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PHYTOCHEMICAL INVESTIGATION AND ANTI-ARTHRITIC ACTIVITY OF FLOWER EXTRACT OF *CYMBIDIUM SARAH*

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ABSTRACT

Medicinal plants are playing an imperative role in the therapy for treating various chronic ailments including arthritis. The present study is aimed to evaluate the in-vivo anti-arthritic activity of ethanolic extract of *Cymbidium sarah* Jean flower using complete freund's adjuvant (CFA)-induced model. The latency of the arthritic secondary response ended after a few days and was characterized by joint swelling and nodule events on the 7th day. Administration of *Cymbidium Sarah Jean* (250 mg/kg) significantly ($P < 0.01$) protected against joint swelling in paws in rats with induced arthritis compared with the arthritis control group. But a significant reduction was observed from day 11 to day 13 in the *Cymbidium sarah* treated (200 mg/kg) group. However, the effects of *cymbidium sarah* at 350 mg/kg were found to be significant ($P < 0.001$) from the initial stage of the secondary response and were maintained throughout the experiment. They were significant ($P < 0.01$) 15-19 days after FCA injection compared with the group treated with the reference standard, indomethacin at 10 mg/kg.

Keywords: *Cymbidium sarah,* Anti-arthritic activity, Extraction, Phytochemical analysis.

INTRODUCTION

It has been estimated that a large number of individuals suffer from Inflammatory and arthritic disorders. However, the magnitude of improvement among the patients with the currently available treatments still disappointing. This prompted many pharmacologists for searching new therapeutic alternatives from natural sources for the treatment of inflammatory arthritic disorders. During the past decades, an increasing number of herbal products have been introduced in arthritic practice and there is large number of herbal medicines whose therapeutic potential has been assessed in a variety of animal models. Under these circumstances, Indian traditional medicine systems (Ayurveda, Siddha and Unani) can play a vital role for providing better therapeutic active drug and/or lead structures for newer drug development from natural sources. With the increasing interest in the field of herbal medicines, traditional systems of

medicine have become a topic of global importance. In many developing countries, a large proportion of the population relies heavily on the traditional practitioners and medicinal plants to meet primary health care needs[1].

Arthritis is a chronic disorder, mainly causing inflammation, in particular or extended joints, in the human body comprising of more than 360 joints. Most of the arthritic, cases are chronic and continuous, accounts for muscular stiffness. If arthritis left untreated, can further lead to joint torpidity and muscle atrophy. In the pre-arthritic or acute stage, the small joints of the hands and feet are usually affected. Around 100 types of arthritis are reported in all the age groups and various drugs are available for its treatment. Juvenile arthritis, rheumatoid arthritis, and gout are inflammatory in nature, while, osteoarthritis is regressive. Arthritis is a condition of mainly inflamed joints affecting single or in group. Arthritis is associated with disruption of cartilage. The main function of cartilage is to protect and behave as a shield just like the brake shoe of the cycle or motor car for joints, to support smooth movement and locomotion activity. Cartilage has a decisive role in balancing the external shock and pressure on the joint[2].

Arthritis is one of the major socio-economic disabilities affecting adult individuals. Recent survey by the world health organization revealed that 5-10% of world and 10% Indian population is arthritic and the numbers may double by 2030[3]. Degeneration of articular cartilage/extracellular matrix (ECM) of cartilage is an irreversible consequence of progressive arthritis[4].

Quite a few approaches and strategies have been applied in the treatment of arthritis since past two decades. All the possibilities were aimed to provide symptomatic relief, apprehend disease activity and progression, and restrain joint and bone damage. Topical pain relievers, NSAIDs, corticosteroid, DMARDs are the common drugs prescribed to arthritic patients. Traditional NSAIDs include celecoxib, diclofenac, ibuprofen, indomethacin, aspirin and naproxen; on the other hand, methotrexate[5].

In India, many ayurvedic practitioners are using various indigenous plants for the treatment of different types of arthritic conditions. Although the application of these medicaments has a sound tradition and a rational background according to the Indian system of medicine, perhaps it is essential to investigate the rationality of their use in modern scientific terms. Medicinal plants are playing an imperative role in the therapy for treating various chronic ailments including arthritis. The present study is aimed to evaluate the in-vivo anti-arthritic activity of ethanolic extract of *Cymbidium sarah* Jean flower using complete freund's adjuvant (CFA)-induced model.

MATERIAL AND METHODS

Extraction of Plant Material

Flowers of *Cymbidium Sarah Jean* were cut into little pieces using sterile scissor, washed under running tap water to remove the dust impurities. Then the plant Flowers was dried at room temperature (under shade). After complete drying, it was powdered using the motor and pestle.

Around 100 gm of air-dried powdered plant material was Placed in Soxhlet apparatus, starting form Petroleum ether then Hydroalcohol (ethanol: water;70:30) for the plant *Cymbidium Sarah Jean*. Every time before removing with next dissolvable, powdered material was air dried beneath 100 ^{O}C. The extracted solvent was evaporated using the water bath at 100 ^{O}C. After the evaporation the extracted samples were stored in cold for further analysis[6].

Phytochemicals Screening of plant *Cymbidium Sarah jean* (Flowers)

Phytochemical screening activity of *Cymbidium Sarah jean* (Flowers) was carried out to analyze the presence of the following compounds present in the plants such as[7]:

1) Alkaloids:

Alkaloids exist as the salts which are organic acids forms. They are easily soluble in water and alcohols. Hence for the study the solvent chemicals which were used were Hydroalcohol (ethanol: water; 70:30). Various tests are there to determine the alkaloid content in the plant extract.

Meyer's Test

Few drops of the Meyer is reagent, 5mg of Hydroalcoholic extract was added. White and pale-yellow precipitate formation indicates the presence of Alkaloids.

Dragendorff'sTest

To 5 mg of the Hydroalcoholic extract 5ml of distilled water was added, few drop of hydrochloric acid was added until the acid reaction occurs. To this few drops of Dragendorff's is reagent was added, formation of orange or orange red precipitate shows the presence of alkaloid.

Wagner's test

5mg of Hydroalcoholic extract with 1.5% of hydrochloric acid. To this few drops of Wagner's reagent was added. Brown ppt indicates the presence of alkaloid.

2) Flavonoids:

Flavonoids are the group of secondary compounds which are present in plants and plays important role in various plant metabolic activities. Qualitative detection of alkaloids can be done by different methods.

Alkaline reagent test

To 10mg of Hydroalcoholic extract 2ml of sodium hydroxide solution is added. Formation of intense yellow colour which on addition of 0.1% HCL gets colorless which indicated the presence of Flavonoid.

Shinoda test

10mg of the Hydroalcoholic extract was dissolved in the irrespective diluents. To this10 drops of dilute HCL was added and small pieces of magnesium was added. Formation of pink, brown or reddish color ppt indicates the presence of Flavonoid.

Carbohydrates:

Carbohydrates are the group of the plant secondary metabolites which are present in the form of sugars. They may be present in the form of monosaccharide, disaccharide and oligosaccharide. There are various tests to determine the carbohydrates in plant extract.

Anthrone test

5mg of Hydroalcoholic extract was shaken with 10ml of water, the solution was filtered and the filtrate was concentrated. 2ml of anthrone reagent solution was added to the solution. Formation of green blue color indicates the presence of carbohydrates.

Benedict's test

5mg of Hydroalcoholic extract was mixed with 10ml of water, filtered and the filtrate was concentrated and to this solution 5ml of Benedict reagent in solution was added and heated for 5mins. Formation of brick red colored ppt confirms the presence of carbohydrates.

Triterpenoids

Triterpenoid are a gathering of mixes which are available in all plants parts. They assume an essential job in the different restorative fields in improvement of anti-toxins. In plants they can be separated utilizing different solvents, for example, ethanol, methanol and watery concentrates. Different test are there to decide the terpenoids nearness and substance of it in plant extricates.

Salkowski's test

5mg of plant extract was dissolved in 2ml of the chloroform and 2 ml of concentrated sulphuric acid was added from the sides of the test tube. Upper layer turns red and lower layer turns yellow with light green color fluorescent which indicating presence of the tri-terpenoids.

Liebermann-Burchard's test

2mg of plant dry extract was dissolved in acetic anhydride, boiling and the cool down fast and to it 1ml of concentrated sulphuric (H_2SO_4) was added along the sides of the test tube slowly. Formation of pink color indicates presence of triterpenoids.

Saponins

Saponins are also a group of secondary metabolites which are present in the plants are grouped under amphipathic which produces foam formation when the aqueous solution is shaken vigorously for 5 to10 mins. Different sources are there from which the saponins have been isolated they may be either plant

source or marine. They possess anti fedants and they also have the activity of plants against microbes and fungi. Various tests are there to identify the presence of saponins.

Honeycomb test

5ml extract was taken in the test tube and to it few drops of 5% sodium bicarbonate solution was added. The test tube was then shaken vigorously and kept for 3 minutes. Honeycomb like froth formation shows the presence of saponins.

Foam test

1 mg of plant extract was taken in a test tube and 20ml of distilled water was added and the tube was shaken vigorously for 15mins. Formation of foam to the length of 1cm indicates the presence of saponins.

Tannins

The tannins are a plant compounds which are distributed in many species of plants, where they play a role in protection from the predators and also plays an important role in plant growth. They are finding in both gymnosperms and angiosperms. The test to determine the plant extract was.

Lead acetate test

5mg of plants extract was mixed with 0.5ml of 1% lead acetate solution. Precipitate formations indicate the presence of the tannin.

Ferric chloride test

5mg of plant extract was dissolved with 0.5ml of 5% ferric chloride solution in the test tube. Development of dark black color indicates the presence of tannins.

Phenols

Phenols are group of plant compounds which are distributed in species of plants, and they also play a very important role in protection from the predators, and also help in plant growth. They are finding in both gymnosperms and angiosperms. The test to determine the phenols in plant extract are.

Sodium hydroxide test

5mg of Hydroalcoholic extract was dissolved with 0.5 ml of 20% sulphuric acid solution. Followed by the addition of 5 drops of aqueous sodium hydroxide solution. Solutions turn blue on addition of aqueous sodium hydroxide which indicates the presence of phenols.

Steroids

Steroids are the gathering of mixes which are available in all plant's parts. They assume an imperative job in the different therapeutic fields being developed of medications and related mixes. In plants they can be removed utilizing different solvents, for example, ethanol, methanol and fluid concentrates. Test to decide the steroids nearness and substance of it in plant extracts.

Salkowski's test

5mg of plant extract was dissolved in 2ml of the chloroform and 2 ml of concentrated sulphuric acid was added from the sides of the test tube without disturbing the ring development at the junction. Upper layer turns red and lower layer turns yellow with light green color which indicating presence of the steroid.

Glycosides

Glycosides are the group of the plant secondary metabolites which are present in the form of sugars. They are the molecules which bound to another functional group with glycosidic bonds. They play important roles in living organisms. Many of the plants stores this chemicals compounds in inactive glycoside which can be made active by enzyme hydrolysis, Test to determine the glycosides is Molisch test.

Anti arthritic activity on Hydroacoholic extract of *Cymbidium Sarah Jean* (Flowers)

Selection of *Cymbidium Sarah Jean* dose for pharmacological studies

Acute toxicity study:

Five groups (n = 5) of male albino mice were used in the acute toxicity study of *Cymbidium Sarah Jean* Hydroalcoholic extract. Animals from all groups were fasted overnight and administered (p.o) with single dose (250, 500, and 3000 mg /kg) of the extract. A group of animals which received equal volume of Normal saline served as control. Changes in the behavior of animals were observed for 24 h after extract administration. For any signs of toxicity and mortality, animals were observed for 14 days[8].

Animals

Healthy 8-week-old female Sprague Dawley (SD) rats (180-200 g) were used in the present study. The animals were procured from College of Veterinary Science and Animal Husbandry Mhow, Indore (M.P), India. They were provided normal diet and tap water ad labium and rats were housed at 23 ± 2°C and 50-65% humidity under a 12:12 ± 1 hour light dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee[9].

Grouping of Animals

The rats were divided into five groups of five animals each as follows.

• Group-1: Vehicle control

• Group-2: Arthritis control

• Group-3: *Cymbidium Sarah Jean* extract 200 mg/kg/day, p.o.

• Group-4: *Cymbidium Sarah Jean* extract 400 mg/kg/day, p.o.

• Group-5: Indomethacin 10 mg/kg/day, p.o.

The method described by (Newbould, 1965) was employed with some modifications. Adjuvant arthritis was induced by subcutaneous injection of Complete Freund's Adjuvant (FCA) (0.1 ml) (Difco Labs, Chennai) into the sub plantar tissue of the right hind paw of each rat. The test groups consisted of FCA -injected rats challenged with their respective doses of the test drug administered orally 24 hours before FCA injection. The vehicle control rats were injected with 0.1 ml of liquid paraffin (Incomplete Freund's Adjuvant) only. The drug treatments were continued for 20 days after inducing arthritis[10].

The swelling in the injected paw and the contralateral hind paw were monitored daily using a mercury displacement Plethysmometer. The increase in the extent of erythema and edema of the tissue shows the severity of the inflammation. The differences between the experimental groups and the arthritis control group were statistically analyzed. The change in body weight was also recorded daily[11].

Biochemical parameters

At the end of the study, blood samples were withdrawn from all groups through retro orbital plexus puncture, and the biochemical parameters were analyzed. (Parasuraman et al.,2010). Hematological parameters such as the hemoglobin (Hb) level, the red blood cell (RBC) count, the white blood cell (WBC) count and the erythrocyte sedimentation rate (ESR) were estimated manually. Liver markers such as SGOT and SGPT were analyzed using an auto analyzer. The liver enzyme levels were estimated using Lab Kit enzymatic kits[12].

Statistical analysis

The mean ± SEM values were calculated for each group. Statistical differences among the groups were determined using one-way ANOVA followed by Tukey's multiple comparison test. $P < 0.05$ was considered to be significant.

Results and Discussion

Administration of the different extractives of Hydroalcoholic extract of *Cymbidium Sarah Jean* in mice at doses of 250, 500 mg/kg and 2000 mg/kg by oral gavages did not reveal any adverse effects or signs of toxicity. Observations twice daily for fourteen days also did not reveal any drug related observable changes or mortality. Accordingly, the acute oral LD_{50} of the extractives was concluded to exceed 2000 mg/kg body weight, the highest dose tested in the study. The arthritic control animals exhibited a significant decrease in body weight compared with the control group. The results showed that indomethacin at 10 mg/kg and *Cymbidium Sarah Jean* at 250 mg/kg and 350 mg/kg ameliorate the weight loss that occurs during arthritis.

The latency of the arthritic secondary response ended after a few days and was characterized by joint swelling and nodule events on the 7th day. Administration of *Cymbidium Sarah Jean* (250 mg/kg) significantly ($P < 0.01$) protected against joint swelling in paws in rats with induced arthritis compared with the arthritis control group. But a significant reduction was observed from day 11 to day 13 in the *Cymbidium Sarah Jean* treated (200 mg/kg) group. However, the effects of *Cymbidium Sarah Jean* at 350 mg/kg were found to be significant ($P < 0.001$) from the initial stage of the secondary response and were maintained throughout the experiment. They were significant ($P < 0.01$) 15-19 days after FCA injection compared with the group treated with the reference standard, indomethacin at 10 mg/kg.

Table 1: Yield of crude extracts

Extracts	**Colour**	**Consistency**	**Yield(% w/w)**
Cymbidium Sarah Jean **(Flowers)**			
Pet ether	Dark brown	Semisolid	10.45%
Ethanol: Water; 70:30	Brown	Semisolid	12.22%

Table 2: Results of Preliminary qualitative phytochemical tests

Sr No.	**Phytoconstituents**	**Test**	**Observations**
1	Alkaloid test	Mayers test	Positive
2	Flavonoid test	Alkaline reagent test	Positive
3	Phenolic content	Sodium hydroxide test	Positive
4	Tannin content	Ferric chloride test	Negative
5	Steroid test	Salkowski's test	Positive
6	Carbohydrate	Benedict's Test	Positive
7	Triterpenoids	Salkowski's test	Positive
8	Saponins test	Saponins test	Positive
9	Glycosides	Glycosides test	Positive

Table 3: Effect of Hydroalcoholic extract of *Cymbidium Sarah Jean* on rodent growth

Group (n=5 in each group)	Body weight (g)		Increase in body weight (%)
	Initial	Final	
Normal control	179±1.62	190.23±2.89	6.27
Arthritis control	183.30±1.66	190.23±2.49	3.78
Cymbidium Sarah Jean, 250 mg/kg	171.80±1.05	181.33±0.35	5.54
Cymbidium Sarah Jean, 350 mg/kg	179±1.52	188.46±7.39	5.28
Indomethacin,10 mg/kg	160.13±0.18	172.16±1.30	7.51

Table 4: Ant arthritic activity of Hydroalcoholic extract of *Cymbidium Sarah Jean* compared with indomethacin in injected paw (swelling volume in ml)

Treatment	Post-insult time of assay (days)						
	1	7	11	15	17	19	21
Normal control	0.13± 0.00	0.11± 0.00	0.11± 0.00	0.11± 0.00	0.11± 0.00	0.12± 0.00	0.11± 0.00
Arthritic control	0.75± 0.00	1.20± 0.00###	0.86± 0.01###	0.87± 0.01###	0.87± 0.01###	0.89± 0.01###	0.92± 0.01###
Cymbidium Sarah Jean, 250 mg/kg	0.73± 0.01	0.89± 0.01***	0.71± 0.00***	0.70± 0.01***	0.74± 0.01***	0.68± 0.00***	0.76± 0.01***
Cymbidium Sarah Jean, 350 mg/kg	0.72± 0.01	0.87± 0.01***	0.81± 0.00***	0.75± 0.01***	0.54± 0.01***	0.52± 0.01***	0.71± 0.01***
Indomethacin,10 mg/kg	0.65± 0.00	0.80± 0.00***	0.68± 0.00***	0.75± 0.00***	0.64± 0.00*** 0	0.63± 0.01***	0.58± 0.01***

Values are expressed as mean±SEM; n=5 rats in each group; ***P<0.001 compared with arthritic control; ###P<0.001 compared with normal control.

Table 5: Effect of the Hydroalcoholic extract of *Cymbidium Sarah Jean* on biochemical and hematological parameters

Group	Biochemical parameter		Hematological parameter			
	SGOT (U/L)	SGPT (U/L)	WBC (cells/cu.mm)	RBC (millions/cu.mm)	ESR (mm/hr)	Hb (gm/dl)
Normal control	106.26±0.13	56.68±0.72	8.31±0.06	5.90±0.05	4.27±0.20	14.05±0.24
Arthritic control	233.68±1.98	159.72±1.72	8.67±0.10##	4.65±0.23###	8.18±0.16###	9.87±0.203###
Cymbidium Sarah Jean, 250 mg/kg	182.86±2.13***	128.86±3.39***	8.25±0.03	5.81±0.22	6.31±0.15***	10.57±0.115***
Cymbidium Sarah Jean, 350 mg/kg	150.91±2.67***	113.64±1.19***	8.29±0.01	5.57±0.14	5.79±0.13***	11.38±0.31***
Indomethacin, 10 mg/kg	127.25±0.77***	94.02±1.62***	8.36±0.01	5.58±0.04	5.15±0.12*	13.14±0.23

Values are expressed as mean±SEM, n = 5 rats in each group, ***P<0.001, **P<0.01 compared with arthritic control, ###P<0.001, ##P<0.01 compared with normal control

Conclusion

The Result of biochemical parameters and hematological parameters shows, elevated SGOT and SGPT levels and reduced RBC, ESR and Hb levels in the arthritic controls compared with the normal controls. Administration of the Hydroalcoholic extract of *Cymbidium Sarah Jean* to arthritic rats (Group-3, Group-4, and Group-5) enhanced the Hb and RBC levels compared with the arthritic control group.

References

1. Mohanty S, Sahoo AK, Konkimalla VB, Pal A, Si SC. Naringin in combination with isothiocyanates as liposomal formulations potentiates the anti-inflammatory activity in different acute and chronic animal models of rheumatoid arthritis. ACS omega. 2020 Oct 26; 5(43):28319-32.
2. Muthukumaran, C., Praniesh, R., Navamani, P., Swathi, R., Sharmila, G. and Kumar, N.M., 2017. Process optimization and kinetic modeling of biodiesel production using non-edible Madhuca indica oil. *Fuel*, *195*, pp.217-225.
3. Newbould, F.H.S. and Neave, F.K., 1965. The recovery of small numbers of Staphylococcus aureus infused into the bovine teat cistern. *Journal of Dairy Research*, *32*(2), pp.157-162.
4. Parekh, J. and CHANDA, S., 2008. Antibacterial activities of aqueous and alcoholic extracts of 34 Indian medicinal plants against some Staphylococcus species. *Turkish journal of Biology*, *32*(1), pp.63-71.
5. Porcheret M, Jordan K, Jinks C, P. Croft in collaboration with the Primary Care Rheumatology Society. Primary care treatment of knee pain—a survey in older adults. Rheumatology. 2007 Nov 1; 46(11):1694-700.
6. Ren SX, Zhang B, Lin Y, Ma DS, Yan H. Selenium nanoparticles dispersed in phytochemical exert anti-inflammatory activity by modulating catalase, GPx1, and COX-2 gene expression in a rheumatoid arthritis rat model. Medical science monitor: international medical journal of experimental and clinical research. 2019; 25:991.
7. Rosen, L.S., Gordon, D., Kaminski, M., Howell, A., Belch, A., Mackey, J., Apffelstaedt, J., Hussein, M., Coleman, R.E., Reitsma, D.J. and Seaman, J.J., 2001. Zoledronic acid versus pamidronate in the treatment of skeletal metastases in patients with breast cancer or osteolytic lesions of multiple myeloma: a phase III, double-blind, comparative trial. *Cancer journal (Sudbury, Mass.)*, *7*(5), pp.377-387.
8. Schett G, Gravallese E. Bone erosion in rheumatoid arthritis: mechanisms, diagnosis and treatment. Nature Reviews Rheumatology. 2012 Nov; 8(11):656-64.
9. Shinde, V.M., Dhalwal, K., Mahadik, K.R., Joshi, K.S. and Patwardhan, B.K., 2007. RAPD analysis for determination of components in herbal medicine. *Evidence-Based Complementary and Alternative Medicine*, *4*(S1), pp.21-23.
10. Tierney M, Fraser A, Kennedy N. Physical activity in rheumatoid arthritis: a systematic review. Journal of Physical Activity and Health. 2012 Sep 1; 9(7):1036-48.

11. Vadell AK, Barebring L, Hulander E, Gjertsson I, Lindqvist HM, Winkvist A. Anti-inflammatory Diet In Rheumatoid Arthritis (ADIRA)—a randomized, controlled crossover trial indicating effects on disease activity. The American journal of clinical nutrition. 2020 Jun 1; 111(6):1203-13.
12. Winkvist A, Bärebring L, Gjertsson I, Ellegård L, Lindqvist HM. A randomized controlled cross-over trial investigating the effect of anti-inflammatory diet on disease activity and quality of life in rheumatoid arthritis: the Anti-inflammatory Diet In Rheumatoid Arthritis (ADIRA) study protocol. Nutrition journal. 2018 Dec; 17(1):1-8.