International Journal of Medical and Pharmaceutical Sciences
Open access peer-reviewed Journal
Volume 1, Issue 11, Page 68-73.
https://doi.org/10.5281/zenodo.17573234

Research Article

Evaluation of Anti-Arthritic Activity of Aqueous Extract of Portulacaria Afra in Formaldehyde Induced Arthritis Model of Rats

Shivam Yadav*[1], Satyam Yadav[2], Dr. Bharat Kumar Taygi[3], Dr. Sandeep Jain[3]

[1]*Assistant Professor BVM College of Pharmacy Gwalior*
[2]*Assistant Professor Vikrant University Gwalior School of Pharmacy*
[3]*IPS College of Pharmacy Gwalior*

Protulacaria afra, a South African plant with a reddish stem and green leaves, is a soft-wooded shrub or tree. It is easy to care for and suitable for sunny locations. Jade plants are used in folk remedies, nausea treatment, epilepsy, diarrhea, corns, and purging the intestines. Protulacaria afra has potential pharmacological properties, including antioxidant and anti-inflammatory effects, which could prevent diseases like cancer and cardiovascular disorders. Further research is needed to fully understand its benefits. The objective of the present study was to assess the anti-arthritic potential of aqueous extract of the leaf of Protulacaria afra and its solvent fractions using the in vitro and in vivo methods. The leaf extract of Protulacaria afra, a dark brown oleo-resinous plant, was found to contain phenolics, terpenoids, flavonoids, and carbohydrates. The crude extract (AEPA) was found to suppress albumin denaturation by 94.30% at a dose of 400 µg/mL, while the standard drug prevented 97.80%. The fractions also reduced paw diameter by 91.99% (EAFPE) and 80.87% (MFPE) at the highest dose studied. The hexane and aqueous fractions did not significantly suppress denaturation. The results obtained from the in vivo studies suggest that the ethyl acetate fraction of the aqueous extract had better anti-arthritic activity compared to the extract itself and the methanol fraction. Further investigations need to be carried out for determining the active principle in extract responsible of the anti-inflammatory action.

Keywords: Protulacaria afra , Formaldehyde induced Arthritis , Aqeous extract of Protulacaria afra , anti-inflammatory , phenolics, terpenoids, flavonoids, and carbohydrates.

INTRODUCTION

Portulacaria afra is a small-leaved succulent plant native to South Africa, known for its reddish stem and green leaves. It is easy to care for and suitable for sunny locations. P. afra is a soft-wooded, semi-evergreen upright shrub or small tree, usually 2.5-4.5 meters tall. Its trunk is stout and juicy-stemmed, with a diameter of 20 cm or more [1]. The leaves are opposite, obovate, glabrous, and blunt green, usually less than 1.3 cm long. Jade plants are not a major component of herbal or alternate medicine, but they are recommended for warts in folk remedies, nausea treatment, epilepsy, diarrhea, corns, and purging the intestines. In Africa, it is used to treat epilepsy, diarrhea, corns, and purge the intestines [2]. In China, a variety with pointed leaves called the stone lotus is used to treat diabetic symptoms. Portulacaria afra, also known as elephant bush or spekboom, is a South African plant with potential pharmacological properties. Studies have shown that it has antioxidant properties, which could prevent diseases like cancer and cardiovascular disorders. It also has anti-inflammatory effects, making it a potential treatment for inflammatory conditions [3]. Additionally, it may have antimicrobial properties, making it effective against pathogens, which could improve overall health. However, more research is needed to fully understand its mechanisms of action and its potential benefits for human health [4]. The present study

aimed to extract secondary metabolites from Portulacaria afrawhole plant using water as a solvent, determine its total phenolic content, obtain various solvent fractions from the crude extract, and explore its anti-arthritic potential in animals.

2 Materials and Methods

2.1 Collection and Identification of the Plant

The plants of *Portulacaria afra*were purchased from Shubham Nursery, Bhopal, Madhya Pradesh and the preliminary identification of the plant was done at RB Science, Bhopal for authentication.

2.2 Preparation for the Extraction of the plant material

The plant leaves were washed with distilled water and dried in shade. The dried leaf was powdered and stored in an airtight container. The extraction was done using distilled water as the solvent using a soxhlet apparatus. The powder was packed in a thimble and heated at 75°C for 11 hours. The extract was filtered and concentrated on a boiling water bath to obtain the oleo-resinous residue. The oleo-resinous extract was collected and stored in desiccators to remove excess moisture [5]. The dried extract was weighed and stored for further analysis. The extract was mixed with distilled water and hexane, then partitioned into hexane, ethyl acetate, and methanol layers. The hexane fraction was separated, while the ethylacetate fraction was separated. The methanol fraction was extracted, and the residual aqueous layer was collected. The solid fraction was evaporated, and the solid fraction was dissolved in distilled water to create the desired strength test solution [6].

2.3 Qualitative and Quantitative estimation of Phytochemical in the extract

Qualitative estimation [7] identifies phytochemicals in plant extracts through chemical tests for the presence of the alkaloids, carbohydrates, glycosides, saponins etc, while quantitative estimation [8] for determines their exact amounts of total phenolics and flavonoids content using spectrophotometric. Both methods provide valuable information but do not accurately measure concentrations, limiting researchers' ability to assess potential health benefits.

2.4 In vitro anti-arthritic activity using egg albumin denaturation inhibition

The reaction mixture (5 ml) included egg albumin (0.2 ml), phosphate buffered saline, 2.8 ml (pH 6.4) and 2 ml of sample (crude extract and fractions respectively) and diclofenac sodium at various concentrations (25, 50, 100, 200 and 400 µg/ml), respectively. Equal volume of double-distilled water served as control. The mixtures were incubated at 37 ± 2 °C in a Biochemical oxygen demand (BOD) incubator for 15 min and then heated at 70 °C for 5 min. Their absorbance was measured at 660 nm [9].

2.5 Pharmacological Study Animals

2.5.1 Animals

Healthy Wistar rats of either sex, weighing 180-250g were used for the study. The animals were housed in cages during the course of experimental period and maintained at 12 day and night schedule with a temperature 17-26°C maintained at standard experimental condition. The animals were fed with standard rodent pellet feed and water ad libitum. The animals were fasted 12 hours before the experiment with free access to only water. The protocol was approved by the institutional ethical committee.

2.5.2 Acute Toxicity Study

A total of three animals were used which received a single oral dose (2000mg/kg) of extract. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a period of 14 days. Once daily observations were made for changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, perspiration, urinary incontinence, and defecation) and central nervous system (drowsiness, tremors and convulsion) changes. Mortality, if any, was also observed over the period of 2 weeks [10].

2.5.3 Anti-arthritic action using formaldehyde induced arthritis method

The study involved animals divided into seven groups: Control, Standard Drug (Aspirin), Aqueous Extract of *Protulacaria afra* (AEPA), Ethylacetate

fraction (EAFPE), and Methanol fraction (MFPE). Arthritis was induced by subplantar injection of 2% formaldehyde solution on day 1 and repeated on day 3. The drug treatment was sustained for 10 days, and arthritis was evaluated by checking the mean increase in paw diameter for 10 days via digital vernier caliper on days 2, 4, 6, 8, and 10 [11]. The results were compared with the control group.

2.6 Statistical Analysis

The study parameters were conducted in triplicate. Mean ± standard deviation was used to express the data. To compare the groups, one-way analysis of variance was applied with the aid of Graph Pad Prism Version 7. Statistical significance was considered as $p<0.05$.

3 Results and Discussion

3.1 Extraction Yield

The study investigated the effect of *Protulacaria afra* extract and solvent fractions on formaldehyde-induced arthritis in rats. The extract was obtained from a dark brown, oleo-resinous leaf and had a dry weight of 13.9% compared to the dry leaf powder. The plant was authenticated at RB Science and MFP-PARC, Bhopal.

3.2 Phytochemical screening of AEPA

A small fraction of the dried extracts were subjected to the phytochemical screening for detecting the presence alkaloids, glycosides, tannins, saponins, flavonoids, terpenoids proteins and carbohydrates (Table 1).

Table 1: Phytochemical screening of AEPA

Phytochemical	Test	Observation	Inference
Alkaloid	*Mayer's reagent*	no precipitate	Alkaloid Absent
	Hager's reagent	no precipitate	
	Wagner's reagent	reddish brown colour not obtained	
	Dragendorff's reagent	reddish brown precipitate not obtained	
Flavonoid	*Shinoda test*	red color	Flavonoid Present
	Alkaline reagent test	Yellow color that turns red on acidification	
	Zinc HCl reduction test	red color	
Glycoside	*Froth Test*	No Frothing	Glycoside absent
	Bontrager's Test	Rose pink or red color in the ammonical layer not found	
	Keller-Kiliani Test	No color in acetic acid layer	
Phenolic and Tannins	*Ferric chloride*	Blue green color	Phenolics and Tannins present
	Gelatin Solution	White precipitate	
	Alkaline reagent test	Yellow to red precipitate	
	Vanillin HCl test	Purplish red color	
Proteins	*Millon's Test*	no precipitate	Protein absent
	Ninhydrin Test	No coloration	
Carbohydrates	*Molisch's Test*	Purple ring	Carbohydrate present
Triterpenoids	*Salkwoski Test*	Yellow color in lower layer	Triterpenes present

3.3 Total Phenolic Content

AEPA was evaluated for quantification of the total phenolic content concentration in extract. Standard curve of gallic acid was plotted in distilled water for determining absorption data. From this Beer's law range and regression coefficient is determined. The linear equation of gallic acid was found to be $y = 0.0048x + 0.0052$ with a R^2 value of 0.9972. The total phenolic content in extracts, expressed as gallic acid equivalents. The total phenolic content of AEPAwas found to be 26.90 ± 1.185 GAEmg/g.

3.4 Evaluation of In vitro anti-arthritic activity using egg albumin denaturation inhibition

The in vitro study of prevention of denaturation of egg albumin was taken as the measure of ability to reduce inflammation, pain and inhibiting arthritic mediators. The results obtained suggested that the crude extract (AEPA) was able to suppress 94.30 % denaturation of albumin at dose of 400 µg/mL while at the same dose standard drug prevented 97.80% denaturation. The fractions were also able suppress denaturation by 91.99 % (EAFPE) and 80.87% (MFPE) at the highest dose studied. The hexane and aqueous fractions could not suppress denaturation significantly (Table 2).

Table 2 Inhibition of albumin denaturation by P. afra aqueous extract and its solvent fractions

Treatment	Inhibition of egg albumin denaturation (%)				
	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL
AEPA	31.51 ± 0.748	47.50 ± 0.435	61.19 ± 0.129	78.85 ± 0.591	94.30 ± 0.241
HFPE	13.25 ± 0.248	18.75 ± 0.387	26.63 ± 0.318	31.59 ± 0.470	35.68 ± 0.195
EAFPE	28.42 ± 0.748	44.70 ± 0.128	59.24 ± 0.210	75.27 ± 0.340	91.99 ± 0.908
MFPE	25.31 ± 0.301	37.50 ± 0.435	47.34 ± 0.285	62.38 ± 1.154	80.87 ± 0.189
AFPE	22.14 ± 0.248	26.98 ± 0.560	31.11 ± 0.106	36.55 ± 0.380	41.36 ± 0.470
Diclofenac	75.63 ± 0.289	76.83 ± 0.560	81.45 ± 0.438	92.98 ± 0.272	97.80 ± 0.673

3.5 Evaluation of Acute toxicity

The acute toxicity test was performed by using the dried AEPA at concentration of 2000 mg/kg to the test animal, administered orally. No animal died and hence the dose of upto 2000 mg/Kg was considered to be safe. As none of the animals died, the LD_{50} was considered to be more than 2000 mg/Kg and any dose less than 2000 mg/Kg would be considered for evaluation of anti-arthritic action. Also, since fractions were also taken from the extract, the same dose (200 mg/kg) was used for extracts and fractions.

3.6 Evaluation of Anti-Arthritis Activity on Formaldehyde Induced Arthritis Study

Formaldehyde-induced arthritis is one of the most suitable test procedures for primary screening of anti-arthritic agents. As shown in the table7.4, AEPA was able to reduce the paw diameter by 67.58% on the 10^{th}day. On the other hand, EAFPE and MFPE reduced the paw diameter by 69.81 % and 62.30% respectively at the same time period. The standard drug was able suppress the paw diameter by 73.41% on the 10^{th} day of treatment (Table 3).

Table 3: Change in paw diameter by extract and solvent fractions

Group	Paw diameter (mm)				
	2 day	4 day	6 day	8 day	10 day
Disease control	8.34 ± 0.029	9.53 ± 0.051	10.81 ± 0.073	12.97 ± 0.042	13.05 ± 0.033
Aspirin	3.98 ± 0.033	3.92 ± 0.010	3.73 ± 0.043	3.71 ± 0.018	3.47 ± 0.042
AEPA	4.31 ± 0.032	4.49 ± 0.102	4.62 ± 0.068	4.56 ± 0.104	4.23 ± 0.051
EAFPE	4.16 ± 0.044	4.11 ± 0.069	4.35 ± 0.074	4.11 ± 0.036	3.94 ± 0.073
MFPE	5.01 ± 0.033	5.15 ± 0.068	5.17 ± 0.102	5.11 ± 0.073	4.92 ± 0.044

Results are reported as mean ± SD (n=5)

The results obtained from the *in vivo* studies suggest that the ethyl acetate fraction of the aqueous extract had better anti-arthritic activity compared to the extract itself and the methanol fraction. The activity of the ethyl acetate fraction was almost similar to standard drug aspirin (Figure 1).

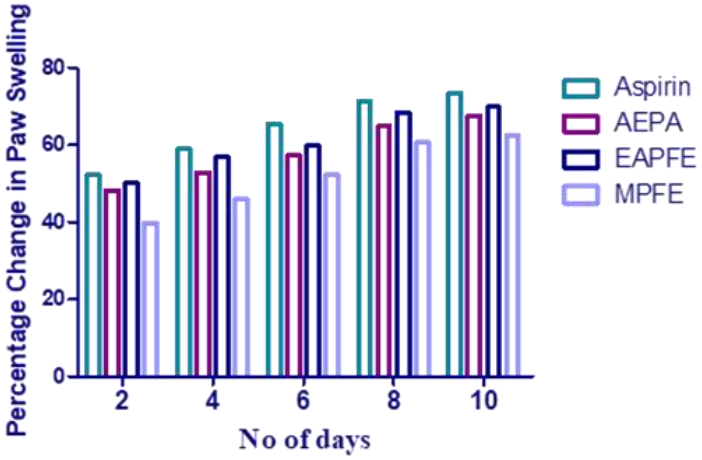


Figure 1: Comparative anti-arthritic effect of aspirin, AEPA, EAFPE and MFPE

CONCLUSIONS

The study investigated the effects of *Protulacaria afra* leaf extract and solvent fractions on formaldehyde-induced arthritis in rats. The extract was dark brown and oleo-resinous, with a dry weight of 13.9%. The extract contained phenolics, terpenoids, flavonoids, and carbohydrates. The crude extract (AEPA) suppressed 94.30% denaturation of albumin at a dose of 400 µg/mL, while the standard drug prevented 97.80%. The fractions also suppressed denaturation by 91.99% (EAFPE) and 80.87% (MFPE) at the highest dose. The study found that AEPA reduced paw diameter by 67.58% on the 10th day, while EAFPE and MFPE reduced it by 69.81% and 62.30%, respectively. The standard drug suppressed paw diameter by 73.41%. In this study, it was found that show ethyl acetate fraction of aqueous extract has superior anti-arthritic activity, requiring further investigation to determine active principle.

Conflict of Interest

None.

REFERENCES

1. De Vos B, Hayeshi RK, Pheiffer W, Nyakudya TT, Ndhlala AR. A Review on the anti-hyperglycaemic potential of Catharanthus roseus and Portulacaria afra. South African Journal of Botany. 2023 Dec 1; 163:1-9.
2. Basson DC, Van Vuuren S, Risenga IM. The effect of elevated carbon dioxide on the medicinal properties of Portulacaria afra. South African Journal of Science. 2024 Feb;120(1-2):1-7.
3. Du Toit A, MacDonald R, Steyn E, Mahlanza ZP, Zulu AB, De Wit M. Review of the underutilized indigenous portulacaria afra (spekboom) as a sustainable edible food source. Agronomy. 2023 Apr 25;13(5):1206.
4. Ijaz S, Iqbal J, Abbasi BA, Kanwal S, Tavafoghi M, Ahmed MZ, Mahmood T. Investigation of bioactive constituents and evaluation of different in vitro antimicrobial, antioxidant, and cytotoxicity potentials of different Portulacaria afra extracts. Journal of King Saud University-Science. 2024 Feb 1;36(2):103033.
5. Basson DC, Teffo TK, Risenga IM. A phytochemical screening, antioxidant and antibacterial activity analysis in the leaves, stems and roots of Portulacaria afra. Journal of Herbmed Pharmacology. 2022 Dec 31;12(1):109-17.
6. Tabassum S, Ahmad S, Rehman Khan KU, Tabassum F, Khursheed A, Zaman QU, Bukhari NA, Alfagham A, Hatamleh AA, Chen Y. Phytochemical profiling, antioxidant, anti-inflammatory, thrombolytic, hemolytic activity in vitro and in silico potential of Portulacaria afra. Molecules. 2022 Apr 7;27(8):2377.
7. Seiyaboh EI, Seiyaboh Z, Izah SC. Environmental Control of Mosquitoes: A Case Study of the Effect of MangiferaIndica Root-Bark Extracts (Family Anacardiaceae) on the Larvae of Anopheles Gambiae. Annals of Ecology and Environmental Science. 2020; 4(1): 33-38.
8. Godhwani S, Godhwani JL, Vyas DS. Ocimum sanctum: an experimental study evaluating its

anti-inflammatory, analgesic and antipyretic activity in animals. J Ethnopharmacol 21(2):1987, 153-163.

9. Leelaprakash G, Mohan Dass S (2011). In vitro anti-inflammatory activity of methanol extract of Enicostemmaaxillare. International Journal of Drug Development & Research, 3: 189-196
10. Jonsson M, Jestoi M, Nathanail AV, Kokkonen UM, Anttila M, Koivisto P, Karhunen P, Peltonen K. Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin. Food and chemical toxicology. 2013 Mar 1; 53:27-32.
11. Alamgeer, Uttra AM, Hasan UH. Anti-arthritic activity of aqueous-methanolic extract and various fractions of Berberisorthobotrys Bien ex Aitch. BMC Complementary and Alternative Medicine (2017) 17:371. DOI 10.1186/s12906-017-1879-9.

Cite: Shivam Yadav*, Satyam Yadav, Dr. Bharat Kumar Taygi, Dr. Sandeep Jain, Evaluation of Anti-Arthritic Activity of Aqueous Extract of Portulacaria Afra in Formaldehyde Induced Arthritis Model of Rats, Int. J. Med. Pharm. Sci., 2025, 1 (11), 68-73. https://doi.org/10.5281/zenodo.17573234