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Research Article

Liquid – Liquid Fractionation and Evaluation of In-Vitro Anti-Inflammatory Activity of Ethanol Extract of *Leucas Aspera*

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ABSTRACT

Inflammation is a biological defence mechanism that enables living cells to protect themselves against diseases such as bacteria, fungi, viruses, physical agents, and defective immune function, inflammation may be acute (initial inflammation or chronic (out of proportion of protection damage) Factors such as redness, swelling, pain, loss of function of cells, and heat are common symptoms of inflammation. Denaturation of proteins can be crucial to the process of inflammation, which is a complex and normal immunological response to damage or infection involving a series of molecular events, the objective of the present study is to evaluate anti-inflammatory properties of various fractions of 70% ethanol extract of *Leucas aspera* plant by In-vitro protein denaturation (egg albumin) method, findings from the studies indicate that ethanol fractions are showing better protection of inflammation by inhibiting the protein (albumin) denaturation may be due to the presence of higher concentration phytoconstituents including polyphenols.

INTRODUCTION

Inflammation is a biological defense mechanism that enables living cells to protect themselves against diseases such as bacteria, fungi, viruses, physical agents, and defective immune function, inflammation may be acute (initial inflammation or chronic (out of proportion of protection damage) types. Factors such as redness, swelling, pain, loss of function of cells, and heat are

common symptoms of inflammation. Human living cells naturally develop protective mechanisms in response to body inflammation due to microbial infections, mechanical injuries, and burns stimuli Both acute and chronic inflammatory responses play a significant role in the natural defense mechanisms of the human body's inborn immune system to maintain human health. Its main aim is to stimulate living cells to eliminate harmful

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agents and remove damaged tissues to heal the affected parts. The inflammatory response is effective by secretion of different mediators responsible for the initiation, progression, persistence, regulation, and resolution of inflammation effects.¹ Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the progressive movement of plasma and leukocyte-like constituents from the blood into the injured tissues/locations. Chronic inflammation leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous breakdown and healing of the tissue from the inflammatory process.² In our body inflammation is regulated by numerous signalling pathways forming a complex system, in general, this complex biological response leads to the restoration of homeostasis, for the treatment of inflammation, people have been using non-steroidal anti-inflammatory drugs (NSAIDs) as medicines. However the increasing side effects such as heart attack and strokes due to these drugs are main reasons needed to replace synthetic drugs with minimal risk-causing plant-based medicines.³ Medicinal plants are the only source for the treatment of diseases in ancient days and since then numerous herbs and plants have been recognized as medicinal plants because of their potency to cure ailments. The newly discovered and the existing medicinal plants are being screened for many diseases and to identify significant therapeutic importance.⁴ *Leucas aspera* is a perennial herb found commonly in tropical regions, it is an herbaceous aromatic weed belonging to the family Lamiaceae (Labiatae), traditionally it is known as “Thumbai” in Tamil and “Dronapushpi” in Sanskrit and It is used as medicinal plant since ancient times. The whole plant is traditionally important because it has many

therapeutic values, *Leucas aspera* flowers are used as stimulant, expectorant (treat coughs), asperients (relieve constipation), and diaphoretic (inducing perspiration), insecticide and the flowers are mixed with honey and given to children to treat cold. The leaves are used as insecticides and mosquito repellent, the leaf juices are considered as a remedy for chronic rheumatism, psoriasis and other chronic skin eruptions, for stomach pain and indigestion the plant extract was given with honey.⁵ The extensive literature review on *Leucas aspera* reveals that the plant has many phytoconstituents like Polyphenols, Flavonoids, Terpenes, Alkaloids and essential oils etc, so the present project work has been taken to screen the anti-inflammatory properties of various fractions of 70% ethanol extract of plant by *In-vitro* protein denaturation (egg albumin) method.

Review of Literature

Plant profile:

L. aspera is a small herbaceous erect plant with a free blooming nature and flowering in the months of August to September. It is pungently aromatic herb and grows abundantly in the high land crop fields, roadsides, homesteads and fallow lands all over India the plant genus *Leucas* belongs to *Lamiaceae* family and embraces about 80 species, which are widely employed to cure many diseases by the traditional healers worldwide.

Vernacular Names

Sanskrit: Dronapushpi, Chitrapathrika, Chitrakshup

Kannada: Tumbi

Punjabi: Guldor

Bengali: Darunaphula, Hulkasha

Gujarati: Kulnnpful

Hindi: Gomamadhupati

Sindhi: Kubo

Maharashtra: Bahupful

Telugu: Thummichittu





Fig.1: Plant Profile.

Distribution

Leucas aspera is an aromatic herb distributed in India, Philippines, The plains of Mauritius and Java, Tropical Asia and Africa, It grows as a competitive weed and as annual herb found in highland crop fields, fallow lands, cultivated fields, sandy soils, wastelands and roadsides, grows upto 15 – 60 cm height with quadrangular stem and branches.⁶

Botanical description: *Leucas aspera* is an annual, branched, herb erecting to a height of 15-60 cm with stout and hispid acutely quadrangular stem and branches. Leaves are sub-sessile or shortly petiolate, linear or linearly lanceolate, obtuse, pubescent up to 8.0 cm long and 1.25 cm broad, with entire or crenate margin; petiole 2.5-6 mm long; flowers white, sessile small, in dense terminal or axillary whorls; bracts 6 mm long, linear, acute, bristle-tipped, ciliate with long slender hairs; calyx variable, tubular, 8-13 mm long; tube curved, contracted above the nutlets, the lower half usually glabrous and membranous, the upper half ribbed and hispid; mouth small, very oblique, not villous, the upper part produced forward; teeth small, triangular, bristle-tipped, ciliate, the upper tooth being the largest. Corolla 1 cm long; tube 5 mm long and pubescent above, annulate in the middle; upper lip 3 mm long, densely white-woolly; lower lip about twice as long, the middle lobe obovate, rounded, the lateral lobes small, subacute. Fruit nutlets, 2.5 mm long,

oblong, brown, smooth, inner face angular and outer face rounded.^{7,8}

METHODOLOGY

Selection and Extraction of Plants Material-

The aerial part of *Leucas aspera* were generally collected around the fields of Harapanahalli, Vijayanagara (D) Karnataka, INDIA. Authentication of Raw Plant Material is the basic Starting Priority in Developing of Botanical/Herbal Product. *Lucas aspera* Plant Material was authenticated by Prof. K Prabhu Sir, Dept. Of Pharmacognacy, S.C.S. College of Pharmacy, Harapanahalli, Herbarium is deposited in the Department of Pharmacognocny. Whole plant of *Leucas aspera* were dried under shade, mixed together & then made in to coarse powder with mechanical grinder and extracted by continuous hot extraction method in Soxhlet apparatus. The percentage yield was calculated for the extracts with reference to the crude material taken using the formula given below.

$$\% \text{ of yield} = \frac{\text{Weight in grams of extract obtained}}{\text{Weight in grams of plant material taken}} \times 100$$

Fractionation of crude extract was carried out by suspending 73.54gm of extract in 200ml of water and then partitioning with hexane, chloroform and acetate in order of increasing polarity by using separating funnel. All the fractions including aqueous fraction were condensed using water bath. Fractions so obtained were hexane (13.61gm),

chloroform (32.3 gm), ethyl acetate (1.23gm), ethanol (13.07gm) and aqueous (3.54gm).

Preliminary phytochemical screening

The obtained fractions of the plant extract were subjected to preliminary phytochemical screening following the standard procedures described in the practical Pharmacognosy by C.K. Kokate⁹ and R.K. Khandelwal¹⁰ results are summarized in table no.

Inhibition of Protein Denaturation.

The anti-inflammatory activity of various fractions of 70% alcoholic extract of *Leucas aspera* were carried out according to the modified methods of Mduranga¹¹ and Sakatet al.¹² The anti-inflammatory activity of various fractions of 70% alcoholic extract can be determined by In- vitro inhibition of the denaturation of egg albumin (protein). 0.2 mL of 1-2% egg albumin solution (from fresh hen's egg/ or commercially available egg albumin powder), 2 mL of sample extract or

standard (Diclofenac) at varying concentrations, and 2.8 mL of phosphate bufferer saline (pH 7.4) were mixed to form a reaction mixture of a total volume of 5 mL.

- A total volume of 5 mL of the control was created by combining 2 mL of triple-distilled water, 0.2 mL of 1-2% egg albumin solution, and 2.8 mL of phosphate-bufferer saline.
- The reaction mixtures were then incubated at $37\pm 2^{\circ}\text{C}$ for 30 min and will be heated in a water bath at $70\pm 2^{\circ}\text{C}$ for 15 min.
- After cooling, the absorbance was measured at 660 nm by a suitable UV/Vis spectrophotometer using triple distilled water as the blank [1].
- The following equation was used to determine the % inhibition of protein denaturation

$$\% \text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Table no 01 Percentage and Colour of the Extract

Sl No	Solvent type	Wt. of the sample before extraction (gm)	Wt. of the extract (gm)	Percentage of yield (%)	Color of the extract	Consistency
01.	70% Ethanolic extract	720gm	181.5gm	25.20 %	Blackish Green	Sticky and Viscous

II. Fractionation

Fractionation of 70% Ethanol extract was carried out with different solvents with increasing order of polarity and fractions so obtained were given in the table no 02

Table no 02: Fractions of extract

Sl.no	Solvent fraction	Out in gms
01	n- Hexane fractions	17.6
02	Chloroform fractions	46.4
03	Ethanol fractions	64.6
04	Aqueous fractions	13.4

III. Qualitative analysis of phytochemicals

The obtained extracts were tested for various chemical constituent The according to standard

procedure and results are shown in the following table no 03

Sl no.	Name of phytochemicals	Name of the Tests	n- Hexane fractions	Chloro form fractions	Ethanol fractions	Aqueous fractions
01.	Carbohydrates	Molish test	++	++	++	+
		Fehling test	++	+	++	++
02.	Glycosides	General test	+	+	+	+
		Legals test	+	+	+	+
04.	Alkaloids'	Mayers test	++	++	-	-
		Hagers test	++	++	++	-
05.	Phenols and Tannins	FeCl ₃ test	+++	+++	+++	++
06.	Saponins	Froth test	+	++	++	-
07.	Flavonoids	Alkaline test	+		++	++
		Lead acetate	+		++	++
08.	Proteins and Amino acids	Millions test	-		++	
		Biuret test	-		++	
			Present +	Absent -	Intensified results ++	

IV. Inhibition of Protein Denaturation

There are many In-Vitro methods to estimate anti-inflammatory actions of the drugs in our present studies inhibition of protein (albumin) denaturation was carried out with different fractions of plant extract and results are represented in table no 04 and fig no 02 From our studies we found that all fractions shows

inhibition of albumin denaturation with increasing order of concentration in that ethanol fractions shows more significant protection of inflammation or prevention of protein denaturation with minimum inhibition 23.4±0.15% to maximum inhibition of 65.14±0.72%.from 20% to 100% concentration respectively.

Table no 04: In vitro protein denaturation of Different fractions of 70% ethanolic extract

Different plant	Concentration of plant extracts / % Protein denaturation				
	20%	40%	60%	80%	100%
Fractions					
n-Hexane	21.38±0.90	30.41±0.19	41.14±0.01	50.21±0.02	60.01±0.02
Chloroform	22.3±0.16	31.32±0.01	41.94±0.12	50.39±0.10	61.12±0.72
Ethanol	23.4±0.15	34.34±0.02	45.54±0.01	55.64±0.02	65.14±0.72
Aqueous	17.41±0.11	20.34±0.04	29.34±0.02	36.34±0.02	45.01±0.02
STD (Diclofenac)	22.34±0.12	33.14±0.52	45.64±0.81	58.39±0.12	73.12±0.02

Values are expressed in mean ± SE of 3 replicate

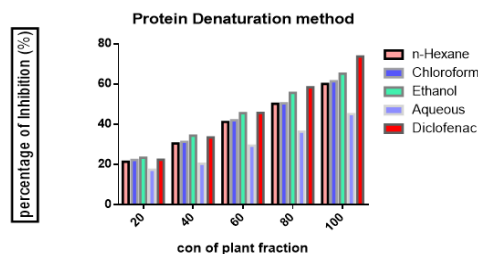


Fig no 02: In vitro protein denaturation of Different fractions of 70% ethanolic extract

DISCUSSION & CONCLUSION

Inflammation is survival mechanism as it is conserved as a process of protection, it is composed of complex sequential changes in the tissue to eliminate the initial cause of the cell injury, Since a large proportion of NSAIDs available in the market have significant undesirable effects, the need for new anti-inflammatory drugs contributes to the advancement of research for newer, safer, effective molecules with fewer side effects and from vegetal sources. Therefore, it can be observed that a significant number of substances of vegetal origin form the part of the therapeutic arsenal of modern medicine. Extraction is the crucial step for the analysis of medicinal plants and choice of solvents is crucial in solvent extraction, solvent should have low toxicity and preservation capabilities, promote extract absorption and low temperature evaporation and also consider the quality of phytochemicals and metabolites recovered, to extract polar and non polar components polar and non polar solvents are typically utilized based on polarity solvents from least polar to polar like n-Hexane to water are used for extraction and usually aqueous mixture of ethanol and methanol are used in extraction as they have better efficiency of salvation so in our studies we selected 70% alcohol as extraction solvent and we got percentage of yield of 25.20 gm % reveals the presence of phytoconstituents in the extract. In fractionation selected solvent is added according to the increasing order of polarity starting from n-Hexane to water, in all combination water is present because of its high polarity and miscibility with organic solvents and we found that highest concentration in ethanol (Quantity) fractions and in Qualitative phytochemical analysis of various solvent fractions shows the presence of various secondary metabolites like alkaloids, saponins proteins except in aqueous fractions and other metabolites

like phenolic compounds and Flavanoids are present in all fractions but in ethanolic fractions more intensified results indicate the presence of more concentration of these compounds. Protein denaturation, which occurs when a protein loses its natural shape and biological function, is strongly related to a number of inflammatory variables and has important consequences for inflammatory responses.¹³ Denaturation of proteins can be crucial to the process of inflammation, which is a complex and normal immunological response to damage or infection involving a series of molecular events. Denaturation of proteins is a well-documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc) have shown dose dependent ability to thermally induced protein denaturation.¹⁴ The denaturation is used loosely to designate the change of proteins from a soluble to an insoluble form brought about by a large variety of chemical and physical agents, including acids, alkalies, alcohol, acetone, salts of heavy metals and dyes¹⁵ and heat, light, and pressure.¹⁶ Some literature reported that denaturation of protein is one of the cause of rheumatoid arthritis due to the production of auto-antigens in certain rheumatic diseases.¹⁷⁻¹⁹ In our studies Inhibition of Protein Denaturation method we found that all fractions shows inhibition of albumin denaturation with increasing order of concentration, in that ethanol fractions shows more significant protection of inflammation or prevention of protein denaturation with minimum inhibition $23.4 \pm 0.15\%$ to maximum inhibition of $65.14 \pm 0.72\%$. from 20% to 100% concentration respectively. Indicating that ethanol fractions are showing better protection of inflammation may be due to the presence of higher concentration phytoconstituents including polyphenols. But further more detailed quantification studies and various in-vitro and In-vivo studies are further required to justify the claim.



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Conflict Of Interest

Author does not have any conflict of interest.

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