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Physicochemical and Antibacterial Activity of Rhizomes of *Curcuma angustifolia* Roxb, (Zingiberaceae)

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Abstract: The antimicrobial properties of methanolic extract of Curcuma angustifolia Roxb. rhizomes were assayed in vitro against human pathogenic bacteria represented by Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Escherichia coli and Shigella flexneri by 96 well-plate method. The extracts studied in the present investigation exhibited varying degree of inhibitory effect against all tested bacteria. The result shows MIC at 2 µl for P. aeruginosa, S. typhi, S. arueus and S. flexneri whereas it was 8 µl for E. coli. The antibacterial activity may due to variety of secondary metabolites present in the plant, such as glycoside, flavonoid, tannin and phytosterol, which have been found in vitro to have antimicrobial properties. Physicochemical analysis of the rhizome powder showed 2.50 % total ash, 0.50 % acid insoluble ash, 2.0 % acid soluble ash, 0.50 % water insoluble ash, water soluble ash 2.0 % and 19.04% loss on drying. Rhizomes are rich source of protein, carbohydrate and minerals. This is the first report on the pharmacognostic studies of C. angustifolia Roxb. rhizome. The results of pharmacognostic analysis were helpful in developing standards for purity, quality and identification of the crude drugs.

Keywords: Curcuma angustifolia, rhizome, physicochemical, antibacterial activity

1. Introduction

The natural plant materials have served as an important source of pharmaceutical products since time immemorial; the modern medicines for human use are required to meet exacting standards that relate to their efficacy, quality and safety [1]. Some of antibiotics have failed to discourage the growth of many bacteria that have genetic ability to resist to particular drugs, and these antibiotics may have the various side effects on human body which can harm to vital organs of body like kidney, liver, pancreas and their impact on the immune system [2]. Plants are rich source of natural products used to cure various kinds of diseases, as it have a wide variety of secondary metabolites, such as alkaloids, flavonoid, phenols, tannins, terpenoids, phytosterol and steroids. As per World Health Organization (WHO) report, approximately 80% of the global population relies on traditional herbal medicines as part of standard healthcare system [3].

Common names of *C. angustifolia* in English: 'narrow-leaved turmeric; Manipuri: yaipan; Hindi: tikhur; Malayalam Koova. The rhizomes of *C. angustifolia* used as ethnic food and for its medicinal values ^[4,5]. It has potential antioxidant, antimicrobial and anti-cancerous activity towards human cervical cancer HeLa cells ^[6]. In Madhya Pradesh (India), it used in the treatment of indigestion, skin diseases, cough, bronchitis, allergy, leucoderma and as general tonic, it has also found beneficial in diarrhoea, dysentery and colitis, typhoid fever, ulceration of bowels, bladder and in painful micturation ^[7]. In the present investigation, an indigenous medicine *Curcuma angustifolia* Roxb is tested against various disease causing human pathogenic bacteria to prove its antibiotic efficiency and physicochemical analysis studied.

2. Material and Methods

The samples of *Curcuma angustifolia* Roxb was collected from Sitakhandi, Nanded district of Maharashtra and identified by using reliable literature [8, 9, 10, 11]. The herbarium specimen deposited in VH Herbarium, department of Botany, Vivekanand Arts, Sardar Dalipsingh Commerce and Science College, Samarth Nagar, Aurangabad.

Preparation of crude extracts

Dried rhizome powder sample (10g) was extracted in a Soxhlet apparatus with methanol used as solvent at 65° C for 18-24 hrs and the sample was stored in amber coloured bottle for the further study.

Test microorganisms and their sources

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

Antibacterial Assay 96-well plates method:

About $100\mu l$ sterile Mueller-Hinton broths was added onto each well along with $2\mu l$ serial diluted human pathogenic bacteria suspension in each well, next different concentrations of methanol extracted samples as 2, 4, 6, 8 and $10\mu l$ was loaded to 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. Finally, the optical density was measured at 540 nm on the spectrophotometer.

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Physicochemical study The methods of $^{[12, 13, 14, 15, 16]}$ were adopted for the study of dried powder and methanolic extract of rhizome of Curcuma angustifolia Roxb.

3. Results and Discussion

The methanolic rhizome extract of C. angustifolia Roxb., in various concentrations were tested against human pathogenic bacteria, it was cleared from the results, that maximum inhibition of P. aeruginosa, S. typhi, S. arueus and S. flexneri were found in 2 µl concentration, whereas in case of E. coli it was 8µl. Regarding minimum inhibitory concentration (MIC) it was 2 µl for bacterium P. aeruginosa, S. typhi, S. arueus and S. flexneri while it was 8 ul for E. coli (table 1).

Table 1: Antibacterial activity of C. angustifola Roxb against human pathogens

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C.	С.	Human pathogenic Bacteria (2 µl)					
Sr. No	angustifola	Р.	S. typhi	S.	E sali	S.	
	(conc. µl)	aeruginosa		arueus	E. coli	flexneri	
1	2 μl	0.28	0.28	0.26	0.29	0.29	
2	4 μl	0.39	0.32	0.27	0.31	0.48	
3	6 µl	0.45	0.50	0.37	0.39	0.45	
4	8 µl	0.45	0.56	0.56	0.25	0.46	
5	10 μl	0.42	0.52	0.43	0.43	0.37	
	MIC	2 μl	2 μl	2 μl	8 μl	2 μl	
	S. E	± 0.031	± 0.056	± 0.054	± 0.033	± 0.035	

Quantitative chemical parameters

The different physicochemical tests were carried out for the determination of dry matter (DM), total ash (TA), acid insoluble ash (AIA), acid soluble ash (ASA), nitrogen (N), crude protein (CP), calcium (Ca) and phosphorus (P) and results are given in (table 2). The various phytochemical tests were done for the detection of chemical constituents (table 3).

Table 2: Physicochemical analysis of C. angustifolia Roxb rhizome

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D M	ΤA	AIA	ASA	WIA	WSA	Ca	P	N	Protein
(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
19.04	8.00	0.75	7.25	0.50	2.00	0.041	0.224	9.83	61.43

Table 3: Phytochemical group test for the rhizome extract of C. angustifolia Roxb.

Sr. no.	Phytoconstituents	Rhizome extract of C. angustifolia Roxb.				
1	Carbohydrate	++				
2	Protein	++				
3	Starch	++				
4	Amino Acid					
5	Steroid					
6	Glyc os ide	++				
7	Flavonoid	++				
8	Tannin	++				
9	Alkaloid					
10	Phenol					
11	Terpenoids					
12	Phytosterol	++				

⁺⁺ Presence, -- Absence

4. Conclusion

The rhizome of Curcuma angustifolia has potential antibacterial agent as it shows significant activity against bacterial pathogens and physicochemical properties has great importance in the nutrition and medicine. The present results will form the basis for selection of Curcuma angustifolia for further investigation in the potential discovery of new herbal bioactive compounds. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts.

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