WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.453
Volume 15, Issue 3, 937-946. Research Article ISSN 2277–7105

INVITRO EVALUATION OF EXTRACT OF BAUHINIA VAHLII

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Article Received on 08 Jan. 2026,
Article Revised on 28 Jan. 2026,
Article Published on 01 Feb. 2026,

https://doi.org/10.5281/zenodo.18430807

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How to cite this Article: T. Jyothirmai*, Dr. Mercy Rani Rahi, M. Sree Sai Manoher, Mahitha Pothugunta, Dupada Amrutha Kumari, K. Arpitha, P. Yagnasri (2026). Invitro Evaluation of Extract of Bauhinia Vahlii. World Journal of Pharmaceutical Research, 15(3), 937–946.

ABSTRACT

The study investigated the anti-inflammatory activity and anti-diabetic activity of the chloroform extract of ***BAUHINIA VAHLII*** *leaf* (Kattumandarai). The anti-inflammatory activity and anti-diabetic activity of chloroform extracts of ***BAUHINIA VAHLII*** leaf was evaluated using five in vitro-based assays heat induced hemolysis inhibition, Inhibition of albumin denaturation, Anti-proteinase action, preparation of red blood cells (RBCS) suspension and α-amylase inhibitory. Results showed that the mechanism of the anti-inflammation activity, ability of ***BAUHINIA VAHLII*** leaf extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 71% was observed at 500 µg/ml. Aspirin, a standard anti-inflammation drug showed the maximum inhibition 68% at the concentration of 100 µg/ml compared with control. Anti-diabetic activity results showed good α-amylase inhibitory activity of the ***BAUHINIA VAHLII*** leaf compared with that of standard acarbose. A maximum inhibition of 88.55% ± 0.43% was achieved at a concentration of 4 µl/ml by the leaf, which was comparable to that of standard acarbose inhibition of approximately90.96% ± 1.81%. The 50% Inhibitory concentration (IC50) of the extract and acarbose was found to be 0.47 and 0.69 µl/ml, respectively. In the present study, results indicate that the chloroform extracts of ***BAUHINIA VAHLII*** possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols, The extract fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin

denaturation, proteinase activity and stabilized the Red Blood Cells membrane. This study gives on idea that the compound of the plant ***BAUHINIA VAHLII*** can be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases such as cancer, neurological disorder, aging and inflammation. ***BAUHINIA VAHLII*** is a good choice for the management of diabetes as it can effectively inhibit the key enzymes of carbohydrate metabolism such as α-amylase thereby decreasing the postprandial hyperglycemia. The anti hyperglycemic activity of ***BAUHINIA VAHLII*** leaf strongly supports its stability to decrease sugar level hence it may be further validated for its use as an anti-diabetic agent.

KEYWORDS: ***BAUHINIA VAHLII*** leaf (Kattumandarai), Anti-inflammatory activity, anti-diabetic activity, chloroform extracts, five in vitro-based assays heat induced hemolysis inhibition, Inhibition of albumin denaturation, Anti-proteinase action, preparation of red blood cells (RBCS) suspension and α-amylase inhibitory.

INTRODUCTION

Inflammation is complex process, which is frequently associated with pain and involves occurrence such as the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cell in the body are damaged by microbes, physical agents or chemical agents, the injury is form of stress.[1] The migration of leukocytes from the venous systems to the site of damage, and the release of cytokines, are known to play a crucial role in the inflammatory response. These chemicals cause widening of blood capillaries (vasodilatation) and the permeability of the capillaries. This will lead to increased blood flow to the injured site. Inflammation can be classified as either acute or chronic.[2] 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.[3] Non-steroidal anti-inflammatory drugs (NSAID) are commonly used for the management of inflammatory conditions. However, these drugs have several adverse side effects, especially gastric irritation, leading to the formation of gastric ulcers. Therefore, the search for natural sources and phytochemical with anti-inflammatory activity has greatly increased in recent years.

Further, several epidemiological studies also indicated that the incidence of chronic diseases, such as cancer, cardiovascular diseases, and inflammation, is inversely correlated with the consumption of fruits and leaf's rich in polyphenols, such as Flavonoids.[4] In the plant kingdom every plant has the potential to produce primary and secondary metabolites which are bioactive in curing many diseases.[5] It includes flavonoids, terpenoids, glycosides, tannins, steroids, and saponinsect.[6] Bioactive compounds from the plants source have the broad spectrum of anti-bacterial, anti-fungal, and ant-oxidant activity. Therefore, the present study was conducted to determine the anti-inflammatory activity of selected Ajwain leaf using several in vitro bioassays, such as inhibition of albumin denaturation, antiproteinase activity, membrane stabilization, and heat induced haemolysis activity.[7]

MATERIALS AND METHODS

MATERIALS

BAUHINIA VAHLII leaf was purchased from the Guntur in Nallapadu. Leaf was subjected to pulverization to get coarse powder. It was stored in air tight container further use.

Reagents

Bovine albumin (HiMedia RM 638, Mumbai), 1N HCL, Visible Spectrophotometer, trypsin, tris HCL buffer, casein, perchloric acids, aspirin, saline, all these were procured from local market NaH_2PO_4,NACL,sodium phosphate buffer pH 7.4α-amylase enzyme (HiMedia RM 638, Mumbai) acarbose, starch, dinitro salicylic acid, and chloroform Herbal Science Trust Bangalore. All the other chemicals were procured of analytic grade.

Preparation of crude extract

30 g of dry power were weighed and transferred to Soxhlet apparatus and the extracted with chloroform at 35°C for 3-4 cycles. The extract was collected and the chloroform was evaporated after extraction by using rotary evaporator connected to a vacuum pump. The final extract in semi-solid form was dried by placing in desiccators. Until used for the anti-inflammatory bioassays, within one week.

Procedure for in vitro anti-inflammatory activity

A) Inhibition of albumin denaturation

The anti-inflammatory activity of ***BAUHINIA VAHLII*** leaf was studied by using inhibition of albumin denaturation technique which was studied according to followed with minor modification.

The reaction mixture was consists of test extracts and 1%aqueous solution of bovine album infraction, p^H of the reaction mixture was adjusted using small amount of 1N HCL. The sample extracted were incubated at 37^{o}c for 20 min and then heated to 51^{o}c for 20min, after cooling the sample the turbidity was measured at 660nm. (UV visible spectrophotometer). The experiment was performed in triplicate. The percentage inhibition of albumin denaturation was calculated as follows.

Percentage inhibition = (abs1 control= abs2 sample) x100/abs control.

Whereas,

A1 = absorption of the control sample, and A2 = absorption of the test sample.

(B) Anti-proteinase action

The test was performed according to the modified method. 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 370 c for 5min 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% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

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

MEMBRANE STABILIZATION

(C) Preparation of Red Blood cells (RBCs) suspension

The Blood was collected from healthy human volunteer who has not taken any NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment and transferred to the centrifuge tubes. The tubes 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 with isotonic buffer solution (10mm sodium phosphate buffer pH 7.4). Composition of the buffer solution (g/L) used was NaH_2PO_4, Na_2HPO_4, and NaH_2PO_4.

(D) Heat Induced Haemolysis

The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (100 - 500 µg/ml) 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 56 ºC for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. The Percentage inhibition of Haemolysis was calculated as follows:

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

Antidiabetic Activity of Ajwain

In vitro α-amylase inhibitory assay

The in vitro α-amylase inhibitory activity was carried out according to the method. The leaf extract from the solvent leaf extracted was allowed to react with 200 µL of α-amylase enzyme (HiMedia RM 638, Mumbai), and 100 µL of 2mm of phosphate buffer (pH, 6.9). After 20-min incubation, 100 µL of 1% starch solution was added. The same was performed for the controls, where 200 µL of the enzyme was replaced by buffer. After incubation for 5min, 500 µL of dinitro salicylic acid reagent was added to both control and test. They were kept in boiling water bath for 5min. The absorbance was recorded at 540nm using spectrophotometer, and the Percentage inhibition of α-amylase enzyme was calculated using the following formula:

% Inhibition = [(Control – Test)/Control] ×100

Suitable reagent blank and inhibitor controls were simultaneously carried out. All values were expressed as mean ± standard error of mean (SEM) (n = 3).

RESULTS AND DISCUSSION

Inhibition of albumin denaturation Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of ***BAUHINIA VAHLII*** leaf extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 71% was observed at 500 µg/ml. Aspirin, a standard anti-inflammation drug showed the maximum inhibition 68% at the concentration of 100 µg/ml compared with control.

Table 1: Effect of sample on inhibition of albumin denaturation.

Treatment	Concentration µg/Ml	Absorbance 210nm	%inhibition of proteinase
Control	-	0.380	-
sample	100	0.260	31
Sample	200	0.200	47
Sample	300	0.160	57
Sample	400	0.130	65
Sample	500	0.110	71
aspirin	100	0.120	68

Each value represents the mean SD. N=3, Experimental group were compared with control ** **p≤0.05, considered significant; ns p≥0.05, non-significant. ***BAUHINIA VAHLII***, chloroform extract.

Table 2: Effect of sample on proteinase inhibitory action.

Treatment	Concentration µg/ml	Absorbance 210nm	%inhibition of proteinase
Control	-	0.380	-
Test	100	0.303	21
Test	200	0.280	27
Test	300	0.240	36
Test	400	0.220	42
Test	500	0.180	53
aspirin	100	0.170	55

Each value represents the mean SD. N=3, Experimental group were compared with control **p0.05, non-significant. ***BAUHINIA VAHLII*** leaf, chloroform Extract.

Table 3: Effect of sample on heat induced haemolysis of erythrocyte.

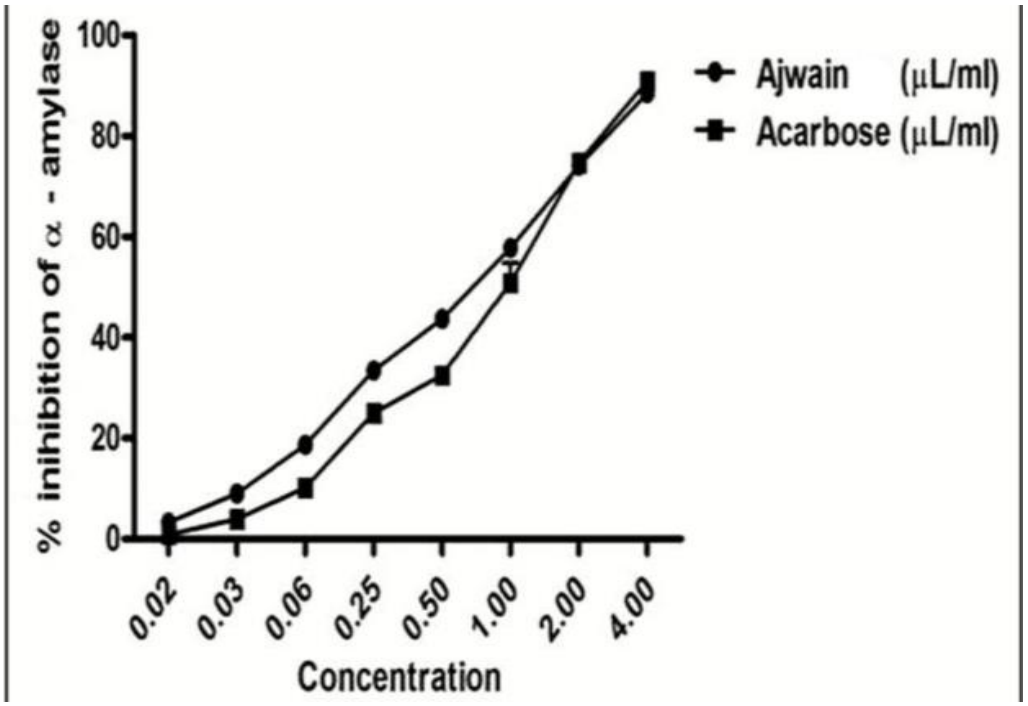
Treatment	Concentration µg/ml	Absorbance 210nm	%inhibition of proteinase
Control	-	0.310	-
Test	100	0.22	21
Test	200	0.210	30
Test	300	0190	36
Test	400	0.170	43
Test	500	0.150	51
aspirin	100	0.090	71

ANTI-DIABETIC ACTIVITY

RESULTS

The results showed good α-amylase inhibitory activity of the Ajwain leaf compared with that of standard acarbose. A maximum inhibition of 88.55% ± 0.43% was achieved at a concentration of 4 µl/ml by the leaf, which was comparable to that of standard acarbose

inhibition of approximately90.96% ± 1.81%. The 50% Inhibitory concentration (IC50) of the extract and acarbose was found to be 0.47 and 0.69 µl/ml, respectively.



CONCLUSION

ANTI-INFLAMMTORY ACTIVITY

In the present study, results indicate that the chloroform extracts of ***BAUHINIA VAHLII*** leaf possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavanoids, tannins, steroids, and phenols, The extract fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation, proteinase activity and stabilized the Red Blood Cells membrane. This study gives on idea that the compound of the plant trachyspermum ammi can be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases such as cancer, neurological disorder, aging and inflammation.

ANTI-DIABETIC ACTIVITY

Ajwain is a good choice for the management of diabetes as it can effectively inhibit the key enzymes of carbohydrate metabolism such as α-amylase thereby decreasing the postprandial hyperglycemia. The anti hyperglycemic activity of Ajwain leaf strongly supports its stability to decrease sugar level hence it may be further validated for its use as an anti-diabetic agent.

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