Available online on 15.06.2020 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Extraction, Phytochemical Screening and Anti-Inflammatory Activity of Hydro-Ethanolic Extract of Roots of *Dactylorhiza hatagirea*

Shailja Sharma*[1], Prabhat Kumar Jain[2], Geeta Parkhe[2]

1- Sagar Institute of Pharmacy & Technology, Gandhi nagar, Bhopal (M.P.), India

2- Scan Research Laboratories, Bhopal (M.P.), India

ABSTRACT

The present work showed phytochemical screening, anti-inflammatory activities and sub-acute toxicity of hydro-ethanolic extract of roots of *Dactylorhiza hatagirea* (*D. hatagirea*) and rhizomes of *Curcuma angustifolia* (*C. angustifolia*). The anti-inflammatory activity was evaluated by Carrageenan-Induced Rat Paw Edema method. Acute toxicity of the extract (2000 mg/kg) was examined in Swiss albino mice for 14 days. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent method and aluminium chloride method respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids. The total phenolics content of roots of *Dactylorhiza hatagirea* and rhizomes of *Curcuma angustifolia* extract was (0.675, 0.456mg/100mg), followed by flavonoids (0.832, 1.091mg/100mg) respectively. Hydro-ethanolic extract up to 2000 mg/kg did not produce any toxic effects. The hydro-ethanolic extract of *Dactylorhiza hatagirea* and *Curcuma angustifolia* (100 and 200 mg/kg) inhibited the inflammation induced by carrageenan in rats in a dose dependent manner. After anti-inflammatory activity tablet formulation was prepared of the extract and evaluated as per IP. The hydro-ethanolic extract of *Dactylorhiza hatagirea* and *Curcuma angustifolia* possesses a strong anti-inflammatory activity and may be considered an interesting source of effective anti-inflammatory compounds.

Keywords: Sub-acute toxicity, anti-inflammatory effect, *Dactylorhiza hatagirea, Curcuma angustifolia,* Herbal tablet formulation

Article Info: Received 21 March 2020; Review Completed 24 May 2020; Accepted 29 May 2020; Available online 15 June 2020

Cite this article as:

Sharma S, Jain PK, Parkhe G, Extraction, Phytochemical Screening and Anti-Inflammatory Activity of Hydro-Ethanolic Extract of Roots of *Dactylorhiza hatagirea*, Journal of Drug Delivery and Therapeutics. 2020; 10(3-s):86-90
http://dx.doi.org/10.22270/jddt.v10i3-s.4092

***Address for Correspondence:**

Shailja Sharma, Sagar Institute of Pharmacy & Technology, Gandhi nagar, Bhopal (M.P.), India

INTRODUCTION

Inflammation is a mechanism of great benefit for maintaining homeostasis in the body. The inflammatory response may be appropriate, physiologic and necessary in the presence of an infection and cellular damage or stress. Conversely, it may be inappropriate, altered homeostasis, pathologic and damaging when it is reacting out of proportion causing undesired effects[1-2] and contribute to diseases. In fact, inflammation is implicated in osteoarthritis, heart disease, Alzheimer's, age-related macular degeneration, chronic obstructive pulmonary disease, multiple sclerosis, stroke and cancer[3-6]. Inflammation is frequently associated with the increase in vascular permeability and mediator release[7], increase of protein denaturation and membrane alteration[8]. Further, leucocyte infiltration, oedema and granuloma formation represent typical features of inflammation[9]. Moreover, the host response has been considered to be mediated mainly by B and T lymphocytes, neutrophils and monocytes/macrophages. These cells are triggered to produce inflammatory mediators, including cytokines, chemokines, arachidonic acid metabolites and proteolytic enzymes, which collectively contribute to tissue degradation by activation of several distinct host degradative pathways[10-11]. Steroidal and non-steroidal anti-inflammatory drugs are known to treat inflammation and pain. However, their prolonged use often leads to serious side-effects such as gastrointestinal tract dyspepsia, peptic ulceration, haemorrhage and perforation leading to death in some patients[12]. Many medicines of plant origin have been used since long time without any adverse effects, and new

medicinal plants are introduced to develop analgesic and anti-inflammatory drugs. The bulbous roots of *Dactylorhiza hatagirea* (D.Don) Soo (Fam. Orchidacea) which are synonymous to the tubers of Orchis macula (Orchidaceae) and serve as source of Salep, are used traditionally in Indian subcontinent specially in the Northern region and Nepal as aphrodisiac and sexual stimulant. It is considered as a nutritive and restorative tonic and also as an alternative source of Salep used very commonly in Europe[13]. *Curcuma angustifolia* Roxb, belonging from *Zingiberceae* family is an important plants used in India as traditional medicine in treatment of various diseases and disorder. The major chemical constituents of the plants are methyl eugenol, camphor, cineol etc.In India many tribal and rural people using this plants in treatments of various diseases .In rural India the *Curcuma angustifolia* is being used in consumption, excessive thirst, jaundice, kidney disorder, fever and for vitality and flattening the body. The rhizome is used in bone fracture, inflammation, intestinal disease etc by tribal[14-15]. Therefore, the present study was designed to investigate anti-inflammatory activities of hydro-ethanolic extract of roots of *Dactylorhiza hatagirea* and rhizomes of *Curcuma angustifolia* by using Carrageenan-Induced Rat Paw Edema model.

MATERIALS AND METHODS

Plant material

Roots of *Dactylorhiza hatagirea* and rhizomes of *Curcuma angustifolia* was collected from rural area of Bhopal (M.P), India. The taxonomical identification and authentication of the plant material was done by Dr. Ravi Upadhyay, Department of Botany, Govt. N.M.V. Hoshangabad (M.P). The specimens of voucher have been submitted and preserved in the herbarium of Govt. N.M.V. Hoshangabad (M.P.).

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction of plant material

Dried powdered roots of *D. hatagirea* and rhizomes of *C. angustifolia* has been extracted with ethanol and water (80:20 v/v) hydroalcoholic using soxhlet apparatus for 48 hrs, filtered and the extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts[16].

Qualitative phytochemical analysis of plant extract

The roots of *D. hatagirea* and rhizomes of *C. angustifolia* extract obtained were subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate[17-18]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from roots of *D. hatagirea* and rhizomes of *C. angustifolia* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

Total Phenol Determination

The total phenolic content was determined using the method of Olufunmiso et al[19]. A volume of 2 ml of roots of *D. hatagirea* and rhizomes of *C. angustifolia* extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The blue colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total Flavonoids Determination

The total flavonoid content was determined using the method of Olufunmiso et al[19]. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity

Acute toxicity study of the prepared extracts of both plants was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines-423[20] the animals were fasted for 4 hr, but allowed free access to water throughout. As per the OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals; and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, The dose level to be used as the starting dose is selected from one of three fixed levels 50, 100, 300 and 2000 mg/kg body weight. Acute toxicity was determined as per reported method[21].

Anti-inflammatory activity

Carrageenan-induced paw edema model

Paw edema was induced by injecting 0.1 ml of 1% w/v carrageenan suspended in 1% CMC into sub-plantar tissues of the left hind paw of each rat. Rats were divided into following groups; each group consisting of six animals[22].

- Group I Carrageenan control
- Group II Hydroalcoholic extract *Dactylorhiza hatagirea* (100 mg/kg/p.o.)
- Group III Hydroalcoholic extract *Dactylorhiza hatagirea* (200 mg/kg/p.o.)
- Group IV Hydroalcoholic extract *Curcuma angustifolia* (100 mg/kg/p.o.)
- Group V Hydroalcoholic extract *Curcuma angustifolia* (200 mg/kg/p.o.)
- Group VI Diclofenac sodium (10 mg/kg) as standard reference

The paw thickness was measured before injecting the carrageenan and after 1, 2, 3 and 4 hour using vernier caliper. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the carrageenan control group.

The percentage (%) inhibition of edema is calculated using the formula

$$\% \text{ inhibition} = \frac{T_o - T_t}{T_o} \times 100$$

Where T_t is the thickness of paw of rats given test extract at corresponding time and T_o is the paw thickness of rats of control group at the same time.

Data Analysis

The data is expressed as mean ± Standard Deviation (SD). Results were analyzed using one-way ANOVA followed by Dunnet's test. Differences were considered as statistically significant at $P < 0.05$, when compared with control.

Formulation of Herbal Tablet

Tablets using extracts as active ingredients were prepared by dry granulation method. The dried powder extract and other ingredients were mixed uniformly and then the mixture was blended and granulated. The granules were compressed into tablets in an 8-station machine.

Table 1: Formulation of Herbal Tablet

Ingredient	Quantity Per Tablet (mg)
Dactylorhiza hatagirea	200
Curcuma angustifolia	200
Talc	250
MCC	40
Magnesium Stearate	10

Herbal Tablet was evaluated by various parameters as per IP

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening of roots of *D. hatagirea* and rhizomes of *C. angustifolia* extracts revealed the presence of various components such as phenolic compounds, carbohydrates, flavonoids, saponins and diterpins among which flavones were the most prominent ones and the results are summarized in table 2.

Table 2: Result of Phytochemical screening of hydroalcoholic extracts

S. No.	Constituents	*Dactylorhiza hatagirea*	*Curcuma angustifolia*
1.	Alkaloids	-ve	-ve
2.	Glycosides	-ve	-ve
3.	Flavonoids	+ve	+ve
4.	Diterpenes	-ve	+ve
5.	Phenolics	+ve	+ve
6.	Amino Acids	-ve	-ve
7.	Carbohydrate	+ve	+ve
8.	Proteins	-ve	-ve
9.	Saponins	+ve	+ve
10.	Oils and fats	-ve	-ve

The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.042X+0.002$, $R^2= 0.999$,** where X is the gallic acid equivalent (GAE) and Y is the absorbance.

The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.06X+0.019$, $R^2= 0.999$,** where X is the quercetin equivalent (QE) and Y is the absorbance. Results was shown in table 3 and fig 1& 2.

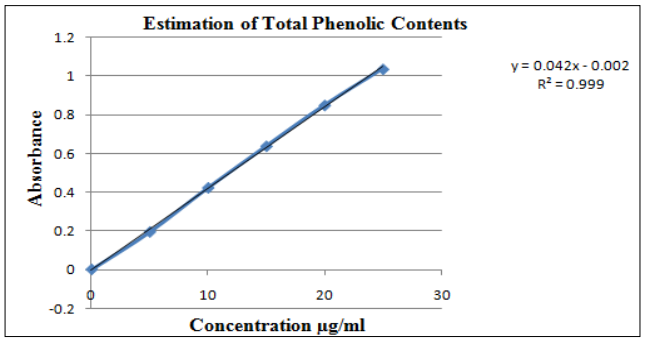

Figure 1: Graph of Estimation of Total Phenolic content

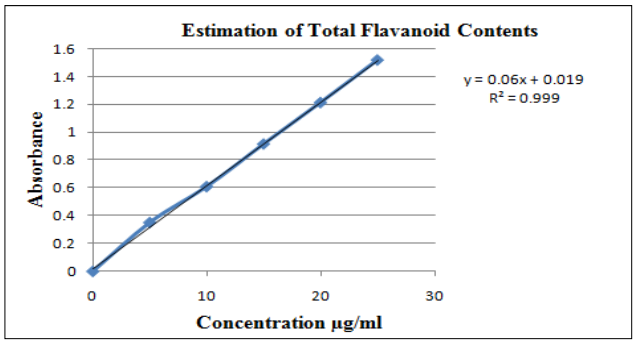

Figure 2: Graph of Estimation of Total flavonoid content

Table 3: Total Phenolic and Total flavonoid content

S. No.	Solvents→ Bioactive compound↓	Hydroalcoholic extracts	
		Dactylorhiza hatagirea	***Curcuma angustifolia***
1.	Total Phenol (Gallic acid equivalent (GAE) mg/100mg)	0.675	0.456
2.	Total flavonoid (Quercetin equivalent (QE) mg/100mg)	0.832	1.091

Results show the effect of Hydroalcoholic extract of *D. hatagirea* and *C. angustifolia* and standard drug as compared to carrageenan control at different hours in carrageenan-induced paw edema model using vernier caliper. Hydroalcoholic extract of *D. hatagirea* and *C. angustifolia* administered at a dose of 100 and 200 mg/kg p.o prevented carrageenan-induced paw edema with a percentage inhibition at 1, 2, 3,and 4 hour, respectively. Diclofenac sodium at a dose of 10 mg/kg p.o. prevented carrageenan-induced paw edema with a percentage inhibition at 1, 2, 3.and 4 hour, respectively.

Table 4: Effect of Hydroalcoholic extract of *D. hatagirea* and *C. angustifolia* at doses of 100 and 200 mg/kg, and diclofenac sodium as compared to carrageenan control group at different hours in carrageenan-induced paw edema model using vernier caliper

Group	Treatment	Change in paw thickness (mm) ± SD			
		1	**2**	**3**	**4**
1	Control (gel base)	1.34 ± 0.1	2.35 ±0.12	3.64 ± 0.15	3.25 ± 0.15
2	Hydroalcoholic extract *Dactylorhiza hatagirea* (100 mg/kg)	1.14 ± 0.13 (14.93%)	1.74 ± 0.22 (26.44%)	2.50 ± 0.15 (31.3%)	1.97 ± 0.16 (39.0%)
3	Hydroalcoholic extract *Dactylorhiza hatagirea* (200 mg/kg)	1.00 ± 0.12(25.6%)	1.54 ± 0.16 (35.02%)	1.81±0.10 (50.09%)	1.42±0.19 (56.34%)
4	Hydroalcoholic extract *Curcuma angustifolia* (100 mg/kg)	1.12 ± 0.09 (16.03%)	1.70 ± 0.28 (27.40%)	2.48 ± 0.10 (32.5%)	1.96 ± 0.11 (40.0%)
5	Hydroalcoholic extract *Curcuma angustifolia* (200 mg/kg)	0.98 ± 0.10 (26.0)%	1.50 ± 0.13 (34.0%)	1.79±0.09 (51.00%)	1.40±0.18 (57.40%)
6	Diclofenac sodium (10 mg/kg)	0.58±0.11 (55.97%)	0.8 ±0.12 (66.24%)	1.14 ± 0.12 (68.82%)	0.9 ±0.11 (72.13%)

All values are expressed as mean ± SD; P < 0.05 v/s carrageenan control

Table 5 shown the result of evaluation parameter of herbal tablet

Table 5: Characteristics of Developed Herbal Formulation

S. No.	Evaluation Parameters	Results
1.	Weight variation	None of the tablets out of the limit
2.	Average hardness	4.25 kg/cm^2
3.	Average % friability	0.897
4.	Average disintegration Time	15 min.

CONCLUSION

The Hydroalcoholic extract of *D. hatagirea* and *C. angustifolia* showed potent anti- inflammatory activity on carrageenan induced paw edema in rats. The anti-inflammatory activity of root extract of *D. hatagirea* and rhizomes extract of *C. angustifolia* demonstrated a dose-dependent response and were comparable to diclofenac (reference drug). The extracts were most active at the dose level of 200 mg/kg body weight in the fourth hour of treatment. The extracts of *D. hatagirea* and *C. angustifolia* could, therefore, be an alternative bio-resource for generating anti-inflammatory agents. However, further studies are necessary to elucidate the mechanism behind this effect and their active compounds. The present study, therefore, scientifically confirms and supports the traditional use of *D. hatagirea* and *C. angustifolia* in the management of inflammation

REFERENCES

1. Miller AH, Maletic V, Raison CL, Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression, Biol Psychiatry, 2009; 65:732–741.
2. Abdelmagid SM, Barbe MF, Safadi FF, Role of inflammation in the aging bones, Life Sci., 2015; 123:25–34.
3. McCarty MF, Interleukin-6 as a central mediator of cardiovascular risk associated with chronic inflammation, smoking, diabetes, and visceral obesity: down-regulation with essential fatty acids, ethanol and pentoxifylline, Med Hypotheses, 1999; 52:465–477.
4. Azeem AK, Dilip C, Prasanth SS, Shahima VJH, Sajeev K, Naseera C, Anti-inflammatory activity of the glandular extracts of *Thunnus alalunga*, Asia Pac J Med, 2010; 3:4–412.
5. Medzhitov R, Inflammation 2010: new adventures of an old flame, Cell, 2010; 140:771–776.
6. Kumar V, Bhat ZA, Kumar D, Bohra P, Sheela S, In-vitro anti-inflammatory activity of leaf extracts of *Basella alba* linn. Var. alba, Int J Drug Dev Res, 2011; 3:124–127.
7. Vane JR, Botting RM, Anti-inflammatory drugs and their mechanism of action, Inflamm Res, 1998;47:78–87.
8. Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Nkeh-Chungag NB, Iputo JE, An experimental evaluation of Albuca setosa aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation, J Med Plants Res, 2010; 4:789–795.
9. Gorzalczany S, Lopez P, Acevedo C, Ferraro G, Anti-inflammatory effect of Lithrea molleoides extracts and isolated active compounds, J Ethnopharmacol, 2011;133:994–998.
10. Birkedal-Hansen H, Role of cytokines and inflammatory mediators in tissue destruction, J Periodont Res, 1993; 28:500–510.
11. Hernandez M, Dutzan N, Garcıa-Sesnich J, Abusleme L, Dezerega A, Silva N, Gonzalez FE, Vernal R, Sorsa T, Gamonal J, Host-pathogen interactions in progressive chronic periodontitis, J Dent Res, 2011; 90:1164–1170.
12. Griffin MR, Epidemiology of nonsteroidal anti-inflammatory drug-associated gastrointestinal injury, Am J Med, 1998; 104:23–29.
13. Bhattarai NK, Some endangered medicinal plants of Nepal. In: Handa SS, Kaul MK (eds). Supplement to Cultivation and Aphrodisiac Activity of *Dactylorhiza hatagirea* Utilization of Medicinal Plants. Jammu: Regional Research Laboratory, 1996; 676–7.
14. Pereira J, The contribution of Jonathan Pereira to plant taxonomy, key volume bulletin, 2011; 66:1804-1853.
15. Bhavna D, Khatri J, *Gentella asiatica*: The elixir of life. Inter J Pharmaceutical Res 2011; 2(2):431-438.
16. Mukherjee PK, Quality Control of Herbal Drugs", 2nd Edition, Business Horizons, 2007; 2-14.
17. Khandelwal KR, Ed. Practical Pharmacognosy Technique and Experiments, 23rd Edn, 2005; 15, 29, 149, 56.
18. Kokate CK. Ed. Practical Pharmacognosy, 4th Edn., Vallabh Prakashan, 1994; 112,120.
19. Olufunmis O, Olajuyigbe, O. and Afolayan, Anthony J, phenolic Content and antioxidant property of the bark extract of *Ziziphus mucronata* wild. Subsp. Mucronata wild, BMC, Complementary and alternative medicine, 2011; 11:130.
20. Guideline Document on Acute oral Toxicity Testing, Series on Testing and Assessment No. 423. Paris: Organization for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications; 1996. Available from: http//www.oecd.org/ehs.
21. Jonsson M, Jestoi M, Nathanail AV, Kokkonen UM, Anttila M, Koivisto P, Peltonen K, Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin, Food and chemical toxicology, 2013; 53:27-32.
22. Winter CA, Risley EA, Nuss GW, Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs, Proc Soc Exp Biol Med, 1962; 111:544–547.