Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E: ISSN 2278-4136
P: ISSN 2349-8234
JPP 2014; 3 (3): 50-53
Received: 06-08-2014
Accepted: 19-08-2014

Anjusha S
Department of Botany, University of Kerala, Thiruvananthapuram, Kerala, 695581, India

Gangaprasad A
Department of Botany, University of Kerala, Thiruvananthapuram, Kerala, 695581, India

Correspondence:
Gangaprasad A
Department of Botany, University of Kerala, Thiruvananthapuram, Kerala, 695581, India

Phytochemical and Antibacterial Analysis of Two Important *Curcuma* species, *Curcuma aromatica* Salisb. and *Curcuma xanthorrhiza* Roxb. (Zingiberaceae)

Anjusha S. and Gangaprasad A.

Abstract
Since ancient times, people have been exploring the nature, particularly plants in search of new drugs. This has resulted in the use of a large number of medicinal plants with curative properties to treat various diseases. Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent step for screening of plants for secondary metabolites. The present study compares two plant species *Curcuma aromatica* and *Curcuma xanthorrhiza* coming under the family Zingiberaceae, two medicinally and industrially important plant species. Phytochemical analysis reveals the presence of major phytochemicals like flavonoids, tannin, saponin, Terpenoids in both the species. A high amount of curcumin was found in *C. xanthorrhiza* (1.0863 g/100 g) as compared to *C. aromatica*. Antibacterial activity of the aqueous rhizome extract of the two species was studied. The highest percentage of zone of inhibition (89%) was recorded against *Pseudomonas aeruginosa* in *Curcuma xanthorrhiza.*

Keywords: *Curcumin*, *Curcuma aromatica*, *Curcuma xanthorrhiza*, Phytochemisty, antibacterial study

1. Introduction
Man since time immemorial has been using plants or natural product as medicine to promote and maintain good health (Parrota, 2001) [11]. Almost all cultures from ancient times to today have used plants as medicine. Today, medicinal plants are important to the global economy. Many plants are major sources of useful secondary metabolites which are used in pharmaceutical, agrochemical, flavour and aroma industries. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. Laboratories of the world have found literally thousands of phytochemicals showing inhibitory effects on all types of microorganisms *in vitro* (Cowan, 1999) [5].
Curcuma aromatica and *C. xanthorrhiza* are two medicinally important species belonging to the family Zingiberaceae. Curcumin is an important phytochemical seen by the members of the genus *Curcuma*. Curcumin is highly medicinal and industrially important secondary metabolite.
Curcuma aromatica is distributed throughout India widely used as a flavouring agent, tonic, carminative and used against snakebite (Chopra *et al.*, 1941) [4]. It has a creamy white rhizome and leaves are pubescent below. *C. xanthorrhiza* is a native of North East India and is widely cultivated in many parts of India, Srilanka and China. It has a deep yellow rhizome and green leaves with brownish purple veins. It is used as anti venom for Indian cobra, used as tonic, to treat digestive problems. Both the species are well listed drug in Ayurveda and other indigenous systems of medicine. In some regions of India *C. xanthorrhiza* is used under the tag of *C. aromatica* due to the non availability of *C. aromatica*.

2. Materials and methods
2.1 Collection of plant materials
Fresh rhizome of *C. aromatica* and *C. xanthorrhiza* were collected from the plants maintained in the greenhouse, Department of Botany, University of Kerala, Thiruvananthapuram, Kerala. The plant was properly identified with the help of authentic literature and documented with their characteristic features. The rhizomes were collected, washed well to remove all the dirt and were shade dried separately until all the water molecules evaporated and rhizome become well dried for grinding. After drying, the rhizome

was ground well using a mixer grinder into fine powder and transferred into airtight containers with proper labelling for future use.

2.2 Preparation of plant extract

The crude plant extract was prepared by Soxhlet extraction method. About 5gm of powdered rhizomes was uniformly packed into a thimble and extracted with 150 ml of methanol and distilled water separately. The process of extraction continues for 24 hours or the solvent in the siphon tube of an extractor become colourless. After that, the extract was taken in a rotary evaporator and the extract and solvent were separated. Then the extract was taken in a beaker and kept in a hot air oven at 40-50 ^{0}C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4 ^{0}C for their future use in phytochemical analysis and antibacterial studies.

2.3 Phytochemical analysis

2.3.1 Preliminary phytochemical screening

Preliminary phytochemical screening of methanolic and distilled water extract of rhizome of *C. aromatica* and *C. xanthorrhiza* were done by using standard procedures (Harbone, 1998; Edeoga *et al*, 2005) [9, 7] for detecting the presence of flavonoid, tannin, alkaloid, phenol, terpenoid, saponin, quinone, sterols, protein and carbohydrate in the rhizome.

2.3.2 Fluorescence analysis

Rhizomes of *C. aromatica* and *C. xanthorrhiza* were shade dried and powdered. Then the powders were treated with various polar solvents like, ethyl acetate, acetonitrile, dimethyl sulfoxide, water and nonpolar solvents like, hexane, cyclohexane, toluene, chloroform and then fluorescence was observed in long UV (365nm), short UV (254 nm) and visible light.

2.3.3 Quantitative analysis of Curcumin

Dissolve 0.2-0.5 g of weighing, moisture free dried rhizome powder of both the species in a 250 ml of absolute ethanol separately. Reflux the contents in the flask fitted with an air condenser over a heating mantle for 3-5 hour; compensate alcohol loss if any due to evaporation by adding alcohol freshly into the flask. Cool and decant the extract into a volumetric flask and make up the volume (250 ml). Dilute a suitable aliquot (1 – 2 ml) to 10 ml with absolute alcohol. Measure the intensity of yellow colour at 425 nm in a spectrophotometer (Shimadzu, Japan) then work out the Curcumin content using the following formula (Sadasivam *et al.*, 2008) [12].

Curcumin content g /100 g =

$$\frac{0.0025 \times A425 \times \text{volume made up} \times \text{dilution factor} \times 100}{0.42 \times \text{weight of the sample (g)} \times 1000}$$

Since 0.42 absorbance at 425 nm = 0.0025 g Curcumin

2.3.4 Determination of antibacterial activity

Pathogenic bacterial strains, four gram negative, were used for the present study. The organisms were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. The strains were maintained as broth culture on nutrient broth. They were grown and kept in refrigerator. The strains used were *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Salmonella typhi*.

Qualitative analysis of both the species reveals that aqueous extract shows maximum number of phytochemicals when compared to methanolic extract so antibacterial analysis were conducted in aqueous extract. Antibacterial activity of the aqueous extract of the rhizome of *C. aromatica* and *C. xanthorrhiza* were compared with the standard Ampicillin by measuring the zone of inhibition diameter. The disc diffusion method was used for screening of antibacterial activity (Benson, 1990) [3]. This method extensively used to investigate the antibacterial activity of natural antibacterial substances and plant extracts. These assays are based on the use of discs are reservoirs containing the solution of substances to be examined.

3. Results and discussion

3.1 Phytochemical analysis

Phytochemical analysis was carried out to check whether there is any pharmaceutically active compound present in the plant. Phytochemical analysis of methanolic and aqueous extracts of the rhizome of *C. aromatica* and *C. xanthorrhiza* were carried out. Major phytochemicals like flavonoids, tannin, Saponin, sterols, terpenoid were detected in both the plant species. The results were listed in Table.1. Phytochemical constituents of Curcuma species were reported by various authors (Sharif *et al*., 2011; Ahmad *et al*., 2011; Deb *et al*., 2013) [14, 1, 6]. Tannins, phenolics, alkaloids and flavonoids have been suggested to be involved in antibacterial activities (Enzo, 2007) [8].

Table 1: Qualitative analysis of the phytochemicals of *C. aromatica* and *C. xanthorrhiza*

	C. aromatica		***C. xanthorrhiza***	
Compounds	**Distilled water**	**Methanol**	**Distilled water**	**Methanol**
Flavonoid	+	-	+	+
Tannin	+	-	+	-
Saponin	+	-	+	-
Carbohydrate	+	+	+	+
Reducing sugar	-	-	-	-
Quinine	-	-	-	-
Terpenoids	+	+	+	+
Sterols	+	+	+	+
Protein	+	+	+	+
Phenols	+	+	+	+

+ = indicates the presence of constituents, - = indicates the absence of constituents

3.2 Fluorescence analysis

Crude drugs show their own characteristic fluorescence when exposed to ultraviolet radiation and is dependent on its chemical constituents. Fluorescence analysis of the drug powder with different solvents is an important pharmacognostic tool in checking adulterants. Fluorescence analysis of rhizome powder of *C. aromatica* and *C. xanthorrhiza* were done in nonpolar solvents like benzene, cyclo benzene, tolune, chloroform and polar solvents like ethyl acetate, acetonitrile, DMSO and distilled water. The colours were identified using a colour chart. The results were listed in table: 2a and 2b.

Table 2a: Fluorescence analysis: Non polar solvents

Powder + Solvents	Plant	Visible light	Short UV	Long UV
Powder alone	*C.a*	Pea green	Pea green	Pancy purple
	C.x	Saffron yellow	Saffron yellow	Pancy purple
Powder + hexane	*C.a*	Veruoese green	Pod green	Barium yellow
	C.x	Sage green	Sulphur yellow	Amber yellow
Powder + cyclo hexane	*C.a*	No colour	No colour	No colour
	C.x	Canary yellow	Sulphur yellow	No colour
Powder + toluene	*C.a*	Aureolin	Veruoese green	Sea blue
	C.x	Dresden yellow	Chartreuqe green	Sage green
Powder + chloroform	*C.a*	Sulphur yellow	Carnation green	Ivy green
	C.x	Barium yellow	Pod green	Amber yellow

C.a- *Curcuma aromatica*, C.x- *Curcuma xanthorrhiza*

Table 2b: Fluorescence analysis: Polar solvents

Powder + Solvents	Plant	Visible light	Short UV	Long UV
Powder + ethyl acetate	*C.a*	Sap green	Veronese green	Cobalt blue
	C.x	Dresden yellow	Aureolin	Lemon yellow
Powder + aceto nitriyl	*C.a*	Sulphur yellow	Pea green	Methyl violet
	C.x	Canary yellow	Sap green	Orange
Powder + DMSO	*C.a*	Aureolin	Pea green	Sea lavender violet
	C.x	Lemon yellow	Agathia green	
Powder + D.water	*C.a*	Sulphur yellow	Agathia green	Butterfly blue
	C.x	Aureolin	Cyprus green	Princess blue

Table 3: Antibacterial activity of *C. aromatica* and *C. xanthorrhiza*

Name of bacteria	Plant	Concentration (µg/ml)	% of inhibition
P. aeruginosa	*C. aromatica*	100	79
		50	70
		25	65
	C. xanthorrhiza	100	89
		50	76
		25	67
E. coli	*C. aromatica*	100	72
		50	61
		25	63
	C. xanthorrhiza	100	61
		50	59
		25	50
P. vulgaris	*C. aromatica*	100	50
		50	44
		25	41
	C. xanthorrhiza	100	59
		50	46
		25	43
S. typhi	*C. aromatica*	100	80
		50	60
		25	46
	C. xanthorrhiza	100	83
		50	72
		25	66

3.3 Quantitative analysis of Curcumin

Curcumin is the major compound present in the rhizome of the both the species. The Curcumin content present in *C. aromatica* and *C. xanthorrhiza* was measured quantitatively. A high amount of Curcumin was found in *C. xanthorrhiza* (1.0863 g/100 g) when compared to *C. aromatica* (0.0175 g/100 g). Curcumin used as a natural dye in food industries, cosmetic and in pharmaceutical industries. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities (Anand *et al*., 2008) [2].

3.4 Antibacterial activity

C. aromatica and *C. xanthorrhiza* showing a high degree of antibacterial activity against the four gram negative bacteria tested. The selection of bacteria was based on the fact that majority of the bacteria pathogenic to human being are gram negative in nature. The highest percentage of zone of inhibition (89%) was recorded against *P. aeruginosa* by *C. xanthorrhiza* rhizome aqueous extract. The results were listed in table.3. *In vitro* antibacterial efficacies of various species of Curcuma were reported by various authors (Kim *et al*., 2005; Wilson *et al*., 2005; Ahmad *et al*., 2011) [10, 15, 1]. Medicinal plants are now being used as model for antimicrobial agents and it is because that plant based drugs cause less or no side effects when compared with synthetic antibiotics (Satish *et al*., 2008) [13].

4. Conclusions

Medicinal plants constitute an effective source of traditional and modern medicine. Phytochemical screening provides knowledge of the chemical constituents of plants not only for the discovery of new therapeutic agents, but also for information in discovering new sources of other economic materials. Phytochemical analysis reveals the presence of major phytochemicals like flavonoids, tannin, saponin, curcumin etc. Both the plant species exhibited *in vitro* antibacterial activity against four bacteria tested. Some of these bacteria are associated with conditions like infection of skin and urinary tract. The phytochemical analysis and antibacterial studies of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies in the manufacturing of the new drugs for treatment of various diseases.

5. Acknowledgement

The authors are thankful to the Head, Department of Botany for encouragement and facility provided.

6. References

1. Ahmad S, Ali M, Ansari S. H, Ahmed F. Chemical constituents from the rhizomes of *Curcuma aromatica* Salisb. Der Pharma Chemica, 2011; 3(2):505-511.
2. Anand P, Thomas SG, Kunnumakkara AB, Sundaram C, Harikumar KB, Sung B. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochem Pharmacol 2008; 76(11):1590-1611.
3. Benson H. J. Microbiological applications. Wm. C. Brown Publishers, USA, 1990, 134.
4. Chopra RN, Gupta JC, hopra GS. Pharmocological action of the essential oil of *Curcuma longa*. Indian J Med Res 1941; 29:769-772.
5. Cowan MM. Plant products of antimicrobial agents. Clin. Microbial Rev 1999; 12(1):564-582.
6. Deb N, Majumdar P, Ghosh AK. Pharmacognostic and Phytochemical Evaluation of the Rhizomes of *Curcuma longa* Linn. Journal of Pharma Sci Tech, 2013; 2(2):81-86.
7. Edeoga HO, Okwu DE, Mabaebi. Phytochemical constituents of some Nigerian Medicinal Plants. African J of Biotechnology 2005; 4:685-688.
8. Enzo AP. Traditional plants and herbal remedies used in the treatment of diarrheal disease: Mode of action, quality, efficacy and safety considerations. In: Ahmad I, Aqil F, Owais M, editors. Modern Phytomedicine: Turning Medicinal Plants in to Drugs. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2007; 248-260.
9. Harborne JB. Phytochemical method – A guide to modern techniques of plant analysis. Chapman and Hall, London, 1998, 108-148.
10. Kim KJ, Yu HH, Cha JD, Seo SJ, Choi NY, You YO. Antibacterial activity of *Curcuma longa* L. against methicillin-resistant *Staphylococcus aureus*. Phytother res 2005; 19(7):599-604.
11. Parrotta JA. Healing plants of Peninsular India, CABI publishing, Walling ford, UK, 2001, 438-442.
12. Sadasivam S, Manickam. Biochemical Methods, Third edition, New Age International Publishers, New Delhi, 2008, 202.
13. Satish S, Raghavendra MP, Raveesha KA. Evaluation of the antibacterial potential of some plants against human pathogenic bacteria. Adv Bio Res 2008; 2(3-4):44-48.
14. Sharif M. Al-Reza, Rahman A, Parvin T, Rahman M. M, Rahman MS. Chemical composition and antibacterial activities of essential oil and organic extracts of *Curcuma aromatica* SALISB. J food safety 2011; 31(4):433-438.
15. Wilson B, Abraham G, Manju VS, Mathew M, Vimala B, Sundaresan S, Nambisan, B. Antimicrobial activity of *Curcuma zedoaria* and *Curcuma malabarica* tubers. J Ethnopharmacol 2005; 99(1):147-151.