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Research Article

COMPARATIVE ASSESSMENT OF *IN VITRO* ANTIMICROBIAL ACTIVITY OF *CURCUMA CAESIA* ROXB. AND *CURCUMA AMADA* ROXB

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ABSTRACT

Objective: The aim of the present study was to compare the *in vitro* antimicrobial activity of *Curcuma caesia* Roxb. and *Curcuma amada* Roxb. family Zingiberaceae. Both plants have been used traditionally for curing a number of diseases and ailments. The plants have been specifically used in skin problems and inflammatory conditions.

Methods: Each plant material was extracted with dichloromethane (DCM) and ethanol. All extracts were subjected to preliminary phytochemical screening. The antibacterial activity of the extracts was tested against two Gram-positive (*Staphylococcus aureus, Streptococcus pyogenes*), and two Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) using the cup-plate method. The standard drug used was ofloxacin (0.2 ml) at a concentration of 5 mg/ml. The antifungal activity was tested against *Aspergillus fumigatus,* and *Candida albicans* using the cup-plate method and clotrimazole (10 mg/ml) was taken as standard reference.

Results: Phytochemical screening performed on the extracts has shown the presence of various constituents such as glycosides, carbohydrates, saponins, phytosterols, resins, flavonoids, and diterpenoids. On comparing both species, *C. amada* was found to be stronger growth inhibitor against both Gram-positive and Gram-negative strains. *C. caesia* ethanol extract at a dose of 200 mg/ml was most effective and *C. amada* DCM extract 100 mg/ml was least effective in case of antifungal activity against *C. albicans*. In case of *A. fumigatus, C. caesia* DCM extract 100 mg/ml was least effective and *C. caesia* ethanol extract 200 mg/ml was most effective.

Conclusion: The present study provides the information on phytochemical screening and antimicrobial activities of extracts prepared from two plants of family Zingiberaceae, i.e., *C. caesia* Roxb. and *C. amada* Roxb.

Keywords: *Curcuma caesia*, *Curcuma amada*, Zingiberaceae, Phytochemical screening.

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INTRODUCTION

Plants have been regarded as living biochemical factories that provide a vast variety of chemical substances displaying some biological activities. About 35000 plant species are used throughout the world for one or other medicinal purpose. Researchers from various fields have screened only a small fraction of the plants for their phytochemical and pharmacological potentials [1].

Family Zingiberaceae is a large family with 46 to 52 genera and more than a thousand species. Plants in this family are found in the tropics of Africa, Asia, and America, with the greatest number in Southeast Asia. These are small to large perennial plants with creeping horizontal or tuberous rhizomes [2]. Curcuma is a genus of about 80 species in the family Zingiberaceae, and about 40 of them are indigenous to India [3].

Curcuma caesia Roxb. is commonly known as Kali haldi (Fig. 1). It is a perennial herb, and in India, it grows in West Bengal, Madhya Pradesh, Orissa, Bihar, and Uttar Pradesh and is used by the tribal people to cure various ailments [4]. The name "black turmeric" holds due to the presence of cells related to black color in the rhizome. The plant is claimed to be useful in treating piles, leprosy, bronchitis, asthma, cancer, and epilepsy. A paste of rhizomes is applied externally for curing wounds, pimples, and allergies [5].

Curcuma amada Roxb. is commonly known as Amba haldi or Mango ginger. Is a unique spice having morphological resemblance with ginger (*Zingiber officinale*) but has raw mango flavor (Fig. 2). It is widely distributed in the tropics from Asia to Africa and Australia [6]. Mango ginger rhizome has been reputed as an appetizer, alexteric, antipyretic, aphrodisiac, and a laxative. It is also used in itching, skin diseases, bronchitis, asthma, and inflammation due to injuries [5].

METHODS

Plant collection

Dried rhizomes of *C. caesia and C. amada* were purchased from local market of Amritsar. The plant materials were authenticated by Dr. Shiddamallayya at Regional Research Institute (Ay), Bangalore.

Preparation of plant extracts

The dried rhizomes (50 g) of both species were coarsely powdered and subjected to successive solvent extraction using Soxhlet Assembly. The extraction was accomplished with different solvents in their increasing order of polarity, namely petroleum ether, dichloromethane (DCM), ethanol, and distilled water. The marc was dried before the extraction with the next solvent. These extracts were evaporated to dryness by rotary vacuum evaporator [7].

Preliminary phytochemical investigation

The phytochemical investigation covers the identification of crude drug with respect to their phytochemical constituents. The extracts were subjected to preliminary phytochemical screening according to the standard procedures [8].

Fig. 1: Rhizomes of *Curcuma caesia*

Fig. 2: Rhizomes of *Curcuma amada*

Antimicrobial activity

Antibacterial activity was tested against two Gram-positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). The antifungal activity was tested against *Aspergillus fumigatus* and *Candida albicans*. The above-mentioned bacterial and fungal strains were revived by plating on nutrient agar and Sabouraud dextrose agar, respectively. Isolated colonies were selected after overnight incubation at 37°C. The cup-plate method was used to check the antibacterial and antifungal activities. Ofloxacin (0.2 ml) at a concentration of 5 mg/ml was taken as a standard reference for antibacterial activity, and clotrimazole (10 mg/ml) was taken as a standard reference for antifungal activity [9].

Determination of antibacterial activity

The suspension of bacteria was prepared as per McFarland standard. An inoculum was prepared by suspending a single isolated colony in about 5 ml of normal saline. This was mixed slowly to achieve a smooth suspension. Boring of wells was done on the medium using a sterile borer. A fixed amount of inoculum (0.25 ml) was added to 30 ml of sterile solidified nutrient agar medium in Petri dishes. Different extracts (0.1 ml) were poured in three concentrations (100, 150, and 200 mg/ml) in different wells marked as 1, 2, and 3 of the solidified seeded nutrient agar layer in Petri dishes. The Petri dishes were incubated at 37°C for 24 h, and zones of inhibition were observed and measured using a scale. The values of zones of inhibition were recorded in triplicate, and the results were reported in mean (±standard error of the mean [SEM]) [9].

Determination of antifungal activity

The suspension of fungus was prepared as per McFarland standard. An inoculum was prepared by suspending a single isolated colony in about 5 ml of normal saline. This was mixed slowly to achieve a smooth suspension [10]. Later, one drop of tween 20 was added for filamentous fungi, and the mold was broken by shaking. Four bores per plate were made using sterile cork borer. The surface of Sabouraud's agar medium plate was streaked with the help of spreader in all the directions for uniform distribution of fungal strain. Different extracts were poured in three concentrations (100, 150, and 200 mg/ml) in different holes marked as 1, 2, and 3 of the solidified seeded nutrient agar layer in Petri dishes. The test solution (0.1 ml) was added to the respective bores. The surface of Sabouraud's agar plate was dried. The above procedure was carried under aseptic conditions. The plates were incubated at 28°C for 48 h. Later, the values of zones of inhibition were recorded in triplicate and reported in mean (± SEM) [11].

Statistical analysis

Data have been summarized as the mean±standard deviation. A statistical significant test with control was done using one-way ANOVA followed by using Dunnett's test, and $p<0.01$ was considered statistically significant.

RESULTS AND DISCUSSION

Medicinal plants are rich sources of antimicrobial agents, which are used medicinally in different countries and are a source of many potent drugs used for traditional medicine. Medicinal plants exhibit antimicrobial activity by different mechanisms. This can be achieved by the inhibition of cell wall synthesis, interference with the permeability of cell membrane, cause membrane disruption, modifying cellular constituents, and cell damage or cell mutation [12]. Most of the solvents such as ethanol, hexane, and methanol, when used for plant extract showed inhibitory effect on Gram-positive and Gram-negative bacteria [13].

The phytochemical screening conducted on the plant extracts revealed the presence of chemical constituents, which are known to exhibit medicinal as well as physiological activities. Both plants showed similar results in phytochemical screening. *C. caesia* and *C. amada* extracts were found to contain carbohydrates, saponins, glycosides, phytosterols, resins, and flavonoids. Test for glycosides was positive in all the extracts. The ethanol extract of both plant materials was rich in glycosides and contained most of the other chemical constituents also.

Many plants with high flavonoid and saponins contents have been reported to exhibit potential antimicrobial activity against pathogenic microorganisms [14].

The cup-plate method is one of the official methods in IP, where test samples diffuse from the cup through an agar layer in a Petri dish to such an extent that the growth of microorganisms is restricted to a circular area or confined zone around the cavity containing the solution of an antibiotic substance [15].

The antibacterial activity of two extracts (DCM and Ethanol) of *C. caesia* and *C. amada* was studied using the cup-plate method, and the results are shown in Table 1. The antibacterial activity of *C. caesia* and *C. amada* was performed against two Gram-positive (*S. aureus, S. pyogenes*) and two Gram-negative bacteria (*E. coli, P. aeruginosa*). In case of antibacterial activity against *S. aureus*, the zone of inhibition was maximum in *C. caesia* DCM extract (200 mg/ml). Similar inhibition was observed in *C. caesia* ethanol extract (200 mg/ml). *C. amada* ethanol extract (100 mg/ml) was least effective. In case of *S. pyogenes, C. amada* DCM extract (200 mg/ml) was most effective and *C. caesia* DCM extract (100 mg/ml) was least

Table 1: Effect of *Curcuma* species on Gram-positive and Gram-negative bacteria

Groups	Dose mg/ml	Zone of inhibition (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
		S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*
C. caesia	100	13±0.57**	11.66±0.67**	12.33±0.33**	12±0.00**
DCM	150	17.33±0.67**	14±0.00**	18.33±0.33**	13.66±0.33**
Extract	200	19.66±0.67**	16.33±0.33**	20±0.57**	15.66±0.33**
C. caesia	100	12.66±0.67**	13.33±0.33**	20.66±0.33**	14±0.00**
Ethanol	150	17.33±0.33**	16±0.00**	22.66±0.33**	16±0.00**
Extract	200	19±0.00**	16.33±0.68**	23.66±0.33**	25±0.00**
C. amada	100	12.6±0.33**	12.3±0.33**	11.33±0.33**	10.66±0.33**
DCM	150	14.6±0.33**	14.3±0.33**	16±0.57**	13.33±0.33**
Extract	200	18.6±0.33**	19.3±0.88**	17.6±0.33**	16.3±0.33**
C. amada	100	12±0.57**	13.33±0.33**	12.33±0.33**	13.33±0.33**
Ethanol	150	13.6±0.33**	17.66±0.33**	14±0.57**	14±0.57**
Extract	200	16.6±0.33**	19.33±0.33**	17.66±0.33**	18.33±0.33**
Standard Ofloxacin	5	25.91±0.95	26.08±0.63	26.415±0.77	28.25±0.32

Each value represents mean±SEM, n=3. Statistical significant test with control was done using one-way ANOVA followed by using Dunnet test, **p<0.01. *S. aureus: Staphylococcus aureus, S. pyogenes: Streptococcus pyogenes, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, C. caesia: Curcuma caesia*, DCM: Dichloromethane

Table 2: Effect of *Curcuma* species on fungal strains

Groups	Dose mg/ml	Zone of inhibition (mm)	
		C. albicans	*A. fumigatus*
C. caesia	100	12.33±0.333**	9±0**
DCM	150	14±0**	15±0**
Extract	200	16.33±0.333**	16±0**
C. caesia	100	16.33±0.333**	11.66±0.333**
Ethanol	150	19.33±0.333**	20.33±0.333
Extract	200	25±0**	25.66±0.333
C. amada	100	10±0**	11.33±0.333**
DCM	150	11±0**	14.83±0.441**
Extract	200	13.66±0.333**	17.33±0.333**
C. amada	100	12.33±0.333**	10±0**
Ethanol	150	15±0**	13.6±0.333**
Extract	200	17±0**	15.33±0.881**
Standard (Clotrimoxazole)	10	21.5±0.27	24.16±1.29

Each value represents mean±SEM, n=3. Statistical significant test with control was done using one-way ANOVA followed by using Dunnet test, **p<0.01. *C. albicans: Candida albicans, A. fumigatus: Aspergillus fumigatus*, DCM: Dichloromethane, *C. amada: Curcuma amada*

effective. In case of antibacterial activity against *E. coli,* DCM extract of *C. caesia* was more effective than *C. amada* at all concentrations; whereas, the ethanol extract of *C. caesia* was more effective than *C. amada* at all concentrations. The activity increases from DCM extract to ethanol extract with increasing dose. Maximum zone of inhibition was shown by *C. caesia* ethanol extract (200 mg/ml) which was nearly similar to that of the standard. Similar results were observed in case of *P. aeruginosa.* The activity increases from DCM extract to ethanol extract with increasing dose. On comparing both species, *C. amada* was found to be stronger growth inhibitor against both Gram-positive and Gram-negative strains.

C. caesia ethanol extract at a dose of 200 mg/ml was most effective, and *C. amada* DCM extract 100 mg/ml was least effective in case of antifungal activity against *C. albicans* (Table 2). Dose-dependent inhibition was observed. The ethanol extract was more effective than DCM extract whereas *C. caesia* showed better antifungal activity when compared to *C. amada*. In case of *A. fumigatus, C. caesia* DCM extract 100 mg/ml was least effective and *C. caesia* ethanol extract 200 mg/ml was most effective. In case of DCM extract, *C. amada* at a dose of 200 mg/ml was most effective.

CONCLUSION

The results of the present investigations suggest that these plant species are important for further investigations on isolation and characterization of the bioactive principles responsible for the antifungal activity. Moreover, these medicinal herbs may afford lead compounds, which could be beneficial for the future drug development. The phytochemical analysis conducted on the plant extracts revealed the presence of various biologically active constituents. The most active extracts can be subjected to the isolation of therapeutic antimicrobial agents to carry further research in this area.

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