

Qualitative estimation of phytochemicals and antimicrobial activity of certain solvent extracts of *Curcuma amada* Roxb. and *Curcuma caesia* Roxb. Rhizomes

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Abstract

Background: Microbes are the causal agents of numerous diseases and the culprits of food stuff contamination and spoilage. Antibiotics have been always the first line of self defense against them but there have been a gradual increase in antimicrobial resistance which is an issue of global concern. Thus there is a need of research in new drug discovery. Since time immemorial plants have been used as medicines. They have been housing numerous secondary metabolites or phytochemicals which are reported to have antimicrobial effect. The present study was designed to screen the phytochemical constituents and antimicrobial activity of various solvent extracts of *Curcuma amada* and *Curcuma caesia*, two well known aromatic rhizomatous medicinal plants of Zingiberaceae family, used in traditional medicines to cure various diseases.

Methods: Extracts of selected rhizomes were prepared in water, ethanol, methanol and chloroform. Phytochemical screening was done following standard methods. Aqueous and different solvent extracts of the two rhizomes were subjected to antimicrobial assay against *Staphylococcus aureus* (Gram positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* (Gram negative bacteria) and a fungus *Aspergillus niger* following standard agar well diffusion method.

Results: Phytochemical study showed the presence of flavonoids, terpenoids, steroids, saponin, glycosides, alkaloids and tannins in all the extracts except for the aqueous in which flavonoids and steroids were not detected. Except the aqueous extracts all the other extracts showed significant antimicrobial activity. Methanol and ethanol extracts were found to be most effective.

Conclusion: The result obtained thus provide a scientific basis for their use in traditional health care system.

Keywords: *Curcuma amada*, *Curcuma caesia*, rhizomes, traditional medicines, phytochemicals and antimicrobial activity

Introduction

Bacteria and fungi are the causal agents of numerous diseases in human, animals and plants. They are also significant destroyers and contaminators of food stuffs. Antimicrobial drugs have always been preferred against them but use and misuse of these drugs have led to the acceleration of emergence of antimicrobial resistance (AMR) which has become a critical health threat and hence a global issue (WHO). Best practice to avoid emergence and spread of resistance is judicious utilization of antibiotics and creating and living in a hygienic atmosphere. Innovative research and development of new drugs is thus the need of the hour.

According to an estimate of WHO, 80% of population residing in developing countries rely on plant based traditional medicines for primary health care while modern pharmacopoeia contain 25% plant derived drugs or synthetic analogues which are prototypes of lead components isolated from plants (Schmincke, 1997). They are used as templates for development of new drugs by pharmaceutical companies (Borris, 1996). Finding healing powers in plants is an ancient idea and of great interest in recent times after scientist have realized that effective life span of antibiotics is limited. Plants are storehouse and have the limitless ability of synthesizing myriads of secondary metabolites or phytochemicals which they have developed through evolution, to defend themselves from insect pests, fungal attack and other pathogenic diseases. (Seters, 1995). Phytochemicals like terpenoids, flavonoids, tannins, saponins, glycosides etc have been reported by numerous authors to possess antimicrobial activity (Scalbert, 1991; Cowan, 1999; Cushnie and Lamb, 2005; Avato *et al.*, 2006, Policegoudra *et al.*, 2011).

Ginger and turmeric belongs to Zingiberaceae family and they have been known for their medicinal properties in the traditional systems. Turmeric is nature's

magical gift to human, having numerous, unmatched medicinal properties. Also called "a classic grandmother's remedy" it has been widely used by people since time immemorial as medicines, as wound healer, body and mind purifier, as spice for flavouring and colouring curries and other food items, as cosmetics for application on skin for beautification and attainment of healthy skin. Turmeric has found its way from traditional and use as home remedy item to food, cosmetic and health industry. Turmeric has a powerful and amazing anti-inflammatory, antiseptic property, antimicrobial activity which is attributed to the presence of the phytochemicals (Mujumdar *et al.*, 2000; Policegoudra *et al.*, 2011; Seth, 2011; Jose and Thomas, 2014). Curcumin - a diphenylheptanoid which gives yellow colour to turmeric was also found to have antimicrobial activity (Schraufstatter and Bernt, 1949).

The genus *Curcuma* is well known as the turmeric genus. *Curcuma caesia* and *Curcuma amada* are two species belonging to genus - *Curcuma*. The rhizomes of *Curcuma caesia* commonly known as black turmeric (locally kola halodhi) has a characteristic bluish black colour, pungent smell due to presence of volatile components (Pandey and Chowdhary, 2003). It is native to North East and Central India (Ravindran *et al.*, 2007) and have been regarded as endangered by the Central Forest Department of India. Traditionally the rhizomes of *C.caesia* have been used in treatment of leucoderma, piles, asthma, wounds, allergies, cough, fever, rheumatic arthritis, gastric disorder etc (Ravindran *et al.*, 2007; Devi *et al.*, 2015).

Curcuma amada also known as Mango ginger (locally Amada) has morphological similarity with ginger. Its buff coloured rhizome has a typical exotic flavor of raw unripe mango and used in preparing pickles and flavouring food. Rhizome of *Curcuma amada* has been used traditionally to treat various skin diseases, itching, asthma,

wounds and respiratory illness (Policegoudra, 2011).

Considering the traditional use of these two rhizomes the present study was aimed to investigate the antimicrobial activity of different solvent extracts of rhizomes of *C.amada* and *C.caesia* and also to screen the phytochemicals present in them. Four bacterial strains, a gram positive bacteria - *Staphylococcus aureus* and three gram negative bacteria - *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and a fungus *Aspergillus niger* were used for the study. *Staphylococcus aureus* causes skin diseases, respiratory infection, bone and joint pains, boils and food poisoning. Certain *Escherichia coli* strains are harmful and cause serious food poisoning. Both *Pseudomonas aeruginosa*, *Proteus mirabilis* cause urinary tract infections and formation of bladder and kidney stones while the fungus, *Aspergillus niger* produces mycotoxins and causes several ailments of lungs, liver, skin and respiratory organs etc.

Materials and methods

Plant collection: Rhizomes of *Curcuma amada* and *Curcuma caesia* used for the study were collected from the Botanical garden of Dibru College, Dibrugarh. They were washed, peeled, cut, shade dried, powdered in a blender and stored in air tight containers until use.

Test organisms: Four bacterial strains , a gram positive bacteria - *Staphylococcus aureus* (MTCC-87), three gram negative bacteria - *Escherichia coli* (MTCC-10312), *Pseudomonas aeruginosa* (MTCC-3542), *Proteus mirabilis* (MTCC-3310) and a fungus *Aspergillus niger* (MTCC-9652), used for the study were collected from MTCC, IMTECH, Chandigarh.

Preparation of Extract: The powdered rhizome samples were extracted with different solvents, viz. water, ethanol, methanol and chloroform with the help of a

Soxhlet apparatus and concentrated in a rotary evaporator and the crude extract obtained was kept in refrigerated condition.

Qualitative phytochemical analysis: The crude extracts prepared with different solvents were initially dissolved in DMSO and used for qualitative screening of phytochemicals following standard methods from Harborne (1998).

Determination of antimicrobial activity: Standard Agar well diffusion method following Perez *et al.*, (1990) was used for study of antimicrobial activity of different crude extracts. Two commercial antibiotics Ampicillin and Cefotaxime were used as positive control and DMSO was used as negative control. Formation of a clear inhibition zone around the well indicated the inhibition of microbial growth. Diameter of the Zone of Inhibition (ZOI) was measured in mm and the results were expressed as Mean \pm Standard Deviation.

Results and discussion

Phytochemical analysis of the aqueous, methanol, ethanol and chloroform extracts of *C. amada* and *C. caesia* is presented in Table1. Flavonoids, terpenoids, steroids, saponins, glycosides, alkaloids, tannins were found to be present. Saponins, terpenoids, glycosides, alkaloids were detected in the aqueous extracts of both *C. amada* and *C. caesia* but steroids and flavonoids were absent. Table 2 represents the antimicrobial activity of aqueous, methanol, ethanol and chloroform extracts of *C. amada* and *C.caesia* on the experimental test microbes. The methanol extract of *C. amada* was found to be most effective on *Proteus mirabilis* among all test organisms with even greater Zone of Inhibition (ZOI) (20.25 ± 0.50) than the ZOI of the positive controls Ampicillin (19 ± 0.14) and Cefotaxime (12.50 ± 0.07). This was followed by the activity of the ethanol extract of *C. amada* on *Proteus mirabilis*

with ZOI (17.25±0.95) and *Staphylococcus aureus* with ZOI (16.25±1.25). Methanol and ethanol extracts of both *C. amada* and *C. caesia* had moderate antimicrobial activity on other test organisms but chloroform extract did not show such good antimicrobial results in comparison to ethanol and methanol extracts. Aqueous and chloroform extracts of both *C. amada* and *C. caesia* was found to be inactive, (did not show any ZOI) against *Pseudomonas aeruginosa* and *Aspergillus niger* but the ethanol and methanol extracts showed ZOI against them. It was seen that the aqueous extracts of both *C. amada* and *C. caesia* did not show any ZOI or antimicrobial activity against the microbes tested for. This might be due to the absence of steroids and

flavonoids in the aqueous extract of both *C. amada* and *C. caesia*. The active components might have been insoluble in those extracts. It was found that *C. amada* had more potent antimicrobial activity than *C. caesia*.

Antimicrobial activity of *C. amada* and *C. caesia* has been reported in many literatures. (Bhardwaj *et al.*, 2011; Jayalakshmi *et al.*, 2011; Policegoudra *et al.*, 2011; Harit *et al.*, 2013; Jose *et al.*, 2014).

The antimicrobial activity of the experimental plants might be due to the presence of different classes of phytochemicals or secondary metabolites which may independently or collectively produce the antimicrobial activity.

Table 1: Qualitative screening of phytochemicals in different solvents.

plant	Extract	Saponin	tanin	terpenoids	Steroids	alkaloids	glycosides	Flavonoids
<i>Curcuma amada</i>	Aq	+	+	+	-	+	+	-
	Eth	+	+	+	+	+	+	+
	Met	+	-	+	+	+	+	+
	Chl	+	+	+	+	+	+	+
<i>Curcuma caesia</i>	Aq	+	+	+	-	+	+	-
	Eth	+	+	+	-	+	+	+
	Met	+	+	+	+	+	+	+
	Chl	+	+	+	+	+	+	+

‘+’ sign denotes presence and ‘-’ sign denotes absence of the phytochemicals.

Table 2: Antimicrobial activity of *Curcuma amada* and *Curcuma caesia* extracts against bacteria and fungal species tested by agar well diffusion method.

Test organism	Zone of inhibition (mm)								Ampicillin	Cefotaxime
	<i>Curcuma amada</i> extract				<i>Curcuma caesia</i> extract					
	Aq	Eth	Met	Chl	Aq	Eth	Met	Chl		
<i>E.coli</i>	NI	13.00± 0.81	12.75± 0.50	8.00± 0.81	NI	13.50± 0.57	9.25± 0.95	NI	16.7± 0.05	11.3± 0.05
<i>P. aeruoginosa</i>	NI	11.75± 0.50	14.00± 0.81	NI	NI	10.25± 0.50	11.00± 1.15	NI	NI	21± 0.10
<i>P.mirabilis</i>	NI	17.25± 0.95	20.25± 0.50	6.75± 0.50	NI	NI	8.50± 0.70	12.00± 1.15	19.00± 0.14	12.50± 0.07
<i>S. aureus</i>	NI	16.25± 1.25	14.50± 1.00	11.50± 0.57	NI	13.25± 0.50	NI	9.50± 0.70	25.0± 0.38	23.7± 0.23
<i>A. niger</i>	NI	10.75± 0.95	12.25± 0.50	NI	NI	9.33± 1.15	10.66± 1.15	NI	NI	27.5± 0.35

Values are mean inhibition zone (mm) ± S.D of four replicates Diameter of well = 6 mm

Abbreviations: Aq: Aqueous, Eth: Ethanol, Met: Methanol, Chl: Chloroform extracts

Numerous studies have reported terpenoids, flavonoids, saponins, tannins, alkaloids to possess antimicrobial activity. Flavonoids have been found to exhibit broad spectrum of antimicrobial activity which has been attributed to its ability to complex with bacterial cell wall and inhibit growth, nucleic acid synthesis, energy metabolism (Cushnie and Lamb, 2011). It was suggested that saponin might disturb the permeability of the bacterial outer membrane (Arabski *et al.*, 2012). Most alkaloids act through efflux pump inhibition. Studies have also described alkaloids to inhibit certain enzymes and nucleic acid synthesis (Cushnie *et al.*, 2014). Tannins are found to inhibit extracellular microbial enzymes, cause deprivation of iron and other substrates required for microbial growth and thus affect the metabolism of microbes (Scalbert, 1991).

The results indicate ethanol and methanol extracts to exert more inhibitory activity and thus it can be suggested that ethanol and methanol would be better solvents for studying antibacterial and antifungal principles. Many studies have reported methanol as potent solvent for extracting phytochemicals (Parekh and Chanda, 2007; Alam *et al.*, 2009; Jayalakshmi *et al.*, 2011). Various species belonging to the genus *Curcuma* are well known for their multiple uses as medicines, cosmetics, dyes, flavorings and nutraceuticals. Indigenous people collect medicinal plants from forest but rapid deforestation and overutilization have posed a hidden risk of loss and extinction of numerous untapped medicinal plants which are yet to be studied for their activity as potential drugs (Lewis and Lewis, 1995). Overexploitation and deforestation have led to their gradual decline of habitat and if left unchecked will surely lead to extinction of these species. Thus these medicinal plants need to be utilized sustainably and conserved using both in-situ and ex-situ methods (Kasagna and Karumuri, 2011).

Conclusion

The results suggest that *Curcuma caesia* and *Curcuma amada* have the potentiality to be used as antimicrobials which can be attributed to the presence of phytochemicals in them. This study to a little extent provides a scientific basis for the use of these rhizome extracts in traditional medicine for the treatment of microbial induced ailments. But further standardizing methods of isolation, identification of bioactive components and their efficacy on model, beneficial and non target organisms are necessary to completely validate the Traditional Knowledge scientifically. *Curcuma caesia* is a critically endangered species dying out fast due to overexploitation, but it cannot be allowed eventually to be extinct. It thus needs to be conserved and used sustainably for the greater benefit of mankind.

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