from the rhizome of *C. caesia* and *C. aeruginosa* were tested against Gram positive (*Staphylococcus aureus, Streptococcus haemolyticus and Bacillus cereus*) and Gram negative (*Salmonella typhi, Enterobacter aerogens, Vibrio cholerae, Pseudomonas aeruginosa and Serratia marcescens*) bacteria. National Committee for Clinical Laboratory Standard (NCCL) standards were strictly followed to perform anti-bacterial disc susceptibility test using disc diffusion method. **Statistical Analysis:** All the values of the results were expressed as means of two independent experiments ± standard deviation. **Results:** Phytochemical screening of these two plants confirmed the presence of various bioactive substances and thus validating its use in herbal remedies. Anti-bacterial studies showed varying degree of inhibitory action against all the tested bacteria. Among the Gram positive bacteria, acetone extract of *C. caesia* showed maximum activity against *S. aureus* and hexane extract of *C. aeruginosa* exhibited maximum activity against *B. cereus*. In Gram negative bacteria, chloroform extract of *C. caesia* showed maximum inhibitory action against *S. marcescens*, whereas the methanol extract of *C. aeruginosa* showed higher inhibitory action against *S. typhi*. **Conclusions:** The findings about present study suggest that the rhizome extract possess excellent anti-bacterial potential that can be used for therapeutic purposes for many bacterial infectious diseases with proper evaluation procedures. The present study validated the use of these plants in traditional medicine and recommends for making new pharmaceuticals for therapeutic needs.

Key words: Anti-bacterial activity, *Curcuma aeruginosa*, *Curcuma caesia*, medicinal plants, plant extracts

INTRODUCTION

Nature has been a source of medicinal agent for thousands of years.[1] Herbal medicine represents one of the most important fields of traditional medicine all over the world.[2] Different extracts from traditional medicinal plants have been tested to identify the source of therapeutic effects.[3] Developing countries still depend mainly on medicinal herbs due to their cheaper cost and their effectiveness in the treatment of various infectious diseases with lesser side effects.[4,5] Traditional tropical medicinal plants could serve as a good supply of new dependable, biodegradable and renewable drugs for the healing of many diseases.[6,7] Over the past 20 years, there has been an increased interest in the investigation of natural materials as source of new anti-bacterial agents. Traditional medicines by means of plant extracts continues to provide health coverage for over 80% of the world's population mainly in the developing countries.[8]

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The family Zingiberaceae comprises advanced monocot plants and is characterised by aromatic, non-tuberous and tuberous rhizomes, which have tremendous ethnomedicinal properties.[9,10] Of the 80 species reported in the genus *Curcuma* from Indo-Malayan region, 40 are indigenous to India.[11] The position of the spike is the major discriminatory trait in the genus *Curcuma*.[12] It may be terminal or lateral. In addition to this, the presence of coma bract and bract colour are additional identifying characters in *Curcuma* genus. However, it is reported that the position of spike and colour characters varied with the season and climatic changes.[13]

Curcuma caesia commonly known as 'Black turmeric' is a perennial underutilised herb of the family

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Zingiberaceae [Figure 1a]. The plant is mainly distributed in the Himalayan region, north-east and central India.[14] It usually grows well in moist deciduous forests.[15] The plant has a characteristic rhizome flesh of bluish-black colour with pungent smell and hot bitter taste [Figure 1c]. The rhizome shows circular display of remnants of scaly leaves, which is often confused as growth rings. The rhizome of the plant is aromatic, contains essential oil and used for a variety of purposes. The characteristic pungent smell of the rhizome is due to the presence of essential oil rich in camphor and starch. The rhizome is traditionally used in the treatment of hemorrhoids, leprosy, asthma, cancer, fever, wounds, vomiting, menstrual disorder, anthelmentic, aphrodisiac, gonorrheal discharges and inflammation.[16,17] Furthermore, the smooth muscle relaxant, anti-tumour and anti-oxidant properties of *C. caesia* rhizome extract had been reported.[18,19] Due to its high medicinal value, the plant is in great demand in central India.

The chemical compounds isolated from *C. caesia* include about 30 volatile constituents of which camphor (28.3%), *ar*-tumerone (12.3%) and (Z)-β-ocimene (8.2%) are the major components.[20] In addition to this, other compounds such as furanodiene, germacrone, isofuranodienone, curzerenone, curcumenone, germacrane 4, 5-epoxide, curcumenol, aerugidiol and zederone were also reported from the rhizome of this plant.[21] In another report sesquiterpenoids 1, 2, 3 had been isolated from the dried rhizome of *C. caesia* by Asem and Laitonjam.[22]

C. aeruginosa is a native tropical plant [Figure 1b] of Southeast Asia, including Myanmar, Cambodia, Vietnam, Malaysia, Indonesia and Thailand and Western Ghats of south India.[23,24] Fresh rhizomes, which is greenish blue in colour [Figure 1d] and mildly aromatic with ginger-like aroma.[25] Rhizome is used as medicine for rheumatic, cough, asthma and anthelmintic.[26] The traditional medical practitioners in India have identified its usage in amebic dysentery, stomach ache, ulcer and indigestion.[20,27] The rhizome of *C. aeruginosa* is a promising source of potential anti-oxidants.[28] It is employed for making various cosmetic items and for sprains and bruises.[29] Anti-androgenic effect of sesquiterpenes isolated from the rhizomes of *C. aeruginosa* had been reported.[30] The other medicinal uses of rhizome includes postcoital contraception, anti-HIV actions, hepatoprotection, reduced platelet-activation and anti-nociceptive effects.[31-34] Due to its high medicinal value and indiscriminate harvest from the wild, the natural population has come down and according to International Union for Conservation of Nature (IUCN) report, the plant is in the critically endangered category.[35]

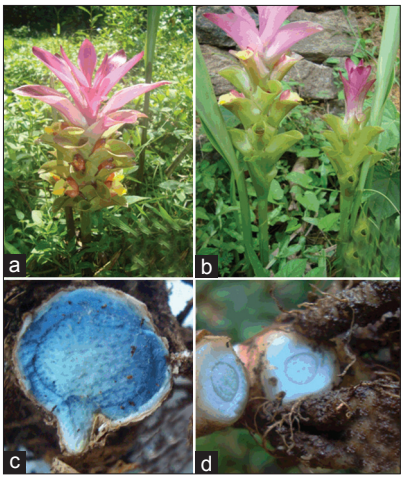

Figure 1: (a) Habit of *C. aeruginosa*; (b) Habit of *C. caesia*; (c) Rhizome of *C. aeruginosa*; (d) Rhizome of *C. caesia*

Several valuable chemical compounds like sesquiterpenes, zedoalactone A, zedoalactone B, zedoarondiol, zedoarol, curcumenol, isocurcumenol, furanodiene and isofuranodiene have been isolated from the rhizome of *C. aeruginosa*.[36-38] The rhizome and leaves of this plant is a good source of several essential oils identified as β-pinene, 1, 8-cineol, curzerenone, furangermenone, furanodienone, camphor, zedoarol, curcumenol, β-elemene and isocurcumenol.[39-43]

The two species of *Curcuma* selected for the present study showed many morphological similarities [Table 1, Figure 1a-d]. In an earlier preliminary study anti-bacterial efficacy of essential oil present in *C. aeruginosa* against two Gram positive and two Gram negative bacteria were investigated.[44] However, detailed study on these two *Curcuma* species is vital for the development of anti-bacterial drugs and their commercial production. The objective of the present investigation was to compare the phytochemical and anti-bacterial properties of two underutilised herbs, *C. caesia* and *C. aeruginosa*.

MATERIALS AND METHODS

Plant Collection

The rhizome of *C. caesia* was collected from Western Ghats of Karnataka, India and the rhizome of *C. aeruginosa* was collected from different localities of Kottayam districts (Kerala, India) during the month of March–April 2011 and identified by

Table 1: Comparison of the various morphological characters of *C. caesia* and *C. aeruginosa*

Plant	Ploidy	Spike position	Colour of calyx	Colour of corolla	Colour of rhizome	Aroma of rhizome	Taste of rhizome	Colour of leaf sheath	Colour of midrib
C. caesia	42	Central/lateral	Purple	Dark pink	Dark blue	Pungent	Bitter	Purple brown	Purple brown
C. aeruginosa	63	Lateral	Purple	Light pink	Greenish blue	Pungent	Bitter	Dark purple	Purple brown

Department of Botany, St. Thomas College Pala, Kerala. Voucher specimen was prepared and deposited in the herbarium of the college for reference.

Preparation of Plant Extract

The collected rhizomes were dried under the room temperature and powdered with a mechanical grinder and stored in air tight container. The 50 g of dried powdered was subjected to soxhlet extraction using hexane, chloroform, ethyl acetate, acetone, methanol and water successively. Before extraction with the next solvent, the powder was air dried to remove the adhering solvent. The extract obtained was filtered and concentrated in rotary vacuum evaporator. The concentrated extract was used for anti-bacterial assays.

Preliminary Phytochemical Screening

The preliminary phytochemical studies were carried out using rhizome powder subjected to successive extraction in soxhlet apparatus with methanol. The rhizome extracts of *C. caesia* and C. *aeruginosa* were then screened for the presence of phytoconstituents such as alkaloids, carbohydrates, proteins, flavonoids, phenols, steroids, lignins, tannins, saponins and aminoacids by using standard chemical methods.[45,46]

Bacterial Strains

A total of eight bacterial strains were tested in the present study. The Gram positive strains include *Staphylococcus aureus*, *Streptococcus haemolyticus* and *Bacillus cereus* and Gram negative consists of *Salmonella typhi*, *Enterobacter aerogens*, *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Serratia marcescens*. The organisms were sub-cultured on Mueller–Hinton Agar (MHA; Himedia, Mumbai, India) medium, incubated at 37°C for 24 h and stored at 4°C in the refrigerator to maintain stock culture.

Screening of Anti-bacterial Activity

Anti-bacterial activity was tested using disc-diffusion method.[47] Petri plates were prepared with 20 ml of sterile MHA media. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The paper discs (6 mm diameter, Whatman no. 1 filter paper) containing different concentrations (1.25, 2.5, 5.0 mg/ml) of crude extracts from *C. caesia* and *C. aeruginosa* with three replicates were dried and placed aseptically on the agar medium with the help of sterile forceps. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Antibiotic streptomycin (10 µg/disc) was used as positive control. The plates were incubated for 24 h at 37°C. The results were recorded by measuring the zone of growth inhibition surrounding the disc and each experiment was repeated three times.

RESULTS

Phytochemical Evaluation

The phytochemcial screening of methanol extracts of *C. caesia* and *C. aeruginosa* rhizome powder demonstrated the presence of several compounds [Table 2]. The main phytochemical constituents include alkaloids, carbohydrates, proteins, aminoacids, flavonoids, phenols, steroids, glycosides and tanins in both plants. However, saponin was not detected in both the plants at the assay conditions.

Anti-bacterial Studies

The anti-bacterial activity of different extracts of *C. caesia* and *C. aeruginosa* rhizome on three Gram positive (*S. aureus, S. haemolyticus, B. cereus*) and five Gram negative (*S. typhi, T. entrobactor, V. cholerae, P. aeruginosa and S. marcescense*) strains of bacteria are tabulated in Tables 3 and 4. The results revealed variability in inhibitory concentration of each extract against a given bacteria. All extracts showed inhibitory action against at least six of the tested bacterial strains in the present study. Both Gram positive and Gram negative bacteria were equally sensitive against each extracts. However, the inhibition of bacterial growth was dose dependent since the inhibitory action of the extract was found to increase with an increase in concentration against all bacterial strains as evidenced by the higher zone of inhibitions at higher concentrations of each extract.

In Gram positive bacteria, acetone extract of *C. caesia* showed maximum activity (zone of inhibition 22 mm) against *S. aureus* and hexane extract of *C aeruginosa* showed

Table 2: Phytochemical screening of methanol extract of *C. caesia* and *C. aeruginosa rhizome*

Constituents	Test	*C. caesia**	*C. aeruginosa**
Alkaloids	Mayer's test	++	++
	Wagner's test		
Carbohydrates	Molisch's test	++	++
	Fehling's test		
Proteins and amino acids	Burette test	++	++
	Millions test		
	Ninhydrin test		
Flavonoids	Lead acetate test	++	++
	$FeCl_3$		
Phenols	Ellagic acid test	++	++
	$FeCl_3$ test		
Steroids	Salkowski test	++	++
	Keller–Kiliani test		
Glycosides test	Anthraquinone test	++	++
Tannins	Ferric chloride test	++	++
	Lead acetate test		
Saponins	Foam test	–	–
	NaOH test		

*++ Present; --Absent

Table 3: Anti-bacterial activity of non-polar and polar extracts of *C. caesia* and *C. aeruginosa*

Extract	Concentration (mg/ml/disc)	Zone of inhibition (mm) Gram positive bacteria								
		Staphylococcus aureus			*Streptococcus haemolyticus*			*Bacillus cereus*		
		C.C*	C.A*	A*	C.C*	C.A*	A*	C.C*	C.A*	A*
Hexane	1.25	-	-	-	-	-	6±1.0	5±0.8	10±1.1	3±0.2
	2.5	-	-	-	-	10±1.3	15±2.1	15±0.1	18±0.6	10±0.5
	5.0	-	-	-	14±0.9	12±1.9	19±2.2	20±1.6	21±2.3	18±0.8
Chloroform	1.25	-	-	6±1.3	-	-	-	5±1.3	4±0.6	3±0.08
	2.5	10±0.7	6±0.4	12±2.2	-	-	-	10±2.3	12±0.9	10±1.2
	5.0	12±2.1	10±1.6	19±2.0	7±0.6	10±0.5	8±0.9	18±1.9	18±0.7	15±1.6
Ethyl acetate	1.25	-	-	-	-	-	-	-	-	4±0.3
	2.5	-	5±1.2	10±1.5	-	-	7±0.7	-	-	12±2.1
	5.0	8±0.9	10±1.1	15±1.3	-	-	14±1.3	-	-	16±1.5
Acetone	1.25	5±1.7	6±1.6	-	5±0.9	4±0.6	5±0.9	4±0.6	3±0.8	4±0.3
	2.5	16±2.0	15±1.0	15±2.0	12±1.8	15±0.7	14±1.4	10±1.2	13±1.0	10±1.3
	5.0	22±1.9	20±1.8	18±2.3	18±2.6	19±0.9	19±1.8	14±1.6	15±1.6	14±1.1
Methanol	1.25	-	-	-	-	-	8±1.5	-	-	5±0.2
	2.5	-	7±1.9	7±1.9	-	7±2.2	12±1.3	-	-	8±0.6
	5.0	10±1.8	10±1.6	15±1.1	-	10±1.2	20±2.3	-	-	14±0.9
Water	1.25	-	-	7±1.9	-	-	7±0.9	-	-	9±0.3
	2.5	-	-	10±0.8	-	-	10±1.3	3±0.6	-	7±0.7
	5.0	-	-	15±0.9	8±0.7	6±0.5	15±2.1	10±0.9	8±0.8	15±2.1

C.C* – *C. caesia*; C.A* – *C. aeruginosa*; A* – Antibiotic

Table 4: Anti-bacterial activity of non-polar and polar extracts of *C. caesia* and *C. aeruginosa*

Extract	Concentration (mg/ml/disc)	Zone of Inhibition (mm) Gram negative bacteria														
		Salmonella typhi			*Enterobacter aerogens*			*Vibrio cholerae*			*Pseudomonas aeruginosa*			*Serratia marcescens*		
		C.C*	C.A*	A*	C.C*	C.A*	A*	C.C*	C.A*	A*	C.C*	C.A*	A*	C.C*	C.A*	A*
Hexane	1.25	9±1.3	10±2.3	7±1.4	-	8±0.4	6±0.8	-	5±0.8	7±0.8	-	-	6±1.2	-	-	3±0.8
	2.5	18±2.3	14±1.9	15±0.9	-	15±2.1	15±2.1	7±1.5	12±2.1	14±1.5	-	6±1.0	14±1.3	-	-	7±1.3
	5.0		16±2.1	21±1.1	-	18±2.3	22±2.4	14±1.9	18±1.9	19±2.1	7±1.2	14±2.1	19±0.7	-	14±1.8	14±1.1
Chloroform	1.25	10±2.3	14±2.1	9±1.3	-	-	-	9±1.8	6±0.8	8±1.2	-	-	7±0.9	10±1.1	8±1.0	5±0.9
	2.5	17±2.1	18±2.8	15±2.1	-	6±0.7	5±0.9	15±2.1	12±1.5	12±2.0	7±0.9	8±1.3	12±1.5	18±1.6	16±2.1	14±1.8
	5.0	18±2.4	20±2.9	20±2.3	8±1.0	14±2.1	11±1.7	20±2.8	19±1.9	15±2.9	14±2.1	12±1.3	18±1.8	27±1.9	19±2.3	20±1.6
Ethyl acetate	1.25	-	-	-	-	-	-	6±2.3	-	3±1.6	-	-	-	-	6±0.6	5±0.7
	2.5	-	-	8±1.3	12±1.7	-	8±2.3	12±1.5	10±1.1	12±2.3	-	-	4±0.7	7±1.0	10±1.0	14±1.1
	5.0	-	-	13±2.0	18±1.3	9±1.3	14±2.1	16±2.2	14±1.5	20±2.4	9±1.6	7±1.4	14±1.0	14±1.2	14±1.6	20±1.2
Acetone	1.25	9±1.4	-	-	-	-	-	-	-	6±1.4	5±1.4	6±1.0	-	4±0.9	5±0.7	8±0.7
	2.5	14±2.1	4±0.7	5±0.7	-	-	-	-	-	10±1.9	16±2.7	14±1.9	7±1.8	12±1.1	10±1.4	14±1.6
	5.0	19±1.6	9±1.3	10±1.2	-	-	8±1.0	-	-	20±2.2	22±2.3	20±2.3	15±1.7	16±1.4	14±1.1	20±1.8
Methanol	1.25	4±1.3	8±1.3	-	8±1.1	-	-	8±1.4	6±0.6	8±1.9	6±0.6	7±1.0	5±1.1	4±0.6	4±0.9	12±1.2
	2.5	10±2.1	15±0.8	12±1.4	12±2.4	10±1.5	11±1.3	12±1.6	10±1.8	12±1.6	13±1.5	14±1.6	12±2.1	10±1.6	8±1.3	17±2.0
	5.0	15±2.3	24±2.1	18±2.1	15±2.1	15±2.2	18±1.5	18±2.2	20±1.3	19±2.7	18±2.2	20±2.1	15±2.3	15±2.0	15±1.8	22±2.3
water	1.25	-	-	8±0.9	-	-	6±0.7	-	-	8±0.8	-	-	6±0.9	6±1.1	3±0.7	9±0.8
	2.5	-	-	12±1.6	-	-	14±2.7	-	-	15±1.7	-	-	12±1.3	12±2.0	8±1.2	14±1.5
	5.0	-	-	18±1.2	-	-	19±2.3	-	-	17±2.3	-	-	15±1.9	16±2.1	14±17	18±1.3

C.C* – *C. caesia*; C.A* – *C. aeruginosa*; A* – Antibiotic

maximum activity (zone of inhibition 21 mm) against *B. cereus* [Table 3]. Chloroform and acetone extracts of both the plants showed inhibition of growth against all the Gram positive bacterial strains tested. The anti-bacterial activity of the acetone extract against *S. aureus* and *S. haemolyticus* was comparable to that of standard antibiotic streptomycin. The hexane extracts of *C. caesia* and *C. aeruginosa* showed almost similar zone of inhibition (i.e., 5-20, 10-21 mm in *C. caesia* and C. *aeruginosa*, respectively) against *B. cereus*.

Among the five Gram negative bacteria tested, chloroform extracts of *C. caesia* exhibited maximum activity (zone of inhibition 27 mm) against *S. marcescense,* but in *C. aeruginosa* showed low zone of inhibition (between 8 and 19 mm)

[Table 4], whereas chloroform extract of two plant samples showed moderate activity against *S. typhi* and *Vibro cholera*. The water extract showed no bacterial activity. The methanol extract of *C. aeruginosa* demonstrated maximum activity (zone of inhibition 8-24 mm) against *S. typhi*, whereas *C. caesia* exhibited low zone of inhibition (between 4 and 15 mm). But also acetone extract of two plants showed almost similar zone of inhibition (5-22 and 6-20 mm in *C. caesia* and *C. aeruginosa*, respectively) against P. *aeruginosa*. Comparatively, the anti-bacterial activity was more prominent on Gram negative bacteria.

DISCUSSION

Plants are vital source of potentially useful bioactive principles for the development of new chemotherapeutic agents.[48] The biological and pharmacological properties of many plants are still unknown. World over, the scientists are exploring the possibilities of utilising pharmacologically active compounds from medicinal plants.[49] Herbal medicines are used by 80% of the people worldwide due to its high efficiency, reduced cost, non-narcotic nature and less side effects.[50,51]

Various species of *Curcuma* are conventionally used as significant ingredients in food and traditional medicines. The present study was carried out with the objective to investigate the phytochemical and anti-bacterial activity of two folklore medicinal plants, *C. caesia* and *C. aeruginosa.* The present work attempts to establish the necessary pharmocognostic standards for evaluating the plant material of *C. caesia* and *C. aeruginosa*. This investigation has opened up the possibility to use this plant for formulating some drugs and human consumption possibly for the treatment of bacterial infections.

Phytochemcial investigations of the rhizome extracts of *C. caesia* and *C. aeruginosa* showed the presence of various bioactive substances such as alkaloids, carbohydrates, proteins, aminoacids, flavonoids, phenols, steroids, glycosides and tanins in both the plants. These active substances may be responsible for the anti-bacterial activities and thereby medicinal properties of *C. caesia* and *C. aeruginosa*. Standardisation of bioactive extracts obtained from a medicinal plant will be carried out on the basis of the phytochemical compounds present in that plant. Therefore, phytochemical screening of medicinal plants is a crucial step in identifying new and effective sources of therapeutically and industrially important bioactive compounds.

In an earlier report, the extracts of *C. caesia, C. aeruginosa* and *C. zedoaria* showed the anti-oxidant, anti-inflammatory and anti-tumor activities of these plants.[52] In *C. aeruginosa* the anti-oxidant activity is due to the polyphenolic compounds present in this plant.[28] Anti-bacterial activity of flavonoids and polyphenolic compounds present in various plants might be attributed to their ability to complex with bacterial cell wall and therefore, inhibit the microbial growth.[7] The presence of anti-microbial substances in higher plants has been well established and it provided a source of inspiration for novel drug compounds as plant-derived medicines have made significant contributions towards human health.[53]

CONCLUSIONS

All the extracts evaluated in the present study showed various levels of anti-bacterial activity and therefore, it was concluded that different secondary metabolites present in both the plants are responsible for this activity. Another aspect of our anti-bacterial study was the dose dependent inhibition of bacterial growth. The increase in antibiotic resistance of microorganism to conventional drugs has necessitated the search for new efficient and cost effective ways for the control of infectious diseases. The results of different anti-bacterial and phytochemical studies on *C. caesia* and *C. aeruginosa* form the foundation for the potential source of new anti-bacterial agents.

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