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Research Article

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF RHIZOME EXTRACTS OF CURCUMA PSEUDOMONTANA J. GRAHAM

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ABSTRACT

The aim of this work is to investigate the phytochemical analysis and antimicrobial activities of different crude extracts from rhizome of *Curcuma pseudomontana* J. Graham. Different organic solvents including hexane, chloroform, ethyl acetate and methanol were used to prepare the crude extracts from rhizome. The extracts of rhizome were tested for their antimicrobial activity by agar well diffusion method, minimum inhibitory concentration and minimum microbial concentration. The phytochemical analysis shows the presence of alkaloid, flavonoid, tannin, steroids, saponins, carbohydrate, proteins, and amino acids in different rhizome extracts. However, not all extracts show presence of these compounds except ethyl acetate. In gram-positive bacteria the *B. subtilis* was found to be the more sensitive than *S. aureus* and *S. scuiri* and in gram-negative, *P. aeruginosa* most sensitive, followed by the *E. coli* and *K. pneumonia.* Among the fungi used, the *C.albicans* was found to be the most sensitive followed by the *A.fumigatus, A.niger* and *A.flavas.* Therefore, in view of these results, the ability of the extracts to inhibit the growth of several bacteria and fungi is an indication of the broad-spectrum antimicrobial potential of *Curcuma pseudomontana* J. Graham. In conclusion, all organic crude extracts from rhizome can be used as sources of new antimicrobial agents but ethyl acetate extract showed most potential and higher antimicrobial property.

INTRODUCTION

Infectious diseases account for 41% of the global disease impede along with noninfectious diseases (43%) and injuries (16%). The main reasons of these infectious diseases are the natural development of microorganism resistance to various antibiotics (Noumedem *et al.,* 2013). Due to the development of multidrug resistance microorganism strains is promoting a renaissance in research of plant bioactive compounds role in antimicrobial activity (Maiyo *et al.,* 2010). The plant products for the treatment of human diseases have certain benefits, they are cheap to produce, biodegradable and readily available. Efficient plant extracts can fight with human pathogenic bacteria without side effects and environmental hazards (Singh, 2004).

Mastitis is an inflammation of mammary glands and characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues (Radostits *et al.,* 2000). Infection of the cow's udder (bovine mastitis) has remained one of the major limitations to growth of the dairy industry in India and abroad. Bovine mastitis is a very serious problem among cattle diseases, which affects the basic income of the dairy industry5 (Al-Qumber *et al.,* 2006). Problems with decreased therapeutic efficacy and the raise of antibiotic-resistant bacteria in livestock production have stimulated the research for new strategies to control mastitis (Varella Coelho *et al.,* 2007; Wu *et al.,* 2007). 6-7

The traditional healers use the genus Curcuma for the treatment of various disorders whereas *Curcuma pseudomontana* J. Graham is comparatively less known. *C. pseudomontana* belongs to family Zingiberaceae. *Curcuma pseudomontana* J. Graham known as Tavaksheera (Ayurveda) Kachura (Hindi), Raan halada, shindalavana or shindalavani (Marathi), Kattu manjal (Tamil), Kattu manjal (Malayalam). *C. pseudomontana* rhizome is beneficial against leprosy, dysentery, cardiac diseases8 (Yoganarasimhan, 1996). Women of Jatapu and Savara tribes eat boiled tubers to increase lactation 9 (Ramarao *et al* 2000). Jatapu and Kaya tribes apply warm tuber paste to treat body swellings. Khand tribes apply the tuber paste on the head for cooling effect, crushed and boiled rhizome are edible

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10 (Patil *et al.,* 2000). The Kukus-Mukus eat fresh tubers as a blood purifier 11 (Bhosle *et al.,* 2006). Savara, Bagata and Valmiki tribes of Andhra Pradesh use tuber extracts to cure jaundice and Bagata tribes use this plant for Diabetes 12 (Panal *et al.,* 2012). Rhizome past used to apply to wounds and cuts 13-14 (Sudhakar Reddy *et al.,* 2009, Poyyamoli *et al.,* 2013). The rhizomes extracts of *C. pseudomontana* are also effective against *Mycobacterium tuberculosis* 15 (Kaliwal *et al.,* 2013) and mineral elements analysis of rhizome powder showed, rich source of protein, available carbohydrate, dietary fibre and minerals 16 (Gurusiddesh *et al.,* 2014). This rhizome can be used for the nutritional requirement of human being attributable to the good qualities of nutrient and it may give adequate protection against diseases arising malnutrition. In this study, we aimed to determine the phytochemical analysis and antimicrobial activity of rhizome extracts of *Curcuma pseudomontana* J. Graham this was the first report on phytochemical analysis and antimicrobial activity of *C.pseudomontana* J. Graham.

MATERIALS AND METHODS

Collection of plant material

Fresh and healthy rhizomes of *Curcuma pseudomontana* J. Graham (Zingiberaceae) was collected from the Western Ghats, Karnataka, India, during September 2012. Taxonomist Prof, G.R. Hegde, Department of Botany, Karnataka University Dharwad, India, authenticated the plant. The rhizomes were dried in hot air oven at 45^0C for 72 hours and powdered for further analysis.

Phytochemical analysis

Phytochemical analysis of rhizome powder was done as described in 17 Khandelwal, (2007). About 100 g of dry rhizome powder was defatted using hexane, followed by chloroform, ethyl acetate and methanol at room temperature and at atmospheric pressure, for 48 h with shaking at 100 rpm/min speed. The extracts were filtered and concentrated by using a rotary evaporator (Buchi Rotavapor R-124).

Culture and maintenance of test microorganisms for antimicrobial studies

Bacterial cultures of *Staphylococcus aureus*, *Bacillus subtilis, S. scuir, E.coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* were maintained in the Department of Biotechnology and Microbiology, which were previously isolated and identified from bovine mastitis infected milk from North Karnataka region and were used for the antimicrobial activity. Fungal cultures of *Aspergillus niger* (MTCC 281), *Aspergillus flavus* (MTCC 2456), *Aspergillus fumigatus* (MTCC 870) and *Candida albicans* (MTCC 3018) were used for antifungal activity and which were obtained from Microbial Type Culture Collection, IMTECH, Chandigarh, India. All the bacterial strains were maintained on nutrient agar (NA, Hi-Media) at 37 $^{\circ}$C and fungi were maintained on Sabouraud's Dextrose agar (SDA, Hi-Media) at room temperature. Mueller-Hinton Agar (MHA, Hi-Media) and Sabouraud's Dextrose Agar (SDA) were used for testing.

Antibacterial activity

Antibacterial activity was carried out by agar well-diffusion method 18 (Mukherjee *et al.* 1995). The plating was carried out by transferring bacterial suspension (10^5 CFU ml^{-1}) to sterile petri plate and mixed with molten nutrient agar medium (bacteria) and sabouraud's dextrose agar (fungi), allowed to solidify. Add 5, 10, 25, 50 and 75 mg/ml of the compound into the respective wells on each plate and allowed to diffuse for 2h Then the plates with bacterial culture and fungal cultures except *Aspergillus flavus* were placed in the incubator at 37 $^{\circ}$C for 24 h. The plates with *A. flavus* were kept at room temperature for 48h. The activity was determined by measuring the diameter of the inhibition zone surrounding the well.

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration was determination by 19 Moore and Goodwin (2007). Nine dilutions of each drug have to be done with brain heart infusion broth (BHI) bacteria and sabouraud's dextrose broth for fungi. In the initial tube 20 µl of drug added into the 380 µl of broth. For dilutions 200 µl of broth added into the next 9 tubes separately. Then from the initial tube 200 µl transferred to the first tube containing 200 µl of broth. This considered as a 10^{-1} dilution. From 10^{-1} diluted tube 200 µl was transferred to the second tube to make 10^{-2} dilution. The serial dilution repeated up to 10^{-9} dilution for each drug. From the maintained stock cultures of required organisms (10^5 CFU/ml), Five micro liter was taken and added into 2 ml of brain heart infusion and sabouraud's dextrose broth. In each serially diluted tube 200 µl of above culture suspension was added. A tube containing nutrient and sabouraud's dextrose broth only inoculated with test organisms as described above to serve as a control. The tubes with bacterial culture and fungal cultures except *Aspergillus flavus* were placed in the incubator at 37 $^{\circ}$C for 24 h. The tube with *A. flavus* was kept at room temperature for 48h. After incubation, the tubes were then examined for microbial growth by observing for turbidity.

Minimum microbial concentration (MMC)

Minimum microbial concentration was determination by 20 Linu Mathew *et al.,* (2012). To determine the MMC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile brain heart infusion agar (for bacteria) and sabouraud's dextrose agar(for fungi) by streaking. Brain heart infusion agar and sabouraud's agar, were only streaked with the test organisms respectively to serve as control. Plates inoculated with bacteria were then incubated at 37 $^{\circ}$C for 24 hours while those inoculated with fungi were incubated at room temperature (28 $^{\circ}$C - 32 $^{\circ}$C) for 48 hours. After incubation the concentration at which no visible growth seen which was noted as the minimum bactericidal concentration.

RESULTS

Phytochemical analysis

Phytochemical analyses show the presence of alkaloid, flavonoid, tannin, steroids, saponins, carbohydrate, proteins, and amino acids in different rhizome extracts of *C.pseudomontana* J. Graham (Table 1).

Antimicrobial analysis

The study of antimicrobial activity was carried out using four different rhizome extracts of *C.pseudomontana* J. Graham against gram-positive and gram-negative bacteria and fungi used by agar well diffusion and micro-dilution methods.

Table 1 Preliminary phytochemical screening of rhizome extracts of *Curcuma pseudomontana* J. Graham

Chemical constituent	Chemical test	Hexane extract	Chlorofom extract	Ethyl acetate Extract	Methanol extract
Alkaloid	Mayers test	+	+	+	+
	Hanger's test	+	+	+	+
	Wanger's test	+	+	+	+
Flavonoid	Shinoda test	-	+	+	+
	Lead acetate test	-	+	+	-
	NaoH test	-	+	+	+
Tannin	Ferric chloride test	-	-	+	-
	Dilute nitric acid test	-	-	+	-
Steroids	Libermann-burchard test	-	-	+	-
	Salkowski test	+	-	+	-
Saponins	Foam formation test	+	+	+	-
Glycoside	Keller-killani test	+	-	+	-
Carbohydrate	Molish test	-	+	+	+
	Fehling's test	-	+	+	+
Proteins	Biuret test	-	+	+	+
	Millon's test	-	+	+	+
Amino acids	Ninhydrin test	-	+	+	+

+: present, -: absent

Almost, all rhizome extracts showed varying degrees of antibacterial activity. An antibacterial activity of all extracts depends largely upon the concentration of extract and type of solvent used for the study. The overall antibacterial study ethyl acetate extract exhibited better antibacterial activities than hexane; methanol and chloroform respectively (Tables 2).

The diameter of inhibition zones for ethyl acetate extract ranged from 15.67±0.5mm (*S.aureus*), 16.33±0.85 mm (*P. aeruginosa*) and 12.66±1.20 mm (*E.coli*), which were nearer to the positive antibiotic standard ciprofloxacin (16±0.05 mm, 21±0.01mm and 17±0.04 mm respectively). The inhibition zone of ethyl acetate extract towards *B. subtilis* (27.33±1.20 mm) was more, compared to the inhibitory zone of the positive standard (20±0.01mm) displaying the potent broad-spectrum activity of the extracts. *K. pneumonia* (13.67±0.66) and *S. scuiri* (10.66±0.33) were less susceptible to ethyl acetate extracts but more susceptible to chloroform and hexane extracts respectively. *C.pseudomontana* J. Graham rhizome extract of hexane were more efficient in inhibiting *B. subtilis*, *S.aureus* and *P. aeruginosa* with the inhibition zones of 22.66±0.33 mm, 15.67±0.33 mm and 15 mm, respectively. *B. subtilis* and *P. aeruginosa* were also more efficiently inhibited by methanol with the inhibition zones of 24.67±1.45 mm and 15.33±0.5 mm, respectively. In all the cases of gram+ve bacteria, rhizome extracts was showing the best result. In case of fungi *A.fumigatus* (25.00±1.52 mm), *C.albicans* (23±0.34 mm) and *A.flavas* (18.67±0.89mm) the diameter of inhibition zone for ethyl acetate extract was nearer to the positive antibiotic standard fluconazole (26±0.10mm, 24±0.05 mm and 26±0.06 mm respectively). The inhibition zone of rhizome extract of chloroform towards *C.albicans* (25±0.59mm) was more compared to the inhibitory zone of the positive standard (24±0.05 mm) displaying the potent broad-spectrum activity of the extracts. *A.niger* (10.66±0.33) were less susceptible to ethyl acetate extracts but were more inhibited by hexane extracts (22.67±1.20). The rhizome extract of hexane were more proficiently inhibiting *C.albicans* and *A.fumigatus* with zones of inhibition of 24±0.57mm and 25.33±0.66 mm respectively. Chloroform and methanol were also more efficiently inhibit the *C.albicans* with zone of inhibition of 25±0.59 mm and 22±0.57 mm, respectively.

Table 2 Antibacterial activity of rhizome extracts of Curcuma pseudomontana J. Graham by agar well diffusion method

ORG	EX	Zone of inhibition (Diameter in mm) 5 (mg/ml)	10 (mg/ml)	25 (mg/ml)	50 (mg/ml)	75 (mg/ml)	SA
S.aureus	H			12.33±0.89	13.67±0.89	15.67±0.33	16±0.05
	CH			10±0.57	12.67±1.2	13.67±0.66	
	EA				15.67±0.5	15.67±0.5	
	ME			9.6±0.31	11.32±0.33	15.33±0.5	
B. subtilis	H		14.66±0.33	21.33±0.88	19.00±0.57	22.66±0.33	20±0.01
	CH		8.66±0.66	9.00±1.00	11±1.15	14.67±1.45	
	EA	14.33±0.88	20.33±0.88	23.33±0.88	24.33±1.20	27.33±1.20	
	ME	15.33±1.45	18.33±0.89	18.33±1.20	21.33±0.89	24.67±1.45	
S. scuiri	H			9±0.57	10.66±0.66	12.66±0.33	20±0.07
	CH					10.66±0.33	
	EA					10.66±0.33	
	ME						
E.coli	H				10.34±0.33	12±1.15	17±0.04
	CH				10.66±0.66	11±1.0	
	EA				11.00±1.00	12.66±1.20	
	ME				6.66±3.33	9.33±0.33	
K. pneumonia	H						18±0.06
	CH			10.33±0.33	10.66±0.67	15.33±0.58	
	EA				10±0.00	13.67±0.66	
	ME			10.67±0.33	11.33±0.66	12±0.57	
P. aeruginosa	H				11 ±0.57	15±0.00	21±0.01
	CH			9.67±0.33	10.67±0.33	13 ±0.00	
	EA			8.67±0.33	12.67±0.89	16.33±0.85	
	ME			11.00±0.58	12.67±0.88	15.33±0.5	

ORG- Organisms, Ex- Extracts, H- Hexane, CH- Chloroform, EA- Ethyl acetate, ME- Methanol; SA-Standard Antibiotics Ciprofloxacin (mg/ml). All values are mean ±standard deviation of triplicates

The response for rhizome extracts of *C.pseudomontana* J. Graham was different towards each of the pathogens (Tables 3).

Table 3 Antifungal activity of rhizome extracts of *Curcuma pseudomontana* J. Graham by agar well diffusion method

Organisms	Ex	Zone of inhibition (Diameter in mm)					
		5 (mg/ml)	10 (mg/ml)	25 (mg/ml)	50 (mg/ml)	75 (mg/ml)	SA
C.albicans	H	10±0.00	10±0.58	18±0.89	20.67±0.66	24±0.57	24±0.05
	CH	9.33±0.56	12.67±0.20	13±0.88	14±0.54	25±0.59	
	EA			16±0.57	18±0.23	23±0.34	
	ME	12±0.00	13.33±0.33	14.66±0.33	18±1..52	22±0.57	
A.fumigatus	H	7.50±0.28	8.66±0.66	8.66±4.33	17.33±0.67	25.33±0.66	26±0.10
	CH			8.00±0.00	12.33±0.33	13.66±1.33	
	EA			10.00±0.00	15.65±0.34	25.00±1.52	
	ME				9.00±0.57	9.62±0.78	
A.niger	H					22.67±1.20	26±0.02
	CH				10.00±0.58	13.66±0.88	
	EA				11.67±1.45	14.67±0.33	
	ME				7.50±0.28	9.67±0.33	
A.flavas	H				9.33±0.33	12±0.57	26±0.06
	CH				14.33±0.33	16±0.58	
	EA			10.65±0.33	14±0.58	18.67±0.89	
	ME				14±0.58	15.33±1.45	

Ex- Extract, H- Hexane, CH- Chloroform, EA- Ethyl acetate, ME- Methanol; SA-Stand Antibiotics Fluconazole (mg/ml).
All values are mean ±standard deviation of triplicates

MIC and MMC values rhizome extracts of *C.pseudomontana* J. Graham shown wide range of concentrations ranging from 0.2 – >6.25 µg/ml towards different pathogens. The ethyl acetate extract was excellent in showing very low values of MIC, in the range of 0.2–0.4 µg/ml with an exception of *S. scuiri* and *A.niger* showing a value of 1.6 µg/ml. The MMC values were in the range of 0.4–3.125 µg/ml. The chloroform extract also had significant MIC and MMC values in the range of 0.2–3.125 and 0.8–3.125 µg/ml respectively. The MIC and MMC results of rhizome extracts of *C.pseudomontana* J. Graham (Tables 4 and 5).

Table 4 Minimum inhibitory concentration (MIC) of rhizome extracts of *Curcuma pseudomontana* J. Graham

Organisms	MIC (µg/ml)			
	Hexane	Chloroform	Ethyl acetate	Methanol
S.aureus	0.2	0.2	0.2	0.2
B. subtilis	0.2	1.6	0.2	0.2
S. scuiri	1.6	1.6	1.6	> 6.25
E.coli	0.8	1.6	0.4	3.125
K. pneumonia	> 6.25	0.4	0.2	1.6
P. aeruginosa	1.6	3.125	0.4	1.6
C.albicans	0.2	0.2	0.2	0.2
A.fumigatus	0.2	1.6	0.2	3.125
A.niger	0.8	1.6	1.6	3.125
A.flavas	3.125	1.6	0.4	0.8

Table 5 Minimum microbicidal concentration (MMC) of rhizome of *Curcuma pseudomontana* J. Graham

Organisms	MMC(µg/ml)			
	Hexane	Chloroform	Ethyl acetate	Methanol
S.aureus	0.8	0.8	0.4	0.8
B. subtilis	0.8	3.125	0.8	1.6
S. scuiri	1.6	3.125	3.125	> 6.25
E.coli	3.125	3.125	0.8	6.25
K. pneumonia	> 6.25	1.6	1.6	3.125
P. aeruginosa	1.6	3.125	0.8	1.6
C.albicans	1.6	1.6	0.8	1.6
A.fumigatus	1.6	3.125	1.6	6.25
A.niger	3.125	3.25	3.125	> 6.25
A.flavas	6.25	6.25	0.8	1.6

DISCUSSION

Most traditional medicinal plants in use today have no scientific data on their bioactivity and levels of safety or even how they are likely to affect each other when used as combinations in medicines. Furthermore research has to be done on their mechanisms of action considering that most are orally consumed. In the phytochemical analysis showed the presence of various phytochemical compounds in the rhizome extracts which are known to have various therapeutic importances. The results of phytochemical analysis revealed the presence of alkaloid, flavonoid, tannin, steroids, saponins, glycoside carbohydrate, and proteins, amino acids in the rhizome extracts of *C.pseudomontana.* The rhizome extracts are good sources of different classes of bioactive compounds but ethyl acetate extract showed the presence of all the compounds. All the rhizome extracts contain alkaloid, which is reported possess the antimalarial and antimicrobial activities. Flavonoids were detected in chloroform, ethyl acetate and methanol extracts of rhizome of *C.pseudomontana*. Flavonoids belong to the group of polyphenolic compounds and are typically known for health promoting properties such as antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties 21 (Aiyelaagbe and Osamudiamen, 2009). They commonly observed in the plants and reported that increased consumption of flavonoids will reduced the risk of cardiovascular and cancer diseases 22 (Yang *et al.,* 2001). Moreover, tannins may have the potential values as cytotoxic agent, which is present in the ethyl acetate extract 23 (Aguinaldo *et al.,* 2005). Saponins good sources of antibacterial agents 24 (Mandal *et al.,* 2005), it is observed in the hexane, chloroform, and ethyl acetate rhizome extracts of *C.pseudomontana*.

Due to the development of resistant strains of microorganism is one of the serious issues for developing country like India. Mastitis, the most important deadly disease of dairy animals is responsible for heavy economic losses due to reduced milk

yield 25 (Bhikane *et al.,* 2000). Herbs are known for their medicinal properties and have been used in traditional medicines from time immemorial 26 (Oussallah *et al.,* 2006). The main benefit of natural agents is that the crude extracts include a mixture of compounds like alkaloids, flavonoid, phenols, saponins etc., for which it is difficult to develop resistance by bacteria unlike the synthetic antibiotics that contain a single compound.

Agar diffusion techniques have been widely used to assay for antimicrobial activity of plant extracts 27 (Das *et al.,* 2010). In the present study, the extracts obtained using non-polar and polar solvents. The rhizome extracts of *C. pseudomontana* were tested against bovine mastitis infecting bacteria and food pathogenic fungi. The results indicated that zone of inhibition increased with increasing concentration of extracts, which may be due to the increase in the content of the bioactive compounds of the plant. Similar findings were reported in the rhizome extract of *C. mangga* plant *28* (Koshy *et al.,* 2009). For minimum inhibitor concentration broth micro-dilution method was commonly used. According to Aligiannis *et al.,* 29 (2001) have reported a classification of plant materials based on MIC results (strong inhibitors: MIC up to 500 µg/ml; moderate inhibitors: MIC between 600 and 1500 µg; weak inhibitors: MIC above 1600 µg). Based on Aligiannis *et al., 29* (2001) report, in the present study rhizome extracts of *curcuma pseudomontana* were showed potent antimicrobial activity. The MMC was determined by sub-culturing the test dilution (used in MIC) on to a fresh solid medium and incubated further for 24 h. The concentration of plant extracts that completely killed the bacteria and fungi were taken as MMC. It was noted that most of the antimicrobial properties in different plant part extractions showed, generally MMC value that is almost two fold higher than their corresponding MIC 30 (Omar *et al.,* 2010), the similar results were observed in the present study.

The overall results of the antimicrobial activity showed that rhizome extracts of *C. pseudomontana* showed varying degrees of activity against most of the gram-positive, gram-negative bacteria and fungal strains. Similarly, results were reported in many species of Zingiberaceae family, such as *C. zedoaria, C. longa, C. aromatic*, *C. malabarica, C. amada*, *C.grandis* and *C.neilgherrensis* 31 - 38 (Yoshioka *et al.,* 1998; Negi *et al.,* 1999; Mujumdar *et al.,* 2000; Wuthi *et al.,* 2000; Wilson *et al.,* 2005, Adongo *et al.,* 2012, Mithra *et al.,* 2012, *Chaithra et al.,* 2013, Harit *et al.,* 2013 and Sharmin *et al.,* 2013).

The antimicrobial activity of the rhizome extracts of *C. pseudomontana* explained by disturbance of the permeability barrier of the bacterial cell membrane 39-40 (Cowan 1999; Kumaraswamy *et al.,* 2002). In general, the cell walls of Gram-negative bacteria, which are more complex than Gram-positive ones and act as a diffusion barrier, which makes them less susceptible to the antimicrobial agents than the gram-positive bacteria. In spite of this permeability difference, however, rhizome extracts of *C. pseudomontana* have still exerted some degrees of inhibition against gram-negative bacteria as well. However, the present study reveals that all the four rhizome extracts showed antimicrobial activity but ethyl acetate extract showed very good activity when compared to other rhizome extracts.

The antimicrobial effect of the rhizome extracts of *C. pseudomontana* may be due to the presence of different phytochemicals such as alkaloid, flavonoid, tannin, steroids, saponins and glycoside in different organic solvents extracts. Indeed, members of these phytochemical groups of compounds are known to possess antimicrobial activities 39 (Cowan, 1999). Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other herbivores 41 (Bonjar *et al.,* 2004). These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and an effective antimicrobial substance against many microorganisms. Their activity is probably due to their ability to act on extracellular and soluble proteins and to complex with bacterial cell walls 42(Marjorie *et al.,* 1999). Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes 43 (Raquel *et al.,* 2007). Alkaloids are present in all the rhizome extract, which are mainly, inhibit the *S. aureus* and the mechanism of action of highly aromatic planar quaternary alkaloids was attributed to their ability to intercalate with DNA 44 (Kumar *et al.,* 2007). Sensitivity of bacteria to saponins has often been reported 45 (Avato *et al.,* 2006), although comparative studies between fungi and bacteria showed that bacteria are less sensitive in general. The mechanisms underlying saponins antibacterial activities are still unclear. Some studies report that hemolytic activity is the result of saponins effects on cell membrane permeability by either forming pores in membranes, altering sodium-potassium, and calcium–magnesium ATPase activity, ability to cause leakage of proteins and certain enzymes from the cell 46- 48 (Menin *et al.,* 2001; Plock *et al.,* 2001; Choi *et al.,* 2001). Tannins bind to proline rich proteins and interfere with the protein synthesis 49 (Shimada *et al.,* 2006).

CONCLUSION

The findings of the present investigation reflect the presence of potential phytochemicals from rhizome of *Curcuma pseudomontana* J. Graham, a natural source in developing novel antimicrobial bioactive compound against bovine mastitis causing bacteria and also fungal species. Ethyl acetate extract showed promising results against all tested microorganisms because of the presence of various phytochemicals. Further studies are going on to evaluate the therapeutic value of the plant.

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Competing interests

The authors declare that they have no competing interests.

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