



Phytochemical Screening And Evaluation Of In Vitro Anti-Inflammatory Activity Of Fruits Extract Of *Sterculia Lychnophora*

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Abstract

When exposed to both infectious and noninfectious stimuli, the innate system's physiological homeostatic reaction is inflammation. One of the finest pharmacological classes for both preventing and treating pain is NSAIDs. But it has unfavorable effects on the kidney, cardiovascular system, and gastrointestinal tract are thought to be the main problems with these medications. Herbal medications function through an orchestrated strategy, in contrast to contemporary allopathic medications and also have zero side effects. Thus, this study will further assess the anti-inflammatory activity of fruits of *S. lychnophora*. The fruits of plant was collected, subjected to extraction & tested for qualitative, quantitative & anti-inflammatory studies. Results showed that the methanol & water extract observed to have brown colour while the chloroform & ethyl acetate extract observed to have sticky brown & light brown colour. It can be seen that with the chloroform & ethyl acetate as solvent, the % yield was found to be 1.6 & 5.4% respectively. The methanolic extract had 7.5 % yield while the highest % yield was found with water that is 10.2%. Further phytochemical screening was performed to know the exact presence or absence of particular phytoconstituents. The maximum number of phytoconstituents was present in methanolic extract. The ethyl acetate extract found to have total flavonoid content of 0.463mg/100mg. in case of methanolic extract the total phenol & flavonoid content was found to be 0.741 & 0.854 mg/ 100 mg respectively. Additionally the phenol & flavonoid content in aqueous extract was found to be 0.639 and 0.596 respectively. The anti-inflammatory activity of extract was performed by using Diclofenac sodium as standard. In this case the IC 50 for standard was observed to be 198.42 while for *Sterculia lychnophora* extract it was observed to be 331.94. From the results it can be stated that fruits of *Sterculia lychnophora* have appreciable anti-inflammatory activity.

Keywords: Inflammation, Medicinal plants, Phytochemicals, Diclofenac sodium, *Sterculia lychnophora*

Introduction

According to reports, physical agents, faulty immunological responses, and infections by bacteria, viruses, or fungi can all cause damage to living tissues and cause inflammation. The first objective of the inflammatory response is to identify and eradicate the pathogenic agents; subsequently, it aims to eliminate damaged tissue components, ultimately leading to the restoration of the impacted tissues, organs, or system. Macrophages and neutrophils are known to release several mediators during an inflammatory response, which are in charge of the beginning, intensifying, prolonging, controlling, and ultimately resolving the acute state of inflammation. Several anti-inflammatory mediators and the recruitment of monocytes for the clearance of tissue or cell debris have an impact on the resolution of inflammation. There's a chance that the acute phase won't resolve and instead becomes a chronic phase. Both developed and developing nations, especially those in Africa, bear the burden of medical disorders due in part to chronic inflammation. For example, chronic inflammation has been linked to the development of diabetes mellitus coupled with obesity that results from insulin resistance (Ahmed *et al.*, 2011; Hotamisligil *et al.*, 2005; Oguntibeju *et al.*, 2018).

NSAIDs are the most widely used medications in the world. For orthopaedic ailments such as osteoarthritis, soft tissue injuries, fractures, etc., they are prescribed. One of the finest pharmacological classes for both preventing and treating postoperative pain is NSAIDs. Although there are numerous adverse effects linked to the use of NSAIDs, the unfavorable effects on the kidney, cardiovascular system, and gastrointestinal tract are thought to be the main problems with these medications. 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. Consequently, it is imperative that pharmaceuticals be developed and screened for their anti-inflammatory properties, and several efforts are being made to identify anti-inflammatory medications from locally grown medicinal plants (Davis and Robson, 2016; Vikrant and Arya, 2011).

Herbal medications function through an orchestrated strategy, in contrast to contemporary allopathic medications that are composed of single active ingredients that target a single physiological system. Many distinct compounds found in plants work together to affect specific components of intricate biological pathways. For many ages, medicinal plants have been a rich source of biologically active chemicals, which are then widely employed as pure or crude materials to treat a variety of medical conditions. The toxicity and adverse effects of allopathic drugs have led to a rise in the usage of herbal remedies. Strong medicinal substances are developed in large part by the use of medicinal plants. More than

1.5 million people follow the traditional medical system, which uses medicinal herbs for therapeutic, promotional, and preventive purposes. With the world's largest collection of medicinal plants, India may continue to play a significant role in the production of raw materials used to make pharmaceuticals and cosmetics, either directly or as bioactive chemicals (Calixto, 2004; Percival, 1999; Kumar *et al.*, 2013).

The dried mature seeds of the deciduous tree *Sterculia lychnophora* Hance, which is a member of the Sterculiaceae family, are used in the traditional Chinese medicine Pangdahai. The tropical regions of Vietnam, India, Malaysia, Thailand, Indonesia, Gwangdong, and the Chinese island of Hainan are where the trees are primarily found. According to the dictionary of traditional Chinese medicine, *S. lychnophora* has no smell, is cool or cold in nature, and tastes slightly sweet or bittersweet. It also has a thick viscosity when eaten for extended periods of time. In most cases, it has been used to treat tussis, constipation, and pharyngitis. *S. lychnophora* is sometimes boiled or steeped in hot water in China and used as a beverage to relieve bloating and sore throats. A recent study demonstrated the neuroprotective impact. However, little is known about *S. lychnophora's* antibacterial or anticariogenic properties. (Oppong *et al.*, 2018; Al Muqarrabun and Ahmat, 2015). Thus, this study will further assess the anti-inflammatory activity of plant extract of *S. lychnophora*.

Materials & Methods

Procurement of plant

Fresh and free of diseases fruits of *Sterculia lychnophora* were collected in separate sterile bags from Bhopal, Madhya Pradesh, and month of March, 2023.

Defatting & Extraction

58 gram shade dried powder of fruits of *Sterculia lychnophora* were extraction with petroleum ether using maceration process. The extraction was continued till the defatting of the material had taken place. Defatted plant materials of *Sterculia lychnophora* were extracted with chloroform, ethyl acetate, methanol and water by maceration process (Azmir *et al.*, 2013).

Percentage yield

The % yield was calculated by dividing weight of extract by weight of powder taken multiplied by 100.

Phytochemical screening

The qualitative estimation of phytochemicals were performed as per the methods available in literature.

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50 μ g/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 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 (Alhakmani *et al.*, 2013).

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10-50 μ g/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% AlCl₃ 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 (Chang *et al.*, 2002).

In vitro anti-inflammatory activity of *Sterculia lychnophora*

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 *Sterculia lychnophora* extract (100-500 μ g/mL) or standard diclofenac sodium (100-500 μ g 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 \pm 1) $^{\circ}$ C for 15 min. Denaturation was induced by keeping the reaction mixture at 70 $^{\circ}$ C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm by using double distilled water as blank (Leelaprakash and Dass, 2011).

Results and Discussion

In total four type of extracts were obtained as four different solvents were used in this study. From the morphological point, each extract was having a characteristic colour. The methnol & water extract observed to have brown coloyur while the chloeoform & ethyl acetate extract observed to have sticky brown & light brown colour. All extracts were solid in nature. The percentage yield of extract differ grateley with respect to solvent used. It can be seen that with the chloroform & ethyl acetate as solvent, the % yield was found to be 1.6 & 5.4% respectively. The methanolic extract had 7.5 % yield while the highest % yield was found to be associated with water that is 10.2%.

Further phytochemical screening was performed to know the exact presence or absence of particular phytoconstituents. It was observed that in chloroform extract glycoside, amino acid and proteins were found to be present. In case of ethyl acetate extract flavonoid, amino acid and proteins were found to be present. The methanolic extract tested positive for glycoside, flavonoid, saponin, phenolics, amino acid, carbohydrate and proteins. Further, the aqueous extract contained flavonoid, saponin, phenolics, carbohydrate & diterpene.

After qualitative screening the extract quantity of some important phytoconstituents like phenol & flavonoid was determined. The ethyl acetate extract found to have total flavonoid content of 0.463mg/100mg. in case of methanolic extract the total phenol & flavonoid content was found to be 0.741 & 0.854 mg/ 100 mg respectively. Additionally the phenol & flavonoid content in aqueous extract was found to be 0.639 and 0.596 respectively.

The anti-inflammatory activity of extract was performed by using Diclofenac sodium as standard. The IC 50 value for extract & standard was calculated. The lower the IC 50 value, better is the antioxidant potential. In this case the IC 50 for standard was observed to be 198.42 while for *Sterculia lychnophora* extract it was observed to be 331.94.

Table 1: Extractive values of extracts of *Sterculia lychnophora*

S. No.	Extracts	Colour	Physical nature	% Yield (W/W)
1	Chloroform	Sticky brown	Solid	1.6%
2	Ethyl acetate	Light brown	Solid	5.4%
3	Methanol	Brown	Solid	7.5%
4	Water	Brown	Solid	10.2%

Table 2: Result of phytochemical screening of extract of *Sterculia lychnophora*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	Alkaloids	- ve	- ve	- ve	- ve
2.	Glycosides	+ ve	- ve	+ ve	- ve
3.	Flavonoids	- ve	+ ve	+ ve	+ ve
4.	Saponins	- ve	- ve	+ ve	+ ve
5.	Phenolics	- ve	- ve	+ ve	+ ve
6.	Amino Acids	+ ve	+ve	+ve	- ve
7.	Carbohydrate	- ve	+ ve	+ ve	+ ve
8.	Proteins	+ ve	+ ve	+ve	- ve
9.	Diterpenes	- ve	- ve	- ve	+ ve

(+ ve= Positive; - ve=Negative)

Result of estimation of total phenol and flavonoids content of extract of *Sterculia lychnophora*

Estimation of total phenolic content (TPC)

Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.011X + 0.011$, $R^2 = 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Estimation of total flavonoids content (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y = 0.032X + 0.018$, $R^2 = 0.998$, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 3: Estimation of total phenol and flavonoids content of extract of *Sterculia lychnophora*

S. No.	Extracts	Total phenol content (mg/ 100 mg of dried extract)	
		Total phenol content	Total flavonoids content
1.	Chloroform	-	-
2.	Ethyl acetate	-	0.463
3.	Methanol	0.741	0.854
4.	Water	0.639	0.596

Table 4: % Inhibition of Diclofenac sodium and *Sterculia lychnophora* extract

Concentration (μ g/ml)	% Inhibition	
	Diclofenac sodium	<i>Sterculia lychnophora</i> extract
100	40.57	32.12
200	47.04	40.26
300	60.56	47.65
400	79.07	54.02
500	94.88	63.78
IC ₅₀	198.42	331.94

Conclusion

In comparison to aqueous, chloroform, and ethyl acetate extracts, the methanolic extract of *Sterculia lychnophora* fruits shown strong anti-inflammatory effect in the current investigation. It can be inferred that inflammatory mediators such prostaglandins, leukotrienes, polymorphonuclear cells, or bradykinins may be inhibited because the anti-inflammatory impact was more pronounced at the later stages of inflammation. Methanolic extract's anti-inflammatory properties could be attributed to the active ingredients' presence, which includes tannins, steroids, alkaloids, and flavonoids. It is still necessary to research the chemical components and mechanism underlying the pharmacological actions.

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