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TO EVALUATE THE ANTI-INFLAMMATORY ACTIVITY OF RHIZOMES EXTRACT OF *ALPINIA GALANGA*

Purnendra Kumar Gupta*, Dr. Jagdish Chandra Rathi and Manish Sahu

NRI Institute of Pharmaceutical Sciences, Bhopal (M P).

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***Corresponding Author**
Purnendra Kumar Gupta
NRI Institute of Pharmaceutical Sciences, Bhopal (M P).

ABSTRACT

Percentage yield of petrolium ether and ethanolic extract of *Alpinia galanga* exhibited in 1.53 and 3.46 % respectively. The phytochemical screening revealed the presence of flavonoids, phenol, carbohydrates, tannins, Proteins and saponins compounds in the *Alpinia galanga* extract. Total phenolic content 0.856 (mg GAE/100mg) and total flavonoid content 0.942 (mg QE/100mg). Ethanolic extract of *Alpinia galanga* possess significant anti-inflammatory potential. Ethanolic extract of *Alpinia galanga* rhizome possess significant antiinflammatory potential. Ethanolic extract of *Alpinia galanga* rhizome prevented formalin-induced paw edema in a dose- dependent manner showing significant anti-inflammatory effect, percentage inhibition shown was found to be 69.17% and 72.27% at dose of 100 and 200 mg/kg, respectively. Hence, it is suggested that ethanolic extract of *Alpinia galanga* rhizome may provide benefits in the management of inflammation. These findings support the use of the extract in traditional system of medicine for the management of inflammatory conditions. These results clearly prove the anti-inflammatory effects of ethanolic extract of *Alpinia galanga* rhizome in experimental animals and support the potential usage of this plant in the management of inflammatory diseases.

KEYWORD: Anti-inflammatory, *Alpinia galanga,* Phytochemical, NSAIDS.

INTRODUCTION

Inflammation is usually a body response to tissue damage and to a number of systemic malfunctions including asthma, atherosclerosis, arthritis, physical injury and infection amongst many others.[1] Medicinal plants comprise of phytochemicals that improves the physiological balance of human beings and the knowledge of thesehealing properties has

been passed down through generations.[2] A vast range of medicinal plants around the globe has not yet been investigated to ascertain the claims made by traditional folks about their usefulness in treating diseases.[3,4] Nonsteroidal antiinflammatory drugs, such as indomethacin, are widely used in the treatment of inflammatory diseases. Although these drugs are highly effective, they have a number of deleterious adverse effects, such as gastrointestinal ulcers.[5,6] Therefore, researchers are continuously looking for new agents with fewer adverse effects for the treatment of inflammation.[7] Formalin- induced paw edema is one of the most suitable test procedures to evaluate chronic anti-inflammation, as it closely resembles human arthritis.[8,9]

Herbal therapy, although still an unwritten science, is well established in some countries and traditions and has become a way of life in almost 80% of population in rural areas.[10, 11] *Alpinia galanga* (Linn.) of *Zingiberaceae* family is one amongst those medicinally important plants.[12] Different parts of the plant are used in the treatment of many diseases for its anti-fungal, anti-tumour, antimicrobial, anti-inflammatory, anti- diabetic, antioxidant, antiulcer and many other properties.[13, 14, 15, 16]

The objective of the present study was to evaluate the anti-inflammatory activity of rhizomes extract of *Alpinia galanga.*

MATERIALS AND METHODS

The entire chemical used for the study was analytical grade purchased from Himedia lab. Pvt. Limited. Beaker, flask, aluminum foil, spatula, butter paper, cotton, forceps, tissue paper, range of glassware's & plastic wares and U.V Spectrophotometer (Lab India, 3000+) were used.

Preliminary Investigation

Collection of Plant material: Rhizomes of *Alpinia galanga* were collected from local market of Bhopal in themonth of February, 2021.

Selection: The plants have been selected on the basis of its availability and folk use of theplant.

Drying: Drying of fresh plant parts was carried out in sun but under the shade.

Storage: Dried rhizomes of *Alpinia galanga* were preserved in plastic bags, closed tightly

andpowdered as per the requirements.

Extraction procedure: Following procedure was adopted for the preparation of extracts from the shadedried and powdered herbs.

Defatting of plant material: 52.85 gram of rhizomes dried powdered of *Alpinia galanga* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by soxhlation method. The extraction was continued till the defatting of the material had taken place.

Extraction by soxhlation process: Defatted dried powdered of *Alpinia galanga* has been extracted with ethanolic solvent using soxhlation method for 48 hrs, filtered and dried using vacuum evaporator at 40°C.

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

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ powdered\ drug} X100$$

Phytochemical Screening: Phytochemical examinations were carried out for all the extracts as per the standard methods.

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

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

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

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

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

2. Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water

and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates was 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.

3. Detection of glycosides: Extract was hydrolysed with dil. HCl, and thensubjected to test for glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extract was 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.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Quantitative studies of phytoconstituents

Total phenol content estimation

Principle: The total phenol content of the extract was determined by the modified folin-ciocalteu method.

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol.

Preparation of Extract: 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 colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Principle: Determination of total flavonoids content was based on aluminium chloride method.

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 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.

Evaluation of *in vitro* anti-inflammatory activity

Anti-inflammatory activity of the *Alpinia galanga* extract was evaluated by protein denaturation method as described by Padmanabhan and Jangle [82]. Diclofenac sodium, a powerful non steroidal anti-inflammatory drug was used as a standard drug. The reaction mixture consisting of 2 mL of different concentrations of *Alpinia galanga* extract (100-500 µg/mL) or standard diclofenac sodium (100-500 µg mL-1) and 2.8 mL of phosphate buffered saline (pH 6.4) was mixed with 0.2 mL of egg albumin (from fresh hen's egg) and incubated at (37±1)°C for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm by using double distilled water as blank. The percentage inhibition of protein denaturation was calculated by using the following formula.

$$\%\ \boldsymbol{Inhibition} = \frac{At - Ac}{Ac}\ X\ 100$$

Where, at=absorbance of test sample; Ac=absorbance of control

The plant concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

***In-vivo* anti-inflammatory activity of *Alpinia galanga* extract**

Animals: Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Drugs & Chemicals: Diclofenac injections (Voveran), formalin (Sigma Chemical) were used in present study.

Toxicity study: Preliminary experiments were carried out on rats (n=6). Ethanolic extract of *Alpinia galanga* rhizomes were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD).[83] Animals were kept fasting providing only water, extract were given p.o. in doses

of 500, 1000 and 2000 mg/kg/p.o. administered orally for 4 days of different groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti- inflammatory effect.

Experimental designs

Group –1: Normal

Group –2: Control

Group –3: Diclofenac sodium (10 mg/kg, bw, Standard)

Group –4: Ethanolic extract of *Alpinia galanga* rhizomes (100mg/kg, p.o.) Group –5: Ethanolic extract of *Alpinia galanga* rhizomes (200mg/kg, p.o.)

Formalin induced hind paw edema

Anti-inflammatory activity was measured using formalin induced rat paw oedema assay. The rats were divided into 5 groups of 6 animals each (plant extract was dissolved and administered per oral at different dose levels). Group 1 was normal treated with distilled water only, Group 2 was treated as formalin (0.2 ml of 2% v/v freshly prepared formalin solution prepared in distilled water) was used as edematogenic agent, Group 3 was administered Diclofenac sodium (10 mg/kg, bw) and considered as standard. Group 4 were treated with ethanolic extract of *Alpinia galanga* rhizomes (100mg/kg, p.o.). Group 5 were treated with ethanolic extract of *Alpinia galanga* rhizomes (200mg/kg, p.o.). The thickness was measured before injecting the formalin and after injecting the formalin everyday at a fixed time. The volumes of oedema of the injected were measured after the induction of inflammation using a plethysomgraph to calculate the percentage of paw oedema inhibition.

$$\%\ \boldsymbol{Inhibition} = \frac{Vc - Vt}{Vc} X100$$

Where, Vc- Edema volume of control groupVt- Edema volume of test group

Statistical Analysis: All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

RESULTS AND DISCUSSION

Determination of Percentage Yield

Yield of Extraction: To obtain the percentage yield of extraction is very important

phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extract obtained from samples using ethanolic solvent is depicted in the table below.

Table no. 1: % Yield of *Alpinia galangal.*

S. No.	Extracts	% Yield (w/w)
1.	Pet. Ether	1.53%
2.	Ethanolic	3.46%

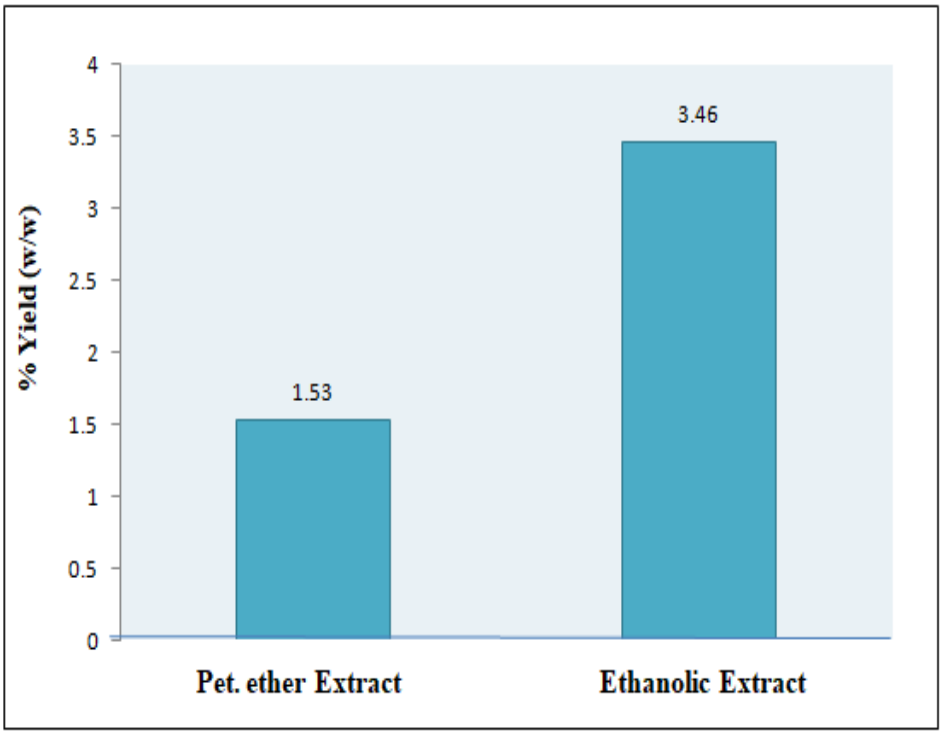

Figure 1: Comparative graph of % Yield of *Alpinia galangal.*

Percentage yield of petrolium ether and ethanolic extract of *Alpinia galanga* exhibited in 1.53 and 3.46 % respectively.

Phytochemical screening of extract

Small portion of the dried extracts was subjected to the phytochemical tests using standard method to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extracts of all samples. Small amount of each extract was suitably resuspended into the distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed in the table below.

Table no. 2: Phytochemical screening of extracts of *Alpinia galangal.*

S. No.	Constituents	Ethanolic extract	Observation
1.	**Alkaloids** Dragendroff's test Hager's test	-ve -ve	Green coloured Not yellow coloured
2.	**Glycosides** Legal's test	-ve	Green coloured
3.	**Flavonoids** Lead acetate Alkaline test	-ve +ve	Yellow colour but no precipitate Yellow colour
4.	**Phenol** Ferric chloride test	+ve	Black coloured
5.	**Proteins** Xanthoproteic test	+ve	Yellow coloured
6.	**Carbohydrates** Fehling's test	+ve	Red colour precipitate
7.	**Saponins** Foam test	+ve	Layer of foam
8.	**Diterpenes** Copper acetate test	-ve	Green coloured
9.	**Tannins** Gelatin Test	+ve	White colour precipitate

Results of phytochemical screening were found flavonoids, phenol, carbohydrates, Tannins, Proteins and saponins were detected in ethanolic extracted of *Alpinia galanga.*

Results of estimation of total phenol and flavonoids content of *Alpiniagalanga* extract

Estimation of total Phenol content (TPC): Total phenol content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **$y = 0.021x + 0.002$, $R^2 = 0.999$,** where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Calibration Curve of Gallic acid

Table no. 3: Preparation of calibration curve of Gallic acid.

Concentration (µg/ml)	10	20	30	40	50
Mean Absorbance	0.227	0.434	0.649	0.855	1.097

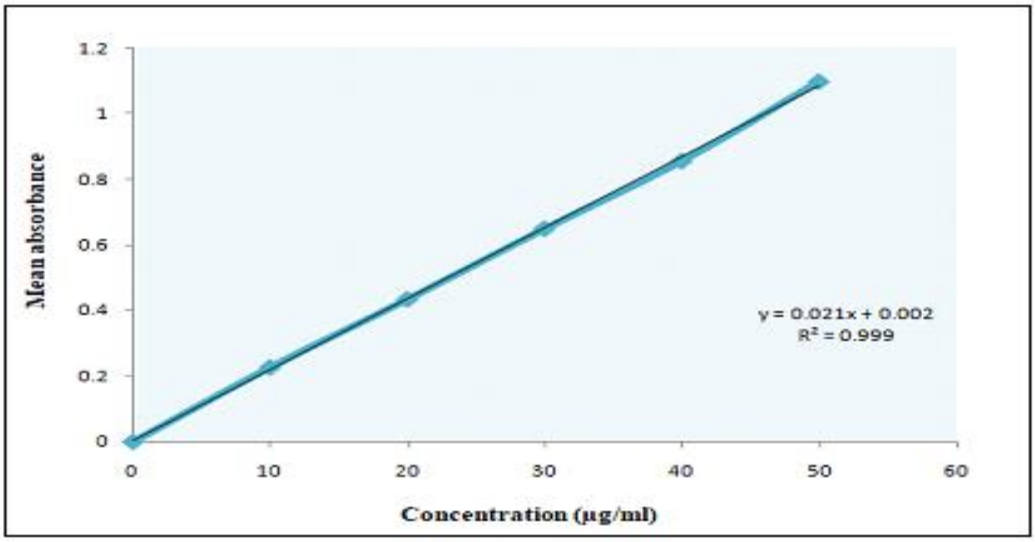

Figure 2: Graph of calibration curve of Gallic acid.

Estimation of Total flavonoids content (TFC): Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **y = 0.036x + 0.002, R^2=0.999**, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table no. 4: Preparation of calibration curve of Quercetin.

S. No.	Concentration (µg/ml)	Absorbance
1	5	0.185
2	10	0.362
3	15	0.543
4	20	0.732
5	25	0.896

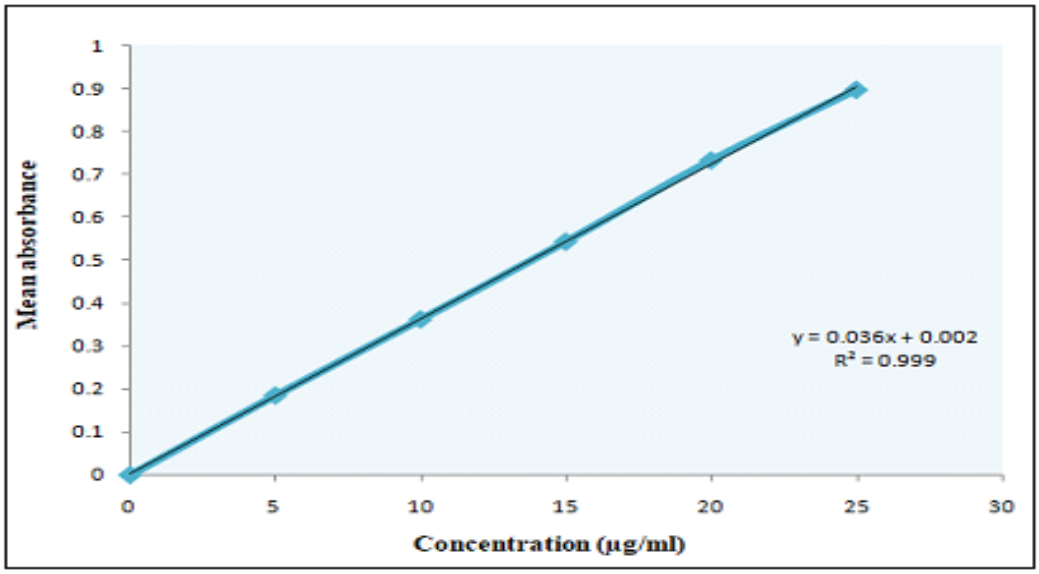

Figure 3: Graph of calibration curve of Quercetin.

Table no. 5: Estimation of total phenolic and flavonoids content of *Alpinia galanga* extract.

S. No.	Extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Ethanolic	0.856	0.942

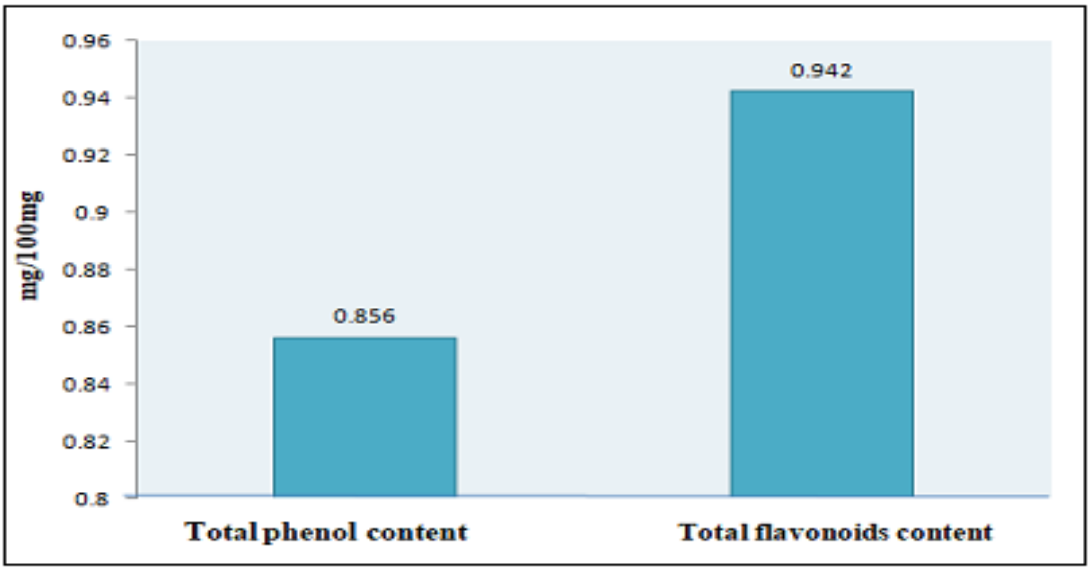

Figure 4: Comparative graph of total phenol and flavonoids content.

The presence of phytochemicals (Phenols, Flavonoids) was quantitatively screened. The extract quantitative analysis revealed total phenolic content (equivalent to gallic acid) of 0.856mg/100 mg. The total content of flavonoid (equivalent to quercetin) was found 0.942mg/100 mg in *Alpinia galanga* extract.

Results of *in vitro* anti-inflammatory activity

Table no. 6: % Inhibition of Diclofenac sodium and *Alpinia galanga* extract.

Concentration (µg/ml)	% Inhibition	
	Diclofenac sodium	Alpinia galanga extract
100	35.45	36.69
200	42.74	40.22
300	57.61	58.42
400	72.22	62.89
500	93.58	70.48
IC_{50}	230.41	259.33

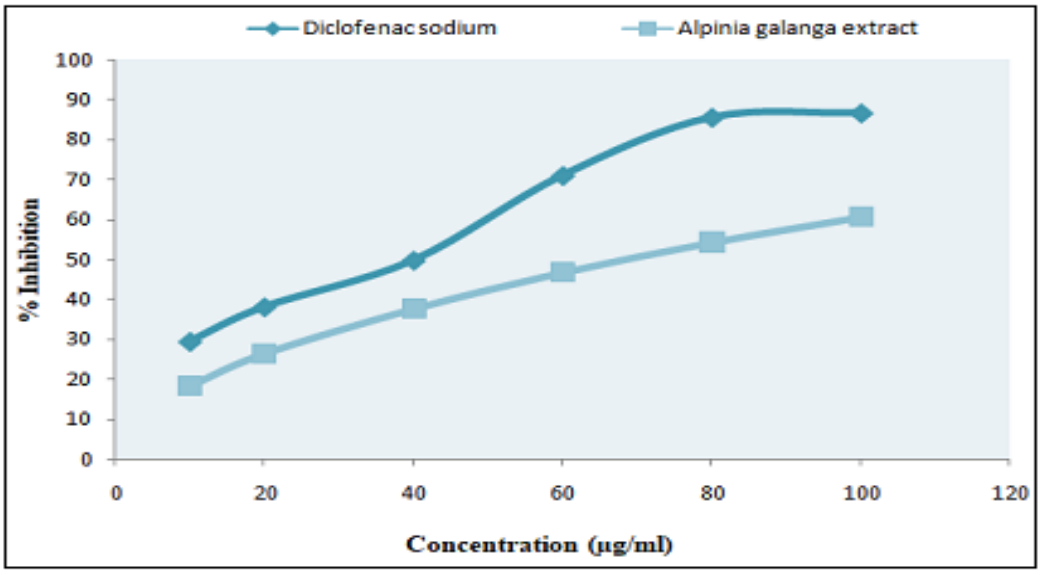

Figure 5: Graph of *in vitro* anti-inflammatory activity.

Results of *in vivo* anti -inflammatory activity

The effect of ethanolic extract of *Alpinia galanga* rhizomes and standard drug as compared to formalin control group in formalin-induced paw edema model using plethysomgraph. Ethanolic extract of *Alpinia galanga* rhizomes administered at a dose of 100 and 200 mg/kg p.o., showed 71.42% and 77.14%, inhibition, respectively while Diclofenac sodium at a dose of 10 mg/kg p.o. prevented formalin induced paw edema with a percentage inhibition of 82.85%.

Table no. 7: Effect of ethanolic extract of *Alpinia galanga* rhizomes on paw edema in rats.

Group	Treatment	Dose (mg/kg)	Mean differences in Paw Volume (ml)	Percentage of Inhibition (%)
Group-I	Normal	-	--	--
Group-II	Control	0.1 ml of 1% (w/v)	3.50 ±0.60	--
Group-III	Diclofenac sodium	10	0.60±0.20 ***	82.85
Group-IV	Ethanolic extract of Alpinia galangal rhizomes	100	1.00±0.50 **	71.42
Group-V	Ethanolic extract of Alpinia galanga rhizomes	200	0.80±0.50 ***	77.14

Values are expressed as mean ± SD.

*P < 0.05-significant compared to formalin treated group.

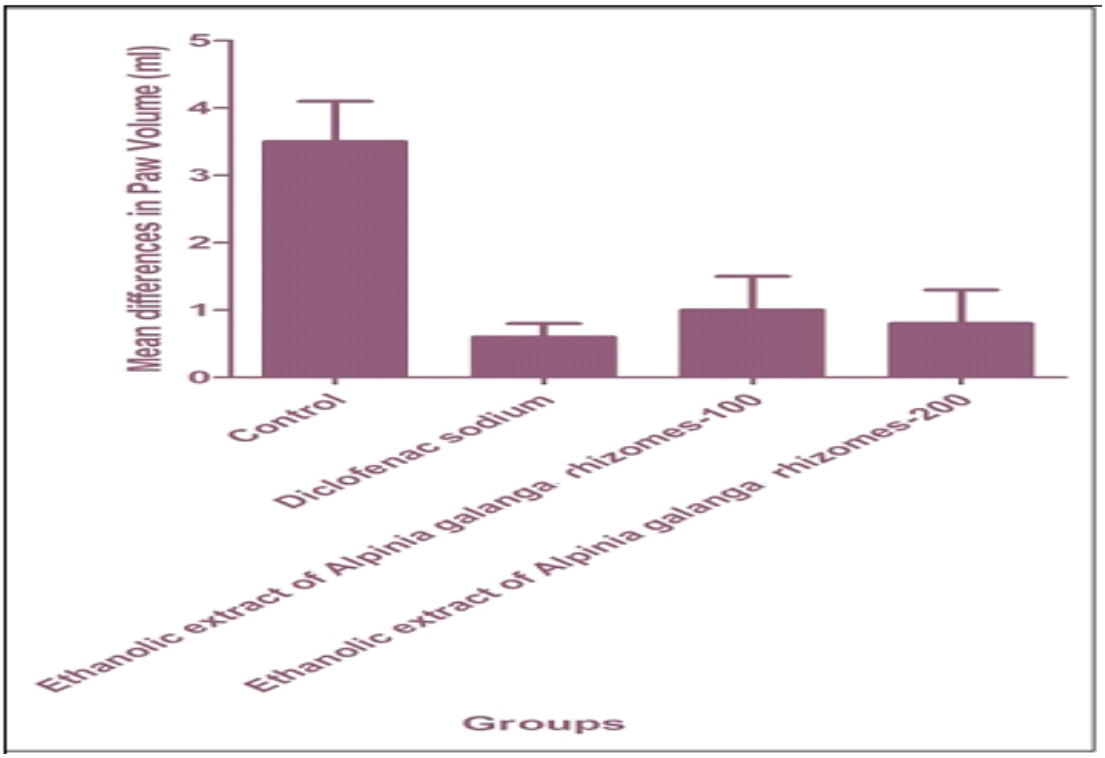

Figure 6: Effect of ethanolic extract of *Alpinia galanga* rhizomes on pawedema induced by formalin in rats.

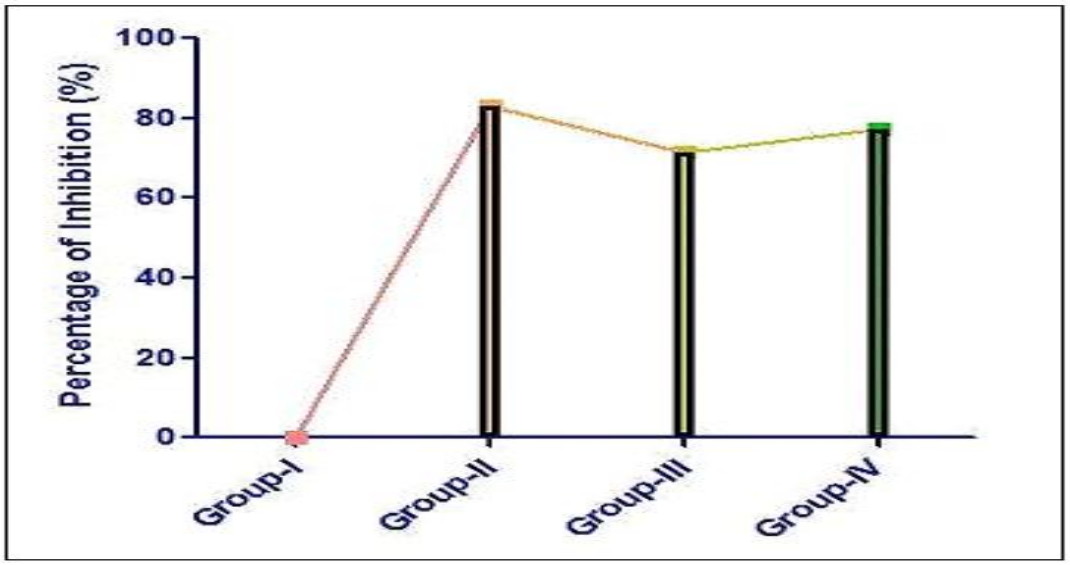

Figure 7: Effect of ethanolic extract of *Alpinia galanga* rhizomes on percentage of inhibitions induced by formalin in rats.

DISCUSSION

Inflammation is usually a body response to tissue damage and to a number of systemic malfunctions including asthma, atherosclerosis, arthritis, physical injury and infection amongst many others. Medicinal plants comprise of phytochemicals that improves the

physiological balance of human beings and the knowledge of thesehealing properties has been passed down through generations. A vast range of medicinal plants around the globe has not yet been investigated to ascertain the claims made by traditional folks about their usefulness in treating diseases. The yield of ethanolic extract was found to be 3.46% w/w. The phytochemical screening revealed the presence of flavonoids, phenol, carbohydrates, tannins, Proteins and saponins compounds in the *Alpinia galanga* extract.

Total phenolic content 0.856 (mg GAE/100mg) and total flavonoid content 0.942 (mg QE/100mg). Ethanolic extract of *Alpinia galanga* possess significant anti-inflammatory potential. These findings support the use of the extract in traditional system of medicine for the management of inflammatory conditions.

Inflammation is an important biological response of the vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation can also cause numerous diseases, such as cancer, autoimmune diseases, cardiovascular diseases, and neurodegenerative diseases. Nonsteroidal antiinflammatory drugs, such as indomethacin, are widely used in the treatment of inflammatory diseases. Although these drugs are highly effective, they have a number of deleterious adverse effects, such as gastrointestinal ulcers. Therefore, researchers are continuously looking for new agents with fewer adverse effects for the treatment of inflammation. Formalin- induced paw edema is one of the most suitable test procedures to evaluate chronic anti-inflammation, as it closely resembles human arthritis. Ethanolic extract of *Alpinia galanga* rhizome prevented formalin-induced paw edema in a dose- dependent manner showing significant anti-inflammatory effect, percentage inhibition shown was found to be 69.17% and 72.27% at dose of 100 and 200 mg/kg, respectively. Hence, it is suggested that ethanolic extract of *Alpinia galanga* rhizome may provide benefits in the management of inflammation.

CONCLUSION

Herbal therapy, although still an unwritten science, is well established in some countries and traditions and has become a way of life in almost 80% of population inrural areas. Chronic anti-inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population. At present, although synthetic drugs are dominating the market but element of toxicity that these drugs entail, cannot be ruled out. Their prolonged use may cause severe adverse effects on chronic administration the most common being

gastrointestinal bleeding and peptic ulcers. Consequently there is a need to develop a new anti-inflammatory agent with minimum side effects. Search for safe and effective anti-inflammatory agents have been given priority in scientific research in herbal system of medicine.

The yield of extract obtained from samples using ethanolic solvent is depicted in the table. Percentage yield of petrolium ether and ethanolic extract of *Alpinia galanga* exhibited in 1.53 and 3.46 % respectively. Results of phytochemical screening were found flavonoids, phenol, carbohydrates, Tannins, Proteins and saponins were detected in ethanolic extracted of *Alpinia galanga*. The presence of phytochemicals (Phenols, Flavonoids) was quantitatively screened. The extract quantitative analysis revealed total phenolic content (equivalent to gallic acid) of 0.856mg/100 mg. The total content of flavonoid (equivalent to quercetin) was found 0.942mg/100 mg in *Alpinia galanga* extract.

Ethanolic extract of *Alpinia galanga* rhizome possess significant antiinflammatory potential. These findings support the use of the extract in traditional system of medicine for the management of inflammatory conditions. These results clearly prove the anti-inflammatory effects of ethanolic extract of *Alpinia galanga* rhizome in experimental animals and support the potential usage of this plant in the management of inflammatory diseases.

Conflicts of interests

There is noconflicts of interests.

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