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IN VITRO STUDY ON ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS EXTRACT OF *VATERIA INDICA* RESIN

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ABSTRACT

Inflammation is a part of immune response. It comprises a complex array of adaptive responses to tissue injury which are both local and systemic. The sample was subject for phytochemical analysis to find the phytochemical constituents. The various phytochemical analysis like alkaloids, Phenol, Flavonoids, tannins, saponins etc., were done with different solvents. The result shows that aqueous sample possess the presence of phytochemical constitiuents. The Anti-inflammatory activity of Vateria indica resin was done by using Inhibition of albumin denaturation, Hypotonicity-induced hemolysis, Anti-lipoxygenase activity, Heat induced hemolysis and Proteinase inhibitory activity at various concentrations (100-500 µg/ml). A standard was used for comparison. The study reveals that the Aqueous extract of Vateria indica resin protects the albumin denaturation. Heat induced hemolysis, hypotonicity induced hemolysis and lipoxygenase activities were inhibited at higher concentration when compared to the standards. Current study demonstrates that the, Vateria indica resin can be used as an effective anti-inflammatory agent. The extracts showed more anti-inflammatory potential as the dose varies. GC-MS was also done to identify the bioactive compounds. The result reveals that sample contains mainly Sesquiterpenoid compounds. Therefore, our studies support the use of Vateria indica resin in treating inflammation.

KEY WORDS

Anti-Inflammatory, Albumin Denaturation, Lipoxygenase, Human Red Blood Cell (HRBC), Membrane stabilization, GC-MS.

INTRODUCTION

Inflammation is a biological response by which the release of chemical substances from tissues. Mostly the inflammation is associated with Leukotrienes, Histamine and Bradykinins etc.[1]It occurs in response to allergen, wounds, infection and auto-immune conditions.[2]Some are inflammatory diseases such as hepatitis rheumatoid arthritis, asthma and colitis are among the causes of death and disorder in the world. In recent the Inflammation is treated by using modern medicines, but the use of synthetic drugs cannot be affording easily by the large population. It has to rely on use of traditional medicines.[3]The use of traditional medicines which produced from the medicinal plants is used for the treatments.[4]

Inflammation response is a normal process towards tissue injury which is caused by trauma and some microbiological agents. Inflammation is the result determined by chemotactic, vasoactive and proliferative factors.[5]

Vateria indica, the white dammar.[6] The plant *Vateria indica resin* belonging to the family of Dipterocarpaceae is commonly called as Vellai Kungiliyam in Tamil. A kind of resinous material known as “Sarja rasa” obtained

from trunk of tree *V.Indica* resin by incising and tapping is used in traditional Indian system of medicine.

The resin finds its use in traditional Indian systems of medicine like Ayurveda and Siddha for health and healing diseases. It is credited with tonic, carminative and expectorant properties and is used for the treatment of respiratory disorders like chronic bronchitis, throat troubles, tubercular gland, boils, piles, diarrhea and rheumatism and so on. A popular Siddha medicinal preparation called Kungiliya parpam is prepared out of this resin with tender coconut water.[7]

Hence, the present study was to determine the anti-inflammatory activity of aqueous extract of *V.Indica* resin by various activities.

MATERIALS AND METHODS

Collection of Plant material

The resins of plant *vateria indica* were collected from Kerala at Kozhikode district and stored at room temperature.

Preparation of the extract

150 gm of *V.Indica* resin was brought from the local market and powdered. The resin was mixed with one tender coconut water (250ml). Boiled the mixed resin started to appear on the surface of the boiling liquid in a molten state. The resin was separated by filtration. Then it was cooled. The melting procedure and recovery was repeated for several times. The final product was dried, ground and then sieved. The final powder was creamy white in color.[8]

Preliminary Qualitative Phytochemical Screening

The qualitative screening of secondary metabolite was carried out by Trease and Evans (1996) and Harborne (1987) using different solvents and aqueous sample extract.

Assessment of invitro anti-inflammatory activity

Inhibition of albumin denaturation

The mixture contains test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl.[9,10] The sample extracts were incubated at 37 ºC for 20 min and then heated to 51º C for 20 min. After cooling the samples, the turbidity was measured at 660nm.

The Percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control

Antiproteinase action

The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100 – 500 µg/ml). The mixture was incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% Per chloric acid was added to arrest the reaction.[10,11] Cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against buffer as blank. The percentage inhibition of proteinase inhibitory activity was calculated.

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

Membrane stabilization

Preparation of Red Blood cells (RBCs) suspension

Fresh whole human blood was collected and transferred to the heparinized centrifuged tubes. The tubes containing the sample were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and re-constituted as 10% v/v suspension with normal saline.[10, 12].

Heat induced hemolysis

The 2.0 ml of reaction mixture contains 1 ml of test sample of 100 - 500 µg/ml concentrations and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56ºC for 30min. then the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant was taken at 560 nm. The Percentage inhibition of Hemolysis was calculated as follows:

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

Hypotonicity-induced hemolysis

Different concentration of extract (100-500µg/ml), reference sample, and control were separately mixed with 1ml of phosphate buffer, 2ml of hypo saline and 0.5ml of HRBC suspension. Diclofenac sodium (100µg/ml) was used as a standard drug. All the assay mixtures were incubated at 37°C for 30minutes and centrifuged at 3000rpm. The supernatant liquid was decanted, and the hemoglobin content was estimated by a spectrophotometer at 560nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%.[14]

Percentage protection = 100- (OD sample/OD control) x 100

Anti-lipoxygenase activity

Anti-Lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme. Test samples were dissolved in 0.25ml borate buffer with a pH of 9.0 and added 0.25ml of lipoxidase enzyme solution. Then it was incubated at 25°C for 5 minutes. After which, 1.0ml of linoleic acid solution (0.6mM) was added, mixed well and absorbance was measured at 234nm. Indomethacin was used as reference standard.[13]The percent inhibition was calculated from the following equation,

% inhibition=

[{Abs control- Abs sample}/Abs control] x 100

Gas Chromatography - Mass Spectroscopy (GC-MS) analysis

GC-MS analysis of aqueous extract of *V.Indica* resin was performed .The detector used is Flame ionization detector. The carrier gas used is nitrogen at flow of 2.5ml/min. The injector operated at 250°C and the oven temperature was at 120°C for 2 minutes then gradually increased to 260°C for 5 min.

Statistical Analysis

The results are expressed in percentage of Inhibition (%).

RESULT AND DISCUSSION

Preliminary Qualitative Phytochemical Screening

The preliminary qualitative phytochemical screening of *V.Indica* resin was carried out and the result was given in the Table I. The aqueous extract shows the presence of Alkaloids, flavonoids, phenol, tannins, steroids, glycosides, saponins, diterpenes.

TABLE I: The preliminary qualitative phytochemical screening of *V.Indica* resin

TEST	ETHANOL	METHANOL	PETROLEUM ETHER	AQUEOUS	AQUEOUS
Alkaloids	_	_	+	_	+
Flavonoids	+	+	+	_	+
Phenols	+	+	+	_	+
Tannins	+	+	+	_	+
Steroids	_	+	_	_	+
Glycosides	+	+	+	+	+
Saponins	_	_	+	_	+
Carbohydrates	_	_	_	_	_
Protein/Amino acids	_	_	+	_	+
Diterpines	_	_	_	_	+

Inhibition of albumin denaturation

Inhibition of albumin denaturation is the main cause of inflammation. The mechanism of anti-inflammatory activity was studied to check the ability of plant resin which inhibits protein denaturation. The *V.Indica* resin shows maximum inhibition of 63% was observed at 500 µg/ml. Aspirin, a standard anti-inflammation drug showed the maximum inhibition 60% at 100 µg/ml concentration compared with control (Table II).

TABLE II: Effect of Aqueous extract of *V.Indica resin* on Inhibition of albumin denaturation

TREATMENT	CONCENTRATION(µg)	% OF INHIBITION
CONTROL	-	00
Vateria indica resin	100	23
	200	35
	300	41
	400	56
	500	63
ASPIRIN	100	60

Proteinase inhibitory activity

Lysosomal granules of Neutrophils are known to be a rich source of Proteinase. The inflammatory response carried during tissue damage is caused by the leukocytes proteinase enzyme. This leukocyte proteinase is protected by proteinase inhibitor.[10] So, Proteinase inhibitor plays a vital role in anti-inflammatory activity.

Table III showed maximum inhibition of 44% at 500 µg/ml and that of standard, Aspirin was found to be 46% at 100 µg/ml concentrations.

Table III: Effect of Aqueous extract of *V.Indica resin* on Proteinase inhibitory activity

TREATMENT	CONCENTRATION(µg)	% OF INHIBITION
CONTROL	-	00
	100	14
	200	21
Vateria indica resin	300	27
	400	35
	500	44
ASPIRIN	100	46

Membrane stabilization

The various disorders or disease produced by the release of lysosomal enzyme. The release of enzyme takes place during any inflammation and it may cause acute or chronic inflammation. The lysosomal constituents were prevented by Lysosomal stabilization.[15]The inhibition of the enzymes acts on NSAID drugs. The Human Red Blood Cell Membrane Stabilization (HRBC) membrane has similar action like of the lysosomal membrane.[16, 17] The study was carried out to check the stability of HRBC membrane by the *V.Indica* resin was compared with the standard.

Heat induced hemolysis

Test extract inhibited the heat induced hemolysis of RBCs to varying degree as shown in Table IV. It shows the maximum inhibition 64% at 500 µg/ml. Aspirin, standard drug showed the maximum inhibition, 71% at 100 µg/ml.

The anti-inflammatory mechanism of *V.Indica* resin was studied using the RBCs membrane Stabilization. The Heat induced Hemolysis was inhibited by the *V.Indica* resin. This resin inhibits the release of neutrophil lysosomal content at the site of inflammation and also include the bactericidal and protease enzyme, which upon extracellular release cause further tissue inflammation and damage.[18]

Membrane stabilization

Table IV: Effect of Aqueous extract of *V.Indica* resin on heat induced hemolysis for anti-inflammatory activity

TREATMENT	CONCENTRATION(µg)	% OF INHIBITION
CONTROL	-	00
	100	21
	200	35
Vateria indica resin	300	48
	400	55
	500	64
ASPIRIN	100	71

Table V: Effect of Aqueous extract of *V.Indica resin* on Hypotonicity-induced hemolysis

TREATMENT	CONCENTRATION(µg)	% OF INHIBITION
CONTROL	-	00
Vateria indica resin	100	14
	200	23
	300	37
	400	46
	500	58
Diclofenac sodium	100	51

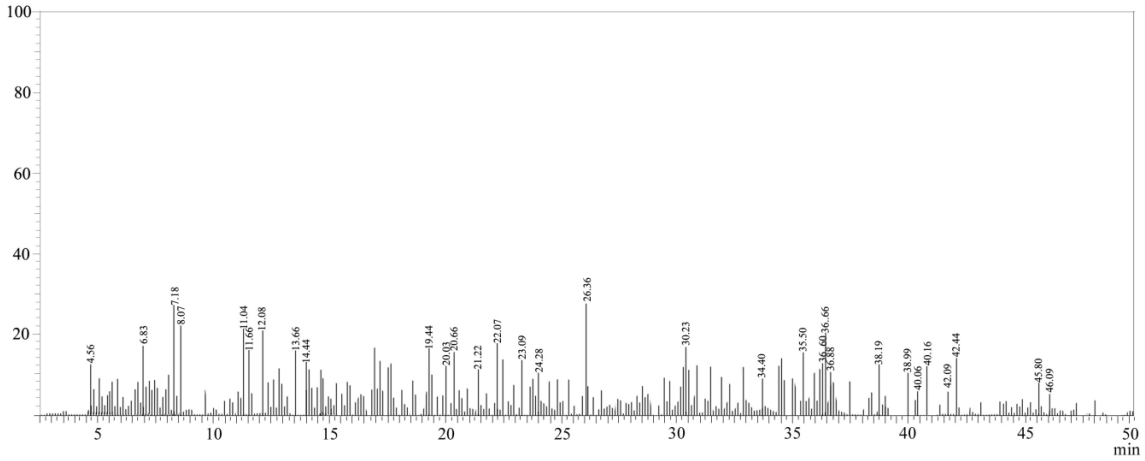
Table VI: Effect of Aqueous extract of *V.Indica resin* on Anti-lipoxygenase activity

TREATMENT	CONCENTRATION(µg)	% OF INHIBITION
CONTROL	-	00
Vateria indica resin	100	09
	200	16
	300	22
	400	36
	500	45
Indomethacin	100	59

Table VII: List of main compounds found in the aqueous extract of *V.indica* resin

S.NO	COMPOUND NAME	CONTENT AS PER INTEGRATED PEAK AREA METHOD	BIOLOGICAL PROPERTIES
1	EUDESMOL	4.40	Cytotoxicity
2	ERIOFLORIN METHACRYLATE	2.60	Anti- inflammatory activity
3	ALPHA HUMULENE	6.90	Anti- inflammatory activity
4	CAMPHENE	9.50	
5	ZERUMBONE	1.90	anti-inflammation, antioxidant agent, and anti-carcinogenic agents
6	NEROLIDOL	1.00	transdermal delivery of therapeutic drugs
7	LINALOOL	2.45	
8	BETA PINENE	0.98	Antibacterial Activity, Antibacterial Activity, Cytotoxic Activity
9	KAEMPFEROL	0.20	Anti-cancer effects, Diabetes Cardiovascular disorders, Anti-bacterial activity, Anti-viral activity, Antioxidant effects
10	Camphor	6.50	Anti- inflammatory activity

Figure I: GC-MS chromatogram of aqueous extract of *V.indica* resin.



Hypotonicity-induced hemolysis

The hemolytic effects of hypotonic solution accumulate fluid excessively within the cell. This results the rupturing of the cell membrane. The injury of red cell membrane reduces the cell susceptibility to secondary damage through free radical induced lipid peroxidation.[19]

The result shows the maximum inhibition takes place at 500 µg/ml concentration (Table V). The resin protects the erythrocyte membrane against lysis induced by hypotonic solution. Diclofenac sodium offered a significant inhibition at 100µg/ml.

Anti-lipoxygenase activity

Lipoxygenases, a non-heme iron- containing dioxygenases. It has been found that these LOX products play a vital role in bronchial asthma, inflammation and tumor angiogenesis.[20, 21]. In human tissues LOX is expressed in platelet, eosinophils, synovial fluid, neutrophils, colonic tissues, lung tissues, monocytes and bone marrow cells. Inhibitors of LOX and leukotrienes receptor antagonist are used in the treatment of asthma.[22] The present study describes the lipoxygenase inhibitory activity of *V.Indica* resin.

The *V.Indica resin* shows maximum inhibition of 45% was observed at 500 µg/ml whereas the standard Indomethacin showed the maximum inhibition 59% at the concentration of 100 µg/ml (Table VI). The *V.Indica* resin inhibits the lipoxygenase enzyme activity.

The results obtained from our studies on *V.Indica* resin have shown a potential anti-inflammatory activity.

GC-MS

The aqueous extract of *V.Indica* resin the compounds were identified using GC-MS. Figure 1 represents the peak area, molecular weight. Table VII represents the identified compounds based on peak area and its pharmacological properties.

Eudesmol, Alpha Humulene, Camphene, Camphor compounds have highest peak value. The sample contains mainly Sesquiterpenoid compounds has the anti-inflammatory property. Especially the Sesquiterpenoid lactam possess anti-inflammatory activity. It also exhibits anticancer activity, Cytotoxicity activity.

CONCLUSION

Inflammation is a reaction of living tissues which acts towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response.

An anti-inflammatory response is the action against inflammation. The anti-inflammatory activities of *V.Indica* resin were evaluated to identify the beneficial effects related to inflammation. In this study, the phytochemical analysis exhibit positive result in aqueous solution and the aqueous extract of *V.Indica* resin inhibited the heat induced albumin denaturation, proteinase activity and stabilized the Red Blood Cells membrane. The GC-MS result of *V.Indica* resin possesses the sesquiterpenoid compound which has an anti- inflammatory activity. It can be concluded that aqueous extract of *V.Indica* resin possessed marked anti-inflammatory activity. The study helps in vivo study for future works. Further studies in future basis of present studies are however needed to isolate the active principle (s) responsible for anti-inflammatory

potential.

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