

Study Of Phytochemical Screening and In Vivo Antiarthritic Activity of Cochlospermum Gossypium

Akash Kumar^{*1}, Santosh Kumar Ojha², Satish Kumar Sarankar³

¹Masters of Pharmacy in Pharmacology Student, Mansarovar Global University Sehore, Madhya Pradesh, India

²Associate Professor, Mansarovar Global University, Sehore, Madhya Pradesh, India.

³Professor & Principal, Mansarovar Global University, Sehore, Madhya Pradesh, India.

ABSTRACT

The present study was undertaken to investigate the phytochemical composition and anti-arthritic potential of the hydroalcoholic leaf extract of *Cochlospermum Gossypium*. Pharmacognostic evaluation was carried out through fluorescence analysis, which provided characteristic identification parameters for the crude drug. The hydroalcoholic extract was obtained with a yield of 8.50% w/w and subjected to preliminary phytochemical screening. The results revealed the presence of alkaloids, flavonoids, phenolic compounds, proteins, carbohydrates, and diterpenes. Quantitative estimation showed total phenolic and flavonoid contents of 0.58 mg/100 mg and 0.96 mg/100 mg, respectively. The anti-arthritic activity of the extract was evaluated using the Freund's Complete Adjuvant (FCA)-induced arthritis model in rats. Arthritic animals exhibited a significant increase in paw volume along with elevated levels of inflammatory mediators such as prostaglandin E₂ (PGE₂), interleukin-1 β (IL-1 β), and tumor necrosis factor- α (TNF- α). Treatment with the hydroalcoholic extract resulted in a marked reduction in paw edema and inflammatory marker levels. Furthermore, the extract improved antioxidant status by increasing reduced glutathione (GSH) and superoxide dismutase (SOD) levels compared with the arthritic control group. The findings demonstrate that *Cochlospermum gossypium* leaf extract possesses significant anti-arthritic activity and supports its traditional use in the management of inflammatory disorders. The observed effects may be attributed to the presence of phenolic and flavonoid constituents in the extract.

Keywords: *Cochlospermum gossypium*, Hydroalcoholic Extract, Phytochemical Screening, Total Phenolic Content, Total Flavonoid Content, Anti-Arthritic Activity, FCA-Induced Arthritis, PGE₂, IL-1 β , TNF- α , GSH, SOD.

INTRODUCTION

The combinatorial word "arthritis" was created by fusing Greek and Latin. "Arthron" in Greek means joint, and "itis" in Latin means inflammation. As a result, arthritis is typically thought of as a condition brought on by inflamed joints. Fundamentally, "Arthritis" refers to a group of related medical conditions rather than a single illness. In the US alone, 300,000 children and over 47 million adults suffer. If appropriate therapies are not given on time, the condition may render a person permanently incapacitated. It places a significant financial strain on people all around the world due to lost wages and rising prescription costs. Although there is already more than one therapy option available to manage the

illness, a quick solution has not yet been discovered. It is important to grasp the workings of a bone-joint in order to properly comprehend the condition.^[1] A bone is typically classified as a joint when it moves or twists on a related piece or pieces to preserve the functional flexibility. Ligaments function as elastic bands to assist hold the bones in place during movement. Ligaments retain them in place at all times, whether they are moving or at rest. In order to shield the bone surfaces from direct contact, cartilage tissue covers them. This soothes limb movement without producing pain or eroding the bone from friction. Synovial fluid, which is aligned with the ligaments inside the joint cavity, is produced by cells

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

from the synovial membrane and fills the cavity inside the joint.^[2]

LITERATURE REVIEW

Das et al., (2025) investigate the pharmacological activities of the methanolic extract of processed roots in Freund's Complete Adjuvant (FCA)-induced rheumatoid arthritis in rats. Rheumatoid arthritis was induced in the rats by intra-articular injection of FCA in right paw on day 0, and treatment of prednisolone and different doses of *Plumbago zeylanica* methanolic extract (PZME) was given from day 1 to 28. Throughout the study, behavioral and physical arthritis parameters were evaluated, while biochemical serum levels of COX-2, inflammatory markers, and oxidative stress parameters were measured at the study's endpoint, and tibio-tarsal joints were subjected to microscopic studies for histological evaluation. PZME treatment significantly attenuated FCA-induced rheumatoid arthritis, evidenced by reduced paw volume, joint diameter, and improved behavioral parameters (gait, weight-bearing). PZME also decreased serum levels of inflammatory markers (TNF- α , IL-6, RF, CRP), oxidative stress parameters (TBARS, catalase), and COX-2 expression. Histological analysis confirmed reduced synovial hyperplasia and cartilage erosion. LC-MS/MS analysis identified several phytoconstituents, which may contribute to the observed effects.^[3]

Kumatia et al., (2024) examined the antioxidant and anti-arthritic properties of A Micraster seed extract and its constituent parts. Next, using an in vitro assay, the arthritic activity of the crude extract (ASE) and its most effective fraction (ASEA) was assessed against rats that had been given CFA-induced arthritis. Based on 100 mg of GAE, the estimated polyphenolic content of ASE was 13.00 ± 0.00 mg. Out of all the extract fractions, ASEA had the most number of polyphenolic components (1076 ± 0.00 mg/100 mg of GAE). In the DPPH free radical scavenging assay, ASE and ASEA had significant antioxidant activity ($IC_{50} = 20.17 \pm 1.291$ and 19.35 ± 0.865 , respectively), which was comparable to ascorbic acid's ($IC_{50} = 17.35 \pm 0.500$). Moreover, ASEA's in vitro antiarthritic activity was 5.75 and 13.63 times greater than that of diclofenac sodium and the crude

extract, respectively. These findings suggest that micraster seed extract and its fractions, which include phytosterols and polyphenols, have strong antiarthritic activity by preventing inflammation, oxidative stress, protein denaturation, inflammatory cell infiltration, and synovium damage.^[4]

Sharma et al., (2023) examined *Nyctanthes arbor-tristis*'s (NAT) potential using in vitro and in silico methods. Reflux method was used to generate hydroethanolic extracts of harsingar, which showed TPC (98.56 ± 0.46 mg GAE/g) and TFC (34.51 ± 0.45 mg CE/g) with alkaloids, phenol, saponin, steroids, proteins, tannins, terpenoids, carbohydrates, and flavonoids. LC-MS/MS examines the extract's active ingredients. At 1 mg/mL, NAT showed the greatest scavenging capacity in terms of anti-oxidant and anti-arthritic action. At 250 μ g/mL, splenocyte proliferation peaked. Studies on in vitro cell splenocytes showed that IL-10 was upregulated and TNF- α were down regulated. Furthermore, bioactive components and targets bind with a favorable binding affinity, according to an in-silico study. Based on in vitro and in silico research, these results highlight *Nyctanthes arbor-tristis*' potential to have anti-arthritic benefits.^[5]

Singha et al., (2023) discussed the problems posed by herbal anti-arthritic medications as well as the pharmacodynamics and pharmacokinetics of these herbal nanomedicines. Joints and arthritic conditions are among the most dangerous illnesses that humans have ever encountered. What's even more worrying is that there is no recognized treatment for arthritis. The use of traditional therapies to treat severe joint inflammation is associated with a number of negative effects. For this reason, researchers have been searching for safer alternatives, such as herbal treatments. By using the diverse and adaptable characteristics of different nanoparticles, herbal medicines based on nanotechnology may be able to get beyond the delivery constraints of different natural ingredients.^[6]

Jamadar et al., (2023) an investigation was initiated to evaluate the potential anti-arthritic properties of *Bauhinia acuminata* Linn's ethanol and aqueous extracts. Significant (** $p < 0.01$) dose-dependent suppression of paw volume edema was seen with



Bauhinia acuminata Linn, and the hematological profile was significantly improved. The ethanolic extract of *Bauhinia acuminata* Linn leaves, at 400 mg/kg, significantly (** $p < 0.01$) decreased paw volume edema by 70.24% on the 28th day in models of arthritis caused by formaldehyde. On the other hand, 61.157% inhibition of the ethanolic extract of *Bauhinia acuminata* Linn leaves at 200 mg/kg, b.w., p.o., appears to indicate substantial efficacy (* $p < 0.05$). On day 28, the paw volume edema was reduced by 58.677% by the aqueous extracts of *Bauhinia acuminata* Linn leaves at a dose of 400 mg/kg, which appears to exhibit considerable effectiveness (* $p < 0.05$). Conversely, on day 28, an aqueous extract of *Bauhinia acuminata* Linn leaves at a dose of 200mg/kg, b.w., p.o., seems to exhibit considerable activity (* $p < 0.05$) with 54.54% inhibition.^[7]

Banik and Das, (2023) focused on using in vitro assays to assess the anti-inflammatory activity of *Alstonia scholaris* (AS) latex in arthritis. Both the AS latex and regular diclofenac sodium were shown to have good anti-arthritic capability. When tested at 250 $\mu\text{g/mL}$, the proteinase enzyme was inhibited by latex and diclofenac sodium in the BSA denaturation technique by 71.59% and 85.23%, respectively. The greatest percentage of inhibition of protein denaturation in the egg albumin denaturation method was seen at 250 $\mu\text{g/mL}$ for both the standard and latex, with 70.42% and 81.32%, respectively. The latex demonstrated significant anti-arthritic potential in a concentration-dependent manner in the HRBC membrane stabilization model. Latex and diclofenac sodium demonstrated membrane stabilization rates of 68.77% and 72.12%, respectively, at 250 $\mu\text{g/mL}$ in hypotonic solution.^[8]

Dogra et al., (2022) examined the anti-arthritic properties of *Euphorbia helioscopia* aqueous and ethanolic extracts. The examination was conducted using the adjuvant-induced arthritis (AIA) approach. Female Wistar rats were injected with arthritis using Complete Freund's adjuvant (CFA). Wistar rats were administered aqueous and ethanolic extracts of the aerial portions of *Euphorbia helioscopia* orally at doses of 300, 150, and 75 mg/kg/day. Significant anti-arthritic efficacy was demonstrated by both extracts in a dose-dependent manner. Among the studied

extracts, *Euphorbia helioscopia*'s ethanolic extract (300 mg/kg) shown possible anti-arthritic action. The investigation has led to the conclusion that *Euphorbia helioscopia* exhibits strong anti-arthritic properties.^[9]

Farooq et al., (2022) aimed at assessing the anti-inflammatory and anti-arthritic properties of Aloe vera (*Aloe barbadensis*), Nux-vomica (*Strychnos nux-vomica*), and Colchicum (*Colchicum autumnale*) in vitro. At concentrations of 125, 250, and 500 $\mu\text{g/mL}$, along with standard, anti-inflammatory activity was assessed using the human red blood cell membrane stabilization method and anti-arthritic activity by protein denaturation method. All of the extracts contained alkaloids, saponins, terpenoids, phenols, and anthraquinones, according to phytochemical screening. FTIR analysis indicated organic acids, amine groups, aromatic or aliphatic compounds, esters and halogens, and phenolics. Using the protein denaturation method, the highest inhibition levels of colchicum, nux-vomica, and aloe vera were found to be 98.5%, 99.6%, and 72.3% at 500 $\mu\text{g/mL}$, respectively, when compared to the standard medication, Diclofenac sodium. Using the membrane stabilization method, the highest protection levels of colchicum, nux-vomica, and aloe vera at 500 $\mu\text{g/mL}$ were found to be 40.20%, 35.67%, and 40.1%, respectively, in comparison to the standard medication.^[10]

Prajapati and Gajera, (2022) examined the anti-arthritic properties of methanolic extract from *Abies webbiana* Lindl. (MEAW) leaves in albino rats with full Freund's adjuvant- induced arthritis. With the exception of group 1, all study groups 2 through 6 had sub- plantar injections of 0.1 milligrams per kilogram of CFA agent into the albino rats' left hind paws. Group 1 has typical control. Group 3 represents the Standard control (methotrexate 0.5 mg kg⁻¹), while Group 2 represents the Arthritic control. As the test control, groups 4-6 are given varying amounts of MEAW (100, 200, and 400 mgkg⁻¹). Following the eighth day of CFA induction, treatment is administered once daily (p.o.) for a total of twenty days. Hematological, biochemical, antioxidant, and histological parameters were evaluated after day 28 while body weight and paw volume were determined in vitro. When compared to the arthritic control group, MEAW showed dose-dependent antiarthritic action,



as evidenced by a decrease in paw volume and an increase in body weight. MEAW (400 & 200 mg/kg) showed noteworthy ($p < 0.001$) anti-arthritic activity by raising RBC and Hb levels and bringing WBC, platelets, ESR, CRP, and RF levels into balance. The modified biochemical measures (AST, ALT, ALP, and Total Protein Levels) and antioxidant parameters (SOD, MDA, and GSH) further supported the anti-arthritic action. Additionally, joint damage was avoided by methotrexate and MEAW 400 mg/kg-1 (Histopathological Analysis). In summary, the results of this study suggest that *A. webbiana* Lindl. could be a useful therapeutic or preventive option for the management of arthritis and inflammation.^[11]

Dharmaraj et al., (2022) the purpose of the study was to examine the anti-arthritic properties of the *Pterocarpus indicus* leaves and bark ethanolic extract. *Pterocarpus indicus* leaf and bark ethanolic extract was found to have anti-arthritic properties using in vitro methods of stabilizing the membrane of human red blood cells (HRBC) and inhibiting protein denaturation. *Pterocarpus indicus* leaf and bark ethanolic extract was tested in vitro at 20, 40, 60, 80, and 100 µg/ml, respectively, to see whether it inhibited protein denaturation. The anti-arthritic activity was also estimated using the HRBC method at different concentrations (20, 40, 60, 80, and 100 µg/ml). At a concentration of 100 µg/ml, the percentage of inhibition for HRBC is 65% for leaves, as well as 69% for bark and 78% for egg albumin or leaves and 82% for bark. *Pterocarpus indicus* leaves and bark ethanolic extract inhibited protein (albumin) denaturation in a concentration-dependent manner. According to the results of this study, *Pterocarpus indicus* bark and leaves had more powerful anti-arthritic properties when extracted using ethanol.^[12]

Plant profile

Cochlospermum Gossypium

Cochlospermum Gossypium tree which belongs to the family Bixaceae. It is a flowering plant and native to India, Thailand and Burma, Southeast Asia grown in forest, hill areas. This tree flowers are used for temple offering. The genus *cochlospermum* compose of 14 species of trees belongs to Bixaceae family and few species are belongs to *Cochlospermaeaceae* family. Which are distributed throughout the world

and native to tropical regions particularly Latin America, Africa, Australia and Indian Subcontinent.^[13] *C. Gossypium* is in unani medicine *Qurs-e-sartaan* is used for antipyretic, tuberculosis, hectic fever and also used for diarrhoea, dysentery and pharyngitis. *C. Gossypium* plant parts like leaves, stem and bark, wood also contain important phytochemicals constituents which are medicinally very useful.^[14]

Scientific classification:

Kingdom: Plantae

Subkingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Dilleniidae

Order: Violales

Family: Bixaceae

Genus: *Cochlospermum*

Species: *Gossypium*

Botanical description: *Cochlospermum gossypium* is a small tree growing to a height of 7.5 m (25 ft) usually found in dry deciduous especially on stony hills;

Bark: Bark is 20-25mm thick, smooth and pale grey in color and surface is dark grey, branchlets are thick.

Leaves: Leaves are glabrous above, densely brownish tomentose below and the arrangement of leaves is alternative distichous. Leaf base is chordate. The leaves appear at the tips of the branch's leaves are simple, palmately 3-5 lobed, alternate, and estipulate.^[15]

Flowers: Flowers of the *C. Gossypium* tree are the most conspicuous part of the tree. Flowers are bisexual, buttercup shaped and colour of the flower is



bright yellow in colour and stamens are in orange. Flowers are large and with prominent sepals.

Fruits: Fruits are brown in colour and oval shaped. Fruits are come in the form of capsules made up of five segments. The capsules are splits and open to release the seeds which embedded in the silky cotton present in the capsules. Wood is soft, light and dark brown color. Plant containing numerous seeds surrounded by pale brown cottony hair.^[16]

Distribution:

The plant habitat is terrestrial and this species is distributed thought to be native to Central and Southern India, globally distributed in Indo-Malaysia. Within India, it occurs almost all over except western coastal districts. It is particularly common in hot, dry and stony regions. This tree is also found in Telangana forests.

Chemical constituents:

Cochlospermum gossypium plant material used in sidda and unani system of medicine from ancient times. This plant material contains many primary and secondary metabolites like flavonoids, tannins, steroids, lipids, starch, phenols and sugars. More amounts of phenols of secondary metabolites were found to be rich in leaves and bark of this plant.^[17]

Chemical constituents like Gallic acid, Ellagic acid, 2, 3-Dihydrobezoic acid, Thymol, Syringaldehyde, Quercetin, cynarine are identified through the LC-MS analysis of dichloromethane leaf extract of *Cochlospermum Gossypium*.

Sasikala A *et al* quantification of primary and secondary metabolites of leaves and stem bark of *Cochlospermum gossypium* describes the presence of chemical constituents like alkaloids, steroids, carbohydrates, flavonoids and phenolic compounds and it determines the high number of phenolic compounds in leaves and stem bark of *C. gossypium*. Physicochemical analysis of gum kondagogu. It revealed that gum kondagogu had higher amount of fiber, protein, tannin, calcium and potassium contents than karaya gum.^[18]

Pharmacological activities:

Cochlospermum Gossypium has several pharmacological activities, and potential to provide health to the society. It is used as anti-microbial, anti-bacterial anti carcinogenic, anti-oxidant, and Hepato protective activity, and also used as stimulant, sedative and is useful in cough, gonorrhea, syphilis and trachoma. Plant is used in the treatment of immunity system and memory power especially below the age of 12 years kids.

Anti-bacterial activity:

G. Bhagavanth, Madhusudhan, A.Ramakrishna, D.Ayodhya Gold nanoparticles (AuNPs) were prepared from H₂AuCl₄ using gum kondagogu, by adopting green synthesis, The arrangement of the gold nano particles was recognized through the adjustment in the shade of the arrangement from yellow to red. The integrated particles were described by different systems. The orchestrated, new gum-based impetus was extremely proficient, simple to plan, stable, savvy and ecoaccommodating. The synthesized gold nanoparticles showed good antibacterial activity. *Cochlospermum gossypium* stem bark and root powder is traditionally used for pregnancy and ash of fruit mixed with coconut is used for the treatment of scabies.^[19]

Uses:

Medicinal Uses:

In Ayurvedic medicine, parts of the *Cochlospermum gossypium* plant, including the gum, have been traditionally used to treat various ailments, such as inflammation, skin diseases, and digestive disorders.

Sedative and Stimulant:

The plant is also known to have sedative and stimulant properties, and is used in traditional practices to treat conditions like cough, gonorrhea, syphilis, and trachoma.

Anti-microbial and Anti-bacterial:

Research has shown that the plant, including its gum, exhibits antibacterial and antifungal activity.

Wound Healing:



The katira gum is also known to have wound-healing properties and is used in traditional medicine for treating various ailments.

Other Uses:

The plant's bark, root, and leaves are used to treat conditions like cough, bone fracture, ulcer, jaundice, and other ailments.

Industrial and Agricultural Uses: Katira Gum:

The gum, known for its water-insoluble and swelling properties, is used in various industrial applications.

Substitutes for Gum Tragacanth:

The katira gum can be used as a substitute for gum tragacanth in various industrial processes.

Adhesive:

The gum is used in making water-borne adhesive pastes, especially when mixed with gum arabic.

Other Applications:

It has been used in cigar and ice-cream manufacture, and may be used in other industrial contexts.

Fibers and Floss:

The seed floss from the plant is used for stuffing pillows.

AIM AND OBJECTIVES

The present study is aimed to evaluate the phytochemical profile and *in vivo* antiarthritic potential of *Cochlospermum Gossypium* leaves extract for treatment of rheumatoid arthritis (RA). The specific objectives are:

1. To collect, and prepare the leaves of *Cochlospermum Gossypium* for experimental analysis.
2. To perform qualitative phytochemical screening of the extract to identify the presence of major bioactive constituents.

3. To estimate the total phenolic and flavonoid content of the extract quantitatively.
4. To assess the *in vivo* antiarthritic activity of the extract using suitable animal models such as Freund's complete adjuvant (FCA)-induced arthritis in rats.
5. To compare the antiarthritic efficacy of the extract with a standard reference drug.

PLAN OF WORK

1. Collection and Identification of plant
2. Extraction of leaves by Soxhlet extraction method
3. Preliminary phytochemical screening of extract
4. Estimation of various phytoconstituents
 - Estimation of total phenol content
 - Estimation of total phenol content
5. *In vivo* anti-arthritis activity of extract
 - Freund's complete adjuvant (FCA)-induced arthritis in rats

MATERIALS AND METHODS

Materials used for study

The materials used in the study consisted of various analytical-grade chemicals procured from reputed suppliers. Potassium mercuric iodide and picric acid were obtained from Thomas Baker, Mumbai. Iodine, potassium iodide, sodium nitropruside, sodium hydroxide, lead acetate, and Folin-ciocalteu reagent were procured from Loba Chemie Pvt. Ltd., Mumbai. Potassium bismuth iodide, pyridine, gelatin, nitric acid, copper acetate, and sodium chloride were obtained from S. D. Fine Chem. Ltd., Mumbai. Organic solvents such as methanol, ethanol, and chloroform were supplied by Qualigens Fine Chemicals, Mumbai. Fehling's solution was procured from Central Drug House Ltd., Delhi. All chemicals and reagents used in the study were of analytical grade and utilized without further purification.



Instruments used for study

The instruments used in the study were obtained from standard manufacturers and suppliers to ensure accuracy and reliability of results. A UV–visible spectrophotometer (Labindia 3000+) from Labindia Instruments Pvt. Ltd. was used for spectrophotometric analysis. A micro centrifuge was procured from REMI Laboratory Instruments, Mumbai. The pH meter was obtained from Accumax India, New Delhi, and an electronic balance from Contech Instruments Ltd., Mumbai. A hot air oven supplied by Oracle Equipments, New Delhi, was used for drying purposes. A vortex apparatus was obtained from Ambros Lab Equipments, Ambala. The rotary vacuum evaporator was supplied by Microtech Scientific Instruments, New Delhi, and a sonicator from Athena Technology, Thane. All instruments were calibrated and maintained as per standard laboratory procedures before use.

Methods

1. Collection of *Cochlospermum gossypium*

- The leaves of *Cochlospermum gossypium* were collected.

The collected plant parts were cleaned, shade-dried, and coarsely powdered using a mechanical grinder.

2. Preparation of Extract

- The powdered leaves were subjected to Soxhlet extraction using Hydro Alcohol (Ethanol water, 80:20 v/v) as solvent.
- The extract was filtered and concentrated under reduced pressure using a rotary evaporator. The yield of extract was calculated and stored in an airtight container at 4°C for further studies.

3. Preliminary Phytochemical Screening

The obtained extract was subjected to qualitative phytochemical tests to identify the presence of:

4. Quantitative Estimation of Bioactive Constituents

- Total Phenolic Content (TPC):** Determined using the Folin–Ciocalteu method, expressed as mg gallic acid equivalent (GAE)/100 mg extract.
- Total Flavonoid Content (TFC):** Determined using the aluminum chloride colorimetric method, expressed as mg quercetin equivalent (QE)/100 mg extract.

5. In vivo Antiarthritic Activity

a. Experimental Animals

b. Induction of Arthritis

- Arthritis was induced by injection of Freund's Complete Adjuvant (FCA) into the subplantar region of the left hind paw of each rat.

c. Grouping and Treatment

- Animals were divided into five groups (n = 6):

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 *Cochlospermum Gossypium* at doses of 100mg/kg p.o. **Group V** received the extract of *Cochlospermum Gossypium* at doses of 200mg/kg p.o.

d. Treatment Schedule

e. Biochemical parameters

EXPERIMENTAL WORK

Selection of plant materials

The plant has been selected on its availability and folk use of the plant. Every part of the plant like bark, leaves, flowers, roots, fruits and seeds may contain active secondary metabolites.

Collection of plant materials



Leaves of *Cochlospermum Gossypium* were collected from ruler area of Bhopal (M.P.) in the month of January, 2026.

Drying

The plant sample were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. Drying of fresh plant parts were carried out in sun but under the shade. Dried leaves of *Cochlospermum Gossypium* were preserved in plastic bags and closed tightly and powdered as per the requirements.

Physicochemical parameters

Organoleptic characters

It included evaluation of plant powder by colour and odour.

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 *Cochlospermum gossypium* has been extracted with hydroalcoholic solvent (ethanol: water; 80:20v/v) using soxhlet extraction process for 48 hrs, filtered and dried using vacuum evaporator at 40°C.^[20]

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. The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Powder drug taken}} \times 100$$

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.^[21]

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

b) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red



precipitate indicates the presence of reducing sugars.

- b) **Benedict's Test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

- a) **Conc. H₂SO₄ Test:** extract dissolved in distilled water and treated with few drops of conc. H₂SO₄. Formation of red color indicates the presence of glycosides.

4. Detection of saponins

- a) **Froth Test:** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric

- a) chloride solution. Formation of bluish black color indicates the presence of phenols.

- b) **Folin Ciocalteu Test:** 1 ml extract was added to 1 ml folin ciocalteu reagent; blue green color indicates the presence of the phenols.

6. Detection of flavonoids

- a) **Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

- b) **Alkaline Reagent Test:** Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

7. Detection of proteins

- a) **Xanthoproteic Test:** The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

8. Detection of diterpenes

- a) **Copper acetate Test:** Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

9. Detection of tannins

- a) **Gelatin test:** The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

10. Detection of Sterols

- a) **Salowski Test:** 3-4 drops of Conc. Sulphuric acid were added to the extract in chloroform. Formation of red color appears at the lower layer indicates the presence of sterols.

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.^[22] 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.^[22] 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% AlCl_3 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 *Cochlospermum Gossypium

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 ($25 \pm 2^\circ\text{C}$). 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).^[23] An extract of *Cochlospermum gossypium* (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.^[24]

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 *Cochlospermum gossypium* at doses of 100mg/kg p.o.

Group V received the extract of *Cochlospermum gossypium* 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 (50 μl) 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. The substrate solution (200 μl) 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 (100 μl), and the optical density was measured at 450 nm.^[25, 26]

Estimation of Cytokines

ELISA kits were used to measure the pro-inflammatory cytokines $\text{TNF-}\alpha$ and $\text{IL-1}\beta$. The quantity of markers was expressed as pg/ml of the serum and determined using standard curves.^[27]

Endogenous Antioxidants

The Ellman method was used to quantify the reduced glutathione (GSH). Superoxide dismutase (SOD) was



measured using the Misra and Fridovich technique. 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.^[27]

Ethical consideration

Permission for conduction of these experiments was obtained, from the relevant ethics committees.

Statistical analysis

The values were expressed as mean ± 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

Results of Fluorescence analysis

Table 1: Fluorescence analysis of leaves of *Cochlospermum gossypium*

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

Results of percentage yield of *Cochlospermum gossypium*

Table 2: % Yield of *Cochlospermum gossypium*

S. No.	Extract	Colour	Odor	% Yield (w/w)
1.	Hydroalcoholic	Dark black	Characteristic	8.50

The hydroalcoholic extract of *Cochlospermum gossypium* was obtained with a percentage yield of 8.50% w/w. The extract appeared dark black in color with a characteristic odor, indicating the successful extraction of phytoconstituents present in the plant material.

Results of phytochemical screening of extract

The phytochemical screening result found to support the results obtained from extractive value as here also

the hydroalcoholic extract of the plants yields maximum positive results for phytochemical test. The preliminary phytochemical screening of the hydroalcoholic extract of *Cochlospermum gossypium* leaves revealed the presence of several important bioactive constituents, indicating the medicinal richness of the plant. The extract tested positive for alkaloids, flavonoids, phenolic compounds, proteins, carbohydrates, and diterpenes, while glycosides, saponins, tannins, and sterols were found to be absent.



Table 3: Result of phytochemical screening of Hydroalcoholic extract of *Cochlospermum gossypium*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids Wagner's test: Hager's test:	+Ve -Ve
2.	Glycosides Conc. H ₂ SO ₄ test	-Ve
3.	Flavonoids Alkaline reagent test: Lead acetate 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]

Preliminary phytochemical screening of the hydroalcoholic extract of *Cochlospermum gossypium* indicated the presence of alkaloids, flavonoids, phenolic compounds, proteins, carbohydrates, and diterpenes. Glycosides, saponins, tannins, and sterols were not detected in the extract. These results confirm that the hydroalcoholic extraction method effectively extracted various phytoconstituents from the plant material.

Results of estimation of total phenol and flavonoid content

Total phenol content estimation (TPC)

The calibration curve of gallic acid for total phenolic content (TPC) estimation showed a steady increase in absorbance with increasing concentration (5–25 µg/ml), indicating a linear relationship between concentration and absorbance. This confirms the reliability and accuracy of the for quantifying phenolic compounds. The obtained calibration data can be effectively used to determine the total phenolic content of the hydroalcoholic extract.

Table 4: Preparation of calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean absorbance
1	5	0.225
2	10	0.439
3	15	0.674



4	20	0.907
5	25	1.124

Total flavonoid content estimation (TFC)

The calibration curve of quercetin for total flavonoid content (TFC) estimation demonstrated a proportional increase in absorbance with increasing concentration

(5–25 µg/ml), indicating good linearity. This confirms the suitability of the for the quantitative determination of flavonoids. The standard curve can be reliably used to calculate the flavonoid content present in the hydroalcoholic extract.

Table 5: Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Mean absorbance
1	5	0.189
2	10	0.387
3	15	0.549
4	20	0.756
5	25	0.924

Table 6: Total phenol and flavonoid content of *Cochlospermum Gossypium*

S. No.	Extract	Total phenol content	Total flavonoid content
		mg/ 100mg	
1.	Hydroalcoholic extract	0.58	0.96

The hydroalcoholic extract of *Cochlospermum gossypium* showed a total phenolic content of 0.58 mg/100 mg and a total flavonoid content of 0.96 mg/100 mg. The results indicate the presence of measurable amounts of phenolic and flavonoid constituents in the extract, with flavonoids being present in a comparatively higher quantity than phenolic compounds.

Results of antiarthritic activity of *Cochlospermum Gossypium* extract

The arthritis control group showed a progressive increase in paw volume throughout the study period compared to the normal control group. Treatment with *Cochlospermum gossypium* extract produced a significant ($P < 0.001$) reduction in paw edema in a dose- dependent manner. The 200 mg/kg-treated group showed greater inhibition of paw swelling than the 100 mg/kg-treated group and demonstrated effects comparable to those of indomethacin by day 28 (Table 7 and Figure 1).

Table 7: Effect of *Cochlospermum gossypium* extract on paw volume in FCA-induced arthritic rats

Paw volume (mL)				
Group	Day 7	Day 14	Day 21	Day 28
Group I	0.22 ± 0.04	0.21 ± 0.03	0.20 ± 0.04	0.20 ± 0.03
Group II	0.74 ± 0.05	0.92 ± 0.04	1.02 ± 0.05	1.08 ± 0.04



Group III	0.60 ± 0.04	0.48 ± 0.03**	0.36 ± 0.04***	0.28 ± 0.03***
Group IV	0.69 ± 0.03	0.61 ± 0.04*	0.54 ± 0.03**	0.46 ± 0.04**
Group V	0.66 ± 0.04**	0.55 ± 0.03**	0.44 ± 0.04***	0.34 ± 0.03***

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

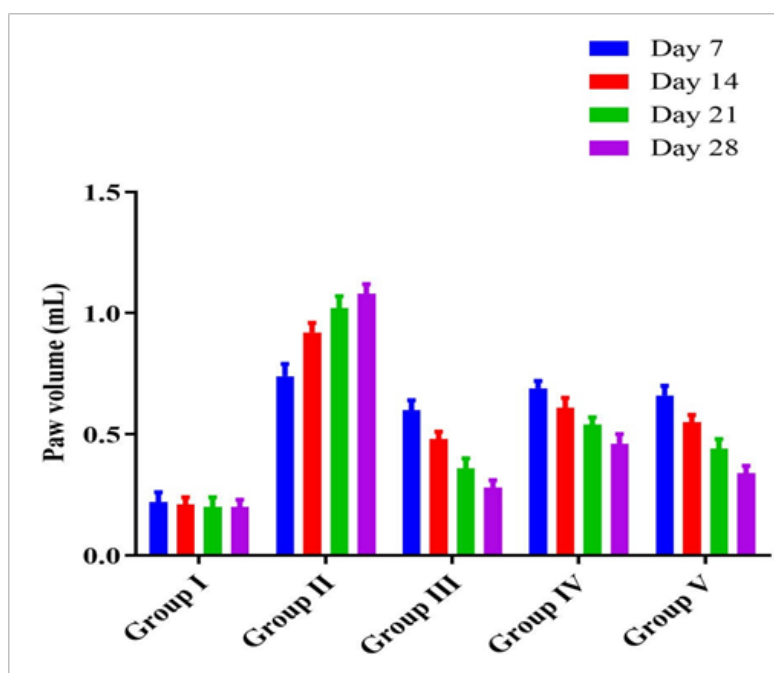


Figure 1: Effect of *Cochlospermum Gossypium* extract on paw volume in FCA- induced arthritic rats

PGE₂ Levels

Treatment with *Cochlospermum gossypium* extract significantly reduced PGE₂ levels in arthritic rats compared to the arthritis control group (Table 8 and Figure 2). The arthritis control group showed a progressive increase in paw volume and elevated

PGE₂ levels, whereas the standard drug indomethacin produced marked inhibition of inflammation. The extract-treated groups showed dose-dependent anti-arthritic activity, with the 200 mg/kg dose exhibiting greater reduction in PGE₂ levels than the 100 mg/kg dose, indicating significant anti-inflammatory potential ($P<0.001$).

Table 8: Effect of *Cochlospermum Gossypium* extract on PGE₂ levels in FCA-induced arthritic rats

Group	PGE ₂ Levels (pg/mL)
Group I	38.5±2.4
Group II	118.6±4.8
Group III	52.4±3.1***
Group IV	74.8±3.6**
Group V	60.2±3.3***

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



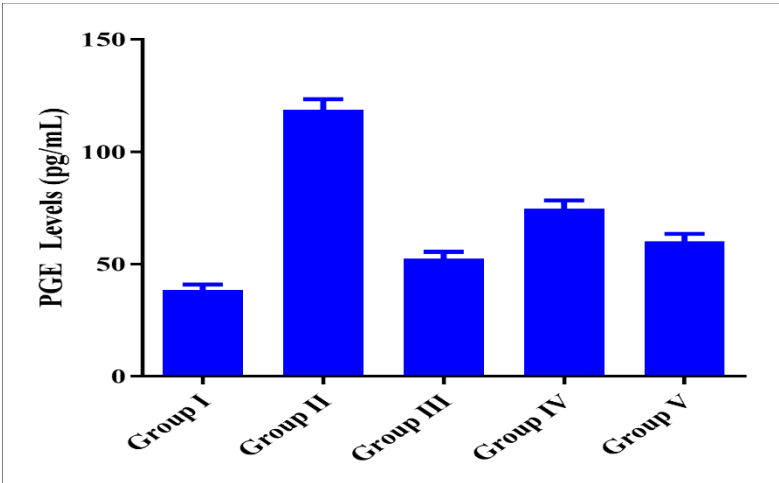


Figure 2: Effect of *Cochlospermum Gossypium* extract on PGE₂ levels in FCA- induced arthritic rats

Cytokine Modulation

The arthritis control group showed markedly elevated IL-1 β levels compared to the normal group, indicating a severe inflammatory response (Table 9 and Figure 3). Treatment with *Cochlospermum Gossypium* extract significantly reduced IL-1 β levels in a dose-

dependent manner. The 200 mg/kg-treated group demonstrated greater suppression of IL-1 β than the 100 mg/kg-treated group and effects comparable to those of the standard drug indomethacin, suggesting potent anti-inflammatory activity of the extract ($P<0.001$).

Table 9: Effect of *Cochlospermum Gossypium* extract on IL-1 β levels in FCA-induced arthritic rats

Group	IL-1 β levels (pg/mL)
Group I	24.6 $\pm$ 1.8
Group II	96.4 $\pm$ 4.5
Group III	38.2 $\pm$ 2.6***
Group IV	58.7 $\pm$ 3.2**
Group V	44.5 $\pm$ 2.9***

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

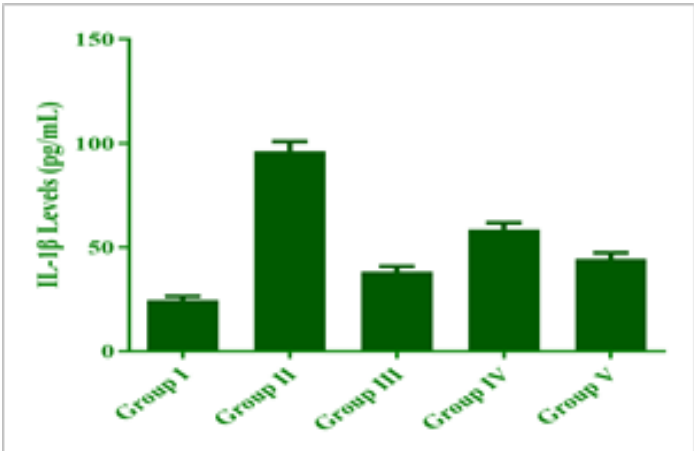


Figure 3: Effect of *Cochlospermum Gossypium* extract on IL-1 β levels in FCA- induced arthritic rats

The arthritis control group exhibited significantly higher TNF- α levels than the normal group, indicating

a severe inflammatory response (Table 10 and Figure 4). Treatment with *Cochlospermum gossypium*

extract notably decreased TNF- α levels in a dose-dependent fashion. The group treated with 200 mg/kg showed greater suppression than the 100 mg/kg group

and effects similar to those of indomethacin, the standard drug, highlighting the extract's potent anti-inflammatory properties ($P<0.001$).

Table 10: Effect of *Cochlospermum Gossypium* extract on TNF- α levels in FCA- induced arthritic rats

Group	TNF- α levels (pg/mL)
Group I	24.6 $\pm$ 1.8
Group II	96.4 $\pm$ 4.5
Group III	38.2 $\pm$ 2.6***
Group IV	58.7 $\pm$ 3.2**
Group V	44.5 $\pm$ 2.9***

Values expressed as mean $\pm$ SEM (n=6) * $P<0.05$.

05, ** $P<0.01$, *** $P<0.001$ as compared to arthritis Control.

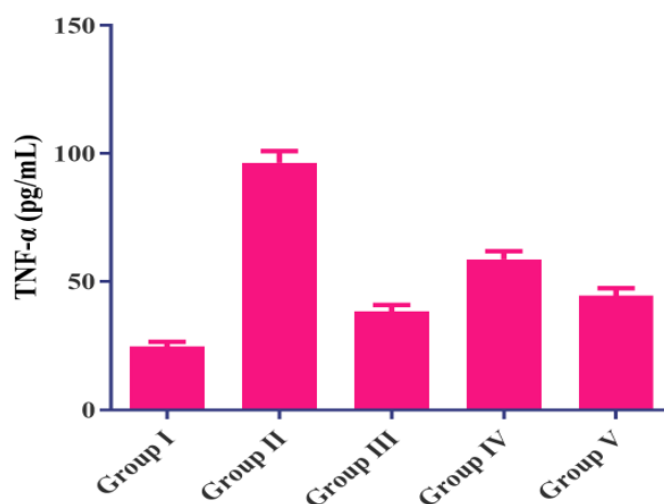


Figure 4: Effect of *Cochlospermum Gossypium* extract on TNF- α levels in FCA- induced arthritic rats

Antioxidant -Restoration

Indomethacin moderately restored GSH and SOD, whereas the extract restored levels. The extract

showed superior antioxidant recovery, indicating added oxidative stress protection beyond anti-inflammatory effects (Table 11 and Fig. 5-6).

Table 11: Effect of *Cochlospermum gossypium* extract on GSH and SOD levels in FCA-induced arthritic rats

Group	GSH levels (μ mol/mg)	SOD levels (U/mg)
Group I	8.62 $\pm$ 0.34	15.8 $\pm$ 0.62
Group II	3.18 $\pm$ 0.21	6.4 $\pm$ 0.38
Group III	7.24 $\pm$ 0.28***	13.9 $\pm$ 0.54***
Group IV	5.86 $\pm$ 0.25**	10.8 $\pm$ 0.46**
Group V	6.74 $\pm$ 0.27***	12.6 $\pm$ 0.49***

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



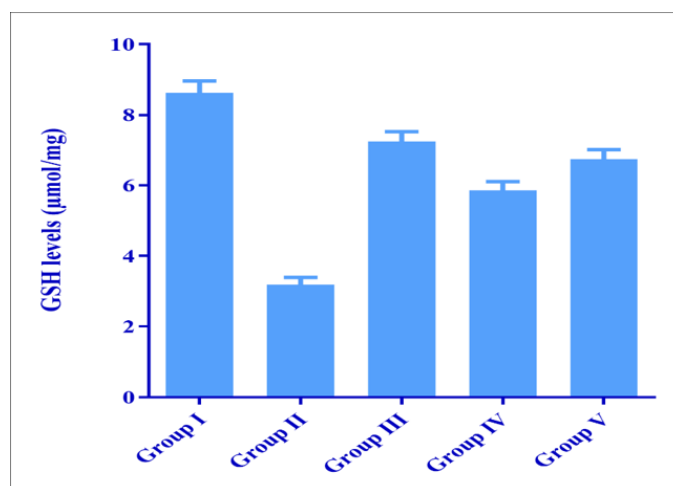


Figure 5: Effect of *Cochlospermum gossypium* extract on GSH levels in FCA-induced arthritic rats

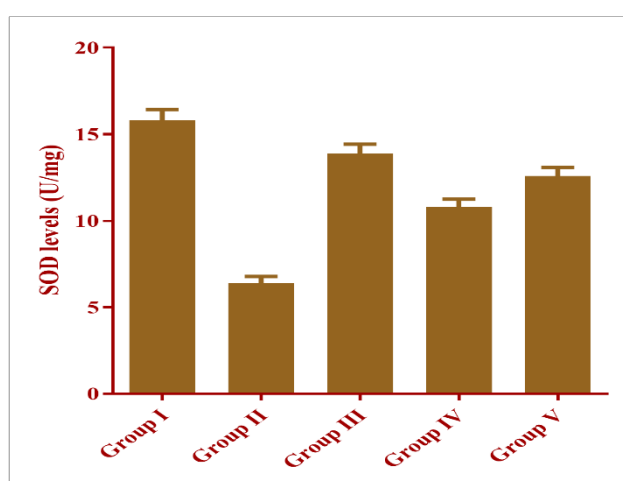


Figure 6: Effect of *Cochlospermum Gossypium* extract on SOD levels in FCA-induced arthritic rats

DISCUSSION

The fluorescence analysis of powdered leaves of *Cochlospermum Gossypium* was carried out by treating the drug with different chemical reagents and observing the color changes under normal daylight, short ultraviolet light, and long ultraviolet light. Fluorescence analysis is an important pharmacognostic evaluation method used for identification, authentication, and detection of adulteration in crude drugs, as many phytoconstituents exhibit characteristic fluorescence behavior when exposed to UV radiation after reaction with specific reagents. The observed fluorescence profile of a plant powder serves as a reliable diagnostic parameter for standardization of herbal materials. In the present study, the untreated powder showed green color under normal light, while it appeared black under both short UV and long UV

light, indicating the presence of naturally occurring chromophoric compounds that undergo quenching under ultraviolet exposure. On treatment with acetic acid, picric acid, ferric chloride, and potassium hydroxide, the powder exhibited dark brown coloration in normal light and remained dark brown under short UV, finally changing to black under long UV light. This suggests the interaction of these reagents with oxidizable phenolic or resinous compounds present in the leaves. Treatment with 1 N hydrochloric acid, 1% sulphuric acid, nitric acid, and aqueous medium produced a brick red color under normal light, dark brown under short UV, and black under long UV. The development of brick red shades in acidic media may be due to the presence of tannin-like polyphenolic substances, flavonoid pigments, or glycosidic compounds that undergo protonation and oxidation, resulting in distinct chromogenic reactions. Similarly, treatment with 1 N sodium hydroxide



produced a coffee-colored appearance in daylight, which may indicate alkaline interaction with phenolic hydroxyl groups and conjugated constituents.

A notable feature of the study is that almost all treated samples appeared black under long UV light, while most showed dark brown under short UV exposure. This consistent ultraviolet fluorescence pattern indicates that the powdered leaves possess several UV absorbing phytoconstituents that exhibit reduced fluorescence intensity after chemical treatment. Such uniform black fluorescence under long UV often suggests the presence of dense phenolic, flavonoidal, or resinous metabolites with strong light absorbing properties.

Thus, the fluorescence characteristics obtained with various reagents provide a characteristic identification profile for *Cochlospermum Gossypium* leaves powder. The distinct changes from green, brick red, coffee, and dark brown under visible light to dark brown and black under UV illumination indicate the presence of diverse chemically reactive phytoconstituents. The fluorescence analysis serves as an important preliminary standardization parameter and can be effectively used for authentication, purity assessment, and quality control of *Cochlospermum Gossypium* crude leaf drug in future pharmacognostic and phytochemical studies. The percentage yield and physical characteristics of the hydroalcoholic extract of *Cochlospermum Gossypium* leaves indicated satisfactory extraction of soluble phytoconstituents from the crude plant material. The obtained hydroalcoholic extract was dark black in colour with a characteristic odor, suggesting the presence of a concentrated mixture of plant metabolites such as phenolic compounds, flavonoids, glycosides, tannins, carbohydrates, and other resinous or pigmented constituents. Dark colored extracts are generally associated with the extraction of oxidized polyphenols, chlorophyll derivatives, and complex secondary metabolites, particularly when hydroalcoholic solvents are used. The hydroalcoholic extract showed a percentage yield of 8.50% w/w, indicating that 4.25 g of dry extract was obtained from every 50 g of powdered leaf material. This extractive value reflects a moderate but appreciable amount of solvent soluble constituents present in the leaves of *Cochlospermum Gossypium*. The percentage yield of

a plant extract depends mainly upon the nature of solvent used, duration of extraction, extraction temperature, particle size of crude powder, and the chemical composition of the plant. Hydroalcoholic solvents are considered highly effective because they dissolve both hydrophilic and moderately lipophilic phytochemicals, thereby increasing the possibility of obtaining a broad spectrum of active

constituents.

The characteristic odor observed in the extract further supports the presence of volatile or semi-volatile aromatic compounds along with non-volatile medicinal metabolites. Such sensory parameters are important preliminary indicators of extract quality and concentration. The dark black appearance may also indicate concentration of tannin-like substances, phenolic pigments, and flavonoidal residues after solvent evaporation.

Thus, the obtained yield of 8.50% confirms that *Cochlospermum Gossypium* leaves contain a considerable quantity of extractable phytochemicals and that the hydroalcoholic extraction procedure adopted was efficient for recovery of medicinally important constituents. The physical appearance and satisfactory extractive value demonstrate the suitability of the prepared hydroalcoholic extract for further phytochemical screening, quantitative estimation, and biological activity studies. This phytochemical profile suggests that the hydroalcoholic solvent was effective in extracting a broad range of polar as well as moderately non-polar secondary metabolites. Among alkaloid tests, Wagner's test showed a positive reaction whereas Hager's test was negative, indicating the probable presence of alkaloidal compounds in lower or selective concentration. Alkaloids are pharmacologically significant nitrogenous compounds known for antimicrobial, anti-inflammatory, analgesic, and protective biological activities. The positive reaction in at least one confirmatory alkaloid test suggests that *Cochlospermum Gossypium* leaves possess certain basic nitrogen containing constituents. Flavonoids were strongly confirmed by both alkaline reagent and lead acetate tests, which showed positive results. The presence of flavonoids is highly important because



these compounds are among the major natural antioxidants responsible for free radical scavenging, anti-inflammatory, Anti-arthritis and wound healing. Similarly, phenolic compounds were confirmed positive by both ferric chloride test and folin-ciocalteu test, indicating a rich occurrence of phenolic hydroxyl containing molecules. Phenols and flavonoids together contribute significantly to antioxidant defense and reduction of oxidative cellular damage. Species of *Cochlospermum* have been reported to contain flavonoidal and phenolic compounds associated with traditional medicinal use, supporting the present findings. Proteins showed positive response in xanthoproteic test, indicating the presence of amino acid-based compounds, while carbohydrates were detected positively by Fehling's test though Benedict's test remained negative, suggesting the presence of certain reducing sugar fractions. Diterpenes were also found present through copper acetate test, signifying the occurrence of terpenoid constituents which are often associated with anti-inflammatory, antimicrobial, and healing properties. On the other hand, glycosides, saponins, tannins, and sterols showed negative results with their respective tests. Their absence indicates either low concentration in the leaves or insufficient extractability under the selected solvent conditions. However, the simultaneous presence of alkaloids, flavonoids, phenols, carbohydrates, proteins, and diterpenes confirms that the hydroalcoholic extract contains multiple classes of biologically active constituents capable of contributing to diverse pharmacological effects. The phytochemical screening establishes that *Cochlospermum Gossypium* hydroalcoholic leaves extract is chemically rich and possesses significant therapeutic potential. The predominance of flavonoids, phenolic compounds, and diterpenes particularly suggests strong antioxidant and medicinal value, thereby justifying its further quantitative estimation, chromatographic characterization, and biological evaluation.

The quantitative estimation of total phenolic and flavonoid contents of the hydroalcoholic extract of *Cochlospermum Gossypium* revealed the presence of significant antioxidant phytoconstituents that are directly associated with anti-arthritis potential. The total phenolic content was found to be 0.58 mg/100

mg gallic acid equivalent (GAE), while the total flavonoid content was recorded as 0.96 mg/100 mg quercetin equivalent (QE) of dry extract. The comparatively higher flavonoid content indicates that flavonoidal compounds constitute the major polyphenolic fraction of the extract and may contribute substantially to its antiarthritic efficacy.

The presence of 0.58 mg/100 mg total phenols suggests that *Cochlospermum Gossypium* extract possesses a good reducing capacity capable of neutralizing reactive oxygen species generated during arthritic progression. Oxidative stress is one of the central mechanisms responsible for destruction of synovial tissues and erosion of cartilage; therefore, the antioxidant action of phenolic compounds can significantly protect joint structures. Likewise, the high total flavonoid content of 0.96 mg/100 mg indicates strong potential for inhibition of inflammatory enzyme pathways and stabilization of collagen proteins. Flavonoids such as quercetin and related polyphenols are widely reported to reduce edema, pain, inflammatory cell infiltration, and autoimmune progression in experimental arthritis models. The predominance of flavonoids over total phenols in the present extract suggests that *Cochlospermum Gossypium* may exert antiarthritic action mainly through flavonoid mediated suppression of chronic inflammation and prevention of protein denaturation.

The effect of *Cochlospermum Gossypium* extract was evaluated in FCA-induced arthritic rats by measuring paw volume, inflammatory mediators, and antioxidant parameters. The arthritic control group showed a progressive increase in paw volume from day 7 to day 28, indicating the development of arthritis. Treatment with the extract reduced paw swelling in a dose-dependent manner, with Group III and Group V showing greater reductions compared to Group IV.

The levels of inflammatory markers, including PGE₂, IL-1 β , and TNF- α , were markedly elevated in the arthritic control group. Administration of the extract significantly lowered these parameters, with higher doses producing greater reductions. Furthermore, the arthritic control group exhibited decreased GSH and SOD levels, whereas treatment groups showed



improved antioxidant status, as evidenced by increased GSH and SOD values. The results indicate that *Cochlospermum Gossypium* extract effectively reduced arthritic manifestations and improved biochemical parameters in FCA-induced arthritic rats.

SUMMARY AND CONCLUSION

Rheumatoid arthritis is a chronic, systemic inflammatory disorder that primarily affects the synovial joints, leading to progressive cartilage destruction, bone erosion, and ultimately disability if not adequately controlled. Unlike osteoarthritis, which results largely from mechanical wear and tear, rheumatoid arthritis is driven by immune dysregulation an inappropriate activation of T cells, macrophages, and synovial fibroblasts that release a cascade of inflammatory mediators. Despite advances in biologic therapies, many patients still experience incomplete responses or intolerable side effects, and access to costly biologics remains limited in resource-poor settings. This reality continues to fuel the search for plant-derived alternatives that are affordable, orally available, and mechanistically broad-spectrum. *Cochlospermum Gossypium*, used traditionally in certain inflammatory conditions, was therefore a rational candidate to evaluate in a well-established animal model of polyarthritis. Freund's complete adjuvant induces arthritis through a carefully orchestrated immunological sequence. When heat-killed *Mycobacterium tuberculosis* suspended in paraffin oil is injected subcutaneously into the rat hind paw, the mycobacterial cell wall components particularly muramyl dipeptide and trehalose dimycolate are recognized by Toll-like receptors (especially TLR2 and TLR4) on resident immune cells. This triggers an intense local innate response, but more importantly, it primes adaptive immunity. Within 10 to 14 days, a T-cell mediated response develops, not only against mycobacterial antigens but also against self-antigens in the host joint through epitope spreading. The result is a symmetrical, progressive arthritis affecting both the injected and the contralateral paw, closely mimicking the chronic relapsing course of human rheumatoid arthritis. The present study on *Cochlospermum Gossypium* leaves demonstrated encouraging pharmacognostic and phytochemical characteristics supporting its potential use as an antiarthritic herbal agent. Fluorescence

analysis of the powdered leaves with various chemical reagents showed characteristic color changes under normal light, short UV, and long UV illumination. The untreated powder appeared green in daylight and black under UV light, while treatment with acidic, alkaline, and other reagents produced brick red, coffee, dark brown, and black shades. These distinct fluorescence behaviors indicate the presence of chemically reactive phytoconstituents and provide a characteristic identification profile useful for authentication and standardization of the crude drug. Hydroalcoholic extraction of the powdered leaves yielded a dark black extract with characteristic odor and a percentage yield of 8.52% w/w, indicating satisfactory extraction of solvent soluble active constituents. The appreciable extractive value confirms that the selected hydroalcoholic solvent system was suitable for obtaining a concentrated medicinal extract rich in bioactive metabolites. Preliminary phytochemical screening of the hydroalcoholic extract revealed the presence of alkaloids, flavonoids, phenolic compounds, proteins, carbohydrates, and diterpenes, whereas glycosides, saponins, tannins, and sterols were absent. Among these, the presence of flavonoids and phenols is particularly important because these compounds are well recognized for their antioxidant, anti-inflammatory, membrane stabilizing, and tissue protective effects, which are directly relevant in the management of arthritic disorders. Diterpenes and alkaloids may further contribute to analgesic and anti-inflammatory activity. Quantitative estimation of polyphenolic constituents demonstrated that the total phenolic content of the hydroalcoholic extract was 0.58 mg/100 mg gallic acid equivalent, while the total flavonoid content was 0.96 mg/100 mg quercetin equivalent. The higher flavonoid concentration compared to total phenols indicates that flavonoids form the dominant antioxidant fraction of the extract. Polyphenolic compounds, particularly flavonoids, are reported to inhibit oxidative stress, suppress inflammatory cytokines, reduce lysosomal enzyme release, prevent protein denaturation, and protect cartilage and synovial tissues from inflammatory destruction. These mechanisms are considered central to antiarthritic activity.

Thus, all the obtained results collectively confirm that *Cochlospermum Gossypium* leaves possess good



pharmacognostic identity, satisfactory extractive efficiency, rich phytochemical composition, and appreciable quantities of phenolic and flavonoidal antioxidants. The predominance of antioxidant and anti-inflammatory constituents provides strong scientific evidence that the hydroalcoholic extract may effectively combat oxidative stress and inflammatory processes involved in arthritis. Therefore, the plant can be considered a promising natural source for further in vivo antiarthritic evaluation and herbal formulation development.

In the present study, the steady increase in paw volume in untreated arthritic rats from day 7 through day 28 confirmed that a robust, persistent inflammatory state had been successfully established. Looking at the paw volume data, the pattern of effect was dose-dependent and progressive. The lower dose (100 mg/kg) produced modest but statistically significant reductions by day 21, while the higher dose (200 mg/kg) showed significant improvement as early as day 14 and continued to widen the gap against the untreated control through day 28. By the end of the experiment, the 200 mg/kg group had achieved paw volumes approaching those of the indomethacin group. Importantly, the extract did not simply blunt the acute flare; it slowed the *progression* of swelling over the two-week treatment period. This temporal pattern suggests disease-modifying potential rather than purely symptomatic relief a distinction that matters greatly in chronic arthritis, where the goal is to prevent irreversible joint destruction, not merely to relieve today's pain. Understanding the mechanism of *Cochlospermum Gossypium* requires looking at the central pathways of joint inflammation. PGE₂, synthesized from arachidonic acid via the cyclooxygenase enzymes, is a principal mediator of vasodilation, increased vascular permeability, and pain sensitization in the arthritic joint. Indomethacin, the reference standard used here, exerts its effect by non-selectively inhibiting COX-1 and COX-2, thereby blocking PGE₂ production. However, the inflammatory cascade does not stop at prostaglandins. Pro-inflammatory cytokines particularly TNF- α and IL-1 β occupy a more upstream position. They are released by activated macrophages and synovial cells and then go on to stimulate COX-2 expression, matrix metalloproteinase production, osteoclast activation, and further recruitment of inflammatory cells.

Between them, TNF- α and IL-1 β create a self-amplifying loop that sustains chronic inflammation. An effective anti-arthritic agent may interrupt this loop at one or several points. Endogenous antioxidants such as reduced GSH and SOD are typically overwhelmed in chronic inflammation, and their depletion serves as both a marker and a mediator of tissue injury.

The results of the present study suggest that *Cochlospermum Gossypium* extract acts through at least three complementary mechanisms. First, it reduced PGE₂ levels. While indomethacin produced the numerically greater reduction, the 200 mg/kg extract achieved a decrease that was statistically significant compared to arthritic controls and not significantly different from indomethacin. This indicates that the plant extract likely interferes with the arachidonic acid pathway, possibly through direct or indirect inhibition of one or both cyclooxygenase enzymes. Second, the extract significantly lowered both TNF- α and IL-1 β . The 200 mg/kg dose reduced TNF- α to levels that slightly exceeded those seen with indomethacin, and it lowered IL-1 β to a comparable degree. This is a particularly meaningful finding because suppressing these upstream cytokines would, in theory, reduce the drive for PGE₂ synthesis rather than merely blocking the end product. In human rheumatology, biologic agents that neutralize TNF- α or IL-1 β have revolutionized treatment; an orally active plant extract achieving similar cytokine suppression in an animal model invites serious attention. Third and perhaps most distinctive the extract restored GSH and SOD levels more effectively than indomethacin.

In conclusion, *Cochlospermum Gossypium* extract demonstrated meaningful anti-arthritic activity in the FCA-induced rat model. The mechanism appears to involve suppression of PGE₂, downregulation of the pro-inflammatory cytokines TNF- α and IL-1 β , and distinct from indomethacin significant restoration of the endogenous antioxidant defenses GSH and SOD. This combined profile suggests that the plant may offer a more balanced approach to chronic inflammatory arthritis, addressing both the immune-driven mediators of inflammation and the oxidative tissue damage that accompanies them. Future work should focus on phytochemical characterization of the



active constituents, dose optimization studies, and ultimately, evaluation in human inflammatory arthritis. The present findings provide a clear experimental basis for considering *Cochlospermum Gossypium* as a promising candidate for further development.

FUTURE PROSPECTS

The findings of the present study indicate that *Cochlospermum Gossypium* possesses promising anti-arthritic potential and warrants further investigation. Future studies may focus on the isolation, purification, and characterization of the active phytoconstituents responsible for the observed pharmacological effects. Advanced analytical techniques such as can be employed for detailed phytochemical profiling. Further research should be conducted to elucidate the precise molecular mechanisms underlying its anti-inflammatory and anti-arthritic activities. Long-term toxicity and safety studies are also necessary to establish its therapeutic applicability. In addition, formulation development and clinical evaluation may help in translating the plant extract into an effective herbal therapeutic agent for the management of rheumatoid arthritis and other inflammatory disorders. The exploration of synergistic effects with existing anti-arthritic drugs may also provide new avenues for improved treatment strategies.

Conflict of interest: No any

ACKNOWLEDGEMENT:

I'm deeply grateful to my guide for expert mentorship, thank you for your constant guidance, patience, and belief in me throughout this pharmacy research journey. Your insights shaped this work. A special thank you to Akanksha Anjali, my incredible friend. Your support, encouragement, and help made every challenge feel lighter.

REFERENCES

1. Maetzel A, Li LC, Pencharz J, Tomlinson F, Bombardier C. The economic burden associated with osteoarthritis, rheumatic arthritis and hypertension: a comparative study. *Ann. Rheum. Dis.* 2004; Vol. 63 (4): 395 – 401.
2. Drake R, Vogl AW, Mitchell AWM in “Grey’s Anatomy” 2nd Ed.; Skeletal muscle system. 2009: p21 – 52.
3. Das, T., Dey, Y.N., Pal, T. et al. Preclinical assessment of anti-arthritic activity of methanolic extract from processed *Plumbago zeylanica* roots. *Sci Rep* 15, 11454 (2025)
4. Emmanuel Kofi Kumatia, Prince Kyei Baffour and Peter Bolah. Antiarthritic and Antioxidant Activities of Antrocaryon micrastrer Seed Extract and Its Fractions. *Hindawi BioMed Research International*, 2024; Volume (2024).
5. Ayushi Sharma, Anjana Goel and Zhijian Lin. In Vitro and In Silico Anti- Rheumatic Arthritis Activity of *Nyctanthes arbor-tristis*. *Molecules*, 2023, 28(16), 1-16.
6. L Ronibala Singha, Sentu Das, Malay Kumar Das. Plant-derived Anti-arthritic Nanomedicines for Effective Therapy in the Management of Inflammatory Diseases. *Pharmacognosy Reviews*, 2023; 17(33):104-122.
7. Jamadar MJ, Khulbe P and Mohite Sk., Evaluation of the anti-arthritic activity of *bauhinia acuminata* (linn) leaves extracts in experimental models. *IJBPAS*, 2023, 12(7): 3430-3439.
8. Bapan Banik and Malay Kumar Das. Therapeutic Potential of *Alstonia scholaris* Latex in the Management of Inflammatory Diseases: An in vitro Approach. *Pharmacognosy Research*, 2023; 15(3) 478-483.
9. Iram Jan, Ashish Dogra, Syed Assim Haq, Sumit Dhiman, Gurdarshan Singh, Mithilesh Kumar. Anti-arthritic Potential of Aqueous and Ethanolic Extracts of *Euphorbia helioscopia* on Adjuvant-induced Arthritis in Rats. *Pharmacognosy Research*, 2022; 14(3), 304-309.
10. Farooq S, Shaheen G, Asif HM, Aslam MR, Zahid R, Rajpoot SR, Jabbar S, Zafar F. Preliminary phytochemical analysis: In-Vitro comparative evaluation of anti-arthritic and anti-inflammatory potential of some traditionally used medicinal plants. *Dose-Response*. 2022; 20(1):1-8.
11. Prajapati, P., & Gajera, V. Anti-arthritic effect of methanol extract of leaves of *Abies webbiana* Lindl. in complete Freund’s adjuvant-induced arthritis in albino rats. *International Journal of Health Sciences*, 2022; 6(S1), 7400-7417.



12. Vithya Dharmaraj, Joseph Sebastin Raj, Sheela.T, Seema S. and Pragyana Paliwal. Evaluation of Anti-Arthritic Activity by Ethanolic Stem Bark and Leaves Extract of *Pterocarpus indicus* Wild. Bulletin of Environment, Pharmacology and Life Sciences Bull. Env. Pharmacol. Life Sci., 2022; Spl. Issue [5]:660-664.
13. Cooke, T. (1903). Bixaceae: The Flora of Presidency of Bombay, Tayler & Francis, London. Vol. I: 55-61.
14. Sasikala A, Lingarao M, Savithramma N. Weekly Sci Res J 2013; 1:1-7
15. Sasikala A and Savithramma N. A medicinal tree species -Phytochemical diversity of *Cochlospermum religiosum* (L.) Alsten. Threats and Concerns to Biodiversity 2012.
16. Kirtikar K, Basu B. Indian Medical Plants, Vol. 1, second ed. Oriental Enterprises, Dehradun, India: 1987;200- 201.
17. Sasikala A, Lingarao M and Savithramma N. Quantification of primary and secondary metabolites from leaves and stem barks of *Cochlospermum religiosum* (L.) Aston. Int Res J Pharm 2013;
18. Janaki, B.; Sashidhar, R. B. From Food Chemistry (1998), 61(1-2), 231-236.
19. Kothale KV, Rothe SP. Ethnomedico-botanical study from tribal regions of west v1darbha (MS). J Comp ToxicolPhysiol 2009; 6(1):030-034.
20. Mukherjee PK. Quality Control of Herbal Drugs, 2 nd Edition, Business Horizons, 2007; 2-14.
21. Kokate CK. Ed. Practical Pharmacognosy, 4 th Edn., Vallabh Prakashan: 1994; 112:120.
22. Arpana Gaur Mishra, Richa Singh, Neha Patil, Geeta Parkhe. Determination of total phenolic, flavonoid content, antioxidant and antimicrobial activity of *Gloriosa superba* seed extract. Asian Journal of Pharmaceutical Education and Research. 2017, 6(2):12-17.
23. Saleem U, Zaib S, Khalid S, Anwar F, Akhtar MF, Ahmad B. Chemical characterization, docking studies, anti-arthritic activity and acute oral toxicity of *Convolvulus arvensis* L. leaves. Asian Pacific Journal of Tropical Biomedicine. 2020;10(10):442-51.
24. Rathore R, Kushwaha S, Patel N, Tyagi C. Phytochemical screening and anti-arthritic activity of hydroalcoholic extract of *Verbascum thapsus*. World Journal of Pharmaceutical Research. Vol 12, Issue 5, 2023; 1322-1331.
25. Akhtar MF, Raza SA, Saleem A, Hamid I, Ashraf Baig MMF, Sharif A, et al. Appraisal of anti-arthritic and anti-inflammatory potential of folkloric medicinal plant *Peganum harmala*. Endocrine, Metabolic & Immune Disorders- Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders). 2022;22(1):49-63.
26. Alzarea SI, Alasmari AF, Alanazi AS, Alzarea AI, Alharbi M, Alshammari A, et al. Butin attenuates arthritis in complete Freund's adjuvant-treated arthritic rats: possibly mediated by its antioxidant and anti-inflammatory actions. Frontiers in pharmacology. 2022;13:810052.
27. Alavala S, Nalban N, Sangaraju R, Kuncha M, Jerald MK, Kilari EK, et al. Anti-inflammatory effect of stevioside abates Freund's complete adjuvant (FCA)-induced adjuvant arthritis in rats. Inflammopharmacology. 2020;28(6):1579-97.

HOW TO CITE: Akash Kumar*, Santosh Kumar Ojha, Satish Kumar Sarankar, Study of Phytochemical Screening and In Vivo Antiarthritic Activity of *Cochlospermum Gossypium*, J. Pharm. Sci., 2026, 2 (7), 1688-1710.
<https://doi.org/10.5281/zenodo.21426899>

