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Phytochemical investigations and antibacterial activity of *Curcuma pseudomontana* J. Grahm (Zingiberaceae)

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Abstract
The extracts of rhizome, root and leaf of *Curcuma pseudomontana* J. Grahm was tested against human pathogenic bacteria viz. *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa* and *Shigella flexneri* by 96 well-plate methods. Extract was screened for the detection of different phytochemical constituents. The rhizome, root and leaf extracts showed significant antibacterial activity at different concentrations against tested organism. The MIC (minimum inhibition concentration) of rhizome and roots are different for bacteria whereas leaf showed MIC at 2µl concentration for all tested bacteria. Phytochemical screening of extracts indicates presence of amino acid, alkaloid, glycoside, steroid, flavonoid, tannin, saponin, phenol, terpenoids and phytosterol. Screening of *C. pseudomontana* extracts for antibacterial activity and for the detection tests of various secondary metabolites forms primary platform for further pharmacological and phytochemical studies.

Keywords: *C. pseudomontana,* antibacterial, phytochemical, 96 well plate

1. Introduction
Plants have been an important source of medicine for thousands of years and are continue to play vital role in the Indian traditional medicine system, the origin effect of the drug is given in our traditional text along with their formulations, keeping this view studies are undertaken for testing of folk medicinal plants for pharmacological prospects (Negi, *et al*., 2013) [6]. Use of plants or plant parts is increasing in many segment of the population, in present hundreds of plant metabolites are being frequently used in the treatment of different kinds of diseases. Near about 80% of the world's population relied upon plants for their medication whereas the use of the medicinal plants and plant products is increasing in many countries, where 35% of drugs contain from natural products (Deb Nilanjana *et al.,* 2013) [2].
Curcuma pseudomontana J. Grahm belongs to family Zingiberaceae which is distributed mainly in tropics of Asia from India to South China, Southeast Asia, Papua New Guinea and Northern Australia. *C. pseudomontana* is endemic to the Western and Eastern Ghats, of peninsular India, the species found in Karnataka, Maharashtra and Andhra Pradesh (Gurusiddesh, *et al.,* 2014) [3]. Dried rhizomes of *C. pseudomontana* J. Grahm., used in skin diseases and impurities of blood. Rhizomes boiled in oil and used as an application to sprain**,** and also useful on snake bite (Nadkarni, 1982) [4]. Rhizome powder are useful in leucoderma, scabies, small pox and intestinal worms as well juice as strong remedy against rheumatism and in combination of ginger used for smooth delivery in North East India (Vedprakash and Mehrotra, 1987) [12]**.** Boiled tubers along with a pinch of salt in oral administration increase the secretion of milk among new mothers and lactating woman in Andhra Pradesh (Ramarao *et al.,* 2000) [8]. The Bagata and Valmiki tribes of Munchingiputtu Mandal, Visakhapatnam district, Andhra Pradesh use *C. pseudomontana* rhizome in the treatment of jaundice and diabetes (Padal *et al.,* 2012) [7].
Vernacular names of the species is Hill turmeric (English), Kachura (Hindi), Raan halad, Shindalavana (Marathi), Kattu manjal (Tamil), Kattu manjal (Malayalam). Herbs, root stock small having ovoid or sub-globose tubers, yellow in center and white periphery. Rhizome is small, conical or cylindrical, 3-6×1-2 cm, pleasantly aromatic, yellowish at center and white periphery. Leafy shoot 70-110 cm tall; leaves 4-6, oblong lanciolate, acuminate, tapering to base; petiole longer than lamina; lamina 22-40×7.5-14 cm. Inflorescence lateral or central up to 15-20 ×3.5-4.5cm; spike 8-10 cm with distinct coma; coma bracts 4-5, elliptic, tip

rounded, bright pink, purple, white or other shades of color; fertile bracts 10-16, bracteole pink, 4-6 mm long; flower longer than bracts, bright yellow; calyx pale yellow, 3-lobed at tip; corolla tube funnel shaped, 3.5-4cm long with oblong acute lobes; labellum 3 lobed, bright yellow, deflexed; anther thecae parallel, 4 mm long, spurred, pointed forward; stigma slightly exerted to anther lobes; style long, filiform; ovary tricarpellary, cylindrical, ellipsoid, 4-5mm long; ovules many; capsule ellipsoid- ovoid; seeds many, globose, pale brown, arilate.

2. Material and Methods

The specimen of *C. pseudomontana* J. Grahm., was collected from Mhaismal, Aurangabad district of Maharashtra state, (GPS readings, latitude 20° 5'32.56"N, longitude 75°10'48.92"E, altitude 2941 ft.) during rainy season and identified with the help of authentic floristic literature (Naik, 1998, Sharma *et al.*, 1996, Sabu, 2006) [5, 11, 9]. Herbarium specimen is deposited in VH Herbarium, department of Botany, Vivekanand Arts, Sardar Dalipsingh Commerce and Science College, Aurangabad.

2.1 Preparation of crude extracts

Dried powder of rhizome, root and leaf sample was extracted in Soxhlet extractor apparatus at 65^0 C for 18-24 hours, methanol used as solvent and prepared sample was stored in amber colour bottle for phytochemical screening and antibacterial activity.

2.2 Test microorganisms

Authentic human pathogenic bacteria culture viz. *E. coli* (NCIM-2931), *Pseudomonas aeruginosa* (NCIM-5029), *Shigella flexneri* (NCIM-5265), *Salmonella typhi* (NCIM-2501) and *Staphylococcus aureus* (NCIM-5021) were obtained from department of Microbiology, Vivekanand Arts, Sardar Dalipsingh Commerce & Science College, Aurangabad, Maharashtra.

2.3 Antibacterial Assay

2.3.1 96-well plates method

The antibacterial assay was carried out by micro dilution method of human pathogenic bacteria in order to determine the antibacterial activity of rhizome, root and leaf extract. About 100µl sterile Mueller-Hinton broths was added onto each well, followed by 2µl serial diluted bacterial suspension, different concentrations as 2, 4, 6, 8 and 10µl of rhizome, root and leaf extract was loaded into each well. Control was prepared by nutrient broth and bacterial suspension without adding plant extract. The prepared 96-well plate was sealed with parafilm and incubated at 37 °C for 24 hours in incubator, optical density was measured on the spectrophotometer at 540 nm (Getachew, *et al.*, 2015) [1].

2.4 Phytochemical Study

The different phytochemical tests were carried out for the detection of secondary metabolites by adopting the methods of (Sahu and Saxsena, 2012) [10].

3. Result and Discussion

The methanol rhizome extract of *Curcuma pseudomontana* J. Grahm in various concentrations were tested against human pathogenic bacteria, it was cleared from the results, that maximum inhibition and MIC (minimum inhibitory concentration) for *P. aeruginosa* (2 µl), *S. typhi* (8µl), *S. arueus* (6µl) whereas for *S. flexneri* and *E. coli* (10µl) respectively (table 1). The various concentrations of root extract showed the maximum inhibition and MIC (minimum inhibitory concentration) for *P. aeruginosa* (4µl), *S. typhi* (2µl), *S. arueus* (4, 8µl) whereas for *S. flexneri* (4µl) and *E. coli* (2, 10µl) respectively (table 2). Whereas leaf extract showed the maximum inhibition and MIC (minimum inhibitory concentration) for *P. aeruginosa*, *S. arueus* and *S. flexneri* (2µl) and that of for *E. coli and S. typhi* (2,4µl) respectively (table 3).

Phytochemical screening of rhizome, root and leaf extract of *C. pseudomontana* J. Grahm., it is cleared from the (table 4), that extract showed the presence of amino acid, steroid, glycoside, flavonoids, alkaloid, tannin, saponins, phenol, terpenoids and phytosterol in more or less. Rhizome extract showed the absence of amino acid, alkaloid and terpenoids whereas test for root extract are negative for glycoside, flavonoids and saponins respectively. The leaf extract have the absence of flavonoids, saponins, phenol and phytosterol.

Table 1: Antibacterial activity of rhizome extract against human pathogens

Sr. No	Rhizome extract (conc. µl)	Pathogenic bacteria (2µl)				
		P. aurignosa	***S. typhi***	***S. arueus***	***E. coli***	***S. flexneri***
1	2 µl	0.82	1.05	1.00	1.00	1.00
2	4 µl	1.09	1.00	1.05	0.82	0.88
3	6 µl	0.85	0.96	0.58	0.68	0.80
4	8 µl	0.82	0.69	1.00	0.88	0.88
5	10 µl	0.82	0.85	0.74	0.65	0.60
	MIC	2 µl	8 µl	6 µl	10 µl	10 µl
	S. E.	± 0.052	± 0.064	± 0.091	± 0.064	± 0.066

Table 2: Antibacterial activity of Root extract against human pathogens

Sr. No	Root extract (conc. µl)	Pathogenic Bacteria (2µl)				
		P. aurignosa	***S. typhi***	***S. arueus***	***E. coli***	***S. flexneri***
1	2 µl	0.22	0.12	0.24	0.18	0.18
2	4 µl	0.21	0.18	0.18	0.16	0.21
3	6 µl	0.26	0.28	0.19	0.22	0.22
4	8 µl	0.22	0.19	0.18	0.20	0.29
5	10 µl	0.24	0.14	0.24	0.21	0.18
	MIC	4 µl	2 µl	4, 8 µl	4 µl	2, 10 µl
	SE	± 0.089	± 0.027	± 0.014	± 0.010	± 0.020

Table 3: Antibacterial activity of leaves extract against human pathogens

Sr. No	Leaves extract (conc. µl)	Pathogenic Bacteria (2µl)				
		P. aurignosa	***S. typhi***	***S. arueus***	***E. coli***	***S. flexneri***
1	2 µl	0.19	0.18	0.14	0.16	0.20
2	4 µl	0.21	0.18	0.18	0.21	0.20
3	6 µl	0.23	0.21	0.22	0.20	0.22
4	8 µl	0.22	0.22	0.24	0.24	0.24
5	10 µl	0.23	0.21	0.26	0.25	0.27
	MIC	**2** µl	**2,4** µl	**2** µl	**2** µl	**2**, 4 µl
	SE	± 0.007	± 0.009	± 0.021	± 0.015	± 0.013

Table 4: Phytochemical analysis of *Curcuma pseudomontana* J. Grahm

Sr. no.	Phytochemical test	Rhizome	Leaves	Root
1	Amino Acid	--	++	++
2	Steroid	++	++	++
3	Glycoside	--	--	++
4	Flavonoid	++	--	--
5	Alkaloid	++	++	++
6	Tannin	++	++	++
7	Saponin	++	--	--
8	Phenol	++	++	--
9	Terpenoids	--	++	++
10	Phytosterol	++	++	--
++, Presence, --, Absence				

***Curcuma Pseudomontana* J. Grahm**

Rhizome

4. Conclusion

The different parts of *Curcuma pseudomontana* J. Grahm viz. rhizome, root and leaf has potential antibacterial properties as it showed significant activity against tested human pathogenic bacteria and it may be due to the presence of various secondary metabolites. Further investigation needs to isolates the individual chemical component for the discovery of new herbal bioactive compounds which may leads to found novel drugs.

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