WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 4, 1725-1733. Research Article ISSN 2277– 7105

ANTIMICROBIAL ACTIVITY OF ETHANOLIC RHIZOME EXTRACT OF *CURCUMA AMADA* ROXB. (MANGO GINGER)

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Article Received on
06 Feb 2015,

Revised on 03 March 2015,
Accepted on 28 March 2015

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ABSTRACT

This study was carried out with an objective to investigate the antibacterial and antifungal potentials of rhizomes of *Curcuma amada*. The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on some bacterial and fungal strains. In the present study, the microbial activity of ethanol extracts of rhizomes of *C. amada* was selected for potential antimicrobial activity against bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar disc diffusion method. The antibacterial and antifungal activities of extracts such as 10 and 20µL (contains 5 and 10mg) of *C. amada* were tested against *Klebsiella pneumonia* NCIM 2883 , *Escherichia coli* NCIM 2931 B2, *Salmonella typhimurium* NCIM 2501, *Shigella flexneri* MTCC 1457, *Candida albicans* MTCC 1637, *Candida glabrata* MTCC 3984, *Cryptococcus* sp. MTCC 7076, *Microsporum canis* MTCC 3270. Zone of inhibition of extracts were compared with that of the standard *Methicillin (10mcg/disc)* for antibacterial activity and *Itraconazole* (*10mcg/disc)* for antifungal activity. The results highlighted that the remarkable inhibition of the bacterial growth was shown against the tested organisms. In antibacterial studies, extract was most effective against *Salmonella typhimurium* NCIM 2501 (B5) – 14mm, while less effect was noticed on *Klebsiella pneumonia* NCIM 2883 (B1) – 0mm. In antifungal studies, the extract was more effective against *Candida glabrata* MTCC 3984 (F2) – 13mm whereas less effect was observed in *Microsporum canis* MTCC 3270 (F4) – 11mm, *Candida albicans* MTCC 1637 (F1) – 11mm, *Cryptococcus* sp. MTCC 7076 (F3) – 11mm.

KEYWORDS: *Klebsiella pneumonia* NCIM 2883 , *Escherichia coli* NCIM 2931 B2, *Salmonella typhimurium* NCIM 2501, *Shigella flexneri* MTCC 1457, *Candida albicans* MTCC 1637, *Candida glabrata* MTCC 3984, *Cryptococcus* sp. MTCC 7076, *Microsporum canis* MTCC 3270.

INTRODUCTION

Antimicrobial resistance is a serious problem of world population. The adverse effects of antibiotics and antibiotics resistance developed by pathogenic microorganisms, so we are in need of plant derived antimicrobial agent. Totally, two-third of active ingredients of plants used as antimicrobial drugs (McChesney et al., 2007). In current days, numerous antimicrobial agents discovered from plant sources are used to treat a wide range of infectious diseases.

Plants are act as a potential source for secondary metabolites, such as flavanoids, terpenoids, alkaloids, tannins, phenols or their oxygen-substituted derivatives, steroids etc., (Geissman**,** 1963), of which at least 12,000 phytocompounds have been isolated (Schultes, 1978) . In many cases, these substances serve as plant defense mechanisms against invasion by microorganisms, insects and herbivores. Some phytocompounds, such as terpenoids give plants their odors; others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavor (e.g., the terpenoid capsaicin from chili peppers) and some of the same herbs and spices used by humans to treat various diseases. Plants are used as potent and powerful drugs (Srivastava et al., 1996). The hundreds of plant species have been used to analyze the antimicrobial properties (Balandrin et al., 1985).

The plants of *Zingiberaceae* composed of 70-80 species family are rich in phytochemicals (Purseglove, 1974 and Aminul, 2004) with various pharmacological activities. C*urcuma amada* Roxb of this family is a very important active spice. It is a unique perennial herb because its rhizome has similar aromatic odour of green mango (*Mangefera indica* L.) and it is morphological resemblance to ginger (*Zingiber officinale* L.). *C. amada* is used in the traditional system of medicine and in ayurveda it is used an appetizer, alexteric, antipyretic, aphorodisiac and laxative (Policegoudra et al., 2010). It is also used to cure biliousness, itching, skin diseases, asthma and inflammation due to injuries (CSIR 1950; Kirtikar and Basu, 1984; Warrier et al., 1994). So the present work, *Curcuma amada* has been selected to investigate for their antimicrobial properties against various infectious microorganisms.

MATERIALS AND METHODS

Collection of plant material

The rhizomes of *C. amada* were collected from Kulumani Village, Tiruchirappalli District, Tamil Nadu, India (**figure -1**). The plant was identified and deposited at The Rapinat Herbarium and Centre for Molecular Systematics, St Joseph's College, Tiruchirappalli, Tamil Nadu. India.

Figure: 1 Rhizome of *C. amada*

Ethanolic rhizome extract of C. *amada* (CAEREt)

The powdered rhizome of *C. amada* weighed (100g) and was soaked in ethanol and kept in Mechanical shaker for 48 hours. The extract was filtered and then filtrate was poured into the Petri plate for complete evaporation of ethanol at room temperature. The yellow residue was obtained and it is used for antimicrobial activity.

Microbial strains

The test strains were: *Klebsiella pneumonia* NCIM 2883 (B1), *Escherichia coli* NCIM 2931 (B2), *Salmonella typhimurium* NCIM 2501 (B3), *Shigella flexneri* MTCC 1457 (B4), *Candida albicans* MTCC 1637 (F1), *Candida glabrata* MTCC 3984 (F2), *Cryptococcus* sp. MTCC 7076 (F3), *Microsporum canis* MTCC 3270 (F4) were used in the study. The cultures were obtained from MTCC, Chandigarh and NCIM, Pune, India.

Disc diffusion method

Microbial strains were tested for antimicrobial sensitivity using the disc diffusion method. (Bauer *et al.*, 1966). This method was used to evaluate *in vitro* antibacterial and antifungal activity of test sample against certain human pathogenic microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively. A sterile cotton swab was used to

inoculate the standardized bacterial suspension on surface of agar plate for even growth. The 10 and 20 µL of test solutions which prepared with 100% of DMSO were poured in each disc (6 mm diameter), separately. One separate disc was used for control study by taking 100% of DMSO without test sample. The plates were incubated at 37±1°C for 24–48 h (bacteria) and 25 ±1°C for 48-72 h (fungus). After incubation, the zone of inhibition was measured in diameter (mm) with ruler/HiAntibiotic Zone Scale-C. The assays were performed in triplicate and the average values are calculated. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) was used as positive control, and DMSO (100%) used as a negative control). All the media, standard discs and HiAntibiotic ZoneScale-C were purchased from Hi-Media (Mumbai, India).

RESULTS AND DISCUSSION

Antibacterial screening

The antimicrobial activity of ethanolic rhizome extract of *C. amada* was examined with various pathogenic microorganisms by the disc diffusion method. The results of antimicrobial activities of CAEREt against selected bacterial species like *Klebsiella pneumonia* NCIM 2883 (B1), *Escherichia coli* NCIM 2931 (B2), *Salmonella typhimurium* NCIM 2501 (B3), *Shigella flexneri* MTCC 1457 (B4), are summarized in Table 1. The two tested concentrations of CAEREt such as 10 and 20µL (contains 5 and 10mg) /disc showed diameter of zone of inhibition on MHA plates for bacteria. In the present study, 10mg (20µL/disc) concentration of extract should more sensitivity than 5mg (10µL/disc) in all the tested microorganisms. In antibacterial studies, extract was most effective against *Salmonella typhimurium* NCIM 2501 (B5) – 14mm, while less effect was noticed on *Klebsiella pneumonia* NCIM 2883 (B1) – 0mm.

Antifungal screening

The antifungal activities of CAEREt against selected fungal strains like *Candida albicans* MTCC 1637 (F1), *Candida glabrata* MTCC 3984 (F2), *Cryptococcus* sp. MTCC 7076 (F3), *Microsporum canis* MTCC 3270 (F4) are presented in Table 1. In antifungal studies, the extract was more effective against *Candida glabrata* MTCC 3984 (F2) – 13mm whereas less effect was observed in *Microsporum canis* MTCC 3270 (F4) – 11mm, *Candida albicans* MTCC 1637 (F1) – 11mm, *Cryptococcus* sp. MTCC 7076 (F3) – 11mm. All the fungal strains depict higher sensitivity to the higher concentration (20µL contains 10mg) of extract for the test sample when compared to the positive control. There is no antimicrobial activity

in solution devoid of extract used as a vehicle control (100% DMSO), which reflect that the antimicrobial activity was directly related to the extract.

Table – 1. Antimicrobial activity of Ethanolic rhizome extract of *Curcuma amada*

S.No	Test Microorganisms	Zone of inhibition (mm) Sample (10 & 20) µL / disc			
Bacteria		**10 µL**	**20 µL**	**PC**	**NC**
1.	*Klebsiella pneumoniae*	0	0	28	0
2.	*Eshsericia coli*	11	13	10	0
3.	*Salmonella typhimurium*	10	14	0	0
4.	*Shigella flexneri*	12	13	32	0
Fungi					
5.	*Candida albicans*	10	11	10	0
6.	*Candida glabrata*	10	13	10	0
7.	*Cryptococcus* sp.	10	11	9	0
8.	Microsporum canis	9	11	9	0

PC - Positive Control

(Using antibiotic disc; Bacteria – Methicillin (10mcg/disc); Fungi – Itraconazole (10mcg/disc)

NC - Negative Control

(Using 100% DMSO – Dimethyl sulfoxide)

Samples - 10 µL / disc & 20 µL / disc

The phytochemicals of plants are of great importance and known to possess antimicrobial and therapeutic properties (Nagesh and Shanthamma, 2009). Indian system of medicine used plant extracts for the treatment of variety of diseases (Kalimuthu et al., 2010). The potent antimicrobial activity of plant extract has been studied by a large number of researchers in different parts of the world (Reddy et al., 2001, Ateb and Erdo, 2003). The crude extracts of medicinal plants are effective against various pathogens (Kubo et al., 1981). The antifungal activity of different plant extracts were studied (Varaprasad et al., 2009). In the present study, all the microbial strains showed higher sensitivity to the plant extract when compared to the positive control except B1 and B4, Which may be due to the presence of phytoconstituents of the plant. Some investigations suggested that the bacteriostatic activity mainly due to the presence carbonylation of terpenoids (Naigre et al., 1996). The researchers reported that the terpenoids of the plant inhibits respiratory enzymes thus affect the energy system of the microbial cell (Sikkema et al., 1992, and Sikkema et al., 1994).

Scientific validation about the antimicrobial properties of the medicinal plants has been widely studied (Cowan et al., 1999). The bacteria such as *Pseudomonas aeruginosa* and enterohemorrhargic *Escherichia coli* developed resistance against the antibiotics such as norfloxacin, ciprofloxacin and amoxicillin-clavulanic acid has been reported (Bassam et al., 2004). Recently Multi-drug resistance is a global problem to the medical field and infections caused by multi-resistant bacteria. The control of diseases by the plant extracts has become popular, biodegradable, cheap and readily available, and the plant extracts fight against human pathogens without toxic side effects (Ray et al., 2004).

The present study showed that the effective antimicrobial properties of *C. amada*. From the results, higher (20 µL/disc) concentration of sample showed greater sensitivity than lower concentration (10 µl/disc) against all the tested microorganisms. It is showed that the higher concentration of CAEREt may act as potent antimicrobial agent against various pathogenic microorganisms. The antibacterial activity of root extract of *C. amada* against *S. aureus* was reported and it exhibited strong antibacterial activity against *S. aureus*. (Attarpour et al., 2004). The antimicrobial activity of purified isolated compounds difurocumenonol was reported and it showed high antibacterial activity against gram-positive bacteria (Policegoudra et al., 2010). The antibacterial activity of leaf extracts four species of Zingiberaceae family such as *C. longa, C. amada, C. aromatica* and *C. caesia* was reported (Ritwiz et al., 2011) and plant the species exhibited potent antibacterial activity against *Bacilus subtilis*. Both antibacterial and antifungal activity of the ethanolic and aqueous extracts of four Curcuma species such as *Curcuma longa* (turmeric), *C. caesia* (Black turmeric), *C. amada* (Mango ginger) and *C. aromatica* (Van turmeric) were also investigated (Harit et al., 2013). The ethanolic extract of *C. longa* and *C. aromatica* was found to have both antibacterial and antifungal activity. The antimicrobial activity of *C. longa* is mainly due to the presence of terpenoids and glycosides (Chhetri, 2008; Okigbo, 2009).

CONCLUSION

Various activities of *C. amada* such as antimicrobial, antioxidant, anti-inflammatory, analgesic, antipyretic, anticancer, and antitumorigenic properties were reported (Rompelberg et al., 1996: Al Rehaily et al., 2002 and Kluth et al., 2007). It contains a multitude of potential bioactive agents that may contribute to health promotion, including flavonoids, phenolic acids, catechins, and several phenylpropanoids (Al Rehaily et al 2002).

The antimicrobial activity of the *C. amad a* was due to the presence the phytocompounds. Hence, the plants can be used to treat many diseases that may serve as leads in the development of novel drugs in modern pharmaceuticals research industries.

Conflict of interest

The authors declare that we have no conflict of interest.

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