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PHYTOCHEMICAL SCREENING AND IN VIVO ANTI-INFLAMMATORY ACTIVITY OF STEM EXTRACT OF *LITSEA GLUTINOSA*

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Abstract

The present study aimed at phytochemical screening and in vivo anti -inflammatory activity of stem extract of *Litsea glutinosa* in formalin induced paw model. The Stems of the plant was collected, processed and tested for qualitative and quantitative parameters. Further by performing its actue toxicity study the extract anti -inflammatory effect was analyzed in Wistar rats. Results showed that the phytochemical tests revealed the presence of flavonoid, phenol, protein, carbohydrate and saponin. Extract found to have total phenolic content (equivalent to gallic acid) of 0.725mg/100 and the total content of flavonoid (equivalent to quercetin) was found 0.811mg/100 mg. From the in vivo test results it was observed that both doses of the hydroalcoholic extract of *Litsea glutinosa* (100 mg/kg and 200 mg/kg) showed a significant decrease in paw volume compared to the control group. In *Litsea glutinosa* 100 mg/kg and 200 mg/kg extract treated rats the Mean differences in Paw Volume was measured to be 1.05±0.50 ml and 0.80±0.50 ml respectively. While in diclofenac treated grou the paw volume was estimated to be 0.65±0.20ml. The percentage of inhibition for Group-IV (72.72%) and Group-V (79.22%) indicates a dose-dependent anti-inflammatory effect. While being a standard drug the % inhibition was observed to be 82.85%. These findings suggest that *Litsea glutinosa* extract possesses anti-edematous properties, and the higher dose exhibits a more pronounced effect. Similar to diclofenac, *Litsea glutinosa* exhibits strong anti-inflammatory effect in formaldehyde induced paw edema model. Thus, it can be concluded that the anti-inflammatory effects observed may be attributed to the presence of bioactive compounds in *Litsea glutinosa* that modulate inflammatory pathways.

Keywords: Medicinal plants, Inflammation, *Litsea glutinosa,* Diclofenac, Formalin induced paw edema, Phytochemicals

Introduction

The immune system kicks in when anything foreign like bacteria, viruses, or hazardous chemicals enters the body or when we sustain an injury. The immune system releases cells to either begin the mending process of wounded tissue or to capture germs and other harmful agents. This reaction is inflammatory. Pain, edema, bruising, or redness may follow. However, inflammation also has an impact on invisible bodily processes. In all cases, the goal is to minimize harm to the host's physiology while facilitating the healing and restoration of damaged tissue. An essential component of stress responses, inflammation arises as a result of them. Acute psychosocial stress can activate the transcription factor kappa beta and cause the release of pro-inflammatory cytokines in the event of a fight-or-flight response, most likely as a result of adrenergic stimulation. In addition to causing the injurious agent to be destroyed or diluted, inflammation also sets off a sequence of events that lead to the healing and reconstitution of damaged tissues. These processes can involve the regeneration of native parenchymal cells, the filling of the defect with fibroblast tissue (scarring), or both. Leukocyte transport to the site of damage is a crucial function of inflammation. This is accomplished by increased blood flow, structural alterations in the microvasculature that allow leukocyte emigration, and leukocyte storage in the injury focus (Furman *et al*., 2019; Trowbridge *et al*., 1997; Black, 2002).

Most remarkably, pathogen-associated molecular patterns (PAMPs), which are evolutionarily conserved structures on pathogens, connect with pattern recognition receptors expressed on innate immune cells to trigger the acute inflammatory response during infection. Damage-associated molecular patterns (DAMPs), which are generated in reaction to unpleasant stimuli that are physical, chemical, or metabolic, can also trigger an acute inflammatory response. After infection, the body produces chemicals such lipoxins, resolvins, maresins, and protectins, which help to reduce inflammation (Serhan, 2014; Ashley, 2012)

Changes in the inflammatory response from short- to long-lived can lead to significant changes in all tissues and organs, as well as normal cellular physiology, and can compromise immune tolerance. These changes can raise the risk of a number of non-communicable diseases in both young and old people. Chronic systemic inflammation

can also compromise healthy immune function, making a person more vulnerable to infections and malignancies as well as less likely to respond well to vaccinations. Moreover, persistent inflammation throughout pregnancy and children might have detrimental effects on development, increasing the lifetime risk of non-communicable diseases, among other things (Kotas, M. E. & Medzhitov, 2015; Fullerton and Gilroy 2016).

In order to control inflammation, both non-steroidal anti-inflammatory medications (NSAIDs) and steroids are frequently utilized. Steroid anti-inflammatory medications prevent leukocytes from migrating and degranulating. NSAIDs, or non-steroid anti-inflammatory medicines, block the activity of the enzyme cyclo-oxygenase. The negative effects of the medications now in use include hypertension, hyperglycemia, renal damage, and G.I. ulceration and bleeding. The biggest drawback of the powerful synthetic medications that are now on the market is their toxicity and recurrence of side effects after stopping use. (Vane and Botting, 1998) Consequently, it is imperative that medications be developed and screened for their ability to reduce inflammation, and numerous attempts are being made to identify anti-inflammatory medications from locally grown medicinal plants (Wang *et al*., 2013).

Primary and secondary metabolites are among the many chemical substances known as phytochemicals that are produced by plants. While secondary metabolites play a variety of roles in species interaction, competition, and disease and damage resistance, primary metabolites are recognized to support plant growth and metabolism. Phytochemicals can be categorized into three main classes based on their metabolic origins: phenolic compounds, terpenoids, and nitrogen-containing alkaloids/sulfur-containing compounds. Plants have been the main source of medicine for thousands of years. Biological properties of phytochemicals with a range of therapeutic applications include anti-inflammatory, anti-allergic, anti-cancer, antibacterial, antiviral, and analgesic effects (Howes, 2018; Zhu *et al*., 2018; Nisar *et al*., 2023).

The plant *Litsea glutinosa* has a wide range of therapeutic uses and is traditionally used to treat a variety of gastrointestinal conditions, including diabetes, edema, traumatic injuries, colds, arthritis, and asthma, as well as symptoms like diarrhea, indigestion, and abdominal pain. The essential oil of the litsea plant is also well-known for its ability to

defend against a wide variety of bacteria. This herb helps prevent many malignancies by eliminating cytotoxicity, acute and genetic toxicity, and parasite and antioxidant effects (Chawra *et al*., 2021; Bhowmick *et al*., 2014).

This study deals with phytochemical screening and in vivo anti -inflammatory activity of stem extract of *litsea glutinosa*. The most used paradigm for assessing the anti-inflammatory and anti-arthritic properties of natural products in the sub-acute phase of inflammation is the formaldehyde-induced paw edema model. The mice developed progressive paw edema in the first and third days of observation after receiving a subcutaneous injection of 2% formalin v/v with distilled water in their palms. This edema was characterized by increased migration of leucocytes and phagocytes, infiltrations of neutrophils and macrophages, and proliferation of fibroblasts into the surrounding area of the injured area. Therefore, one of the most innovative ways to assess anti-inflammatory and anti-proliferative effectiveness during the sub-acute phase of inflammation is to block formaldehyde-induced edema (Arzi, *et al*., 2015; John and Shobana, 2012).

Materials and Methods

Collection of Plant material

The plants have been selected on the basis of its availability and folk use of the plant. Stem *of Litsea glutinosa* were collected from local market of Bhopal in the month of March, 2023.

Extraction by maceration process

63 gram of stem dried powdered of *Litsea glutinosa* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration method. The extraction was continued till the defatting of the material had taken place. Defatted dried powdered of *Litsea glutinosa* has been extracted with hydroalcoholic solvent (ethanol: water; 70:30) using maceration method for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Chen *et al*., 2016).

Quantitative studies of phytoconstituents

Total phenol content estimation

The determination of the total phenol content in the extract was conducted using the modified Folin-Ciocalteu method. To prepare standard solutions, 10 mg of Gallic acid was dissolved in 10 ml of methanol, and various aliquots ranging from 10 to 50 µg/ml were prepared in methanol. Additionally, 10 mg of the dried extract was dissolved in 10 ml of methanol and filtered. For the estimation of phenol, 2 ml of the extract solution (1 mg/ml) was utilized. In a separate set, 2 ml of both the extract solution and each standard solution were mixed with 1 ml of Folin-Ciocalteu reagent, which had been previously diluted with distilled water at a ratio of 1:10 v/v. To this mixture, 1 ml of sodium carbonate solution (7.5 g/l) was added. The resulting solution was vortexed for 15 seconds and allowed to stand for 10 minutes to facilitate color development. The absorbance of the developed color was measured at 765 nm using a spectrophotometer. This absorbance reading provides information about the phenol content present in the extract, with Gallic acid serving as the standard reference. The higher the absorbance, the greater the phenolic content in the sample (Rover and Brown, 2013).

Total flavonoids content estimation

The determination of the total flavonoids content was conducted using the aluminum chloride method as outlined by Parkhe and Bharti in 2019. To prepare standard solutions, 10 mg of quercetin was dissolved in 10 ml of methanol, and various aliquots ranging from 5 to 25µg/ml were prepared in methanol. Additionally, 10 mg of the dried extract was dissolved in 10 ml of methanol and filtered. For the estimation of flavonoids, 3 ml of the extract solution (1 mg/ml) was used. In a separate set, 1 ml of a 2% aluminum chloride solution was added to 3 ml of both the extract solution and each standard solution. The mixtures were allowed to stand for 15 minutes at room temperature to facilitate the reaction between flavonoids and aluminum chloride. The absorbance of the resulting solutions was measured at 420 nm using a spectrophotometer. This method allows for the quantification of total flavonoids in the sample, with quercetin serving as the standard reference. The absorbance readings at 420 nm provide information about the flavonoid content present in the extract (Parkhe and Bharti, 2019).

In-vivo anti-inflammatory activity of *Litsea glutinosa* extract using formalin induced rat paw oedema assay

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.

Toxicity study

Preliminary experiments were carried out on rats (n=6). Ethanolic extract of *Litsea glutinosa* 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, 2001). 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 (Joshi *et al*., 2007).

Experimental designs

Table 1: Experimental designs for anti-inflammatory activity

Groups	Treatment
Group –1	Normal
Group –2	Control
Group –3	Diclofenac sodium (10 mg/kg, bw, Standard)
Group –4	Ethanolic extract of *Litsea glutinosa* stem (100mg/kg, p.o.)
Group –5	Ethanolic extract of *Litsea glutinosa* stem (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 *Litsea glutinosa* (100mg/kg, p.o.). Group 5 were treated with ethanolic extract of *Litsea glutinosa* (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 (Singh *et al.,* 2010).

$$\text{Percentage Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc- Edema volume of control group

Vt- 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

Since ancient times, medicinal plants have been a significant source of compounds used to treat human illness. Even with the significant advancements in contemporary medicine, creating novel medications from natural sources is still seen as crucial.

The phytochemical tests revealed the presence of flavonoid, phenol, protein, carbohydrate and saponin. The presence of phytochemicals (Phenols, Flavonoids) was quantitatively screened. The extract quantitative analysis revealed total phenolic

content (equivalent to gallic acid) of 0.725mg/100 mg. The total content of flavonoid (equivalent to quercetin) was found 0.811mg/100 mg in *Litsea glutinosa* extract.

The presented data illustrates the impact of the hydroalcoholic extract of *Litsea glutinosa* on paw edema induced by formalin in rats, as compared to a control group and a standard anti-inflammatory drug, Diclofenac sodium.

The normal group represents the baseline condition without any treatment. This group provides a reference for the natural state of the animals' paws. The control group, treated with formalin, exhibited a significant increase in paw volume compared to the normal group, indicating the successful induction of paw edema. This serves as a baseline for assessing the effectiveness of interventions.

Diclofenac sodium, a standard anti-inflammatory drug, demonstrated a notable reduction in paw volume compared to the control group. The percentage of inhibition (82.85%) suggests a strong anti-inflammatory effect, validating the model's sensitivity to detect anti-edematous activity.

Both doses of the hydroalcoholic extract of *Litsea glutinosa* (100 mg/kg and 200 mg/kg) showed a significant decrease in paw volume compared to the control group. In *Litsea glutinosa* 100 mg/kg and 200 mg/kg extract treated rats the Mean differences in Paw Volume was measured to be 1.05±0.50 ml and 0.80±0.50 ml respectively. While in diclofenac treated grou the paw volume was estimated to be 0.65±0.20ml.

The percentage of inhibition for Group-IV (72.72%) and Group-V (79.22%) indicates a dose-dependent anti-inflammatory effect. While being a standard drug the % inhibition was observed to be 82.85%. These findings suggest that *Litsea glutinosa* extract possesses anti-edematous properties, and the higher dose exhibits a more pronounced effect.

The percentage of inhibition values for *Litsea glutinosa* extracts, especially at the higher dose, are comparable to the standard drug Diclofenac sodium. The main source of discomfort brought on by formalin is inflammation of the peripheral tissues. Acute inflammation can range in duration from a few minutes to a few days, which is comparatively shorter. Typical alterations include fluid and plasma protein exudation, leukocyte emigration, with neutrophils making up the majority of the emigrant population.

Anti-inflammatory drugs mostly work by inhibiting the cyclooxygenase enzyme, which is in charge of turning arachidonic acid into prostaglandin (PG). It is believed that both acute and chronic inflammation are correlated with these enzymes' extracellular activity. Cyclooxygenase is one of the lysosomal enzymes that NSAIDs block. According to the results, during the phagocytic phase of formalin-induced inflammation, the test medication *Litsea glutinosa* (200 mg/kg) becomes significant.

The capacity of *Litsea glutinosa* to prevent the production of eicosanoid molecules may be a potential mechanism by which it reduces inflammation. It has been demonstrated that *Litsea glutinosa* hydroalcoholic extract is strong inhibitor of eicosanoid production, specifically thromboxane B and leukotrienes B4, through their respective inhibition of COX and LOX. It was also discovered that *Litsea glutinosa,* an active ingredient in *Litsea glutinosa*, inhibits lipid peroxidation. Oxidative stress and neutrophil and monocyte/macrophage infiltration are linked to inflammation. Tissue injury might then result from macrophages releasing proinflammatory cytokines like nitric oxide.

The results suggest that *Litsea glutinosa* extract has the potential to attenuate formalin-induced paw edema in rats, supporting its anti-inflammatory properties. The anti-inflammatory effects observed may be attributed to the presence of bioactive compounds in *Litsea glutinosa* that modulate inflammatory pathways. The extract may inhibit mediators of inflammation, thereby reducing paw edema.

Table 2: Phytochemical screening of extracts *of Litsea glutinosa*

S. No.	Constituents	Hydroalcoholic extract	Observation
1.	Alkaloids		
	Dragendroff's test	-ve	Brown coloured
	Hager's test	-ve	Brown coloured
2.	Glycosides		
	Legal's test	-ve	Dark brown coloured
3.	Flavonoids		
	Lead acetate	-ve	Yellow precipitate
	Alkaline test	+ve	Colourless
4.	Phenol		
	Ferric chloride test	+ve	Bluish 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	Brown coloured

Results of estimation of total phenol and flavonoids content of *Litsea glutinosa* extract

Estimation of total Phenol content (TPC)

The equation obtained from the calibration curve, **$y = 0.021x + 0.002$, $R^2 = 0.999$**, was used to describe the total phenol content of the dry extract sample as mg/100mg of gallic acid equivalent. Here, X stands for gallic acid equivalent (GAE) and Y for absorbance.

Table 3: Preparation of calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean Absorbance
1	10	0.227
2	20	0.434
3	30	0.649
4	40	0.855
5	50	1.097

Estimation of Total flavonoids content (TFC)

The equation based on the calibration curve, **$y = 0.036x + 0.002$, $R^2=0.999$**, was used to quantify the total flavonoid content as quercetin equivalent (mg/100mg), where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 4: Preparation of calibration curve of Quercetin

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

Table 5: Estimation of total phenolic and flavonoids content of *Litsea glutinosa* extract

S. No.	Extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.725	0.811

Table 6: Effect of hydroalcoholic extract of *Litsea glutinosa* on paw edema induced by formalin 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)	3.85 ±0.60	--
Group-III	Diclofenac sodium	10	0.65±0.20 ***	82.85
Group-IV	Hydroalcoholic extract of *Litsea glutinosa*	100	1.05±0.50 **	72.72
Group-V	Hydroalcoholic extract of *Litsea glutinosa*	200	0.80±0.50 ***	79.22

Values are expressed as mean ± SD.

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

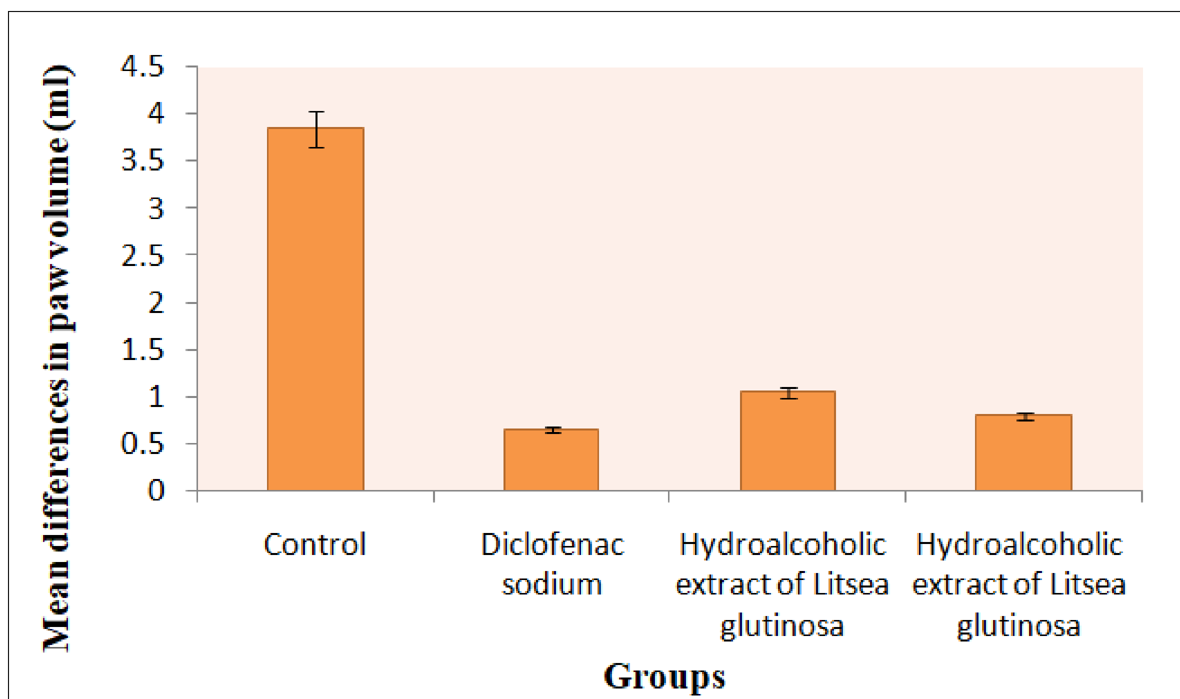

Figure 1: Effect of hydroalcoholic extract of *Litsea glutinosa* on paw edema induced by formalin in rats

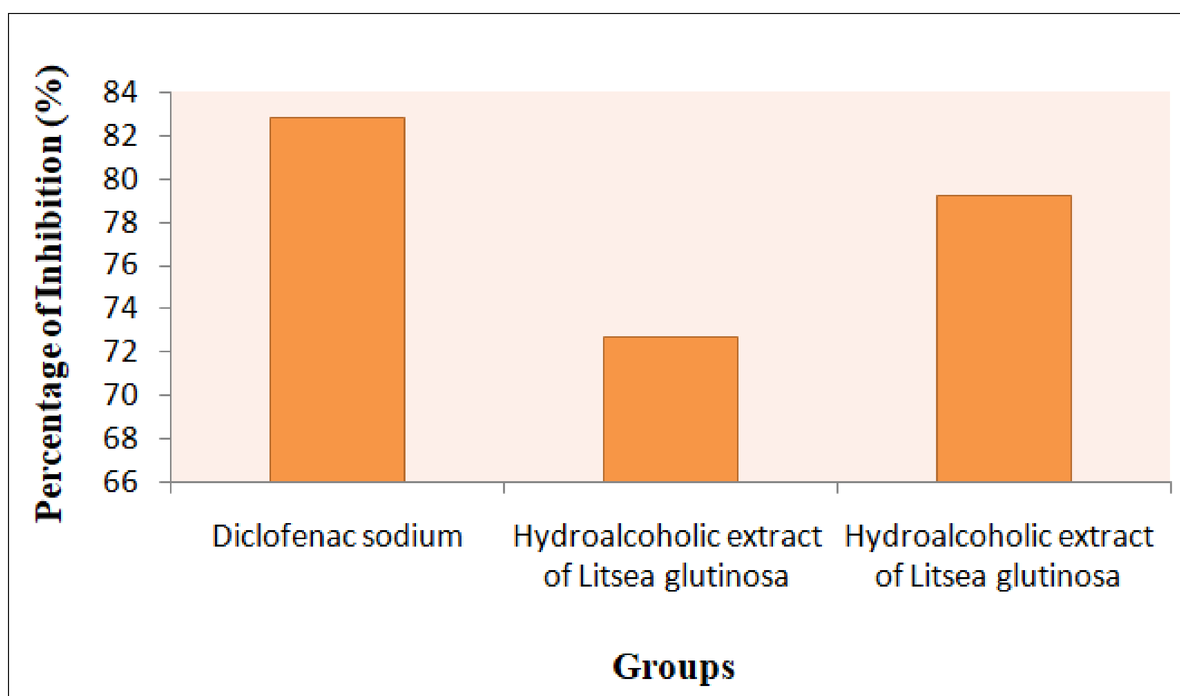

Figure 2: Effect of hydroalcoholic extract of *Litsea glutinosa* on percentage of inhibitions induced by formalin in rats

Conclusion

The ethnomedical history of the plant *Litsea glutinosa* served as the basis for the study's execution. *Litsea glutinosa* anti-inflammatory properties may be attributed to the inclusion of lipids, triterpenoids, flavanoids, and fixed oils. Rat granuloma development was considerably suppressed by hydroalcoholic extract. The in vivo results also support the plant *Litsea glutinosa* possible anti-inflammatory properties, and these plants may provide a source of lead compounds with anti-inflammatory properties that are useful for medicine. This is the initial documentation regarding *Litsea glutinosa* anti-inflammatory properties. Similar to diclofenac, *Litsea glutinosa* exhibits strong anti-inflammatory properties in an inflammatory model of rats caused by formaldehyde.

The best opportunities for utilizing plant potential and their safe application are attained with current understanding. Herbal medicine has the potential to become fully recognized in areas where side effects have limited conventional medication because of its complex composition, synergistic action of various components, and lower number of adverse effects. To summarize, the aforementioned information presents the prospect of utilizing the valuable properties of natural plants to isolate the active principles and conduct additional clinical trials that could uncover the advantages and constraints of their use, as well as potential synergistic effects with conventional therapy.

References

- Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, Ferrucci L, Gilroy DW, Fasano A, Miller GW, Miller AH. Chronic inflammation in the etiology of disease across the life span. Nature medicine. 2019 Dec;25(12):1822-32.
- Trowbridge HO, Emling RC, Fornatora M. Inflammation. A review of the process. Implant dentistry. 1997 Oct 1;6(3):238.
- Black PH. Stress and the inflammatory response: a review of neurogenic inflammation. Brain, behavior, and immunity. 2002 Dec 1;16(6):622-53.
- Serhan, C. N. Pro-resolving lipid mediators are leads for resolution physiology. *Nature* **510**, 92–101 (2014).
- Ashley NT, Weil ZM, Nelson RJ. Inflammation: mechanisms, costs, and natural variation. Annual Review of Ecology, Evolution, and Systematics. 2012 Dec 1;43:385-406.

- Kotas, M. E. & Medzhitov, R. Homeostasis, inflammation, and disease susceptibility. *Cell* **160**, 816–827 (2015).
- Fullerton, J. N. & Gilroy, D. W. Resolution of inflammation: a new therapeutic frontier. *Nat. Rev. Drug Discov.* **15**, 551–567 (2016).
- Vane JR, Botting RM. Anti-inflammatory drugs and their mechanism of action. Inflammation Research. 1998 Oct;47:78-87.
- Wang Q, Kuang H, Su Y, Sun Y, Feng J, Guo R, Chan K. Naturally derived anti-inflammatory compounds from Chinese medicinal plants. Journal of ethnopharmacology. 2013 Mar 7;146(1):9-39.
- Howes MJ. Phytochemicals as anti-inflammatory nutraceuticals and phytopharmaceuticals. InImmunity and inflammation in health and disease 2018 Jan 1 (pp. 363-388). Academic Press.
- Zhu F, Du B, Xu B. Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. Critical reviews in food science and nutrition. 2018 May 24;58(8):1260-70.
- Nisar A, Jagtap S, Vyavahare S, Deshpande M, Harsulkar A, Ranjekar P, Prakash O. Phytochemicals in the treatment of inflammation-associated diseases: the journey from preclinical trials to clinical practice. Frontiers in Pharmacology. 2023 May 9;14:1177050.
- Chawra HS, Gupta G, Singh SK, Pathak S, Rawat S, Mishra A, Gilhotra RM. Phytochemical constituents, ethno medicinal properties and applications of plant: Litsea glutinosa (lour.) CB robinson (lauraceae). Research Journal of Pharmacy and Technology. 2021;14(11):6113-8.
- Bhowmick R, Sarwar MS, RahmanDewan SM, Das A, Das B, NasirUddin MM, Islam MS, Islam MS. In vivo analgesic, antipyretic, and anti-inflammatory potential in Swiss albino mice and in vitro thrombolytic activity of hydroalcoholic extract from Litsea glutinosa leaves. Biological research. 2014 Dec;47:1-8.
- Jones, C. K., Peters, S. C., and Shannon, H. E. (2005). Efficacy of Duloxetine, a Potent and Balanced Serotonergic and Noradrenergic Reuptake Inhibitor, in Inflammatory and Acute Pain Models in Rodents. *J. Pharmacol. Exp. Ther.* 312 (2), 726–732.
- Arzi, A., Olapour, S., Yaghooti, H., and Sistani Karampour, N. (2015). Effect of Royal Jelly on Formalin Induced-Inflammation in Rat Hind Paw. *Jundishapur J. Nat. Pharm. Prod.* 10 (1), e22466. doi:10.17795/jjnpp-22466

- John, N. A. A., and Shobana, G. (2012). Anti-inflammatory Activity of *Talinum Fruticosum* L. On Formalin Induced Paw Edema in Albino Rats. *J. Appl. Pharm. Sci.* 2 (1), 123–127.
- Chen Q, Fung KY, Lau YT, Ng KM, Lau DT. Relationship between maceration and extraction yield in the production of Chinese herbal medicine. Food and Bioproducts Processing. 2016 Apr 1;98:236-43.
- Rover MR, Brown RC. Quantification of total phenols in bio-oil using the Folin–Ciocalteu method. Journal of Analytical and Applied Pyrolysis. 2013 Nov 1;104:366-71.
- Parkhe G, Bharti D. In vitro antioxidant activity, total phenolic and flavonoid contents of hydroalcoholic extract of leaves of Lagerstroemia Parviflora Roxb. Journal of Drug Delivery and Therapeutics. 2019 Aug 30;9(4-A):708-11.
- Joshi CS, Priya ES, Venkataraman S. Acute and subacute toxicity studies on the polyherbal antidiabetic formulation Diakyur in experimental animal models. Journal of health science. 2007;53(2):245-9.
- Singh M, Kumar V, Singh I, Gauttam V, Kalia AN. Anti-inflammatory activity of aqueous extract of Mirabilis jalapa Linn. leaves. Pharmacognosy research. 2010 Nov;2(6):364.