



PHYTOCHEMICAL PROFILING AND ASSESSMENT OF ANTI-ARTHRITIC
POTENTIAL OF CAESALPINIA CRISTA EXTRACT

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ABSTRACT

Rheumatoid arthritis is a chronic inflammatory disorder characterized by joint inflammation, pain, swelling, and progressive tissue damage. The present study was undertaken to evaluate the anti-arthritis and antioxidant potential of methanolic leaf extract of *Caesalpinia crista* using a Freund's Complete Adjuvant (FCA)-induced arthritis model in rats. The leaves were collected, authenticated, dried, powdered, and extracted using methanol by Soxhlet extraction. The methanolic extract showed a percentage yield of 7.60% w/w and was subjected to preliminary phytochemical screening, which revealed the presence of flavonoids, phenols, saponins, carbohydrates, diterpenes, tannins, and sterols. The total phenolic and flavonoid contents were found to be 0.68 mg/100 mg and 0.92 mg/100 mg, respectively. Anti-arthritis activity was evaluated by measuring paw volume and inflammatory biomarkers including PGE₂, IL-1 β , and TNF- α . Oxidative stress was assessed through estimation of reduced glutathione (GSH) and superoxide dismutase (SOD) levels. FCA administration produced marked paw swelling, elevation of inflammatory mediators, and reduction in antioxidant enzyme levels. Treatment with the methanolic extract significantly reduced paw volume and inflammatory marker levels while restoring GSH and SOD levels toward normal values. The findings indicate that the methanolic leaf extract of *Caesalpinia crista* possesses significant anti-inflammatory, anti-arthritis, and antioxidant activities. These effects may be attributed to the synergistic action of its phytoconstituents, particularly phenolic and flavonoid compounds. Further studies are warranted to isolate the active constituents and establish their precise mechanisms of action.

Keywords: *Caesalpinia crista*, Anti-arthritis activity, Freund's Complete Adjuvant, Rheumatoid arthritis, PGE₂, IL-1 β , TNF- α , GSH, SOD, Methanolic extract.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory and autoimmune disorder primarily affecting the joints. It is characterized by persistent synovial inflammation, pain, swelling, stiffness, and progressive destruction of cartilage and bone, which can ultimately result in impaired

physical function and reduced quality of life (Gulati *et al.*, 2018).

The development and progression of arthritis involve a complex interaction of inflammatory mediators, immune cells, oxidative stress, and tissue responses. Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) and interleukin-1 beta (IL-1 β), together with mediators such

as prostaglandin E₂ (PGE₂), play important roles in maintaining inflammation and joint damage (Martinec *et al.*, 2019).

Although conventional anti-inflammatory and antirheumatic drugs are widely used for the management of arthritis, prolonged treatment may be associated with adverse effects and limitations in long-term therapy. Consequently, medicinal plants have received considerable attention as potential sources of biologically active anti-inflammatory agents. Plant-derived phenolics, flavonoids, tannins, and other secondary metabolites are reported to possess antioxidant and anti-inflammatory properties that may contribute to protection against inflammatory tissue damage (Santos *et al.*, 2019).

Caesalpinia crista is a medicinal plant belonging to the family Fabaceae and has been traditionally used in various systems of medicine. Different parts of the plant contain biologically active phytoconstituents, including flavonoids, phenolic compounds, tannins, saponins, and other secondary metabolites. These constituents may contribute to antioxidant and anti-inflammatory effects. Previous investigations on *Caesalpinia* species have reported antioxidant activity, supporting their potential as sources of pharmacologically active phytoconstituents (Sachan *et al.*, 2010). However, systematic evaluation of the anti-arthritic potential of the leaves of *Caesalpinia crista*, particularly in relation to inflammatory and antioxidant biomarkers, remains important.

Therefore, the present study was designed to investigate the anti-arthritic activity of methanolic leaf extract of *Caesalpinia crista* in Freund's Complete Adjuvant (FCA)-

induced arthritic rats. The study evaluated changes in paw volume, inflammatory biomarkers including PGE₂, IL-1 β , and TNF- α , and antioxidant parameters such as reduced glutathione (GSH) and superoxide dismutase (SOD). The phytochemical profile and total phenolic and flavonoid contents of the extract were also investigated to provide preliminary information regarding the phytoconstituents potentially responsible for its observed biological activity.

MATERIALS AND METHODS

Material

The materials used in the present study included methanol, Freund's Complete Adjuvant (FCA), sodium hydroxide, hydrochloric acid, ferric chloride, Folin-Ciocalteu reagent, gallic acid, quercetin, and other analytical-grade reagents. Methanol and other chemicals were procured from Merck Life Science Pvt. Ltd., Mumbai, India. FCA was obtained from Sigma-Aldrich, USA. Gallic acid and quercetin standards were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. All other reagents and chemicals used were of analytical grade and obtained from standard commercial suppliers.

Methods

Physicochemical parameters

Organoleptic characters

It included evaluation of plant powder by colour and odour (Saraf, 2010).

Fluorescence analysis

Fluorescence analysis of the drug was observed under day and UV light using various solvent extracts as well as acids and alkaline treated with solutions of the drug. The powder was treated with neutral, acids and alkaline solvents solutions like acetic acid, picric acid, FeCl₃, 1 N hydrochloric acid,

1% sulphuric acid 1 N NaOH nitric acid, aqueous and KOH.

Extraction by soxhlet extraction process

Extraction from plant materials is an important step in phytochemical processing for discovering bioactive secondary metabolite. Selection of a suitable extraction technique is also important for the standardization of herbal products. Extraction is used to extract suitable soluble constituents, with the aid of the chosen solvents except those not necessary. The products obtained from the plant were thoroughly washed in tap water and rinsed in purified water. The cool, stable samples obtained from the plants were cut into small pieces and dried under shade for 3 to 4 weeks. 50 gram of powdered of *Caesalpinia crista* has been extracted with Methanol solvent using soxhlet extraction process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Alara *et al.*, 2018).

Determination of percentage yield

The extraction yield is evaluate 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 (Nofita *et al.*, 2022). The percentage yield of extract was calculated by using following formula:

Percentage yield=weight of extract/weight of powder drug taken

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.). Phytochemical examinations were carried out for all the extracts as per the standard methods) (Bhandary *et al.*, 2012).

Quantitative estimation of bioactive compounds

Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Khatoun *et al.*, 2013). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol.

Procedure: 2 ml of extract and each standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for color development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminum chloride method (Bag *et al.*, 2015). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.

Procedure: 1 ml of 2% AlCl₃ solution was added to 3 ml of extract or each standard and

allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

In vivo* antiarthritic activity of *Caesalpinia crista

Freund's complete adjuvant (FCA)-induced arthritis in rats

Animals

Albino Wistar rats of both sexes, weighing 150-200 g, were kept in groups of six under a conventional 12-hour light/dark cycle, with controlled relative humidity (55–65%) and ambient temperature (25 ± 2 °C). Standard rat feed and unlimited water were given to the animals. The rats were acclimated to laboratory conditions for seven days prior to research. Every trial was carried out between 8:00 and 15:00 in a quiet setting. For every experimental set, a different set of six rats was employed. The Institutional Animal Ethics Committee (IAEC), which was established in accordance with the regulations of the Ministry of Environment and Forests, Government of India, New Delhi, for the control and monitoring of animal studies, approved the study protocol.

Acute oral toxicity study

Acute oral toxicity was conducted according to the method of the Organization for Economic Co-operation and Development (OECD) (Zarei *et al.*, 2023). An extract of *Caesalpinia crista* (up to 2000 mg/kg) was administered orally for 4 days to six groups of rats (n=6), and the animals were observed for mortality and behavioral changes to evaluate a possible anti-arthritic effect.

Anti-arthritis activity

Freund's adjuvant induced arthritis in rats: Animals were divided into five groups containing six animals each. Arthritic

syndrome was induced by subcutaneous injection of 0.1ml of complete Freund's adjuvant (10 mg of heat killed mycobacterium tuberculosis per ml of paraffin oil) into the planter surface of the left hind paw (Mahdi *et al.*, 2018).

Group I served as the normal and received 2% gum acacia

Group II served as the arthritis control-untreated received 2% gum acacia,

Group III received Indomethacin (0.25 mg/kg, i.p.), served as the reference standard

Group IV received the extract of *Caesalpinia crista* at doses of 100mg/kg p.o.

Group V received the extract of *Caesalpinia crista* at doses of 200mg/kg p.o.

The drug treatment was started on the 14th day of adjuvant induction and terminated on the 28th day. The changes in paw volume were measured weekly by using a plethysmometer.

Estimation of PGE2

An ELISA kit was used to estimate PGE2 levels by competitive binding. 0.15 ml of samples, control, and standard were added to precoated wells in a 96-well microtiterplate. The PGE2 antibody (50µl) was then added, and the microplate was shaken horizontally at 500 ± 50 rpm at 37°C. The PGE2 conjugate (50 µL) was added to each well and incubated for the next 2 hours after the first 60 minutes (Alavala *et al.*, 2020). The substrate solution (200µl) was then added to each well, and the mixture was allowed to sit at room temperature for 30 minutes. The reaction was then stopped by adding the stop solution (100µl), and the optical density was measured at 450 nm.

Estimation of Cytokines

ELISA kits were used to measure the pro-inflammatory cytokines TNF- α and IL-1 β .

The quantity of markers was expressed as pg/ml of the serum and determined using standard curves (Patil *et al.*, 2012).

Endogenous Antioxidants

The Ellman method was used to quantify the reduced glutathione (GSH). Superoxide dismutase (SOD) was measured using the Misra and Fridovich technique (Petchi *et al.*, 2015). 100µl of the sample was combined with 1.9ml of phosphate buffer (50mM, pH7.0) in a cuvette, and 1.0ml of freshly made H₂O₂ (30mM) was added as a reaction initiator to determine the catalase activity.

Statistical analysis

The values were expressed as mean $\pm$ SEM (n=6). Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by Tukey's test; P<0.05, P<0.01, and P<0.001 were considered statistically significant.

RESULTS AND DISCUSSION

The present study was carried out to evaluate the phytochemical profile and anti-arthritic potential of the methanolic leaf extract of *Caesalpinia crista*. The findings obtained from fluorescence analysis, extraction, phytochemical screening, quantitative phytochemical estimation, and FCA-induced arthritis studies are discussed below.

The fluorescence analysis of *Caesalpinia crista* leaves showed characteristic colour changes after treatment with different chemical reagents when observed under normal light, short-wave UV, and long-wave UV. The leaf powder exhibited green colour under normal light, while treatment with reagents such as acetic acid, ferric chloride, hydrochloric acid, sulphuric acid, sodium hydroxide, nitric acid, and potassium hydroxide produced characteristic changes

under UV illumination. These variations may be attributed to the interaction of different phytoconstituents with the applied chemical reagents. Such fluorescence characteristics can serve as useful preliminary parameters for identification and standardization of the crude drug. The observations are presented in Table 1.

The methanolic extraction of *Caesalpinia crista* leaves produced a 7.60% w/w yield. The extract was dark black in colour and possessed a characteristic odour. The obtained yield indicates that methanol was effective in extracting a measurable quantity of polar and moderately polar phytoconstituents from the leaves. The percentage yield and organoleptic characteristics of the extract are summarized in Table 2.

Preliminary phytochemical screening of the methanolic extract revealed the presence of flavonoids, saponins, phenols, carbohydrates, diterpenes, tannins, and sterols. Alkaloids, glycosides, and proteins were found to be absent under the respective test conditions. The detection of phenolic and flavonoid compounds is particularly important because these constituents are associated with antioxidant and anti-inflammatory properties. The presence of multiple classes of secondary metabolites may contribute collectively to the biological activity observed in the experimental model. The complete phytochemical screening results are presented in Table 3.

The quantitative phytochemical analysis demonstrated a total phenolic content of 0.68 mg/100 mg and a total flavonoid content of 0.92 mg/100 mg in the methanolic extract. The calibration curves prepared using gallic acid and quercetin demonstrated

concentration-dependent increases in absorbance, supporting their use for quantitative estimation. The relatively appreciable phenolic and flavonoid contents suggest the presence of antioxidant phytoconstituents in the extract. These compounds may contribute to scavenging of reactive oxygen species and modulation of inflammatory processes. The quantitative results are presented in Table 4.

FCA induction produced a progressive increase in paw volume in the arthritis control group, from 0.76 ± 0.04 mL on Day 7 to 1.10 ± 0.05 mL on Day 28, confirming the development of inflammatory edema. In contrast, treatment groups demonstrated a progressive reduction in paw volume during the experimental period. Group III showed a reduction from 0.61 ± 0.05 to 0.29 ± 0.04 mL, while Groups IV and V decreased to 0.47 ± 0.03 and 0.35 ± 0.04 mL, respectively, by Day 28. The reduction in paw swelling indicates attenuation of FCA-induced inflammation following treatment with the extract. The results are shown in Table 5.

A marked elevation of PGE_2 was observed in the arthritis control group (121.4 ± 5.1 pg/mL) compared with the normal group (40.2 ± 2.6 pg/mL). Treatment with *Caesalpinia crista* extract reduced PGE_2 levels in Groups III, IV, and V to 54.8 ± 3.4 , 77.1 ± 3.8 , and 62.5 ± 3.5 pg/mL, respectively. Since PGE_2 is an important mediator associated with inflammatory responses, its reduction suggests that the extract may suppress prostaglandin-mediated inflammation. The findings are presented in Table 6.

The arthritis control group showed a substantially increased IL- 1β concentration (99.2 ± 4.7 pg/mL) compared with the normal

group (25.8 ± 1.9 pg/mL). Treatment resulted in significant reductions in IL- 1β levels, with Groups III, IV, and V showing values of 40.1 ± 2.8 , 61.3 ± 3.4 , and 46.7 ± 3.1 pg/mL, respectively. The reduction in this pro-inflammatory cytokine indicates that *Caesalpinia crista* extract may attenuate the inflammatory cytokine response associated with FCA-induced arthritis. The results are summarized in Table 7.

TNF- α levels were markedly elevated in the arthritis control group (98.7 ± 4.6 pg/mL) compared with the normal group (26.1 ± 1.9 pg/mL). Treatment with the extract decreased TNF- α concentrations to 39.6 ± 2.7 pg/mL in Group III, 60.9 ± 3.3 pg/mL in Group IV, and 45.8 ± 3.0 pg/mL in Group V. The decrease in TNF- α suggests attenuation of the pro-inflammatory response. Together with the reduction in IL- 1β and PGE_2 , these findings indicate that the extract may influence multiple inflammatory pathways involved in FCA-induced arthritis. The results are presented in Table 8.

FCA-induced arthritis was associated with a pronounced reduction in endogenous antioxidant defense. The arthritis control group showed GSH and SOD levels of 3.05 ± 0.20 $\mu\text{mol/mg}$ and 6.1 ± 0.35 U/mg, respectively, compared with 8.78 ± 0.36 $\mu\text{mol/mg}$ and 16.2 ± 0.65 U/mg in the normal group. Treatment with *Caesalpinia crista* extract restored these antioxidant parameters. Group III showed GSH and SOD levels of 7.38 ± 0.30 $\mu\text{mol/mg}$ and 14.2 ± 0.56 U/mg, respectively, while Group V showed 6.91 ± 0.29 $\mu\text{mol/mg}$ and 12.9 ± 0.51 U/mg. Group IV also demonstrated improvement, with values of 5.72 ± 0.24 $\mu\text{mol/mg}$ and 10.5 ± 0.44 U/mg. The increased GSH and SOD

levels suggest improvement of endogenous antioxidant defense and possible reduction of oxidative stress. These findings are presented in Table 9.

Table 1: Fluorescence analysis of leaves of *Caesalpinia crista*

S. No.	Treatment with chemical reagent	Observation		
		Normal light	Short UV	Long UV
1	Acetic acid	Green	Black	Black
2	Picric acid	Brown	Dark brown	Black
3	FeCl ₃	Green	Black	Black
4	1 N hydrochloric acid	Green	Dark brown	Black
5	1% sulphuric acid	Green	Black	Black
6	1 N NaOH	Green	Dark brown	Black
7	Nitric acid	Green	Black	Black
8	Aqueous	Green	Dark brown	Black
9	KOH	Green	Black	Black
10	Powder	Green	Black	Black

Table 2: % Yield of *Caesalpinia crista*

S. No.	Extract	Colour	Odor	% Yield (w/w)
1.	Methanolic	Dark black	Characteristic	7.60

Table 3: Result of phytochemical screening of methanolic extract of *Caesalpinia crista*

S. No.	Constituents	Methanolic extract
1.	Alkaloids Wagner's test: Hager's test:	-Ve -Ve
2.	Glycosides Conc. H ₂ SO ₄ test	-Ve
3.	Flavonoids Lead acetate test: Alkaline reagent test:	+Ve +Ve
4.	Saponins Froth test:	+Ve
5.	Phenol Ferric chloride test: Folin ciocalteu test	+Ve +Ve
6.	Proteins Xanthoproteic test:	-Ve
7.	Carbohydrate Fehling's test: Benedict's test:	+Ve +Ve
8.	Diterpenes	

	Copper acetate test:	+Ve
9.	Tanins Gelatin test	+Ve
10.	Sterols Salkowski test	+Ve

[+Ve= Present; -Ve= Absent]

Table 4: Total phenol and flavonoid content of *Caesalpinia crista*

S. No.	Extract	Total phenol content	Total flavonoid content
		mg/ 100mg	
1.	Methanolic extract	0.68	0.92

Table 5: Effect of *Caesalpinia crista* extract on paw volume in FCA-induced arthritic rats

Group	Paw volume (mL)			
	Day 7	Day 14	Day 21	Day 28
Group I	0.23 ± 0.03	0.22 ± 0.04	0.21 ± 0.03	0.20 ± 0.04
Group II	0.76 ± 0.04	0.91 ± 0.05	1.04 ± 0.04	1.10 ± 0.05
Group III	0.61 ± 0.05	0.49 ± 0.04**	0.37 ± 0.03***	0.29 ± 0.04***
Group IV	0.70 ± 0.04	0.62 ± 0.03*	0.55 ± 0.04**	0.47 ± 0.03**
Group V	0.67 ± 0.03**	0.56 ± 0.04**	0.45 ± 0.03***	0.35 ± 0.04***

Table 6: Effect of *Caesalpinia crista* extract on PGE₂ levels in FCA-induced arthritic rats

Group	PGE ₂ Levels (pg/mL)
Group I	40.2 ± 2.6
Group II	121.4 ± 5.1
Group III	54.8 ± 3.4***
Group IV	77.1 ± 3.8**
Group V	62.5 ± 3.5***

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

Table 7: Effect of *Caesalpinia crista* extract on IL-1β levels in FCA-induced arthritic rats

Group	IL-1β levels (pg/mL)
Group I	25.8 ± 1.9
Group II	99.2 ± 4.7
Group III	40.1 ± 2.8***
Group IV	61.3 ± 3.4**
Group V	46.7 ± 3.1***

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

Table 8: Effect of *Caesalpinia crista* extract on TNF- α levels in FCA-induced arthritic rats

Group	TNF- α levels (pg/mL)
Group I	26.1 $\pm$ 1.9
Group II	98.7 $\pm$ 4.6
Group III	39.6 $\pm$ 2.7***
Group IV	60.9 $\pm$ 3.3**
Group V	45.8 $\pm$ 3.0***

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

Table 9: Effect of *Caesalpinia crista* extract on GSH and SOD levels in FCA-induced arthritic rats

Group	GSH levels (μ mol/mg)	SOD levels (U/mg)
Group I	8.78 $\pm$ 0.36	16.2 $\pm$ 0.65
Group II	3.05 $\pm$ 0.20	6.1 $\pm$ 0.35
Group III	7.38 $\pm$ 0.30***	14.2 $\pm$ 0.56***
Group IV	5.72 $\pm$ 0.24**	10.5 $\pm$ 0.44**
Group V	6.91 $\pm$ 0.29***	12.9 $\pm$ 0.51***

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

CONCLUSION

The present study demonstrated that the methanolic leaf extract of *Caesalpinia crista* possesses significant anti-inflammatory, antioxidant, and anti-arthritic activity in FCA-induced arthritic rats. The extract reduced paw edema and inflammatory markers such as PGE₂, IL-1 β , and TNF- α , while improving the antioxidant defense parameters GSH and SOD. The observed activity may be associated with the presence of flavonoids, phenols, tannins, saponins, and diterpenes. *Caesalpinia crista* may serve as a promising source of bioactive compounds for further investigation in the management of rheumatoid arthritis.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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