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Anti-arthritic potential of jatropha curcas flowers extract using complete freund's adjuvant (CFA)-induced arthritis model

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ABSTRACT

Jatropha curcas is a member of the euphorbiaceae family and has a number of therapeutic benefits. The anti-arthritic potential of Jatropha curcas flowers extracted using various solvents is the focus of the current investigation. The use of several antioxidant assays has been researched for qualitative and quantitative evaluation of isolated chemicals and antioxidant activity. By administering crude extract orally at dose concentrations of 200 mg/kg and 400 mg/kg to rats that had Complete Freund's adjuvant (CFA)-induced arthritis, it was possible to assess the anti-arthritic effectiveness of the chosen solvent, ethanolic extract. On the 28th day of treatment, paw volume analysis and haematological parameters were analysed. The results show that extract from Jatropha curcas flowers has a sizable anti-arthritic effect.

1. Introduction

The immune system in our bodies is extremely important because an overactive immune system can cause fatal diseases due to various hypersensitive or allergic reactions that can cause many abnormalities and a loss of the ability to distinguish between self and non-self, which can lead to autoimmune diseases. Due to their unclear aetiologies, some prevalent autoimmune disorders, including reactive arthritis, pernicious anaemia, myasthenia gravis, and serum sickness, pose serious problems for the medical and pharmaceutical industries. Rheumatoid arthritis (RA) affects 0.3–1% of the world's population, and of those who have the condition, women are three times more likely than men to have it. A systemic, inflammatory, and persistent autoimmune condition, RA.[1] Pain, swelling, and bone and cartilage degradation are the main signs and symptoms of RA, which can lead to permanent disability. Although the actual cause is unknown, numerous theories claim that a mix of genetic predisposition and exposure to environmental factors like viruses causes it to manifest.[2] Although the specific pathophysiology is still unknown, the generation of several free radicals, including nitrous oxide and superoxide radicals, which are metabolic byproducts of cells, is a known factor. As a result of the release of these free radicals, T-cells may produce interleukins (IL) and tumour necrosis factor (TNF-), which may then affect the production of growth factors, cytokines, and adhesive molecules on immune cells.[3] These molecules may then cause tissue destruction and inflammation. Synovial membrane hyperplasia, inflammatory cell infiltration, and neovascularization are pathological alterations in RA that cause cartilage degradation and articular degeneration.[1] Eliminating symptoms, slowing the disease's course, and improving quality of life are the three main objectives of treatment for rheumatoid arthritis sufferers.[4] Therefore, before beginning the treatment for RA, certain objectives

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such as easing analgesia, reducing inflammation, protecting articular structure, maintaining function, and controlling systemic involvement must be kept in mind.[3] Currently, non-steroidal anti-inflammatory medicines (NSAIDs) or disease-modifying antirheumatic drugs (DMARDs) are being replaced by innovative biological agents, such as TNF monoclonal antibody, in the treatment of RA. Five clinical strategies are used to treat RA. The primary strategy is the use of NSAIDs followed by low doses of glucocorticoids to reduce inflammatory symptoms and disease development. DMARDs, such as methotrexate, sulfasalazine, gold salts, or D-pencillamine, can be used in the treatment of chronic patients. It is possible to treat chronic conditions using TNF-neutralizing medications like infliximab, etanercept, etc., IL-1 neutralising medications like anakinra, and medications that prevent T-cell activation like abatacept. The treatment of chronic patients also includes the use of immunosuppressive and cytotoxic medications including cyclosporine, azathioprine, and cyclophosphamide.[3,5] The therapeutic medications indicated above lessen joint damage and inflammation, although it is yet unclear what hazards they may have in the long run. Drugs can cause gastrointestinal ulcers, cardiovascular issues, hematologic toxicity, nephrotoxicity, pulmonary toxicity, myelosuppression, cirrhosis, diarrhoea, immunological reactions, and local injection-site reactions as long-term side effects. Additionally, ongoing monitoring is necessary due to higher expenses and adverse effects, which include significant risks of infections and cancer. It is not hyperbole to claim that the usage of herbal remedies dates back to the dawn of humankind.[6] They have long been used to treat a variety of illnesses. Herbal medications are created by combining the therapeutic knowledge of generations of doctors who have been practising an old system of medicine for more than a thousand years.[7] Because the currently available medications either have undesirable side effects or are very expensive, researchers are now very interested in the therapeutic compounds originating from plants.[8] As a source of therapeutic substances for the prevention and treatment of many diseases, nature has endowed us with an enormous richness of herbal plants that are widely scattered across the world.[9] WHO estimates that 80% of people worldwide use herbal medicines for their basic medical requirements. Since the birth of civilisation, herbal medicines have been used to treat illness.[10] Chemical components that create the required physiological impact on the body make up these herbal plants' medicinally significant parts.[11] Ayurveda, Unani, Sidha, Homeopathy, and Naturopathy are a few of the legally recognised alternative medical systems that have been practised in India since antiquity.[12] There are over 2500 plant species in India that are now used to make herbal medicines. Herbal remedies have been used for more than three thousand years, either directly as traditional medicine or indirectly in the creation of modern pharmaceuticals.[13] Thus, by using what is known about conventional plants, new, potent medications may be developed that are also more affordable. We have made an effort to cover all of the ayurvedic approaches used to treat RA in this article without mentioning any potential negative effects. Future RA treatments ought to offer greater alleviation.[3] The flowering plant species known as Jatropha curcas, a member of the Euphorbiaceae spurge family, is indigenous to the American tropics, most likely in Mexico and Central America. It is originally from the tropical parts of the Americas, from Mexico to Argentina. Since then, it has migrated to other tropical and subtropical regions of the world, where it has either naturalised or become invasive.[1] Garcia de Orta, a Portuguese physician, coined the term "curcas" for the first time more than 400 years ago.[2] The physic nut is also known as the Barbados nut, the poison nut, the bubble bush, or the purging nut.[1] It is sometimes referred to as "castor oil plant" or "hedge castor oil plant" in parts of Africa and Asia, including India, however it is not the same as the typical castor oil plant, Ricinus communis (they are in the same family but different subfamilies). J. curcas is a tiny, semi-evergreen shrub or tree that can grow to a height of at least 6 metres (20 feet). It can thrive in deserts because it can withstand extreme aridity.[3,4] It includes hazardous phorbol esters, which are found in it. However, there are other edible (non-toxic) indigenous plants to Mexico, among them the piónmanso, xuta, chuta, and aishte that are popular among the locals.[5,6] Additionally, J. curcas contains substances such phytate, saponins, trypsin inhibitors, and a lectin class known as curcin.[7,8]

2. Materials and Methods

2.1. Sample collection

Flowers of the Jatropha curcas plant were harvested in the Bhopal region of Madhya Pradesh, India, and verified by Dr. Zia-Ul-Hasan, Head & Botonist, Safia College of Science.

2.2. Extraction & phytochemical screening

The powdered plant materials were extracted in stages utilising a continuous hot percolation process and a Soxhlet apparatus, first with petroleum ether, then ethanol, and finally water (hydroalcoholic). The yield of a solid mass was obtained by evaporating the solvent at 40^0C. Using conventional techniques, preliminary phytochemical screening, total phenolic content, and total flavonoid content were calculated.[14]

2.3. Total phenol content

Using gallic acid as a reference, TPC was calculated using the Folin-Ciocalteu reagent. A test sample containing 0.5 ml of various Gallic acid concentrations, 2 ml of Folin-

Ciocalteu reagent (1:10 in deionized water), and 4 ml of sodium carbonate solution was also made. This solution was then incubated for 30 minutes at room temperature with periodic shaking. At 765 nm, absorbance was measured. Gallic acid's standard curve was created.[15]

2.4. Total flavonoid content

A diluted sample volume of 0.5 ml was combined with 2 ml of distilled water before being added to 0.15 ml of a 5% NaNO2 solution. After incubating for 6 minutes, 0.15 ml of a 10% AlCl3 solution was added. The stock then received 2 ml of a 4% NaOH solution. Bring the final volume up to 5 ml right away, then leave it alone for an additional 15 minutes. Standard curves for various Rutin concentrations were developed after measuring the absorbance at 510 nm.[16]

3. In-vitro Antioxidant Activity of Extracts

3.1. DPPH assay

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to determine the extracts' in vitro free radical scavenging capability. Test samples in concentrations of 10-100 μg/ml were prepared along with a 0.1 mM methanolic solution of DPPH. 1ml of DPPH solution and 2ml of test material were combined, then incubated in the dark for 10 minutes. Using a double-beam UV-Vis spectrophotometer, absorbance at 515 nm was measured, and the percentage of inhibition was calculated.[17]

3.2. Super oxide scavenging assay

In order to evaluate absorbance at 560 nm against a blank and a reaction mixture without extract as a control, a reaction mixture containing Nitro blue tetrazolium (NBT), 0.3 ml of extract, and 1 ml of alkaline DMSO was made.[18]

3.3. Reducing power assay

In a 0.5 ml sample of various concentrations, 0.5 ml of potassium ferricyanide was added, and the mixture was then incubated at 50 °C for 20 minutes. After cooling, 1.5 ml of trichloroacetic acid was added, and when 0.5 ml of ferric chloride was added, absorbance at 700 nm was measured. Ascorbic acid served as a benchmark substance.[19]

4. In-vivo studies of Jatropha Curcas Flowers Extract

4.1. Experimental animals

The Institutional Animal Ethical Committee (IAEC) at the Pinnacle Biomedical Research Institute in Bhopal, India, approved the use of animals for the current investigation. The Control and Supervision of Trials on Animals (CPCSEA) guidelines were followed during the animal experiments. In the current investigation, anti-arthritic activity was tested on healthy albino wistar rats that were 8–10 weeks old and weighed 150–200g. The animals were acclimated in cages with regular environmental conditions, including temperature (23± 1°C) and 12-hour light/dark cycles. The animals were fed a regular pellet diet and had unlimited access to water.

4.2. Acute oral toxicity

In accordance with OECD (423) recommendations, the acute toxicity investigation for the extract of Jatropha curcas flowers was carried out. The Wistar rats were chosen at random. Four treatment groups with dosages of 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg body weight are included in the test groups. Based on the animal's body weight on the day of treatment, individual doses were computed. The animals were closely monitored for behavioural changes, mortality, and appearance for the first four hours, then frequently for the next twenty-four hours, and finally every day for two weeks.

4.3. In-vivo chronic inflammatory disorders

The effect of plant drugs on chronic inflammatory diseases was assessed using the Complete Freund's adjuvant (CFA) and Monoiodoacetate (MIA) generated Arthritis models.

4.4. Complete freund's adjuvant (CFA) induced Arthritis

Wister rats were utilised to test the anti-arthritic efficacy of the Jatropha curcas extract using Freund's adjuvant-induced arthritis paradigm. The extract with the highest antioxidant findings was chosen for further in-vivo testing. Four groups of six animals each were formed using a random number generator (n=6). Freund's adjuvant (0.01 ml) was given to Group I animals as an arthritic control; Indomethacin (10 mg.kg-1 p.o.) was given to Group II animals as a reference standard; Group III animals received a lower dose of 200 mg/kg of crude extract; and Group IV animals received a higher dose of 400 mg/kg of crude extract. The size of the paws is a sign of arthritis. Giving the extracts to the animal 30 minutes before administering Freund's adjuvant and continuing until the 28th day allowed researchers to evaluate their anti-arthritic effectiveness. On days 0, 7, 14, 21, and 28, the size of each paw was measured using electronic digital callipers. Blood samples were taken from the retro-orbital plexus after 28 days and analysed for total leukocyte counts (TLC), differential leukocyte counts, and other factors (DLC).

5. Results and Discussion

5.1. Qualitative and quantitative phytochemical screening

The phytochemical screening of the flowers of Jatropha curcas revealed the highest concentrations of alkaloids, flavonoids, terpinoid, phenols, and carbohydrates, while the ethanolic extract also contains glycosides.

Table 1: Preliminary phytochemical screening ofJatropha curcas flowers extract

S.No.	Phytoconstituents	Petroleum ether	Ethanol	Water
1	Alkaloid	Present	Present	Present
2	Flavonoid	Absent	Present	Present
3	Terpinoid and steroids	Absent	Present	Present
4	Saponins	Absent	Absent	Absent
5	Tannin/Phenols	Absent	Present	Present
6	Carbohydrates	Absent	Present	Present
7	Reducing sugars	Absent	Absent	Absent
8	Glycosides	Absent	Present	Absent
9	Protein & amino acids	Absent	Absent	Absent

Total phenolic and flavonoid content

By using their respective standards gallic acid and rutin, the phenolic and flavonoid content of the ethanolic and aqueous extracts of Jatropha curcas was calculated using the linear regression equation (y =0.0663x-0.6409) r2 = 0.9924.

Table 2: Total flavonoid and total phenol content of flower extracts of Jatropha curcas

S. No.	Extract	Total Phenol	GAE/g	Total Flavonoid	RE/g
1	Ethanol	0.352±0.003	121.02mg	0.453± 0.004	301.341mg
2	Aqueous	0.215± 0.003	63.157mg	0.299± 0.002	104.41mg

Values were performed in triplicates and represented as mean±SD

6. Antioxidant activity of Jatropha Curcas Flowers Extracts

6.1. DPPH and super oxide dismutase assay

By using the DPPH, Super oxide dismutase, and reducing power assays, the antioxidant activity of petroleum ether extract, ethanolic extract, and water extract was evaluated. Table 3 figure 1 displays the IC50 value for the DPPH and superoxide assays to display percent inhibition values.

6.2. Reducing power assay

The dose-response curves for the extracts of Jatropha curcas are shown in the diagram. It was discovered that when concentrations rose, so did the reducing power activity.

Table 3: DPPH and super oxide dismutase free radical scavenging activity of flowers extracts of Jatropha curcas

Extracts	IC_{50} value μg/ml	
	DPPH assay	SOD assay
Ascorbic acid (Standard)	26.08	36.27
Petroleum ether	173.44	227.24
Ethanolic	118.20	167.50
Aqueous	115.12	153.22

6.3. Acute oral toxicity

Three single-sex Wistar rats were given ethanol extract of Jatropha curcas flowers in accordance with OECD guidelines. Even at the maximum dose of 2000 mg kg-1, the extract did not exhibit any toxicity or death. Therefore, in the current investigation, ethanolic medication extract dosages of 200 mg (low) and 400 mg (high) were chosen to assess their anti-arthritic effectiveness.

6.4. In-vivo anti-arthritis determination

The more effective antioxidant activity is seen in the ethanolic extract of Jatropha curcas. In order to find out whether the extract from Jatropha curcas flowers has anti-arthritic properties, a Complete Freund's adjuvant (CFA) induced arthritis model was used.

6.5. Complete Freund's adjuvant (CFA) induced arthritis

With the aid of electronic digital callipers, the effect of ethanolic plant extract on Freund's adjuvant (CFA)-induced arthritis was measured, and the total and differential leukocyte counts in blood samples were estimated.

6.6. Paw volume determination

A 200mg/kg and 400mg/kg oral treatment of Jatropha curcas ethanolic extract demonstrates the efficacy on Freund's adjuvant-induced chronic arthritic mice. The notable effect was seen on day 28 at both lower and higher extract concentrations, with means and standard deviations of 1.65±0.02 and 1.33±0.03 respectively, compared to 0.61±0.04 for standard medication Indomethacin.

On the final day of treatment, blood was drawn from the retro-orbital plexus and placed into heparinized capillary vials for analysis. Total leukocyte counts (TLC) and differential leukocyte counts were determined (DLC).

7. Discussion

In the current study, the phytochemical composition of a sample of Jatropha curcas flowers was determined

Table 4: Measurement of paw volume in Freund's adjuvant (CFA) induced arthritis animal model

Day	Standard (Indomethacin)	Group-I	Group-II	Group-III	Group-IV
00	1.70±0.032	1.90± 0.012	0.88± 0.028	1.31± 0.041	1.31± 0.027
07	2.95±0.053	3.55± 0.033	1.71± 0.038	2.31± 0.041	2.09± 0.019
14	3.87±0.035	4.17± 0.045	1.81± 0.019	3.09± 0.013	2.61± 0.002
21	4.86±0.022	5.16± 0.057	1.51± 0.012	2.81± 0.034	2.89± 0.025
28	5.53±0.056	5.92± 0.062	1.03± 0.013	2.28± 0.028	3.31± 0.027

Values were performed in triplicates and represented as mean±SD

Table 5: Effect of Jatropha curcas flowers extract on Haematological parameters

Groups	Total Leukocytes Count	Differential Leukocyte Count
Group I	12318.31±31.041	4368.34±31.552
Group II	5781.31±35.760	2222.36±31.050
Group III	9090.33±41.613	3788.01±30.772
Group IV	7941.27±32.641	3152.84±22.932

Each value represents mean ±SD

using petroleum ether, ethanol, and water as solvents. All three extracts passed a preliminary screening that identified the presence of phytochemicals that may have been in charge of the biologicalactivity. With their wide availability, low toxicity, low cost, and biological properties like anti-oxidant activities, anti-microbial effects, modulation of detoxification enzymes, immune system stimulation, reduction of platelet aggregation, modulation of hormone metabolism, and antineoplastic properties, phytochemicals play an important role.[20] The largest amount of active components are present in the ethanolic extracts in this investigation, followed by the aqueous and petroleum ether extracts, Table 1. Using established methods, the contents of total phenol and total flavonoids were determined. Rutin and gallic acid were utilised as reference materials for phenol and flavonoid detection. Phenol concentration was calculated to be 121.02 mg GAE/g of dry extracts for ethanol and 63.157 mg GAE/g of dry extracts for aqueous extracts. For aqueous and ethanolic extracts, the total flavonoid content was 301.341 mg RE/g and 104.41 mg RE/g, respectively, according to Table 2. Phytochemical components have been found to further the in-vitro antioxidant and in-vivo anti-inflammatory activities of extracts from Jatropha curcas flowers. Numerous techniques, including metal chelation, free radical scavenging effects (DPPH), total antioxidant activity, and reducing power test, have been developed to assess the antioxidant activity of plant compounds. Hydrogen peroxide, super oxide anion radicals, and hydroxyl radicals are frequently utilised to destroy oxygen species. The DPPH assay, superoxide dismutase assay, and reducing power assay of crude extracts were used to measure the in vitro antioxidant activity. Hydrogen peroxide, super oxide anion radicals, and hydroxyl radicals are frequently utilised to destroy oxygen species. The DPPH assay, superoxide dismutase assay, and reducing power assay of crude extracts were used to measure the in vitro antioxidant activity. For the DPPH and super oxide assays, ascorbic acid's determined IC50 values are 26.08 and 36.27 μg/ml, respectively. Jatropha curcas petroleum ether extract had a higher IC50 value (173.44 μg/ml and 227.24 μg/ml) in both assays. The ethanolic and aqueous extracts demonstrated DPPH free radical scavenging activity at 118.20 and 115.12 μg/ml and super oxide scavenging activity at 167.50 μg/ml and 153.2 μg/ml, respectively. Figure displays the IC50 value for the DPPH and superoxide dismutase assay graphically. It was discovered that when concentrations rose, so did the reducing power activity. Comparing ethanolic extract to aqueous and petroleum ether extracts, the maximal reduction power is visible. The in-vivo anti-inflammatory efficacy of Jatropha curcas flowers was therefore determined using the ethanolic extract, which outperformed petroleum ether and aqueous leaf extracts in terms of antioxidant activity. On the basis of OECD recommendations, dose-dependent activity of the extract was tested using single-sex wistar rats. On an animal model of fully developed, adjuvant-induced arthritis, 200 mg/kg at the lower dose and 400 mg/kg at the higher dose were used for the in-vivo detection of anti-arthritic activity. Paw volume measurement seems to be an easy and quick operation to perform in order to gauge the severity of the inflammation and evaluate the impact of the drugs. On day 28, the paw volume determination activity produced a discernible result at both lower and higher extract concentrations, i.e., 1.90±0.012 and 5.92±0.062, respectively, in contrast to standard drug indomethacin, which produced results of 1.70±0.032 and 5.53± 0.056. Following treatment with an ethanolic extract of Jatropha curcas leaves, a haematological parameter's total leukocyte count and differential leukocyte count were also calculated. In comparison to the usual medicine Indomethacin, extract concentrations of 400 mg/kg and 200 mg/kg demonstrated meaningful results.

8. Conclusion

It is possible to draw the conclusion that Jatropha curcas ethanolic extract has effective anti-oxidant and anti-arthritic potential on the basis of the current investigation and the obtained results.

9. Conflict of Interest

The authors declare no conflict of interest

10. Source of Funding

None.

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