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PHARMACOLOGICAL INVESTIGATION OF ANTI-INFLAMMATORY ACTIVITY OF COMBINED EXTRACT OF OPHIOPOGON JABURAN AND PANDANUS AMARYLLIFOLIUS USING EXPERIMENTAL ANIMAL MODEL

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ABSTRACT

The present study aimed to develop and evaluate polyherbal suspension of *Ophiopogon jaburan* and *Pandanus amaryllifolius* extracts for antioxidant and anti-inflammatory activity. Methanolic extracts were prepared by Soxhlet extraction and evaluated for percentage yield, phytochemical constituents, total phenolic content (TPC): total flavonoid content (TFC): and antioxidant activity using the DPPH assay. The extracts showed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, terpenoids, and steroids. *Pandanus amaryllifolius* exhibited higher phenolic (46.11 mg GAE/g) and flavonoid content (28.16 mg RE/g) with stronger antioxidant activity (IC_{50} 23.94 µg/mL) compared to *Ophiopogon jaburan*. Three suspension formulations were prepared and evaluated for physicochemical properties. All formulations showed acceptable pH, viscosity, redispersibility, and sedimentation characteristics. Acute oral toxicity study according to OECD 423 revealed that all formulations were safe at 2000mg/kg dose without mortality or toxic effects. The anti-inflammatory activity was evaluated using the

carrageenan-induced paw edema model in rats. The polyherbal suspension (Formulation III) showed maximum inhibition of edema compared with individual formulations, indicating synergistic anti-inflammatory activity. The study concludes that the developed *Ophiopogon jaburan–Pandanus amaryllifolius* polyherbal suspension possesses promising antioxidant, safety, and anti-inflammatory potential.

KEYWORDS: *Ophiopogon jaburan*; *Pandanus amaryllifolius*; Polyherbal suspension; Phytochemical screening; Antioxidant activity; DPPH assay; Acute toxicity; Anti-inflammatory activity.

1. INTRODUCTION

Inflammation is defined as a local or systemic response to tissue damage or different stimuli, such as biological, chemical, physical, and thermal factors. Increased inflammatory response and elevated concentration of proinflammatory markers, such as cytokines and inflammatory chemokines, introduce the organism into a state of hyperinflammation **(Hasan *et al.*, 2022).** Hyperinflammation in combination with reactive oxygen species (ROS) produced by oxidative stress is an important factor contributing to the development of various diseases, such as arthritis, cardiovascular disease, cancer, diabetes, etc. Arachidonic acid (AA) is a polyunsaturated ω-6 fatty acid that can be metabolized through various catabolic pathways. The two most important metabolic pathways of arachidonic acid are catalyzed by the enzymes cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) **(Wang *et al.*, 2021).** Two isoforms of COX enzyme are COX-1, which is constitutively expressed, and COX-2, which is induced by inflammatory factors, hormones, and growth factors. Two steps in the biosynthesis of leukotrienes are catalyzed by 5-LOX: the biotransformation of AA to the intermediate 5-hydroperoxyeicosatetraenoic acid and its further transformation to leukotrienes. On the other hand, 5-LOX is also involved in the catalysis of lipid peroxidation by producing lipid peroxides. These peroxides promote membrane rupture and cell death, leading to the inflammatory response through the release of damage-associated molecular patterns (DAMPs) **(Krieg and Fürstenberger, 2014).** Inhibitors of this enzyme are being developed for the treatment of numerous human diseases and pathological conditions, such as asthma, allergic reactions, atherosclerosis, diabetes, and Alzheimer's disease. There is strong evidence that increased 5-LOX activity is associated with a process of neuroinflammation that causes memory deficits, while decreased enzyme expression promotes cognitive recovery **(Nejatian *et al.*, 2019).**

Ophiopogon jaburan is a perennial medicinal herb belonging to the family Asparagaceae and genus *Ophiopogon*. It is an evergreen, grass-like plant characterized by narrow, elongated leaves and an underground root system with tuberous structures. The plant is widely cultivated as an ornamental species and has also attracted considerable interest because of its traditional medicinal applications and diverse phytochemical composition **(Lei *et al.*, 2021).** Different parts of *O. jaburan*, particularly the roots and leaves, contain several biologically active constituents that may contribute to its therapeutic properties. The presence of multiple bioactive constituents makes *O. jaburan* a potentially valuable medicinal plant for pharmacological investigation **(Tsakem *et al.*, 2025).** However, the biological activity of the plant may depend on the plant part used, extraction solvent, extraction conditions, phytochemical composition, and concentration of active constituents. Therefore, systematic phytochemical characterization and experimental evaluation are necessary to establish its anti-inflammatory potential and determine the mechanisms responsible for its activity **(Ferraz *et al.*, 2020).**

Pandanus amaryllifolius, commonly known as pandan, is an aromatic perennial plant belonging to the family Pandanaceae. It is widely distributed and cultivated in tropical and subtropical regions and is particularly recognized for the characteristic fragrance of its leaves **(Bhuyan and Sonowal, 2021).** The leaves are commonly used as a flavoring and aromatic ingredient in food preparations and have also been traditionally utilized for various medicinal purposes. In addition to its traditional importance, *P. amaryllifolius* has attracted scientific interest because of its diverse phytochemical composition and potential pharmacological activities. The potential anti-inflammatory activity of *P. amaryllifolius* may be associated with modulation of important inflammatory pathways **(Khoo *et al.*, 2026).** Its phytoconstituents may influence the synthesis and release of inflammatory mediators, including prostaglandins, leukotrienes, nitric oxide, and pro-inflammatory cytokines. Flavonoids and phenolic compounds may help regulate cyclooxygenase and lipoxygenase-related pathways, while antioxidant constituents may reduce oxidative stress associated with inflammatory tissue damage. These combined effects may result in decreased vascular permeability, reduced inflammatory cell infiltration, and attenuation of edema **(Sharma *et al.*, 2019).**

Experimental animal models provide an important preliminary approach for evaluating the anti-inflammatory potential of medicinal plants. The carrageenan-induced paw edema model

is one of the commonly used models for assessing acute inflammation. Carrageenan produces a characteristic biphasic inflammatory response, with the early phase involving mediators such as histamine, serotonin, and bradykinin, while the later phase is predominantly associated with prostaglandins, nitric oxide, and pro-inflammatory cytokines. Reduction in carrageenan-induced paw edema following administration of a plant extract provides evidence of its potential anti-inflammatory activity **(Patil *et al.*, 2019).**

Therefore, the present study, entitled "Pharmacological Investigation of Anti-Inflammatory Activity of Combined Extract of *Ophiopogon jaburan* and *Pandanus amaryllifolius* Using Experimental Animal Model," is undertaken to investigate the anti-inflammatory potential of the combined plant extracts. The study focuses on evaluating their phytochemical characteristics and pharmacological activity using an established experimental model of acute inflammation.

2. METHODS AND MATERIALS

2.1 Chemical

Ethanol, Methanol, and Hydrochloric Acid was procured from Merk. Folin–Ciocalteu Reagent and Carrageenan was received from Himedia. Ferric Chloride, Sodium CMC, Ascorbic Acid, Sodium Carbonate and Aluminium Chloride was acquired from Loba Chemie Pvt. Ltd., India. Gallic Acid, Rutin, and DPPH was received from Fizmerck. Baxter India Pvt. Ltd., India produce Normal Saline.

2.2 Plant Collection and Authentication

For the present study, fresh plant materials of *Ophiopogon jaburan* (300 g) and *Pandanus amaryllifolius* (370 g) were collected. The collected plant materials were thoroughly washed with clean water to remove adhering soil, dust, and other extraneous matter. The cleaned plant materials were then shade-dried at room temperature for a period of three days to prevent the degradation of thermolabile constituents. Subsequently, the partially dried materials were further dried in a hot air oven maintained at 45°C until complete dehydration was achieved.

The fully dried plant materials were stored in airtight glass containers under cool and dry conditions to protect them from moisture, microbial contamination, and environmental degradation, thereby preserving their phytochemical integrity. Botanical identification and authentication of *Ophiopogon jaburan* and *Pandanus amaryllifolius* were carried out by a

qualified taxonomist to confirm the identity, authenticity, and purity of the plant materials prior to their use in the study.

2.3 Soxhlet Extraction Procedures (Zakaria *et al*., 2020).

The Soxhlet extraction method employing continuous hot percolation was utilized for the extraction of bioactive constituents from the plant materials of *Ophiopogon jaburan* (300 g) and *Pandanus amaryllifolius* (370 g). The dried plant materials were finely powdered and accurately weighed before being placed into a cellulose thimble of the Soxhlet apparatus. Extraction was carried out at a controlled temperature of 60°C using a suitable solvent.

The extraction process was continued until the solvent in the siphon tube became colorless, indicating complete extraction of the phytoconstituents. After completion of the extraction cycle, the residual plant material (marc) was removed, air-dried to eliminate residual solvent, and subjected to re-extraction using methanol as the solvent to ensure maximum recovery of active compounds. The extraction process was repeated until complete exhaustion of the plant material was achieved, as confirmed by the absence of solvent residue upon evaporation.

The combined solvent extracts were concentrated under reduced pressure using a rotary evaporator maintained at 40°C to remove the solvent completely and obtain the crude extracts. The dried extracts were weighed, and the percentage yield was calculated to determine the extraction efficiency of *Ophiopogon jaburan* and *Pandanus amaryllifolius* extracts.

$$\% \ \text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

2.4 Quantitative Phytochemical Estimation

A. Total Phenolic Content (TPC) Estimation

The phenolic compounds present in the extracts react with Folin–Ciocalteu reagent under alkaline conditions to form a blue-coloured complex. The intensity of the colour developed is directly proportional to the concentration of phenolic compounds present in the extract. Different concentrations of ethanolic extracts of *Ophiopogon jaburan* and *Pandanus amaryllifolius* were prepared and mixed with Folin–Ciocalteu reagent, followed by the addition of sodium carbonate solution. The reaction mixture was incubated for sufficient time to allow colour development, and the absorbance was measured at 765 nm using a UV–

Visible spectrophotometer. Gallic acid was used as the standard reference compound, and a calibration curve was prepared using different concentrations of gallic acid. The total phenolic content of the extracts was calculated from the corresponding calibration curve and expressed as mg Gallic Acid Equivalent (GAE) per gram of extract (mg GAE/g extract) **(Quyen *et al.*, 2020).**

B. Total Flavonoid Content (TFC) Estimation

Flavonoid compounds present in the extract form a yellow-coloured complex with aluminium chloride. The intensity of the colour developed is proportional to the concentration of flavonoids present in the extract. Different concentrations of ethanolic extracts of *Ophiopogon jaburan* and *Pandanus amaryllifolius* were prepared and treated with aluminium chloride and potassium acetate reagents. The reaction mixtures were incubated for adequate colour development, and the absorbance was measured at 510 nm using a UV–Visible spectrophotometer. Rutin was used as the standard reference compound, and a calibration curve was prepared using different concentrations of rutin. The total flavonoid content was calculated from the calibration curve and expressed as mg Rutin Equivalent (RE) per gram of extract (mg RE/g extract). The quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) provided valuable information regarding the concentration of antioxidant phytoconstituents present in the ethanolic extracts. The presence of phenolic and flavonoid compounds may contribute to the antioxidant and anti-inflammatory properties of the extracts and provides a scientific basis for their further pharmacological evaluation **(Padhi *et al.*, 2024).**

2.5 DPPH (2,2-Diphenyl-1-picryl-hydrazyl) Radical Scavenging Assay Method

The antioxidant activity of ethanolic leaf extracts of *Ophiopogon jaburan* and *Pandanus amaryllifolius* was evaluated using the DPPH radical scavenging method. The assay is based on the ability of antioxidant compounds present in the extract to reduce the stable DPPH free radical into a yellow-coloured non-radical form, resulting in a decrease in absorbance. A fresh DPPH solution was prepared by dissolving DPPH in methanol to obtain the required concentration. Different concentrations of ethanolic extracts were prepared using methanol as a solvent. The prepared extract solutions were mixed with DPPH solution and incubated in the dark at room temperature for a specific period to allow the reaction to occur. The absorbance of the reaction mixture was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid was used as a standard antioxidant for comparison. A

control containing DPPH solution without extract was used to determine the maximum absorbance. The percentage of free radical scavenging activity was calculated using the following formula.

$$\%\text{Inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

Where,

$A_{(C)}$ = Absorbance of control

$A_{(S)}$ = Absorbance of sample

The IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was calculated from the percentage inhibition versus concentration curve. A lower IC_{50} value indicates stronger antioxidant activity of the extract. The obtained results were used to assess the antioxidant potential of *Ophiopogon jaburan* and *Pandanus amaryllifolius* leaf extracts **(Mensor *et al.*, 2001).**

2.6 Preparation of Polyherbal Suspension of Extracts (Mahajan *et al.*, 2021)

The polyherbal suspensions were prepared by the wet dispersion method. The required quantities of *Ophiopogon jaburan* and *Pandanus amaryllifolius* extracts were accurately weighed as per the formulation. Sodium Carboxymethyl Cellulose (CMC) (0.8 g) was slowly added to a portion of distilled water with continuous stirring and allowed to stand for 30 minutes to ensure complete hydration and formation of a uniform suspension base.

Sucrose (4.0 g) and sorbitol (2.0 g) were dissolved separately in distilled water and added to the hydrated CMC solution. Methyl paraben (0.1%): previously dissolved in a small quantity of warm distilled water, was then incorporated into the mixture as a preservative. The weighed plant extract(s) were mixed with Tween 80 (0.05%) to obtain a smooth, lump-free paste. The paste was gradually incorporated into the suspension base with continuous stirring until a uniform and homogeneous dispersion was obtained. The final volume was adjusted to 40 mL with distilled water and mixed thoroughly to ensure complete uniformity of the suspension.

The prepared suspensions were filled into clean, dry, amber-colored bottles, tightly closed, labeled, and stored at room temperature until further evaluation.

2.6.1 Preparation of Suspension Formulations

❖ **Formulation I (*Ophiopogon jaburan* Suspension):** Prepared using 2.76 g of *Ophiopogon jaburan* extract along with Sodium CMC (0.8 g): Tween 80 (0.05%): Methyl Paraben (0.1%): Sucrose (4.0 g): Sorbitol (2.0 g): and distilled water q.s. to 40 mL.

❖ **Formulation II (*Pandanus amaryllifolius* Suspension):** Prepared using 2.76 g of *Pandanus amaryllifolius* extract with the same quantities of Sodium CMC (0.8 g): Tween 80 (0.05%): Methyl Paraben (0.1%): Sucrose (4.0 g): Sorbitol (2.0 g): and distilled water q.s. to 40 mL.

❖ **Formulation III (Polyherbal Suspension; 1: 1 Ratio):** Prepared using *Ophiopogon jaburan* extract (2.0 g) and *Pandanus amaryllifolius* extract (2.0 g) in a 1: 1 ratio, together with Sodium CMC (0.8 g): Tween 80 (0.05%): Methyl Paraben (0.1%): Sucrose (4.0 g): Sorbitol (2.0 g): and distilled water q.s. to 40 mL.

The prepared formulations were subsequently subjected to physicochemical evaluation, including pH, viscosity, Redispersibility and sedimentation volume followed by pharmacological evaluation for anti-inflammatory activity in experimental animal models **(Shete *et al.*, 2010).**

Table 1: Composition of Polyherbal suspensions (Formulation I, Formulation II and Polyherbal Formulation III) (Tripathi *et al.*, 2013).

Name of Ingredient	Formulation I (*Ophiopogon jaburan*)	Formulation II (*Pandanus amaryllifolius*)	Formulation III (Polyherbal Combination 1: 1 ratio)
Ophiopogon jaburan Extract	2.76 g	—	2g
Pandanus amaryllifolius Extract	—	2.76g	2g
Sodium Carboxymethyl Cellulose (CMC)	0.8 g	0.8 g	0.8 g
Tween 80 (Wetting Agent)	0.05%	0.05%	0.05%
Methyl Paraben (Preservative)	0.1%	0.1%	0.1%
Sucrose	4.0 g	4.0 g	4.0 g
Sorbitol	2.0 g	2.0 g	2.0 g
Distilled Water	q.s. to 40 mL	q.s. to 40 mL	q.s. to 40 mL

2.7 Evaluation of Polyherbal Suspension Formulations

1. Determination of pH

The pH of Formulations I, II, and III was measured using a calibrated digital pH meter after calibration with standard buffers (pH 4.0, 7.0, and 9.2). About 10 mL of each formulation

was analyzed in triplicate at 25 ± 2°C, and the mean pH value was recorded **(Chandel *et al.*, 2011).**

2. Determination of Viscosity

The viscosity of each suspension was determined using a Brookfield Digital Viscometer at 25 ± 2°C and 50 rpm. Approximately 50 mL of the formulation was used, and the viscosity was recorded after obtaining a constant reading. Measurements were performed in triplicate and expressed in centipose (cP) **(Dandagi *et al.*, 2008).**

3. Redispersibility Test

The Redispersibility of the prepared suspensions was evaluated after allowing the formulations to remain undisturbed for 24 hours. Each bottle was gently shaken manually, and the number of inversions required to redisperse the sediment completely was recorded. A suspension requiring fewer shaking cycles to achieve complete and uniform redispersion was considered to possess good Redispersibility. The observations were recorded for all three formulations **(Roopa *et al.*, 2015).**

4. Determination of Sedimentation Volume

The sedimentation volume of the suspension formulations was determined by transferring 25 mL of each suspension into a 50 mL graduated measuring cylinder. The cylinders were kept undisturbed at room temperature, and the height of the sediment (Hu) and the total height of the suspension (Ho) were recorded at predetermined time intervals (0, 1, 2, 4, 8, 24, and 48 hours). The sedimentation volume (F) was calculated using the following equation: **(Bisht *et al.*, 2021).**

$$F = \frac{H_u}{H_o}$$

2.8 Acute Oral Toxicity Study

The acute oral toxicity study of Formulations I (*Ophiopogon jaburan*): II (*Pandanus amaryllifolius*): and III (polyherbal suspension, 1: 1 ratio) was conducted according to OECD Guideline 423. Healthy Wistar albino rats received a single oral dose and were observed for14 days for mortality, behavioral and locomotor changes, skin and fur abnormalities, respiratory or neurological signs, gastrointestinal effects, food and water intake, body weight changes, and general health. The formulations were considered safe

when no significant toxic symptoms, adverse effects, or mortality were observed during the observation period.

2.9 *In vivo* anti-inflammatory activity (Baek et al., 2016)

2.9.1 Animals

Healthy rats were randomly selected from local area of Bhopal and housed in propylene cages with sterile bedding under 22 ± 2°C, 30.7% humidity, and a 12: 12 h light-dark cycle. They were fed standard pellets with water ad libitum and acclimatized for 7 days before experiments. Each experimental group included six rats (n = 6). All procedures were approved by the Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal.

Animals used

Strain	-	Albino Wistar rats
Age	-	5-6 weeks
Sex	-	either sex
Body weight	-	200±30 g

2.9.2 Experimental setup

Thirty minutes after oral administration of the test extracts or standard drug, 0.1 mL of 1% carrageenan in distilled water was injected into the subplantar region of the right hind paw of all experimental groups. To ensure consistent measurement, a mark was placed at the malleolus of each paw for uniform dipping during subsequent readings. The paw volume was measured using a vernier caliper at 1, 2, 3, 4 and 5 hours post-injection. The actual edema volume at each time point was calculated as the difference between the 1-hour reading and the readings at 2 and 3 hours, providing a quantitative measure of inflammation and the anti-inflammatory effect of the administered extracts or standard drug.

Experimental design

Group No.	Treatment
Group I	Negative Control (Carrageenan-induced inflammation; received vehicle only)
Group II	Standard Control – Ibuprofen (50 mg/kg, p.o.) + Carrageenan
Group III	Carrageenan + Formulation I (*Ophiopogon jaburan* Suspension)
Group IV	Carrageenan + Formulation II (*Pandanus amaryllifolius* Suspension)
Group V	Carrageenan + Formulation III (Polyherbal Suspension; *Ophiopogon jaburan* + *Pandanus amaryllifolius*, 1: 1 ratio)

2.9.3 Carrageenan-Induced paw Edema in Rats

The anti-inflammatory activity of the prepared suspensions was evaluated using the carrageenan-induced paw edema model in Wistar albino rats. The animals were randomly divided into five groups (n = 6 animals/group). Group I served as the negative control and received vehicle treatment with carrageenan-induced inflammation. Group II was treated as the standard control and received Ibuprofen (50 mg/kg, p.o.) along with carrageenan. Group III received *Ophiopogon jaburan* Suspension, Group IV received *Pandanus amaryllifolius* Suspension, and Group V received polyherbal suspension containing *Ophiopogon jaburan* and *Pandanus amaryllifolius* in a 1: 1 ratio, followed by carrageenan induction. The respective treatments were administered orally 30 minutes prior to the induction of inflammation. Acute paw edema was induced by subplantar injection of 0.1 mL of 1% carrageenan solution in normal saline into the right hind paw of each rat. The paw volume was measured before carrageenan administration and subsequently at 1, 2, 3, 4 and 5 hours after inflammation induction using a vernier caliper. The increase in paw volume was calculated by determining the difference between the inflamed right paw and the non-inflamed left paw **(Suebsasana *et al.*, 2009).**

3. RESULTS AND DISCUSSION

3.1 Percentage Yield

Table 2: Percentage Yield of crude extracts.

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
Ophiopogon jaburan	Methanol	300	8.35	2.783
Pandanus amaryllifolius	Methanol	370	15.58	4.210

3.2 Preliminary Phytochemical study

Table 3: Phytochemical Screening of *Ophiopogon jaburan* Leaves Extract.

Phytoconstituent	Test Performed	Observation	Result
Alkaloids	Dragendorff's test	Orange-red precipitate observed	+
Flavonoids	Shinoda test	Pink/red coloration observed	+
Phenolic compounds	Ferric chloride test	Bluish-green coloration observed	+
Tannins	Ferric chloride test	Greenish-black coloration observed	+
Saponins	Foam test	Persistent froth formation observed	+
Glycosides	Keller–Killiani test	Brown ring formation observed	+
Terpenoids	Salkowski test	Reddish-brown coloration observed	+
Steroids	Liebermann–Burchard test	Green coloration observed	+

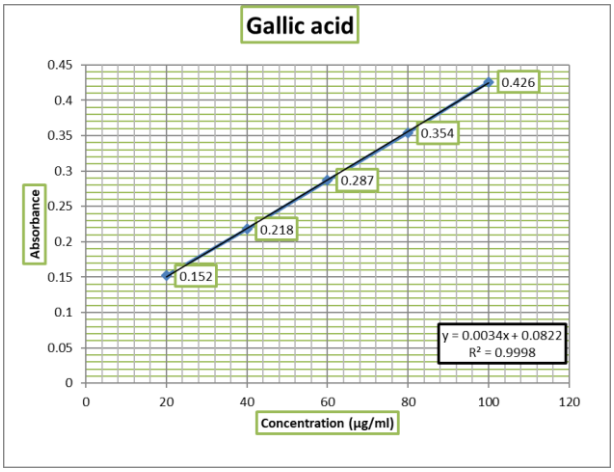
Carbohydrates	Molisch's test	Violet ring formation observed	+
Proteins	Biuret test	Violet coloration observed	–

Table 4: Phytochemical analysis of extracts of *Pandanus amaryllifolius.*

Phytochemical Constituents	Test Performed	Observation	Result
Alkaloids	Dragendorff's Test	Orange-red precipitate	+
Flavonoids	Alkaline Reagent Test	Intense yellow color	+++
Phenolic Compounds	Ferric Chloride Test	Dark blue-green color	+++
Tannins	Gelatin Test	White precipitate	++
Saponins	Foam Test	Persistent froth	++
Glycosides	Keller-Killiani Test	Brown ring formation	+
Terpenoids	Salkowski Test	Reddish-brown interface	++
Steroids	Liebermann-Burchard Test	Green coloration	+
Carbohydrates	Molisch's Test	Violet ring	++
Proteins	Biuret Test	Violet coloration	+
Amino Acids	Ninhydrin Test	Purple color	+
Quinones	Borntrager's Test	Pink coloration	-

3.3 Quantitative Analysis

3.3.1 Total Phenolic content (TPC) estimation



Graph 1: Represent standard curve of Gallic acid.

3.3.1.1 Total Phenolic Content in extract

Table 5: Total Phenolic Content in leaves extracts of *Ophiopogon jaburan.*

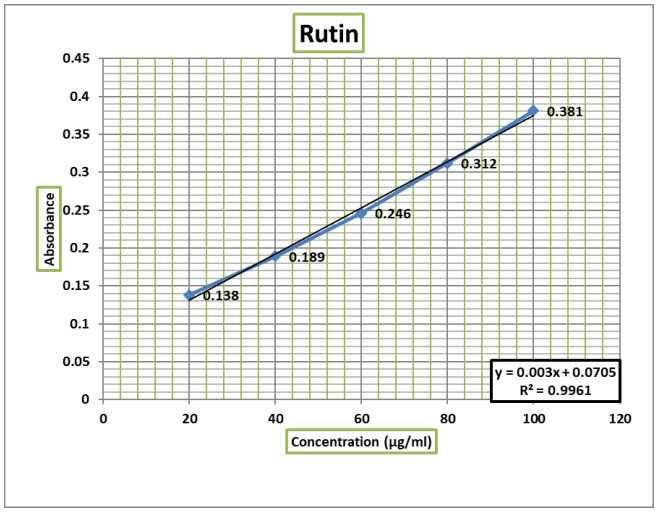
Absorbance	Mean±SD	TFC in mg/gm equivalent of Rutin
0.218	0.230±0.012503	**43.47 mg/gm**
0.231		
0.243		

Table 6: Total Phenolic Content in leaves extracts of *Pandanus amaryllifolius.*

Absorbance	Mean±SD	TFC in mg/gm equivalent of Rutin
0.228		

0.240	0.239±0.011504	**46.11 mg/gm**
0.251		

3.3.2 Total Flavonoids content (TFC) estimation



Graph 2: Represent standard curve of Rutin.

3.3.1.2 Total Flavonoid Content in extract

Table 7: Total Flavonoid Content in *Ophiopogon jaburan* leaves extract.

Absorbance	Mean±SD	TFC in mg/gm equivalent of Rutin
0.138	0.149±0.012583	**26.16 mg/gm**
0.148		
0.163		

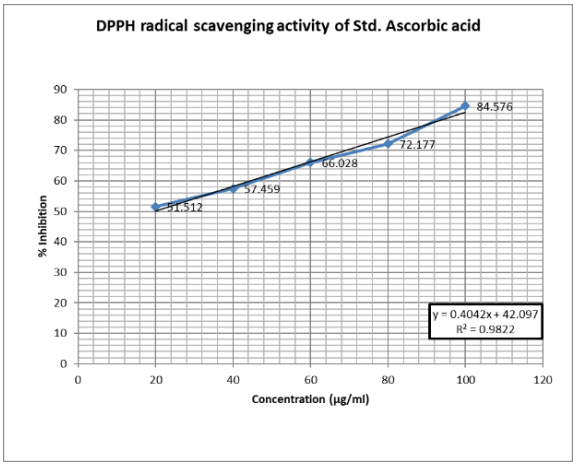
Table 8: Total Flavonoid Content in *Pandanus amaryllifolius* leaves extract.

Absorbance	Mean±SD	TFC in mg/gm equivalent of Rutin
0.141	0.155±0.01249	**28.16 mg/gm**
0.159		
0.165		

3.3.3 DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

Table 9: DPPH radical scavenging activity of Std. Ascorbic acid.

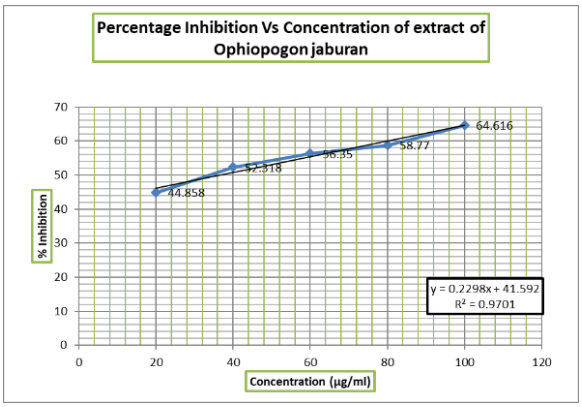
Concentration (µg/ml)	Absorbance	% Inhibition
20	0.481	51.512
40	0.422	57.459
60	0.337	66.028
80	0.276	72.177
100	0.153	84.576
Control	0.992	
IC50	19.55	



Graph 3: DPPH radical scavenging activity of Std. Ascorbic acid.

Table 10: DPPH radical scavenging activity of methanolic leaves extract of *Ophiopogon jaburan.*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.547	44.858
40	0.473	52.318
60	0.433	56.350
80	0.409	58.770
100	0.351	64.616
Control	**0.992**	
IC50	**36.58**	

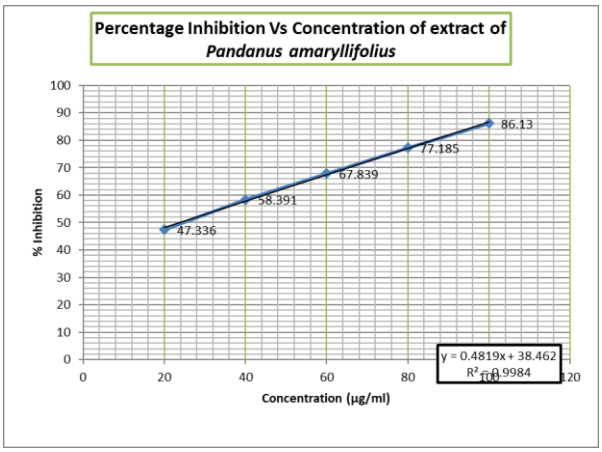


Graph 4: Represents the Percentage Inhibition *Vs* Concentration of extract of *Ophiopogon jaburan.*

Table 11: DPPH radical scavenging activity of methanol extract of *Pandanus amaryllifolius.*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.524	47.336
40	0.414	58.391
60	0.321	67.839

80	0.227	77.185
100	0.138	86.130
Control	**0.995**	
IC50	**23.94**	



Graph 5: Represents the Percentage Inhibition *Vs* Concentration of extract of *Pandanus amaryllifolius.*

3.4 physicochemical parameters of Polyherbal Suspension Formulations

3.4.1 Measurement of pH, Viscosity, Redispersibility and Sedimentation volume

Table 12: pH, Viscosity, Redispersibility and Sedimentation volume test.

Formulation	pH	Viscosity Determination (cps)	Redispersibility	Sedimentation Volume
Formulation I (*Ophiopogon jaburan*)	6.4	1420	Good	0.88
Formulation II (*Pandanus amaryllifolius*)	6.5	1465	Good	0.91
Formulation III (Polyherbal Combination, 1: 1 ratio)	6.6	1480	Good	0.93

3.5 Acute Oral Toxicity Study Result of Suspension Formulations

Table 13: Formulation I: *Ophiopogon jaburan* Suspension.

Toxicological Parameters	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 14
Skin & Fur	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Eye	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mucous Membrane	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Stool	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Urine	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Behaviour	Normal	Normal	Normal	Normal	Normal	Normal	Normal

S.M. Activity	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mortality	Alive	Alive	Alive	Alive	Alive	Alive	Alive
Body Weight	177 g	179 g	181 g	185 g	188 g	193 g	198 g
Food Intake	43 g	40 g	42 g	45 g	47 g	50 g	52 g

Table 14: Formulation II: *Pandanus amaryllifolius* Suspension.

Toxicological Parameters	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 11**	**Day 14**
Skin & Fur	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Eye	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mucous Membrane	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Stool	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Urine	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Behaviour	Normal	Normal	Normal	Normal	Normal	Normal	Normal
S.M. Activity	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mortality	Alive	Alive	Alive	Alive	Alive	Alive	Alive
Body Weight	176 g	178 g	182 g	186 g	190 g	194 g	200 g
Food Intake	42 g	39 g	43 g	46 g	48 g	51 g	53 g

Table 15: Formulation III: Polyherbal Combination (1: 1 Ratio).

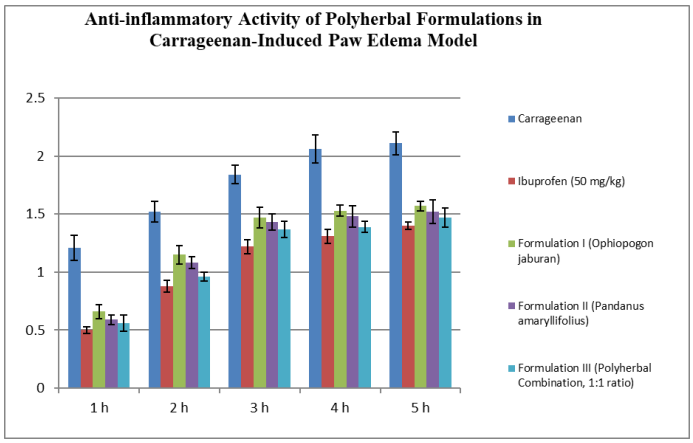
Toxicological Parameters	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 11**	**Day 14**
Skin & Fur	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Eye	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mucous Membrane	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Stool	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Urine	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Behaviour	Normal	Normal	Normal	Normal	Normal	Normal	Normal
S.M. Activity	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mortality	Alive	Alive	Alive	Alive	Alive	Alive	Alive
Body Weight	178 g	181 g	184 g	188 g	192 g	197 g	202 g
Food Intake	44 g	41 g	45 g	48 g	50 g	53 g	55 g

3.5 *In vivo* anti-inflammatory activity

3.5.1 Carrageenan-Induced Edema in Rats

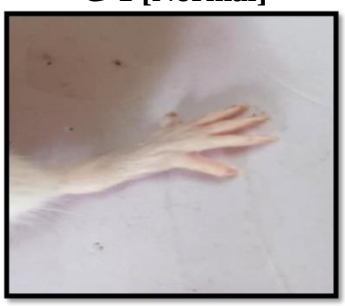
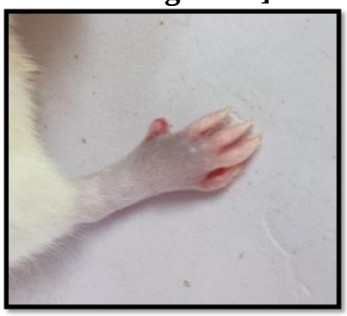
Table 16: Effect of Formulation I (*Ophiopogon jaburan*) Formulation II (*Pandanus amaryllifolius*) Formulation III (Polyherbal Combination, 1: 1 ratio) against carrageenan induced paw edema in rats (n=6).

Group	1 h	2 h	3 h	4 h	5 h
Carrageenan	1.21 ±0.11	1.52 ± 0.09	1.84 ±0.08	2.06 ±0.12	2.11 ± 0.10
Ibuprofen (50 mg/kg)	0.50 ± 0.03***	0.88 ± 0.05***	1.22 ± 0.06***	1.31 ± 0.06**	1.40 ± 0.03**
Formulation I (*Ophiopogon jaburan*)	0.66 ± 0.06***	1.15 ±0.08***	1.47 ± 0.09**	1.53 ±0.05**	1.57 ± 0.04**
Formulation II (*Pandanus amaryllifolius*)	0.59 ± 0.04***	1.08 ± 0.05**	1.43 ± 0.07**	1.48 ± 0.09**	1.52 ± 0.10**
Formulation III (Polyherbal Combination, 1: 1 ratio)	0.56 ± 0.07***	0.96 ± 0.04***	1.37 ± 0.07**	1.39 ± 0.05**	1.47± 0.08**



Graph 6: Effect of Formulation I, Formulation II, and Formulation III on Carrageenan-Induced Paw Edema Volume.

3.6 Images

G-1 [Normal]	G-2 [inducer Carrageenan]	G-3 [standard Ibuprofen 50 mg/kg]
		
G-4 [Formulation I (*Ophiopogon jaburan*)]	**G-5 [Formulation II (*Pandanus amaryllifolius*)]**	**G-6 [Formulation III (Polyherbal Combination,**

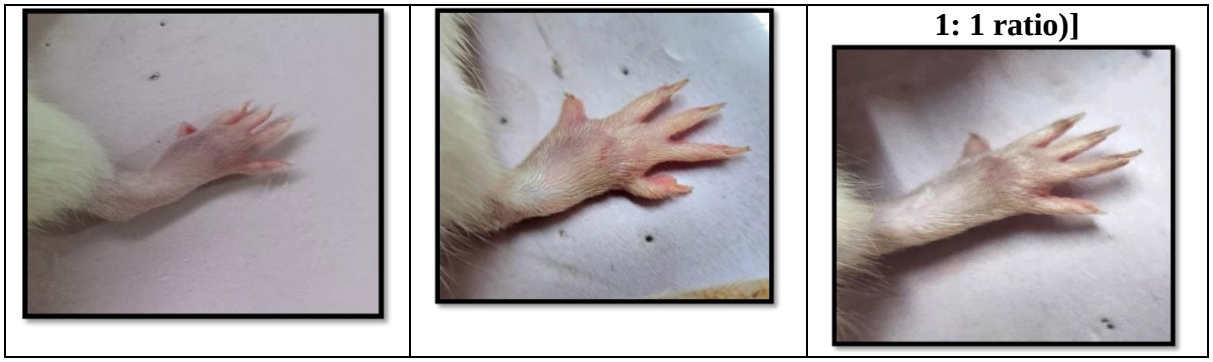


Figure 1: Anti-inflammatory Activity of Formulation III (Polyherbal Combination, 1: 1 Ratio) in Carrageenan-Induced Paw Edema in Rats.

DISCUSSION

The carrageenan-induced paw edema model demonstrated that all three formulations possessed appreciable anti-inflammatory activity. The control group showed progressive edema, confirming successful induction of acute inflammation, while ibuprofen produced the greatest inhibition. Among the herbal formulations, Formulation III (polyherbal combination, 1: 1 ratio) showed the highest activity, followed by Formulation II (*Pandanus amaryllifolius*) and Formulation I (*Ophiopogon jaburan*). The superior activity of the polyherbal formulation may be attributed to synergistic effects of flavonoids, phenolics, saponins, tannins, alkaloids, and other bioactive constituents. These compounds may reduce oxidative stress and modulate inflammatory mediators, including prostaglandins and pro-inflammatory cytokines. The findings suggest that combining both plant extracts enhances the anti-inflammatory potential compared with individual formulations, supporting further pharmacological investigation of the polyherbal suspension.

4. CONCLUSION

The present study successfully developed a polyherbal suspension containing *Ophiopogon jaburan* and *Pandanus amaryllifolius* extracts in a 1: 1 ratio. Phytochemical and quantitative analysis confirmed the presence of phenolic and flavonoid compounds, with *P. amaryllifolius* showing comparatively higher antioxidant activity. All formulations exhibited satisfactory physicochemical properties, while the polyherbal suspension showed superior stability and uniformity. Acute toxicity studies confirmed safety at 2000 mg/kg without mortality or significant toxic effects. The polyherbal formulation demonstrated the highest anti-inflammatory activity against carrageenan-induced paw edema, suggesting possible synergistic effects of the combined phytoconstituents. Overall, the formulation shows

promising antioxidant, anti-inflammatory, and safety profiles and warrants further pharmacological and clinical investigation.

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