International Journal of Pharmacy and Biological Sciences-IJPBS™ (2020) 10 (3): 150-153
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | OA Journal | MCI Approved | Index Copernicus

Phytochemical Analysis and *in vitro* Anti-Inflammatory and Anti-Bacterial Activities *Curcuma Pseudomontana J.Graham*

Pinky Pavani, Yashasvi Jagapathi, Ram Mohan Manda*, Baba Shankar Rao Garige and Vasudha Bakshi

Department of Pharmacognosy and Phytochemistry, School of Pharmacy, Anurag Group of Institutions, Venkatapur, Ghatkesar, Medchal, Telangana, India.

Received: 14 Mar 2020 / Accepted: 6 Apr 2020 / Published online: 1 Jul 2020
***Corresponding Author Email:** rammohanpharmacy@cvsr.ac.in

Abstract

Aim: The present study is aimed to evaluate the Phytochemical analysis and *In-vitro* Anti-inflammatory and Anti-bacterial activity of *Curcuma pseudomontana* J.Graham. **Materials and methods:** In the present investigation, the rhizomes of *curcuma pseudomontana* powder was extracted by successive soxhlation extraction method with ethyl acetate and methanol. The ethyl acetate extracts responded positively to all the tests for carbohydrates and also to the tests for flavonoids and glycosides and methanolic extracts produced positive test for the presence of carbohydrates, proteins, steroids, flavonoid glycosides, tannins and phenol compounds. The extracts were used for testing the *in-vitro* anti-inflammatory activity by using albumin denaturation assay, proteinase inhibitory activity at a concentration of 20, 40, 60, 80 and 100mg/ml and Anti-bacterial activity against two gram positive microorganisms (*Bacillus subtillus and Staphylococcus aureus*) and two gram negative microorganisms (*Salmonella typhi* and *Escherichia coli*) at concentrations 100 mg/ml and 200 mg/ml by adopting cup plate method.

Results: The extracts exhibited significant *in-vitro* anti-inflammatory effect and inhibited the growth of both Gram positive and Gram-negative microorganisms at 100 mg/ml and 200 mg/ml concentrations. **Conclusion:** The findings of this study showed that the effectiveness of methanol extract shows more Anti-inflammatory and Anti-bacterial activity compared to ethyl acetate extract. Because of methanol extract contains more bioactive compounds comparatively then ethyl acetate extract and bioactive components justifying its traditional use.

Keywords

Curcuma pseudomontana, Anti-inflammatory activity, Anti-bacterial activity, Phytochemical analysis.

*****

INTRODUCTION

Curcuma pseudomontana J. Graham is an extremely rare Zingiberaceae species found so far only in the Naikongchhari forest area of Bandarban district in the southeastern hilly area of Bangladesh[1]. *C. pseudomontana* is endemic to the Western and Eastern Ghats, of peninsular India, the species found in Karnataka, Maharashtra and Andhra Pradesh in English it is known as hill turmeric[2]. Dried rhizomes of *C. pseudomontana* J. Grahm., used in skin diseases and impurities of blood[3]. Rhizomes boiled in oil and used as an application to sprain and useful on snake bite[4]. Rhizome powder are useful in leucoderma, scabies, smallpox, and intestinal worms as well as

juice strong remedy against rheumatism and in combination of ginger used for smooth delivery in North East India[5]**.** 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[6] , The Bagata and Valmiki tribes of Munchingiputtu Mandal, Visakhapatnam district, Andhra Pradesh use *C. pseudomontana* rhizome in the treatment of jaundice and diabetes [7]. The rhizome are used for skin problems and coughs by the tribals of Achampet Forest Division in Nallamalais, Telengana, India[8], The Kattunaikan tribe of Malappuram district in Kerala, India, uses the rhizomes for cardiac disorders[9]. The rhizomes are used for muscle pain, leprosy and debility by tribal communities residing in Gundlabrahmeswaram Wildlife Sanctuary (Eastern Ghats), Andhra Pradesh, India[10].

MATERIAL AND METHOD

Plant Material

The fresh rhizomes are collected from the chikmagalur, Western Ghats of Karnatana, India and authenticated by Dr. Madhavashetty, Dept of Botanty; Sri Venkateshara University, Trupathi, Andhra Pradesh and the Voucher specimen was deposited in the herbarium of School of Pharmacy, Anurag Group of Institutions, Venkatapur, Ghatkesar, Medchal, Telengana.

Preparation of Extract:

Freshly collected rhizomes were dried at room temperature and coarsely powdered. The rhizomes powdered 500 gm were extracted successively with ethyl acetate and methanol using Soxhlet apparatus. The crude extract was evaporated to dryness and found to be 30 and 20gms respectively. Preliminary phytochemical screening was performed. ethyl acetate extract of *Curcuma psedomonota* revealed the presence of carbohydrates, steroids, Phenol compounds and flavonoids, methanol extract tested positive for carbohydrates, proteins, glycosides, Phenol compounds and Flavonoid. The constituents present in the ethyl acetate extract and methanol extracts are carrying out the In-vitro Anti-inflammatory and Anti-bacterial activity.

In- vitro Anti-inflammatory

Inhibition of albumin denaturation

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of the extract was added to reach final concentrations (20, 40, 60, 80, and 100 mg/ml). Similar volume of double distilled water served as control. Then the mixtures were incubated at 37±2ºC in an incubator for 15minutes and then heated at 70ºC for 5 minutes. After cooling down, their absorbance was measured at 660 nm using vehicle as blank. The Diclofenac sodium at the final concentration of (20, 40, 60, 80, and 100 mg/ml) was used as reference drug and treated similarly for determination of absorbance[11].

Anti-proteinase action

The reaction mixture (2 ml) include 0.06 mg trypsin, 1 ml 20Mm Tri HCL buffer (pH 7.4) and 1 ml test sample of different concentrations (20-100 µl/ml).The mixture was incubated at 37°C for 5 minutes and then 1ml of 0.8% casein was added. The mixture was incubated for an additional 20 minutes.2ml of 70% perchloric acid was added to arrest the reaction. The cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory was calculated[12].

In vitro Anti-bacterial activity

Test organisms

Two strains of gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, and two strains of gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* were used in our experiment to evaluate the Anti-bacterial activity.

Disc diffusion Method

Disc diffusion method for antimicrobial susceptibility testing was carried out to assess the presence of antibacterial activities of the ethyl acetate and methanol extract.[9,10].

RESULTS

In-vitro **Anti-inflammatory activity effect of Curcuma pseudomontana on heat induced protein denaturation**

Concentration (µg/ml)	% inhibition of protein denaturation		
	Diclofenac sodium (standard)	Ethyl acetate extract of *Curcuma pseusdomontana*	Methanol extract of *Curcuma pseusdomontana*
20	58.24±3.12	33.14±3.16	35.48±5.18
40	65.32±1.24	35.18±2.35	38.74±3.24
60	72.40±2.24	48.90±2.04	59.20±2.42

80	76.65±3.54	62.05±1.40	72.10±3.4
100	83.15±2.26	68.60±1.53	78.12±1.82

Each value represents the mean ± SD. N=3, Experimental group were compared with control **p <0.01considered extremely significant; *p < 0.05, non-significant.

***In-vitro* anti-inflammatory activity *Curcuma psedomontana* on heat induced protein denaturation**

Concentration (µg/ml)	% inhibition of proteinase action		
	Diclofenac sodium (standard)	Ethyl acetate extract of *Curcuma psedomontana*	Methanol extract of *Curcuma psedomontana*
20	60.25±3.64	34.44±2.52	35.98±.84
40	70.02±4.62	22.4±1.48	26.5±2.88
60	71.49±2.54	31.6±3.62	33.00±2.84
80	76.35±4.12	42.2±2.84	48.1±2.60
100	84.10±2.20	54.6±1.84	58.8±2.64

Each value represents the mean ± SD. N=3, Experimental group were compared with control **p <0.01considered extremely significant; *p < 0.05, non-significant.

STATISTICAL ANALYSIS

Results are expressed as Mean ± SD. The difference between experimental groups was compared by One Way Analysis of Variance (ANOVA) followed by Dunnet Multiple comparison test (control Vs test).

In-vitro* anti-bacterial activity of *Curcuma psedomontana

Name of organism	Zone of inhibition (mm)					
	Streptomycin		Ethyl acetate extract of *Curcuma psedomontana*		Methanol extract of *Curcuma psedomontana*	
	100µg/ml	200µg/ml	100mg/ml	200mg/ml	100mg/ml	200mg/ml
E. coli	10.8±0.22	12.2±0.27	6.6±0.24	8.2±0.12	8.0±0.40	9.2±0.50
K. pneumonia	9.5±0.4	11.2±0.12	6.1±0.24	8.0±0.32	8.1±0.22	9.0±0.54
B. subtilis	10.4±0.32	12.6±0.12	5.6±0.12	7.2±0.12	7.1±0.40	8.1±0.42
S. aureus	10.6±0.74	11.8±0.44	5.5±0.22	6.8±0.16	7.2±0.72	8.4±0.30

ZI were expressed as mean+ standard deviation of three replicates.
Low activity (1-6 mm), moderate activity (7-10mm), high activity (11-15 mm).
Represents mean ± S.D. mm; p < 0.05.

CONCLUSION

The above results of preliminary phytochemical analysis and anti-inflammatory and anti-bacterial activity of *Curcuma psedomontana* rhizomes extracts confirmed as a useful Anti-inflammatory and Antimicrobial agent. The present study provides evidence that *Curcuma psedomontana* rhizomes extracts contains medicinally important bioactive compounds like carbohydrates, proteins, alkaloids, glycosides, flavonoids and phenol compounds and this justifies the use of plant species as traditional medicine for treatment of inflammation and bacterial infections.

REFERENCES

1. Rashed Reza, Sayef Shahriad Ripon, Mohammed Rahmatullah Curcuma pseudomontana - a rare Zingiberaceae species plant of Bangladesh and its medicinal uses, Asian Journal of Pharmacognosy, 1(2): 31-32, 2017
2. Gurusiddesh B Hiremath, Basappa B Kaliwal. Pharmacognostic Evaluation of Rhizome of *Curcuma pseudomontana* J. Graham, Int. J. Pharm Bio Sci., 5(2):242-250, 2014
3. 3.Bhosle SV, Ghule VP, Aundhe DJ, Jagtap SD (2009) Ethnomedicinal knowledge of plants used by the tribal people of Purandhar in Maharashtra, India. Ethnobot. Leaflets. 13: 1353-1361.
4. Nadkarni AK. Indian Materia Medica Popular Prakashan, Bombay, 1982, I, II
5. Vedprakash,Mehrotra BN.Ethanobotanical studies on flora of Khandala Maharashta State.j.Eon.Tex.Bot, 9(1); 205-208,1987
6. Ramarao N, Rajendra A, Henry AN. increasing the secretion of breast milk-indigenous practices in Andhra Pradesh. Ancient Science of Life, XIX:176-178.2000
7. Padal SB, Ramakrishna H, Devender R. Ethnomedicinal studies for endemic diseases by the tribes of Munchingiputtu Mandal, Visakhapatnam district, Andhra Pradesh, India. Int. J. Med. Arom. Plants. 2(3): 453-459. 2000
8. Reddy DS. Ethnomedicinal plants used by the tribals of Achampet Forest Division In Nallamalais, Telengana, India. Int. J. Plant Animal Environ. Sci. 5(2): 65-73. 2015

9. Chitra M, Kumar KMP, Geetha SP. A comparative study on ethnobotanical usage of plants for twenty selected diseases by six tribal communities in Malappuram district. Int. J. Herbal Med. 4(4): 108-113. 2016
10. Kanneboyena O, Suthari S, Raju VS (2015) Ethnomedicinal knowledge of inhabitants from Gundlabrahmeswaram Wildlife Sanctuary (Eastern Ghats), Andhra Pradesh, India. American J. Ethnomed. 2(6): 333-346. 2015
11. Sangita C, Priyanka C, Protapaditya D, Sanjib B. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein, Asian Pacific Journal of Tropical Biomedicine; 178-180.2012
12. Oyedepo O, Femurewa AJ. Anti-protease and membrane stabilizing activities of extracts of Fagrazanthoxiloides, Olaxsubscorpioides and Tetrapleuratetraptera. Int J of Pharmacong, 33: 65-9.1995
13. Nyiligira E. Viljoen VM, Vuuren SF. Essential oil composition and *in vitro* antimicrobial and anti-inflammatory activity of South African *Vitex* species, South African Journal of Botany, 70: 611–17.2004
14. Ashraf A. Mostafa A. Khalid S. Turki M. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases, Saudi Journal of Biological Sciences 25: 361–6, 2018