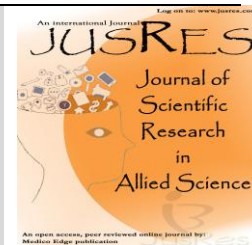




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## Extraction, Phytochemical Screening and Anti-arthritic Potential of Putranjiva Roxburghii

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### ABSTRACT

Using a rat model of Freund's adjuvant-induced arthritis, this study sought to assess the anti-arthritis potential of Putranjiva roxburghii's hydroalcoholic extract by looking at how it affected paw volume, a crucial indicator of inflammation. In order to provide a thorough understanding of the extract's bioactive components and potential therapeutic effects, the research also evaluated the extraction yield, phytochemical profile, and chemical analysis of the extract. The hydroalcoholic extract's high yield was also noted in the study, indicating that it might be a useful source of bioactive substances. In light of the noteworthy anti-arthritic benefits seen in this animal model, Putranjiva roxburghii shows promise as a possible treatment for inflammatory conditions like arthritis. In order to assess the extract's safety and effectiveness in clinical trials, as well as to investigate the precise mechanisms by which it works, more research is required. The findings of this investigation add to the increasing amount of data demonstrating Putranjiva roxburghii's therapeutic benefits and possible use in the management of long-term inflammatory diseases.

### ORIGINAL RESEARCH ARTICLE

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## INTRODUCTION

### Arthritis

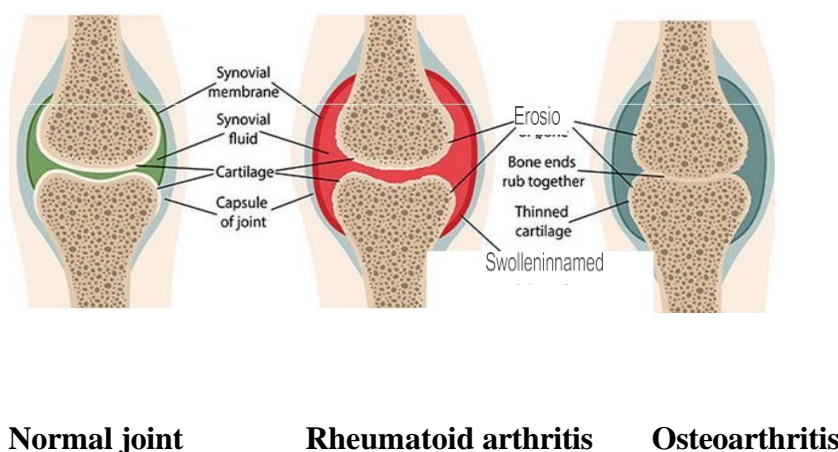
Arthritis is derived from the Greek term "disease of the joints." It is defined as an acute or chronic joint inflammation that often co-exists with pain and structural damage. Arthritis is not synonymous with arthralgia, which refers to pain localized to a joint, regardless of the origin of the pain (which may or may not be due to joint inflammation). Arthritis affected both the Neanderthals and ancient Egyptians, but it was not until 1886 that Dr. John K. Spencer coined the term "osteoarthritis." More than

100 different types of arthritis have been described, the most common being osteoarthritis or degenerative arthritis which is non-inflammatory arthritis. Inflammatory arthritis can occur in several settings, and inflammation can be caused by autoimmune processes (rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, etc.), crystal deposition induced inflammation (gout, pseudogout, basic calcium phosphate disease) or infections (septic arthritis, Lyme's arthritis). Inflammatory arthritis can also accompany other autoimmune connective tissue diseases such as systemic lupus

erythematosus, Sjogren syndrome, scleroderma, myositis, inflammatory bowel disease, celiac disease, etc.

**Epidemiology** During the last 30 years numerous scientists have extensively studied variation of the prevalence and incidence of RA. These studies have demonstrated that RA is a global disease distributed worldwide, regardless of race, sex, ethnicity, nationality, age, etc. However, the results of prevalence and incidence measurements vary depending on the population characteristics and have

changed over time. The prevalence of RA has been rising almost unanimously since 1990 up to date. The largest increase was observed in the Spanish population. However, in Japan and Argentina the prevalence ratios have decreased over the years. Nowadays, the global prevalence ratio of RA is about 1% and it is more common in women, with small continuous fluctuations and an apparent growth from south to north, and from countryside to metropolitan areas.



**Figure 1: Pathophysiology of arthritis**

## EXPERIMENTAL WORK

### Collection of Plant material

In the month of April 2024, leaves of *Putranjiva roxburghii* have been obtained from a local area in Bhopal.

### Drying

Drying of fresh plant parts was carried out in under the shade. Dried leaves of *Putranjiva roxburghii* were preserved in plastic bags, closed tightly and powdered as per the requirements.

### Extraction procedure

Extraction is an essential step in phytochemical processing for the finding of bioactive secondary metabolite from plant materials. For the standardization of herbal products, selection of a suitable extraction technique is also important. Extraction is

used in the removal of desirable soluble constituents, exclusion those not required with the help of the selected solvents. The collected plant materials were thoroughly washed in tap water and rinsed in distilled water. The cleaned, healthy collected plant samples were cut into small pieces and dried under shade for 3 to 4 weeks. Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs: **Defatting of plant material**

60 g of dried *Putranjiva roxburghii* were coarsely pulverised and extracted with petroleum ether using the soxhlation technique. The extraction was continued till the defatting of the material had taken place.

### Extraction by soxhlation process

Defatted dried powdered of *Putranjiva roxburghii* has been extracted with hydroalcoholic solvent (ethanol: water; 75:25) using soxhlation method for 48 hrs., filtered and dried using vacuum evaporator at 40°C.

#### **Determination of percentage yield**

The extraction yield is evaluated of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage.

#### **Phytochemical Screening**

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (Chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (Alkaloids, terpenoids, phenols, flavonoids etc.). All of the extracts were subjected to phytochemical analysis using conventional procedures.

### **RESULTS AND DISCUSSION**

#### **Determination of percentage yield**

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#### **Determination of percentage yield**

To measure the standard extraction efficiency for a given plant, different portions of the same plant, or different solvents utilised, obtaining the percentage yield of extraction is a very essential phenomenon in phytochemical extraction. Table 7.1 shows the yield of extract obtained from samples using hydroalcoholic solvent.

**Table 1: % Yield of *Putranjiva roxburghii***

S. No.	Extracts	% Yield (w/w)
1.	Pet. Ether	0.64%
2.	Hydroalcoholic	9.52%

#### **Results of estimation of total phenol and flavonoids content of *Putranjiva roxburghii* extract Estimation of total phenol content (TPC)**

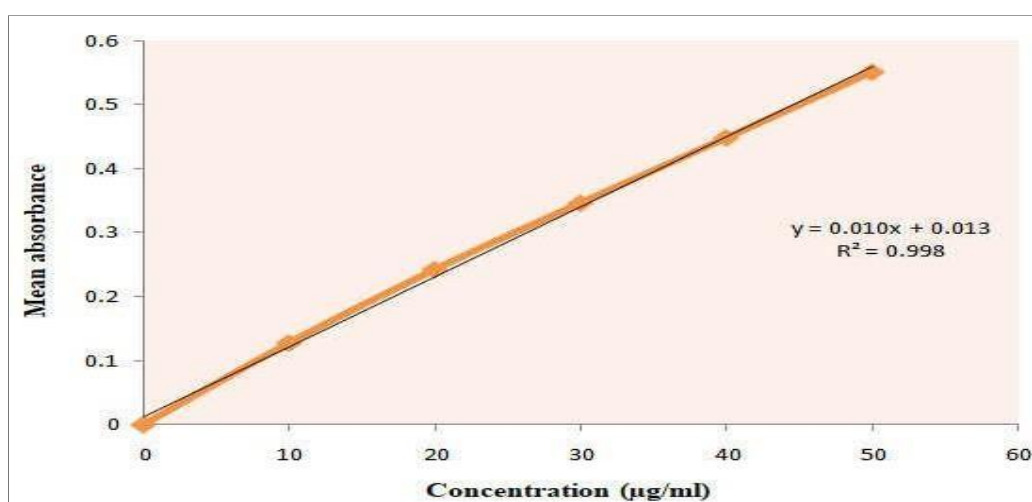
Using the equation obtained from the calibration curve:  $y = 0.010x + 0.013$ ,  $R^2 =$

0.998, where X is the gallic acid equivalent (GAE) and Y is the absorbance, total phenol content was represented as mg/100mg of gallic acid equivalent of dry extract sample.

**Table 2: Preparation of calibration curve of Gallic acid**

S. No.	Concentration (µg/ml)	Absorbance* (Mean ± S.D)
1	10	0.128±0.001
2	20	0.243±0.002
3	30	0.347±0.003
4	40	0.449±0.002
5	50	0.552±0.002

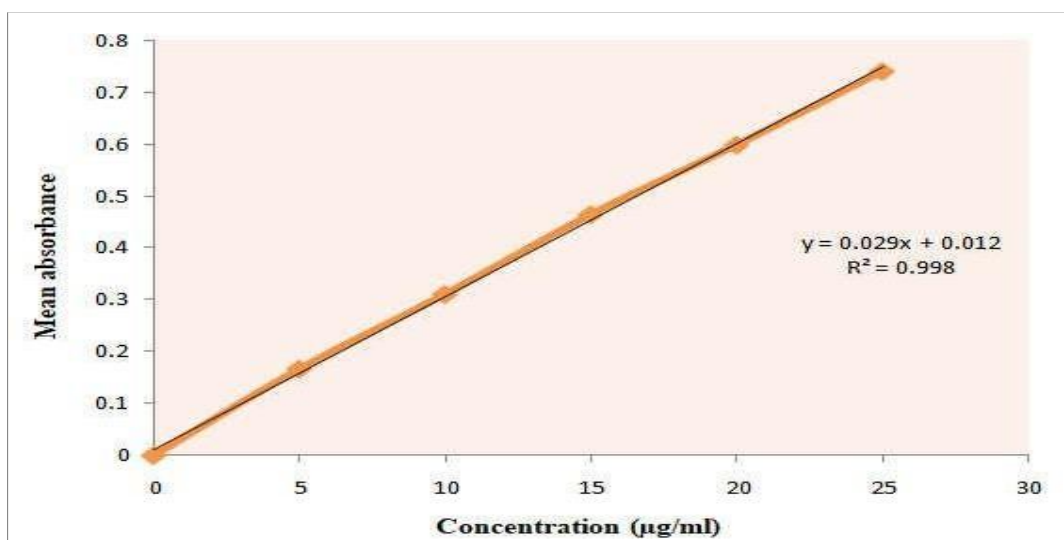
N=3 Average of three determination

**Figure 2: Graph of calibration curve of Gallic acid Estimation of total flavonoids content (TFC)**

Total flavonoids were estimated as quercetin equivalent (mg/100mg) using the equation  $y = 0.029x + 0.012$ ,  $R^2=0.998$ , where X is the quercetin equivalent (QE) and Y is the absorbance.

**Table 3: Preparation of calibration curve of Quercetin**

S. No.	Concentration (µg/ml)	Absorbance* (Mean ± S.D)
L	5	0.167±0.001
2	10	0.311±0.002
3	15	0.465±0.002
4	20	0.599±0.001
5	25	0.741±0.001



N=3 Average of three determination  
Figure 3: Graph of calibration curve of Quercetin

Table 4: Estimation of total phenolic and flavonoids content of Putranjiva roxburghii extract

S. No.	Extract	Total phenol content (mg/ 100 mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.255	0.687

The estimation of total phenolic and flavonoid content in the hydro alcoholic extract of Putranjiva roxburghii, as presented in Table 7.6, provides quantitative insights into the bioactive profile of the extract. The results show that the hydroalcoholic extract contains

0.255 mg of total phenolic content per 100 mg of dried extract and 0.687 mg of total flavonoid content per 100 mg of dried extract.

The relatively higher content of flavonoids compared to phenolic compounds indicates that the extract is particularly rich in flavonoid constituents. Flavonoids are known for their strong antioxidant, anti-inflammatory, and potential therapeutic properties, which could contribute to the medicinal benefits associated with Putranjiva roxburghii. The presence of phenolic compounds, although lower in quantity, also

adds to the extract's antioxidant profile, supporting its potential use in combating oxidative stress and related disorders.

#### Results in vivo anti-arthritis activity

The study investigates the anti-arthritic activity of a hydro alcoholic extract of Putranjiva roxburghii in rats with Freund's adjuvant-induced arthritis, using paw volume as a measure of inflammation. The data show significant differences in paw volume across different treatment groups, with the hydro alcoholic extract of Putranjiva roxburghii demonstrating dose-dependent anti-inflammatory effects.

In the vehicle control group (Group I, 2% gum acacia), there was no significant change in paw volume over the 28-day study period, indicating that the vehicle itself had no impact on inflammation. Paw volume remained consistently low and stable, with a

minor variation between measurements (Day 7:  $0.24 \pm 0.50$  mL, Day 28:  $0.24 \pm 0.25$  mL). This suggests that the vehicle does not exacerbate or alleviate inflammation, serving as an appropriate baseline comparison for the treatment groups.

In comparison, the aspirin-treated group (Group III) showed significant reductions in paw volume starting from Day 14. Aspirin, at

a dose of 200 mg/kg, exhibited a clear anti-inflammatory effect, with paw volume decreasing from  $0.64 \pm 0.10$  mL on Day 7 to  $0.38 \pm$

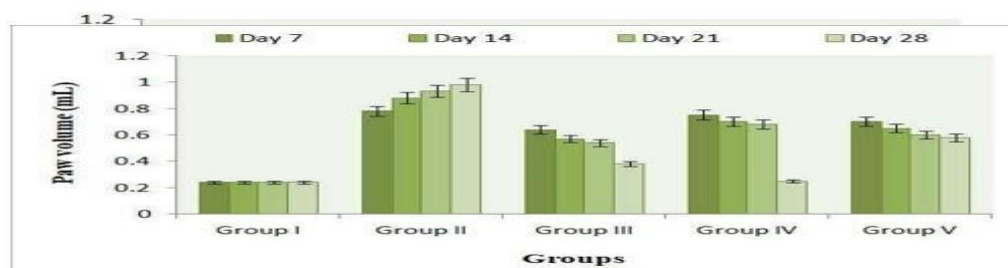
$0.35$  mL on Day 28. These changes were statistically significant (\*\* $p < 0.05$ , \*\*\* $p < 0.001$ ), indicating that aspirin was effective in reducing inflammation and controlling the symptoms of arthritis in this model.

**Table 5: Anti-arthritis activity of hydro alcoholic extract of *Putranjiva roxburghii* against Freund's adjuvant induced arthritis in rats**

Paw volume (mL)					
Group		Day7	Day 14	Day21	Day28
Group I	2% gum acacia	$0.24 \pm 0.50$	$0.24 \pm 0.45$	$0.24 \pm 0.35$	$0.24 \pm 0.25$
Group II	Arthritis control	$0.78 \pm 0.20$	$0.88 \pm 0.35$	$0.93 \pm 0.20$	$0.98 \pm 0.30$
Group III	Aspirin (200 mg/kg p.o)	$0.64 \pm 0.10$	$0.57 \pm 0.15$ ••	$0.54 \pm 0.30$ ••	$0.38 \pm 0.35$ ** *
Group IV	Hydroalcoholic ext ract of <i>Putranjivaroxburghii</i> 100 mg/kg p.o	$0.75 \pm 0.20$	$0.70 \pm 0.10$ *	$0.68 \pm 0.25$ *	$0.25 \pm 0.15$
Group V	Hydroalcoholic extract of <i>Putranjiva</i> <i>roxburghii</i> 200mg/kg p. o	$0.70 \pm 0.32$ .	$0.65 \pm 0.21$ ••	$0.60 \pm 0.25$ ** *	$0.58 \pm 0.4s$ ** *

Values expressed as mean  $\pm$  SEM (n=6) \* $p < 0.05$ , \*\* $P < 0.01$ , \*\*\*  $P < 0.001$  as compared to arthritis Control

**Figure 4: Anti-arthritis activity of hydro alcoholic extract of *Putranjiva roxburghii* against Freund's adjuvant induced arthritis in rats**



## SUMMARY AND CONCLUSION



This study aimed to evaluate the anti-arthritis potential of the hydro alcoholic extract of *Putranjiva roxburghii* in a rat model of Freund's adjuvant-induced arthritis, examining its effect on paw volume as a key marker of inflammation. The research also included an assessment of the extraction yield, phytochemical profile, and chemical analysis of the extract, providing a comprehensive understanding of its bioactive components and their possible therapeutic effects.

The extraction yield of *Putranjiva roxburghii* revealed that the hydro alcoholic solvent provided a much higher yield (9.52% w/w) compared to petroleum ether (0.64% w/w). This indicates that the hydroalcoholic extract is more efficient in extracting the active compounds from the plant, making it a more suitable solvent for future pharmacological studies. The higher yield of the hydroalcoholic extract also suggests that it may contain a greater variety of bioactive compounds that could contribute to its medicinal properties.

In terms of phytochemical composition, the hydro alcoholic extract of *Putranjiva roxburghii* was screened for several classes of bioactive compounds. Alkaloids and glycosides were found to be absent, as both Dragendorff's and Hager's tests for alkaloids, and Legal's test for glycosides, gave negative results. On the other hand, the extract was positive for flavonoids, phenols, proteins, saponins, diterpenes, and tannins. These compounds are well-known for their anti-inflammatory, antioxidant, and analgesic properties, suggesting that the extract may offer therapeutic benefits for conditions like arthritis. Notably, the presence of flavonoids and phenols is of particular interest, as these compounds have been widely studied for their ability to reduce inflammation and oxidative stress, both of which play crucial roles in the pathogenesis of arthritis.

#### **Anti-Arthritis Activity**

The core focus of the study was to assess the anti-arthritis activity of the hydroalcoholic extract of *Putranjiva roxburghii* in a rat model of arthritis induced by Freund's adjuvant. Paw volume, a common indicator of inflammation in arthritis models, was measured over a period of 28 days. The data showed that the hydroalcoholic extract exhibited a dose-dependent reduction in paw volume, suggesting its potential as an effective anti-inflammatory agent.

In the vehicle control group (Group I, 2% gum acacia), there was no significant change in paw volume throughout the study. This group served as a baseline, confirming that the vehicle did not contribute to any reduction in inflammation. In contrast, the arthritis control group (Group II), which received no treatment, showed a steady increase in paw volume, reflecting the progression of inflammation and the establishment of arthritis following the injection of Freund's adjuvant.

The aspirin-treated group (Group III) showed significant reductions in paw volume starting from Day 14, with continued improvements through Day 28. Aspirin, at a dose of 200 mg/kg, significantly reduced inflammation in this model (\*\* $p < 0.05$ , \*\*\* $p < 0.001$ ), which is consistent with its well-documented anti-inflammatory properties. This group served as a positive control and demonstrated the validity of the experimental model and the effectiveness of a known anti-inflammatory drug. The hydro alcoholic extract of *Putranjiva roxburghii* showed a promising anti-arthritic effect. In Group IV (100 mg/kg), paw volume decreased progressively from  $0.75 \pm 0.20$  mL on Day 7 to  $0.25 \pm 0.15$  mL by Day 28. While the reduction in paw volume was statistically significant (\* $p < 0.05$ ) compared to the arthritis control group, the effect was less pronounced than that of aspirin. This suggests that the lower dose of the hydroalcoholic

extract has a moderate anti-inflammatory effect.

### CONCLUSION

In conclusion, the hydro alcoholic extract of *Putranjiva roxburghii* exhibits significant anti-arthritic activity in a rat model of Freund's adjuvant-induced arthritis, with a dose-dependent effect. The extract demonstrated a reduction in paw volume, which is indicative of reduced inflammation, particularly at the higher dose of 200 mg/kg. This effect can likely be attributed to the presence of various bioactive compounds, including flavonoids, phenols, saponins, and tannins, all of which have known anti-inflammatory and antioxidant properties.

The study also highlighted the high yield of the hydroalcoholic extract, suggesting that it may be an efficient source of bioactive compounds. Given the significant anti-arthritic effects observed in this animal model, *Putranjiva roxburghii* holds promise as a potential therapeutic agent for managing inflammatory diseases, such as arthritis. Further studies are needed to explore the precise mechanisms by which the extract exerts its effects, as well as to evaluate its safety and efficacy in clinical trials. The results of this study contribute to the growing body of evidence supporting the medicinal value of *Putranjiva roxburghii* and its potential application in the treatment of chronic inflammatory conditions.

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