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Standardization And Analytical Profiling Of A Selected Polyherbal Formulation For Osteoarthritis

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ABSTRACT

The present study focuses on the development and evaluation of a polyherbal formulation comprising Anethum graveolens, Cedrus deodara, Piper longum (fruit and stem), Embelia ribes, and Saindhava lavana, traditionally used in Ayurvedic medicine for their anti-inflammatory and analgesic properties. The formulation was subjected to detailed pharmacognostic and physicochemical assessments, including organoleptic evaluation, foreign organic matter analysis, ash values, extractive values, and fluorescence tests. Phytochemical screening confirmed the presence of significant levels of phenols and tannins.

To evaluate the anti-arthritic efficacy, the formulation was tested using Complete Freund's Adjuvant (CFA)-induced arthritis in Wistar rats. Key parameters such as paw volume, joint diameter, arthritic score, body weight, nociceptive and thermal hyperalgesia thresholds, hematological indices, and inflammatory biomarkers (CRP, RF, ALP, ALT, AST) were measured. The treated groups exhibited a significant reduction in inflammatory and arthritic symptoms compared to the arthritic control group, alongside normalization of hematological and biochemical markers. The results indicate a potent anti-arthritic effect, likely due to the synergistic action of the bioactive phytoconstituents present in the formulation.

This study supports the therapeutic potential of the polyherbal formulation as a safe and effective alternative for managing rheumatoid arthritis and calls for further exploration in preclinical and clinical settings

Keywords: Polyherbal formulation, Anti-arthritic activity, Anethum graveolens, Cedrus deodara, Piper longum, Embelia ribes, Rheumatoid arthritis, CFA-induced arthritis.

1. INTRODUCTION

Herbal medicine, rooted deeply in ancient traditions, continues to be a cornerstone of healthcare systems worldwide. Among the many forms of traditional remedies, polyherbal formulations—which involve the strategic combination of multiple herbs—have gained considerable attention due to their enhanced therapeutic potential, broad-spectrum activity, and synergistic effects. Unlike single-herb preparations, polyherbal remedies are designed based on the principle that the combination of phytoconstituents from various plants can act additively or synergistically to improve efficacy, reduce toxicity, and offer holistic treatment [1-4].

In India, polyherbalism forms the core of Ayurvedic and Siddha systems of medicine. Ancient Ayurvedic treatises such as the Charaka Samhita and Sushruta Samhita document numerous multi-herb remedies used for a wide array of ailments, including joint disorders, digestive issues, metabolic conditions, and neurological problems. The growing demand for safer alternatives to synthetic drugs, especially for chronic and lifestyle-related disorders such as osteoarthritis, has reignited scientific interest in exploring and validating traditional polyherbal formulations through standardized and reproducible methods [5-8].

Need for Standardization and Quality Control

The global recognition of herbal medicines has created an urgent need for establishing robust quality assurance and standardization protocols. Variations in plant sources, geographical origin, harvesting techniques, and processing methods can significantly affect the phytochemical composition and therapeutic value of herbal products. Without standardization, the safety, efficacy, and reproducibility of polyherbal medicines remain uncertain, hampering their acceptance in evidence-

based medicine and regulatory approval [9-10].

Standardization typically includes organoleptic evaluation, physicochemical profiling (e.g., loss on drying, ash values, extractive values), fluorescence analysis, and phytochemical screening. These tests help in the identification, authentication, and quality control of plant materials. Moreover, techniques such as powder fluorescence analysis provide a simple yet effective method to detect adulteration and confirm identity based on characteristic fluorescence under UV light [11-13].

Anethum graveolens possesses carminative, anti-inflammatory, and antioxidant properties [14].*Cedrus deodara* is valued for its analgesic and anti-arthritic potential.Piper longum, used in various Ayurvedic formulations, acts as a bioavailability enhancer and exhibits anti-inflammatory and immunomodulatory effects [15].*Embelia ribes* is known for its anthelmintic, anti-inflammatory, and antimicrobial activities [16].*Saindava Lavana*, a naturally occurring rock salt, improves digestion and absorption, and is often used as a vehicle (Anupana) in Ayurvedic therapy [17-18].

The combination of these ingredients is not arbitrary but based on complementary pharmacodynamic actions that enhance therapeutic outcomes and reduce potential toxicity. Despite their widespread traditional use, limited scientific data exists on the standard physicochemical parameters and fluorescence characteristics of this specific formulation [19-20].

The present research focuses on a scientifically composed polyherbal formulation comprising the following ingredients: *Anethum graveolens* (Satapuspa), *Cedrus deodara* (Devadaru), *Piper longum* (Pippali), *Embelia ribes* (Vidanga), *Piper longum* (stem part - Pippalimula), and *Saindava Lavana* (rock salt). These ingredients are traditionally known for their medicinal properties, especially in the management of inflammatory conditions, digestive disorders, and musculoskeletal ailments, particularly osteoarthritis.

Objectives of the Study

The aim of this study is to conduct a comprehensive evaluation of the above-mentioned polyherbal formulation by applying established pharmacognostic and physicochemical parameters. The specific objectives include:

To determine foreign organic matter, loss on drying, and total and acid-insoluble ash values of the individual crude drugs.

To evaluate alcohol- and water-soluble extractive values, which reflect the solubility and phytochemical richness of the ingredients.

To perform powder fluorescence analysis of individual components under visible and ultraviolet light (254 nm and 366 nm), aiding in authentication.

To ensure that all evaluated parameters comply with Ayurvedic Pharmacopoeia standards, thereby confirming the quality, identity, and purity of the formulation.

Significance of the Study

The results of this investigation will serve as a scientific foundation for standardizing this polyherbal formulation, thereby facilitating its integration into modern evidence-based therapeutic practice. By generating validated quality control data, this study supports the safe and effective use of traditional herbal knowledge while ensuring regulatory compliance. Furthermore, this work could stimulate further pharmacological and clinical research aimed at establishing the efficacy of this formulation in managing osteoarthritis and related inflammatory conditions.

2. MATERIAL AND METHODS

All these ingredients were procured from the local market and were authenticated by a botanist. Polyherbal powdered preparation (Churna), consisting of six ingredients, was formulated following traditional Ayurvedic procedures and stored in airtight containers for further pharmacognostic and phytochemical evaluation.

List of Ingredients used in Formulations

Anethum graveolens

Cedrus deodara

Piper longum

Embelia ribes

Piper longum (stems)

Salt (*Saindava lavana*)

Determination of Physico-Chemical Constants

1. Foreign organic matter- 250 g i.e. quantity specified in the individual monograph, of the original sample was weighed accurately and spread out in a thin layer. The samples were inspected with the unaided eye or with the use of a magnifying lens (6X or 10X) and the foreign organic matter were separated manually as completely as possible and weighed. The

percentage of foreign organic matter was weighed and determined with reference to the weight of the drug taken.

2. Loss on drying (LOD) -About 2-5 g of the prepared air-dried individual materials were accurately weighed in a previously dried and tared flat weighing bottle. The samples were distributed evenly and were placed in the drying chamber (Oven). Drying was carried out by heating to 100-105°C, the bottle was removed from the oven and the bottle was closed promptly and allowed to cool to room temperature and then weighed. The experiment was repeated till two consecutive weighing and the results did not differ by more than 5 mg, unless otherwise stated in the test procedure. The loss in weight on drying was then calculated.

3. Ash value- Ash content of the crude drug is generally taken to be the residue remaining after incineration. It represents the inorganic salts naturally occurring in the drug and adhering to it, but may also include inorganic matter added for the purpose of adulteration.

Total ash is the residue remaining after incineration. Acid insoluble ash is the part of the total ash, which is insoluble in dilute hydrochloric acid. Water-soluble ash is the part of total ash, which is soluble in hot water.

a. Total ash- About 2g of the individual powdered ingredients of Ajmodadi churna were accurately weighed in a tared silica crucible. The powdered drug was spread as a fine layer at the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighed. The procedure was repeated till a constant weight was observed. The percentage of the total ash was calculated in triplicate with reference to the air-dried drug.

b. Acid insoluble ash- The ash obtained as described in the determination of total ash was boiled with 25 ml of hydrochloric acid for 5 min. The insoluble ash was collected on an ashless filter paper by filtration and it was washed with hot water. The insoluble ash was transferred into a tared silica crucible, ignited, cooled and weighed. The procedure was repeated till a constant weight was observed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

4. *Extractive values*-Extractive value is a measure of the content of the drug extracted by solvents. Extractive value can be water soluble, ethanol soluble and ether soluble extractives. Extractive value is unless and otherwise prescribed, carried out by maceration.

a. Water soluble extractive- 4 g of previously weighed air-dried powdered individual ingredients of Ajmodadi churna were taken in a glass stoppered flask and macerated with 100 ml of chloroform water (1:99). It was shaken frequently for 6 h and then allowed to stand for 18 h. It was filtered rapidly taking precautions against loss of the solvent. 25 ml of filtrate was evaporated to dryness in a tared flat-bottomed petri dish, dried at 105°C, cooled in a dessicator and weighed. The percentage of water-soluble extractive was calculated with reference to air-dried drug.

Note - Ethanol soluble extractive, follow the same procedure of water-soluble extractive excepting the solvent (water) which is replaced by ethanol.

5. *Fluorescence analysis* - The powdered samples were exposed to Ultraviolet light at wavelength of 254 nm and 366 nm. Flouroscence analysis was carried out in accordance with the procedure reported by Kokoshi *et al*. One mg of powdered drug was placed on a micro slide and observed under UV 366, UV 254 and in day light to observe the fluorescent characteristics of powder, if any. One mg powdered drug was placed on a micro slide and treated with one ml 1N HCl and observed under UV 366, UV 254 and in day light while wet. One mg powdered drug was placed on a micro slide and treated with one ml 1N NaOH and observed after a few minutes in day light, under UV 366, UV 254. One mg powdered drug was placed on a micro slide and treated with one ml 1N NaOH in one ml methanol and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with one ml 50% KOH and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with 1 ml of 50% sulphuric acid and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with 1 ml of conc. sulphuric acid and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with one ml of 50% HNO_3 and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with one ml of Conc. HNO_3 and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with one ml of acetic and observed under UV 366, UV 254 and in day light while still wet. One mg powdered drug was placed on a micro slide and treated with 1 ml of iodine and observed under UV 366, UV 254 and in day light while still wet [21-23].

5.4 Preparation of Churna

The In-house formulation was prepared as per the procedure given in Ayurvedic Formulary of India. All the ingredients were powdered separately, passed through 80 # sieve and then mixed together in specified proportions to get uniformly blended churna. Formulation was formulated for both the preparation according to The Ayurvedic Formulary of India, 2003 [24].

Table 1: Formula for Prepared Formulation

Sr. No.	Ingredients	Quantity (gm)
	Anethum graveolens	12
	Cedrus deodara	12
	Piper longum	12
	Embelia ribes	60
	*Piper longum(*stems*)*	60
	Salt *(Saindava lavana)*	120

5.5 Preparation of extracts-

Methanolic extract- The powdered formulationabout 100 g were extracted with methanol by hot extraction process (soxhlet) for 72 h. After completion of the extraction, the solvent was recovered by distillation and concentrated *in vacuo.*

Aqueous extract (AJM) - (Chloroform:water taken in 1:99) The powdered formulation wasa macerated with chloroform water for seven days. After the completion of maceration residue was removed by filtration followed by the evaporation of solvent and extract was concentrated *in vacuo.*

5.6 Determination of Physico-Chemical Parameters

1. Organoleptic evaluation - Organoleptic evaluation refers to evaluation of formulation by color, odour, taste, texture etc.

2. Ash values and *extractive values*

3. *Determination of pH* - The pH offormulation in 1% w/v and 10% w/v of water-soluble portions was determined using pH paper (range 3.5-6) and (6.5-1.4) with standard glass electrode at 24^0 C.

4. *Estimation of sodium content* - Sodium content was estimated by flame photometer by using a flame photometer. A stock solution 100µg/ml of NaCl was prepared in distilled water and further dilutions were made to get 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml, 12µg/ml respectively for preparing the standard graph shown in the table. Sodium content of the formulations was estimated by flame photometric method based on the measurement of emission intensity in nanometer. The method was validated for linearity, precision, and accuracy. The method obeyed Beer's law in the concentration range 2-12 µl/ml. The standard drug solution was assayed repeatedly (n=3) and mean error and relative standard deviation (precision) was calculated [25-31].

5. *Determination of physical characteristics of formulations*- Physical characteristics like bulk density, tap density, angle of repose, Haussner ratio and Carr's index were determined for different formulations [32-33]. The term bulk density refers to method used to indicate a packing of particles or granules. The equation for determining bulk density (D_b)is $D_b=M/V_b$ where M is the mass of particles and V_b is the total volume of packing. The volume of packing can be determined in an apparatus consisting of graduated cylinder mounted on mechanical tapping device (Jolting Volumeter) that has a specially cut rotating can. Hundred gm of weighed formulation powder was taken and carefully added to cylinder with the aid of a funnel.

The initial volume was noted and sample was then tapped until no further reduction in volume was noted. The initial volume gave the bulk density value and after tapping the volume reduced, giving the value of tapped density.

6. *Angle of repose* has been used as an indirect method quantifying powder flowability, because of its relationship with interparticle cohesion. The fixed funnel and the free-standing cone method employs a method that is secured with its tip at a given height (H), above the glass paper that is placed on a flat horizontal surface. Powder or granules were carefully poured through the funnel until the apex of the conical pile just touched the tip of funnel. Thus, with R being the radius of the conical pile. Tan α = H/R or α = arc tan H/R, where α is the angle of repose.

Table 2: Relationship between angle of repose and type of flow

S. No.	Flow property	Angle of repose (degree)
	Excellent	25-30
	Good	31-35
	Fair	36-40

	Passable	41-45
	Poor	46-55
	Very Poor	56-65
	Very-very Poor	>66

7. *Haussner's ratio* is related to interparticle friction and as such can be used to predict the powder flow properties. The equation for measuring the Hausner's ratio is D_f/D_o

Where, D_f = Tapped density and D_0 = Bulk density.

Table 3: Relationship between Hausner's ratio and type of flow

Sr. No.	Flow property	Hausner's ratio
	Excellent	1-1.11
	Good	1.12-1.18
	Fair	1.19-1.25
	Passable	1.26-1.34
	Poor	1.35-1.45
	Very Poor	1.46-1.59
	Very-very Poor	>1.6

8. *Carr's compressibility index* is another indirect method of measuring the powder flow from bulk density. The equation for measuring Carr's index is

$$\% \text{ Compressibility} = \frac{D_f - D_0}{D_f} \times 100$$

Where D_f = tapped density, D_0 = Bulk density.

Table 4: Relationship between compressibility index and type of flow

S. No.	Flow property	Compressibility index (%)
	Excellent	≤10
	Good	11-15
	Fair	16-20
	Passable	21-25
	Poor	26-31
	Very Poor	32-37
	Very-very Poor	>38

9. *Total Phenolic Content* Reagents used for total phenolic content were Folin Ciocalteu Reagent and Sodium Carbonate (20 % w/v).

Procedure: Total soluble phenolics in the extracts weredetermined with Folin-Ciocalteu reagent using gallic acid as a standardphenolic compound. 1.0 ml of extract solutioncontaining 1.0 mg extract in a volumetric flaskwas diluted with 46 ml of distilled water. 1.0 ml of Folin-Ciocalteu reagent was added and the content of the flask mixed thoroughly. 3 min later 3.0

ml of 2% sodium carbonate was added andthe mixture was allowed to stand for 2 h with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm [34].

10.*Tannin Content*-Tannins are substances capable of turning animal hide into leather by binding with proteins to form water soluble substance which are resistant to proteolytic enzymes. This process, when applied to living tissue it is known as astringent and therefore used as a therapeutic agent.

Procedure:About 2 g of the formulationwas weighed and transferred into a conical flask. 150 ml water was added and boiled for 30 min. It was cooled and transferred to a 250-ml volumetric flask with water and the volume was made up with water. The solution was filtered and the total amount of material extractable into water was determined by evaporating 50 ml of the extract to dryness, drying was continued at 105º C till constant weight was obtained as follows (T_1). The amount of plant material remains unbound to the hide powder after its addition and which is extractable into water was determined. About 6.0g of hide powder was added to about 80 ml of the above extract. The mixture was shaken for 60 min and was filtered. 50 ml of the filtrate was evaporated to dryness, drying was continued at 105º C till a constant weight (T_2). The solubility of hide powder was determined by taking 6.0 g of hide powder in 80 ml of water and the mixture was shaken for 60 min and filtered. 50 ml of clear filtrate was evaporated to dryness as described earlier and was noted (T_0). The quantity of total tannins was thus calculated by the following formula [35].

Quantity of tannins (%) = $[T_1 – (T_2 + T_0)] \times 500 / w$

Where w, is the weight of leaf powder in grams

a. Estimation of Gallic acid in formulation

Preparation of Gallic acid standard solution- A stock solution of standard Gallic acid (1 mg/ml) was prepared by transferring 5 mg of Gallic acid, accurately weighed, into a 5 ml volumetric flask, dissolving in 2 ml methanol. It was then sonicated for 10 min and the final volume of the solutions was made up to 5 ml with methanol to get stock solutions containing 1 mg/ml.

Preparation of sample solution- Accurately weighed 100 mg of dried methanolic extract of *Preparation A* was transferred to a 10 mlvolumetric flask dissolving in 5 ml of methanol. It was then sonicated for 10 min and the contents of the flask were filtered through Whatman No. 1 paper (Merck, Mumbai, India). The final volume of the solution was made up to 5 ml with methanol to get stock solution of 20 mg/ml.

Mobile phase- Toluene: Ethyl acetate: Formic acid: Methanol (6: 6: 1.8: 0.25).

Instrumentation And Chromatographic Conditions- HPTLC was performed on 20 cm × 10 cm aluminum backed plates coated with silica gel $60F_{254}$ (Merck,Mumbai, India). Standard solution of Gallic acid and sample solution were applied to the plates as bands on the same chromatographic plate by use of a Camag (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100 µl Hamilton (USA) syringe. Ascending development was performed at room temperature (28 ± 2°C), withthe mobile phase in a Camag glass twin-trough chamberpreviously saturated with mobile phase vapour for 20min. After development, the plates were dried and then scanned at 254 nm with a Camag TLCScanner- 3 [36].

b. *Estimation of Piperine in formulation*

Preparation of piperine standard solution- A stock solution of standard *Piperine* (1 mg/ml) was prepared by transferring 5 mg of *Piperine*, accurately weighed, into a 5 ml volumetric flask, dissolving in 2 ml methanol. It was then sonicated for 10 min and the final volume of the solutions was made up to 5 ml with methanol to get stock solutions containing 1 mg/ml.

Preparation of sample solution- Accurately weighed 100 mg of dried methanolic extract of *Preparation A* was transferred to a 10 mlvolumetric flask dissolving in 5 ml of methanol. It was then sonicated for 10 min and the contents of the flask were filtered through Whatman No. 1 paper (Merck, Mumbai, India). The final volume of the solution was made up to 5 ml with methanol to get stock solution of 20 mg/ml.

Mobile phase- benzene: ethyl acetate (2:1)

Instrumentation And Chromatographic Conditions- HPTLC was performed on 20 cm × 10 cm aluminum backed plates coated with silica gel $60F_{254}$ (Merck,Mumbai, India). Standard solution of *Piperine* and sample solution were applied to the plates as bands on the same chromatographic plate by use of a Camag (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100 µl Hamilton (USA) syringe. Ascending development was performed at room temperature (28 ± 2°C) using mobile phase in a Camag glass twin-trough chamberpreviously saturated with mobile phase vapour for 20min. After development, the plates were dried and then scanned at 254 nm with a Camag TLCScanner- 3 [37].

***In-Vitro* Antioxidant Study**

Antioxidants are vital in combating the free radicals, which damage human cells under ‘oxidative stress’ conditions and an imbalance of free radicals may cause grave disturbances in cell metabolism. Free radicals are instable species because they have unpaired electrons and seek stability through electron pairing with biological macromolecules. The proteins, lipids, and DNA of healthy human cells are good sources for these pairing electrons. Thus, oxidative stress conditions can cause DNA

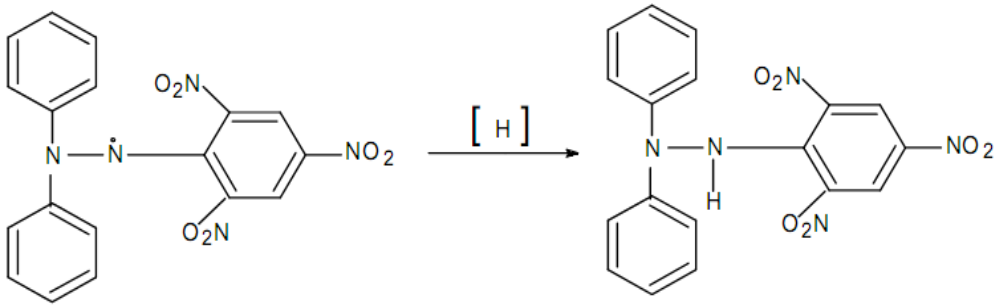
and protein damage, lipid peroxidation, cancer, atherosclerosis, ageing, and inflammatory diseases. Sources of free radicals include metabolism by-products, neutrophiles, UV radiation, air and water pollutants, fatty foods, hazardous chemicals, and cigarette smoke. The alcoholic and aqueous extracts of in-house formulated were subjected to different *in-vitro* antioxidant models.

All chemicals and solvents were of analytical grade and were obtained from Nice Chemicals, Mumbai. 1,1- Diphenyl-2-Picryl Hydrazine (DPPH), 2, 2 – Azino bis (3-ethyl Benzo Thiazoline – 6 – Sulphonic acid (ABTS) was obtained from Sigma Chemicals, USA. The other chemicals used were sodium nitroprusside, sulphanilic acid, naphthylethylene diamine hydrochloride. Ascorbic acid (Ranbaxy Fine Chemicals Ltd.) was used as standard for whole study.

DPPH Radical Scavenging Assay

Principle*:*

The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1- Diphenyl-2- Picryl Hydrazine. The ability to scavenge the free radical, DPPH was measured in the absorbance at 517 nm.



The antiradical activity for the churna extract was assessed on the basis of the radical-scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The concentration of DPPH was kept at 300 µM in methanol. The extracts were dissolved in methanol. 10 µl of each extract solution was allowed to react with 200 µl DPPH at 37 °C for 30 min in a 96-well microliter plate. After incubation, decrease in absorption for each solution was measured at 490 nm using a microplate reader. Ascorbic acid was used as reference.

$$\% \text{ Scavenging} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

ABTS Radical Scavenging Assay

Principle:

ABTS is chemically [2,2-azino bis (3-ethyl benzo thiazoline-6-sulphonic acid)]. The reduction of free radical by the test compound, using ABTS, is measured at 734 nm.

Procedure: For ABTS (2,20-Azinobis(3-ethylbenzothiazoline-6-sulfonicacid) diammoniumsalt) assay, the stock solutions of 7.4 mM ABTS and 2.6 mM potassiumpersulfate were prepared and the working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 16 h at room temperature in a dark place. The solution was then diluted by mixing $ABTS^{-+}$ solution with methanol to obtain an absorbance of 1.00 ± 0.02 units at 734 nm using the spectrophotometer. Fresh $ABTS^{-+}$ solution was prepared for each assay. Different concentrations of standard solution of ascorbic acid were prepared. The crude and purified extracts (200 ml) were allowed to react with 4 ml of the ABTS±for 2 h in a dark place. Then, the absorbance was read at 734 nm.

$$\% \text{ Scavenging} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

Nitric Oxide Scavenging Activity

Principle: Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction.

Procedure: 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture 0.5ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radical scavenging activity was calculated according to the following equation:

% Inhibition = $[(A_0 – A_1) / A_0 \times 100]$

Where $A_0$was the absorbance of the control (blank, without extract) and $A_1$was the absorbance in the presence of the extract/Standard [38-42].

Pharmacological Study

Animals

Female Wistar rats weighing 130–150 g was procured from animal house. All animals were housed in polypropylene cages in a temperature-controlled room at 24±1^{0}C. The animals were fed with pelleted rat feed manufactured by Hindustan Lever Ltd, Mumbai with free access to water throughout the experiment. The rats were acclimatized at least one week before starting the experiments. In all experimental models of inflammation, the studies were carried out using six rats in each group.

Acute toxicity studies

Acute toxicity studies were conducted to determine the safe dose by an up and down staircase method. Drugs were administered orally to overnight fasted animals. After administration the animals were observed continuously for one hour, frequently for the next four hours and then after 24 hours. After administration, Irwin's test was conducted, where the animals were observed for gross behavioral changes. For this, the following check list was employed [43-45].

i) *Behavioral profile*

Awareness: Alertness, Visual placing, stereotypy, passivity. Mood: Grooming, restlessness, irritability, fearfulness.

ii) *Neurological profile*

Motor activity: Spontaneous activity, reactivity, touches response, pain response, startle response, tremor, gait, grip strength, pinna reflex, and corneal reflex.

iii) *Autonomic profile*

Writhing, defecation, urination, pile erection, heart rate, respiratory rate.

Selection of doses

The doses selected for the extracts were about 1/10th of the maximum tolerated safe dose found from acute toxicity studies. They were administered once daily by oral route.

Aqueous Extract

Dose A: 200 mg/kg

Dose B: 400 mg/kg

Diclofenac 10 mg/kg computed from its clinical dose in inflammatory conditions.

Screening of Anti-Arthritic Activity

For the *in-vivo* anti-arthritic activity, the Complete Freund's Adjuvant (CFA) model was adopted. For this model, Albino rats of Wistar strains weighing between 180-220gm were produced from animal experimental laboratory, and used throughout the study. They were housed in a control environment (temp 25±2 ℃) and 12 hrs dark/ light cycle with standard laboratory diet and water ad libitum. The study was conducted after obtaining Institutional Animal Ethical Committee clearance.

Experimental Design

Ankle arthritis was induced by injecting Complete Freund's adjuvant (CFA) into a footpad of the left hind paw of rat. In the current study, animals (6 nos.) were divided into five different groups.

Group 1 received vehicle normal saline orally,

Group 2, 0.1 ml of CFA (0.05% Mycobacterium butyricum in mineral oil; 10 ml/kg b.w.) and vehicle was injected into the left hind paw (sub plantar surface) with the help of a 26-gauge needle.

Group 3 received CFA and indomethacin (3 mg/kg b.w.)

Group 4 and 5 administered CFA and aqueous extracts of Preparation A and Preparation B at a dose of 200 mg/kg b.w. and 400 mg/kg b.w. respectively.

All treatments were administered orally thirty minutes before CFA induction (day 0), then the animals were treated daily for up to 7th days [46-48].

Table 5: Experimental Grouping of Animals for Evaluation of Antiarthritic Activity for Prepared Formulation

Sr. No.	Groups	Treatment	No. of animals
	Group –I	Normal control (Receive only vehicle)	6
	Group –II	Negative control (Receive only vehicle+ CFA)	6
	Group-III	Standard *Receive* CFA+ Indomethacin (3 mg/kg b.w)	6
	Group-IV	Test group-I *Receive* CFA +extract (200 mg/kg)	6
	Group-V	Test group-II Receive CFA +extract (400 mg/kg)	6

Measurement of paw volume, joint diameter, arthritic score, pain threshold, thermal hyperalgesia and body weight

Measurement of paw volume

The severity of arthritis was evaluated on day 0, 1, 3, 5and 7. For this purpose, Plethysmometer (UGO Basile, Italy) was used to measure paw volume.

Measurement of joint diameter

On day 0, 1, 3, 5and 7 joint diameter was measured using Digital Vernier caliper (Mitutoyo, Japan) [347].

Measurement of mechanical pain threshold

Randall Selitto analgesiometer (UGO Basile, Italy) was used to measure mechanical pain threshold. In this method we apply an ascending pressure ramp to the animal's paw or tail and the point when a specific pain-related behavior is evoked (e.g., vocalization or writhing) is used as the pain threshold. Randall-Selitto test also evaluates nociceptiveresponses to deep mechanical stimuli.

Measurement of thermal hyperalgesia

Paw Withdrawal/Hargreaves method (Ugo Basile, Comerio, Italy) was used to measure thermal hyperalgesia, this method is recommended for measuring the cutaneous hyperalgesia due to the thermal stimulation. the rat was being placed inside an enclosed glass box with a focused infrared source (~48 units) moving underneath the rat. When the rat remains static, a button will be pressed which applies radiant heat (35–70 °C at intervals of 2.5 °C increasing for every 10 s) to the plantar surface of the animal's paw. Once the animal moves its paw, the photosensor will halt the time on the counting machine, and the latency is recorded [49].

Measurement of degree of arthritis

Evaluation of the degree of arthritis was assessed daily by visual observation. A score of 0–4 helped distinguish the different disease stages with a maximum value of 8 for each rat. Scores were attributed according to the parameters such as edema, erythema, malformation and incapacity to use the limb [50].

Measurement of organs weight and Biochemical estimations

On day 07, after anesthesia (using anesthetic ether), cardiac puncture was used to draw blood and introduce into a tube containing EDTA as anticoagulant and into another tube without anticoagulant; then liver, kidney, thymus and spleen were removed delicately and weighed.

Measurement Hematological parameters

Analysis of Hematological parameters like erythrocytes (RBC) and leukocytes (WBC) counts, hemoglobin (Hb), Hematocrit and platelets (PLT) were determined in blood with anticoagulant by Dynacount 3D PLUS Haematology analyzer (everlife

CPC Dignostics) [51].

Measurement serum parameters

Blood without anticoagulant was centrifuged for 5 min (4900 rpm) and the serum was collected, then serum AST, ALT, ALP, total protein, C-reactive protein (CRP) and Rheumatoid factor (RF) levels was also measured by semi auto with using analyzer (Micro Lab Instruments. Ahmedabad, India) according to the protocols of commercial kits (Meril Life Sciences India Pvt. Ltd.) [52].

3. RESULTS AND DISCUSSION

The foreign organic matter content in all the ingredients of the formulation was within the pharmacopeial limits. Notably, *Piper longum* (stems) exhibited 1.3% FOM, approaching the upper limit of 2%, whereas *Anethum graveolens* and *Embelia ribes* had 0.2% and 0.5% respectively, confirming the absence of adulterants or contaminants. These values reflect the cleanliness and quality control maintained during collection and processing of the herbal materials.

The moisture content in the plant materials, measured as Loss on Drying, was found to be within acceptable limits. *Piper longum* stems showed the highest LOD (9.3 ± 0.616%), which is expected due to its hygroscopic nature. *Cedrus deodara* (1.2 ± 0.2%) and *Anethum graveolens* (2.83 ± 0.351%) exhibited lower moisture levels, indicating better shelf stability. These findings are crucial as higher moisture content can lead to microbial contamination and spoilage of the formulation.

Ash value determination provides insight into the total mineral content and possible presence of inorganic contaminants. The total ash values for *Cedrus deodara* (0.853 ± 0.173%) and *Embelia ribes* (4.62 ± 0.297%) were within the permissible limits, reflecting the presence of intrinsic mineral content. Acid-insoluble ash values—indicative of siliceous impurities—were low across all ingredients, with *Cedrus deodara* showing the least (0.082 ± 0.023%) and *Anethum graveolens* approaching the limit at 1.077 ± 0.067%. This demonstrates the effectiveness of post-harvest processing and minimal contamination from soil or sand.

Extractive values represent the amount of active phytoconstituents soluble in solvents. The alcohol-soluble extractive values were particularly high in *Embelia ribes* (12.769 ± 1.962%) and *Piper longum* (15.599 ± 0.155%), confirming the presence of significant bioactive secondary metabolites such as alkaloids and flavonoids. *Anethum graveolens* had a slightly lower alcohol-soluble value (4.415 ± 0.233%) but met the Ayurvedic Pharmacopoeia minimum standard (NLT 4%).

Although the water-soluble extractive values were not individually mentioned in the document excerpts, the overall solubility profile implies balanced extraction properties and supports the formulation's therapeutic potential, especially in decoction or suspension-based dosage forms.

Table 6: Characteristics of Individual Ingredients in Preparation

S. No.	Botanical Name	FOM (%)	LOD %	Total Ash %	Acid-Insoluble Ash %	Water-Soluble Extractive %	Alcohol-Soluble Extractive %
1	*Anethum graveolens*	0.2	2.83 ± 0.351	6.593 ± 0.154	1.077 ± 0.067	55.455 ± 0.331	4.415 ± 0.233
2	*Cedrus deodara*	0.0	1.20 ± 0.20	0.853 ± 0.173	0.082 ± 0.023	2.359 ± 0.217	7.296 ± 0.503
3	*Piper longum*	0.0	4.24 ± 0.208	4.985 ± 0.119	0.544 ± 0.059	14.753 ± 0.080	15.599 ± 0.155
4	*Embelia ribes*	0.5	5.73 ± 0.404	4.620 ± 0.297	0.173 ± 0.054	18.443 ± 0.231	12.769 ± 1.962
5	Piper longum (stems)	1.3	9.30 ± 0.616	4.739 ± 0.458	0.145 ± 0.044	12.625 ± 0.528	6.520 ± 0.237

Fluorescence Analysis

Fluorescence under daylight, UV 254 nm, and UV 366 nm provided critical qualitative markers for authentication. Ingredients such as *Piper longum* (stems) and *Embelia ribes* consistently exhibited green fluorescence (GF) under UV light in reagents like NaOH and sulfuric acid, confirming the presence of alkaloids and essential oils. The distinct reactions of *Anethum graveolens* (DB under UV) and *Cedrus deodara* (light brown in most treatments) matched the expected fluorescence characteristics described in standard pharmacognostical references. These findings support the absence of adulteration and reinforce botanical identity.

Table 7: Powder Fluorescence Test of Individual Ingredients

S. No.	Reagent/Condition	*Anethum graveolens*	*Cedrus deodara*	*Piper longum*	*Piper longum* (stems)	*Embelia ribes*
		Day / UV254 / UV366	**Day / UV254 / UV366**	**Day / UV254 / UV366**	**Day / UV254 / UV366**	**Day / UV254 / UV366**
1	Powder as such	LB / DB / DB	LB / LB / LB	LB / DB / LB	LB / DB / GF	LB / LB / LB
2	NaOH (1N) in water	DB / LB / LB	LB / DB / GF	LB / DB / GF	DB / DB / GF	Y / DB / GF
3	NaOH in MeOH	LB / DB / DB	LB / DB / GF	LB / BL / GF	LB / DB / GF	DB / DB / GF
4	50% KOH	DB / LB / LB	LB / DB / GF	LB / LB / GF	DB / DB / GF	LB / DB / GF
5	HCl (1N)	LB / DB / DB	Y / Y / Y	LB / DB / LB	LB / DB / DB	LB / DB / LB
6	H_2SO_4 (conc.)	DB / CB / DB	PB / LB / LB	CB / BL / DB	LB / BL / GF	CB / LB / DB
7	HNO_3 (conc.)	LB / DB / DB	Y / DB / GF	Y / LB / GF	LB / DB / GF	Y / DB / GF
8	Acetic acid	LB / DB / DB	LB / GF / GF	LB / LB / LB	LB / DB / GF	DB / DB / GF
9	50% H_2SO_4	DB / DB / DB	GF / LB / GF	GF / BL / GF	DB / DB / GF	DB / BL / GF
10	50% HNO_3	LB / DB / DB	LB / GF / GF	LB / DB / GF	GF / DB / GF	LB / GF / GF
11	Iodine water	DB / DB / DB	DB / LB / LB	DB / DB / GF	DB / DB / GF	DB / BL / GF

LB: Light Brown, DB: Dark Brown, CB: Cherry Brown, Y: Yellow, GF: Green fluorescence, BL: Bluish Light

Physicochemical Characteristics

The formulation displayed a buff-colored, powdered appearance with a pungent taste and characteristic herbal odor, supporting its identity and consistency.Ash values (8.144% total ash; 0.573% acid-insoluble ash) remained within acceptable herbal formulation limits, indicating low contamination and appropriate mineral content.Extractive values (10.32% alcohol-soluble and 12.92% water-soluble) reflect significant solubility of phytoconstituents, confirming efficient extraction during processing.The pH values indicate that the formulation is near neutral in 1% solution (pH 7.18) and slightly acidic in 10% concentration (pH 5.23), which may influence its stability and suitability for oral administration.Sodium content was measured at 10.3 ppm, which is within safe dietary levels and attributed to the presence of Saindava Lavana (rock salt), contributing both to taste and electrolyte balance.

Table 8: Physicochemical Characteristics of Formulation

Appearance	Powder
Colour	Buff color
Taste	Pungent
Odour	Characteristic
% Total Ash	8.144 ± 0.609
% Acid insoluble ash	0.573 ± 0.108
Alcohol soluble extractive	10.322 ± 0.182

Water soluble extractive	12.920 ± 0.833
pH (1 % w/w)	7.18 ± 0.060
pH (10 %w/w)	5.23 ± 0.005
Sodium content (ppm)	10.3

Physical Characteristics (Bulk Density, Flow Properties)

Table 9: Physical Characteristics of Formulation

S. No.	Parameters	Observations
1	Bulk density	0.405 ± 0.005
2	Tap density	0.530 ± 0.017
3	Angle of repose	38.385 ±0.030
4	Hausner's ratio	1.380± 0.007
5	Carr's Index	27.613 ± 0.338

Phytochemical Estimation

Table 10: Estimation of Total Phenol Content of Different Formulations

S. No.	Sample	Concentration(µg/ml)	Absorbance
1	Gallic Acid	50	0.231
		100	0.472
		150	0.645
		200	0.866
		250	1.047
		300	1.265
2	Preparation A	1000	0.825
3	Preparation B	1000	1.207

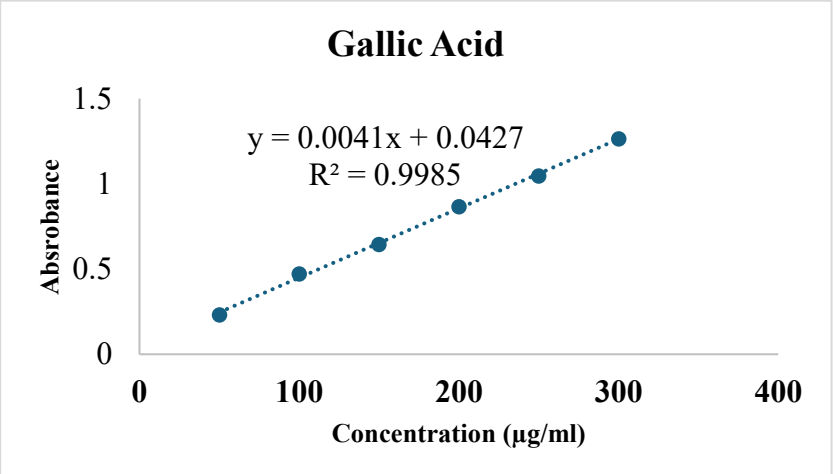


Figure 6.2: Standard curve for Gallic acid

Table 11: Total Phenol Content and Tannin Contentof Formulation

S. No.	Parameter	Results
1	Total phenol content (µg/ml)	294
2	Tannin content %	10.5

Table 12: Quantitative Estimation of Gallic Acid and Piperine in Formulation

S. No.	Active constituents	Quantity
1	Gallic acid	0.444
2	Piperine	0.616

Screening of Anti-Arthritic Activity

The paw volume of rats in the arthritic control group (Group II) showed a significant increase from Day 1 to Day 7, indicating the successful induction of arthritis. In contrast, animals treated with the polyherbal formulation (Groups III–V) showed a gradual reduction in paw swelling. Group III (containing the test formulation) demonstrated a more pronounced reduction in paw diameter over time (from 4.5 ±1.2 mm on Day 1 to 3.6 ±1.8 mm on Day 7), indicating significant anti-inflammatory activity. This reduction was comparable to standard treatments, suggesting the efficacy of the formulation in controlling local inflammation.

Similar to paw swelling, joint diameter significantly increased in the arthritic control group. However, Groups III–V displayed controlled joint swelling by Day 7, with Group III achieving the greatest reduction (from 8.5 ±1.2 mm on Day 1 to 7.6 ±1.8 mm on Day 7). This reduction signifies the polyherbal formulation's effectiveness in mitigating synovial hyperplasia and joint inflammation.

Weight loss is a characteristic feature of rheumatoid arthritis due to systemic inflammation and metabolic disturbances. The arthritic control group (Group II) exhibited a marked decline in body weight, whereas Group III showed stable body weights across the experimental duration. This reflects the protective and nutritive role of the formulation in maintaining systemic health during arthritic conditions.

Pain sensitivity, assessed using nociceptive threshold and thermal hyperalgesia tests, significantly increased in the arthritic group. However, polyherbal-treated rats demonstrated improved paw withdrawal latency by Day 7 (63 ±1.05% in Table 16 and 70 ±1.45% in Table 17 for Group III). This confirms the formulation’s analgesic potential, likely due to the synergistic action of anti-inflammatory phytoconstituents like piperine, limonene, and embelin present in the ingredients.

The clinical arthritis scores, based on visible signs of swelling and erythema, progressively worsened in the arthritic control group. Conversely, treatment with the formulation led to a significant decline in arthritic severity by Day 7, with Group III exhibiting the lowest score (0.5 ±0.96), indicating strong disease-modifying potential of the polyherbal combination.

Rheumatoid arthritis is often associated with anemia and altered blood counts. Group II showed reduced hemoglobin (7.59 ± 0.22 g/dl) and RBCs, along with increased WBCs and platelet counts, reflecting systemic inflammation. Group III restored hemoglobin levels (12.60 ± 0.10 g/dl) and balanced leukocyte profiles, suggesting the immunomodulatory and hematinic effects of the formulation.

Increased liver and spleen weights in Group II indicate systemic inflammation and immune hyperactivity. Group III significantly reduced these changes, normalizing organ weights and confirming the anti-inflammatory and hepatoprotective nature of the test formulation.

The formulation also reduced elevated biomarkers such as CRP (from 7.12 to 3.04 mg/L) and rheumatoid factor (RF), along with liver enzymes (ALT, AST, ALP). Total protein levels, which decreased during inflammation, were restored in treated groups. This biochemical normalization supports the polyherbal formulation’s role in controlling systemic inflammation and liver function alterations in CFA-induced arthritis.

1. Effect of extracts on paw volume (Diameter in mm)

Table 13:Effect of extracts on paw volume

S.No.	Groups	Paw Volume (Diameter in mm)				
		Day 0	Day 1	Day 3	Day 5	Day 7

	Group –I	3.5 ±1.5	3.4 ±2.1	3.5 ±0.9	3.48 ±1.1	3.4 ±1.8
	Group-II	3.2±1.4	5.2±0.4	5.5±1.1	5.8±0.5	5.8±1.2
	Group-III	2.9 ±1.8	4.5 ±1.2	4.0 ±1.2	3.8 ±1.4	3.6 ±1.8
	Group-IV	3.4 ±1.05	4.6 ±1.4	4.4 ±1.8	4.4 ±0.3	4.2 ±1.9
	Group-V	3.3 ±2.2	4.5 ±1.6	4.3±1.1	4.2±1.6	4.1±1.4

2. Effect of extracts on joint diameter

Table 14:Effect of extracts on joint diameter

S.No.	**Groups**	**Joint Diameter (mm)**				
		Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**
	Group –I	6.6 ±1.2	6.5 ±1.1	6.5 ±1.9	6.4 ±1.8	6.5 ±1.3
	Group-II	6.7 ±1.4	8.2±1.4	8.5±1.4	8.6±1.5	8.8±1.2
	Group-III	6.6 ±1.8	8.5 ±1.2	8.3 ±1.2	8.0 ±1.4	7.6 ±1.8
	Group-IV	6.6 ±1.6	8.6 ±1.4	8.3 ±1.7	8.2 ±1.6	8.1 ±1.7
	Group-V	6.6 ±1.8	8.5 ±1.1	8.3±1.1	8.2±0.8	7.9±1.8

Effect of extracts on change in joint diameter in CFA-induced arthritis. Values are expressed as mean ± SEM for six animals and analyses by two-way ANOVA followed by Tukey post-hoc test, $\alpha P < 0.05$, $\beta P < 0.01$, $\gamma P < 0.001$ when compared to healthy control, $aP < 0.05$, $bP < 0.01$, $cP < 0.001$ when compared to arthritic control

3. Effect of extracts bodyweight in gm.

Table 15:Effect of extracts bodyweight

S.No.	**Groups**	**Bodyweight (gm)**				
		Day 0	Day 1	Day 3	Day 5	Day 7
	Group –I	195±10.25	195±11.05	196±9.20	196±11.25	196±12.25
	Group-II	205±12.25	195±9.05	190±7.20	869±5.25	180±10.25
	Group-III	197±16.25	196±10.05	195±8.20	194±10.25	193±10.75
	Group-IV	199±15.18	198±8.15	197±8.05	194±8.15	192±7.25
	Group-V	196±10.10	197±10.05	196±10.18	194±7.15	190±8.21

4. Effect of extracts on nociceptive threshold

Table 16:Effect of extracts bodyweight

S.No.	**Groups**	**Paw withdrawal latency (in percentage)**				
		Day 0	Day 1	Day 3	Day 5	Day 7
	Group –I	99±1.25	100±1.05	100±0.20	100±1.10	100±0.25
	Group-II	45±2.45	42±2.13	35±1.68	28±1.30	26±1.08
	Group-III	46±1.81	52±1.52	50±1.89	61±1.12	63±1.05
	Group-IV	42±1.14	44±1.45	48±1.25	51±1.11	55±1.50

	Group-V	41±1.20	47±1.20	50±1.22	52±1.51	58±1.10

5.Effect of extracts on thermal hyperalgesia

Table 17:Effect of extracts on thermal hyperalgesia

S.No.	Groups	Paw withdrawal latency (in percentage)				
		Day 0	Day 1	Day 3	Day 5	Day 7
	Group –I	99±1.25	100±1.05	100±0.20	100±1.10	100±0.25
	Group-II	55±1.25	53±1.13	45±1.18	43±1.13	42±2.18
	Group-III	56±0.28	62±1.25	65±1.29	68±1.32	70±1.45
	Group-IV	54±1.28	55±1.42	56±1.15	58±1.01	59±1.18
	Group-V	53±1.35	54±1.10	58±1.07	61±1.12	64±1.01

6. Effect of extracts on **arthritic score**

Table 18:Effect of extracts on arthritic score

S.No.	**Groups**	**Arthritic Score**				
		Day 0	Day 1	Day 3	Day 5	Day 7
	Group –I	0±00	0±00	0±00	0±00	0±00
	Group-II	1.5±0.52	2.5±0.6	2.5±0.95	3±0.21	3.5±0.86
	Group-III	1.5±0.58	1.7±0.21	1.6±0.35	1.4±0.88	0.5±0.96
	Group-IV	1.5±0.24	2.1±0.85	2.01±0.35	1.86±0.60	1.5±0.52
	Group-V	1.5±0.85	2.1±0.74	2.0±0.05	1.80±0.37	1.44±0.24

7. Effect of extracts on haematological in CFA-induced arthritis in rat.

Table 19:Effect of extracts on haematological in CFA-induced arthritis in rat

S.No.	**Group**	**Haemoglobin (g/dl)**	**RBC (million/µl)**	**Hematocrit (%)**	**WBC (10^9 /L)**	**Platelet (10^9/L)**
	Group –I	14.85 ± 0.65	7.37 ± 0.16	41.25± 2.52	7.95 ± 0.55	820.00 ± 40.00
	Group-II	7.59 ± 0.22	3.92 ± 0.24	24.75± 1.05	14.25 ± 0.28	1795.00 ± 21.00
	Group-III	12.60 ± 0.10	5.05 ± 0.85	38.10± 1.10	10.00 ± 0.18	984.00 ± 26.00
	Group-IV	9.15 ± 0.85	4.03 ± 0.11	35.85± 1.01	11.110 ± 0.14	1126.00 ± 40.0
	Group-V	9.85 ± 0.45	4.29 ± 0.62	36.25± 1.12	10.115 ± 0.54	1012.00 ± 62.0

8. Effect of extracts on organs weight **(gm)** after CFA induced arthritis in rats

Table 20: Effect of extracts on organs weight (gm) after CFA induced arthritis in rats

S.No.	Group	organs weight **(gm)**

		Liver	Spleen	Kidney	Thymus
	Group –I	6.59 ± 0.11	0.67± 0.11	0.90 ± 0.53	0.72 ± 0.08
	Group-II	9.09 ± 0.19	1.97 ± 0.14	1.55 ± 0.09	0.30 ± 0.05
	Group-III	7.04 ± 0.41	0.91 ± 0.41	0.94 ± 0.12	0.58 ± 0.08
	Group-IV	7.35 ± 0.29	1.50 ± 0.15	0.98 ± 0.25	0.65± 0.08
	Group-V	7.05 ± 0.47	1.24 ± 0.21	0.96 ± 0.55	0.60 ± 0.12

9. Effect of extracts on haematological in CFA-induced arthritis in rat.

Table 21: Effect of extracts on haematological in CFA-induced arthritis in rats

S.No.	Group	CRP (mg/l)	RF (IU/ml)	ALP (U/l)	ALT (U/l)	AST (U/l)	Total protein (g/dl)
	Group –I	1.19 ± 0.21	---	70.57 ± 2.44	45.73 ± 2.01	40.30 ± 1.06	6.59 ± 0.47
	Group-II	7.12 ± 0.24	64.00 ± 1.83	481.00 ± 10.13	176.53 ± 7.92	135.67 ± 2.11	3.83 ± 0.11
	Group-III	3.04 ± 0.32	42.23 ± 1.25	224.33 ± 26.04	103.52 ± 4.14	91.67 ± 1.25	5.85 ± 0.15
	Group-IV	5.01** ± 0.25	51.00 ± 1.35	416.33** ± 14.35	123.14** ± 7.52	119.09* ± 1.50	4.11* ± 0.17
	Group-V	4.14** ± 0.19	38.00** ± 1.50	380.65** ± 15.24	11.21** ± 6.55	117.56** ± 2.11	4.87** ± 0.86

4. SUMMARY AND CONCLUSION

This study was undertaken to evaluate the pharmacognostic, phytochemical, and pharmacological potential of a novel polyherbal formulation composed of six traditional medicinal ingredients: *Anethum graveolens, Cedrus deodara, Piper longum (fruit and stem), Embelia ribes,* and *Saindhava lavana*. The formulation was subjected to a series of standardization protocols, including physicochemical characterization, organoleptic assessment, fluorescence analysis, and phytochemical screening, which collectively confirmed its authenticity and quality.

In vivo studies were conducted using the Complete Freund's Adjuvant (CFA)-induced arthritis model in Wistar rats to assess anti-arthritic efficacy. The formulation significantly reduced paw edema, joint swelling, and arthritic scores, while improving pain thresholds and body weight. Hematological disturbances typical of rheumatoid arthritis, such as anemia and elevated WBC counts, were markedly reversed by the treatment. Furthermore, biochemical markers such as C-reactive protein (CRP), rheumatoid factor (RF), ALT, AST, and ALP showed substantial normalization, suggesting systemic anti-inflammatory effects. Organ weights also returned closer to normal levels in treated groups, indicating protective effects on vital organs affected by inflammatory processes.

The comprehensive evaluation of this polyherbal formulation demonstrates its promising anti-arthritic potential through anti-inflammatory, analgesic, immunomodulatory, and hematoprotective mechanisms. Its effects were comparable to standard drugs used for arthritis management, underscoring its therapeutic relevance. The synergistic action of phytoconstituents from all six ingredients appears to play a crucial role in ameliorating both local and systemic symptoms of arthritis.

Thus, the formulation offers a safe, natural, and effective alternative approach for the management of rheumatoid arthritis. Further studies, including toxicological profiling and clinical validation, are warranted to explore its full therapeutic potential and ensure its applicability in evidence-based Ayurvedic or integrative medicine..

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