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INVITRO EVALUATION OF EXTRACTION OF ABUTILON INDICUM

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ABSTRACT

The study investigated the anti-inflammatory activity and anti-diabetic activity of the chloroform extract of Abutilon indicum seed. The anti-inflammatory activity and anti-diabetic activity of chloroform extracts of Abutilon indicum seed 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 Abutilon indicum seed 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 100µg/ml compared with control. Anti-diabetic activity results showed good α-amylase inhibitory activity of the Abutilon indicum seed compared with that of standard acarbose. A maximum inhibition of 88.55% ± 0.43% was achieved at a concentration of 4µl/ml by the seed, which was comparable to that of standard acarbose inhibition of approximately 90.96% ± 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 trachyspermum ammi 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. Abutilon indicum 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 Abutilon indicum seed strongly supports its stability to decrease sugar level hence it may be further validated for its use as an anti-diabetic agent.

KEYWORDS: Abutilon indicum, Anti-inflammatory activity, anti-diabetic activity, chloroform extracts, and 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

INVITRO: In vitro (meaning in glass, or in the glass) studies are performed with microorganisms, cells, or biological molecules outside their normal biological context. Colloquially called "test-tube experiments", these studies in biology and its sub disciplines are traditionally done in lab ware such as test tubes, flasks, Petri dishes, and microtiter plates. Studies conducted using components of an organism that have been isolated from their usual biological surroundings permit a more detailed or more convenient analysis than can be done with whole organisms; however, results obtained from in vitro experiments may not fully or accurately predict the effects on a whole organism. In contrast to in vitro experiments, in vivo studies are those conducted in living organisms, including humans, known as clinical trials, and whole plants.

DEFINITION

In vitro (Latin: in glass; often not italicized in English usage) studies are conducted using components of an organism that have been isolated from their usual biological surroundings, such as microorganisms, cells, or biological molecules. For example, microorganisms or cells can be studied in artificial culture media, and proteins can be examined in solutions. Colloquially called "test-tube experiments", these studies in biology, medicine, and their subdisciplines are traditionally done in test tubes, flasks, Petri dishes, etc. They now involve the full range of techniques used in molecular biology, such as the omics.

In contrast, studies conducted in living beings (microorganisms, animals, humans, or whole plants) are called in vivo.

Medicinal plants are the nature's gift to human beings to make disease free healthy life. It plays a vital role to preserve our health. India is one of the most medico- culturally diverse countries in the world where the medicinal plant sector is a part of time- honoured tradition that is a respected even today. Here, the main traditional systems of medicine include Ayurveda, Unani and Siddha.1 with the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems, the evaluation of the rich heritage of traditional medicine is essential.[1] In India different parts of medicinal plants have been used for curing various diseases from ancient times. In this regard, one such plant is Abutilon indicum. The Abutilon L. genus of the Malvaceae family comprises about 150 annual or perennial herbs, shrubs or even small trees widely distributed in the tropical and subtropical countries of America, Africa, Asia and Australia.[2] Some of the plants belonging to the species are amongst much acclaimed Ayurvedic herbs and in the recent past there has been a renewed scientific interest in exploring the specie.

ANTI-INFLAMMATORY

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 leafs 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 flavanoids, 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 abutilon indicum fruit using several in vitro bioassays, such as inhibition of albumin denaturation, antiproteinase activity, membrane stabilization, and heat induced haemolysis activity.[7]

Inflammation usually occurs when infectious microorganisms such as bacteria, viruses or fungi invade the body, reside in particular tissues and/or circulate in the blood. Inflammation may also happen in response to processes such as tissue injury, cell death, cancer, ischemia and degeneration. Mostly, both the innate immune response as well as the adaptive immune response are involved in the formation of inflammation. The innate immune system is the foremost `defense mechanism against invading microorganisms and cancer cells, involving the activity of various cells including macrophages, mast cells and dendritic cells. The adaptive immune systems involves the activity of more specialized cells such as B and T cells who are responsible for eradicating invading pathogens and cancer cells by producing specific receptors and antibodies.[8] Numerous inflammatory mediators are synthetized and secreted during inflammatory responses of different types. Inflammatory substances are usually divided to two main categories: pro- and anti-inflammatory mediators. Nevertheless, some mediators such as interleukin (IL)-12 possess both pro- and anti-inflammatory

properties. Among the inflammatory mediators and cellular pathways that have been extensively studied in association with human pathological conditions are cytokines (e.g., interferons, interleukins and tumor necrosis factor α), chemokines (e.g., monocyte chemoattractant protein 1), eicosanoids (e.g., prostaglandins and leukotrienes) and the potent inflammation-modulating transcription factor nuclear factor κ B.

Tumor necrosis factor (TNF)-α is an important pro-inflammatory cytokine which is secreted from various cells and exerts many cellular effects. TNF-α has been associated with multiple illness states in humans, including immune and inflammatory diseases, cancer, psychiatric disorders, among others.[9] Another cytokine which mostly exerts a pro-inflammatory activity is IL-1α. It stimulates the secretion of pro-inflammatory cytokines such as IL-1β and TNF-α. However, IL-1α has also been associated with anti-inflammatory activity. Similar to IL-1α, IL-6 usually acts as a pro-inflammatory cytokine but it also has some anti-inflammatory effects. As mentioned above, the IL-12 family of cytokines (including IL-12, IL-23, IL-27 and IL-35) possess both pro- and anti-inflammatory functions. On the other hand, IL-10 is a potent anti-inflammatory cytokine the activity of which impedes the action of many pro-inflammatory mediators. By weakening and controlling the inflammatory response IL-10 helps to maintain tissue homeostasis and attenuates the damage that may result from an exaggerated inflammatory response.

Plant profile *Abutilon indicum*

Scientific classification

KINGDOM: Plantae

CLADE: Tracheophytes

CLADE: Angiospores

CLADE: Eudicots

CLADE: Rosids

ORDER: Malvales

FAMILY: Malvaceae

GENUS: Abutilon

Abutilon indicum

MATERIALS AND METHODS

Plant material

Abutilon indicum (L) fruit samples were collected from surrounding area near to Guntur (Andhra Pradesh) and authenticated by Dr.P. Satyanarayana Raju garu, Acharya Nagarjuna University. A voucher specimen was deposited in the Department of Botany and Microbiology, Acharya Nagarjuna University. Guntur Andhra Pradesh.

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, dinitrosalicylic acid, and chloroform Herbal Science Trust Bangalore. All the other chemicals were procured of analytic grade.

EXTRACTION WITH CHLOROFORM

Abutilon indicum (L) fruits were collected, washed under running tap water and dried at room temperature for 20 days. For preparing dried powder of fruit extract prepared. The shade dried fruits sample were rushed to get 200 gm powder sample and successively extracted with 150ml chloroform in a soxhlet extractor for 18 to 20 hrs. Excess solvent was evapourater using rotary evapourater. This process was continued and carried out until the siphon tube was emptied. The collected extract was then evapourated by rotavapor to remove the solvent completely and crude

Procedure for in vitro anti-inflammatory activity

A) Inhibition of albumin denaturation

The anti-inflammatory activity of abutilon indicum 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^oc for 20 min and then heated to 51^oc 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 abutilon indicum

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 seed 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 dinitrosalicylic 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:

$$\% \text{ Inhibition} = [(\text{Control} - \text{Test})/\text{Control}] \times 100$$

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

Preliminary phytochemical screening results of Abutilon indicum

S.NO	**Screening Tests**	**Chloroform**
1	Steroids	Positive
2	Triterpenoids	Negative
3	Saponins	Negative
4	Steroidal saponin	Negative
5	Triterpenoid saponin	Negative
6	Alkaloids	Negative
7	Carbohydrates	Negative
8	Flavonoids	Positive
9	Phenols	Negative
10	Amino acids	Negative
11	Fixed oils and fats	Negative
12		

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 abutilon indicum fruit 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. Abutilon indicum, 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. Abutilon indicum, 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 abutilon indicum fruit compared with that of standard acarbose. A maximum inhibition of 88.55% ± 0.43% was achieved at a concentration of 4µl/ml by the fruit, 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

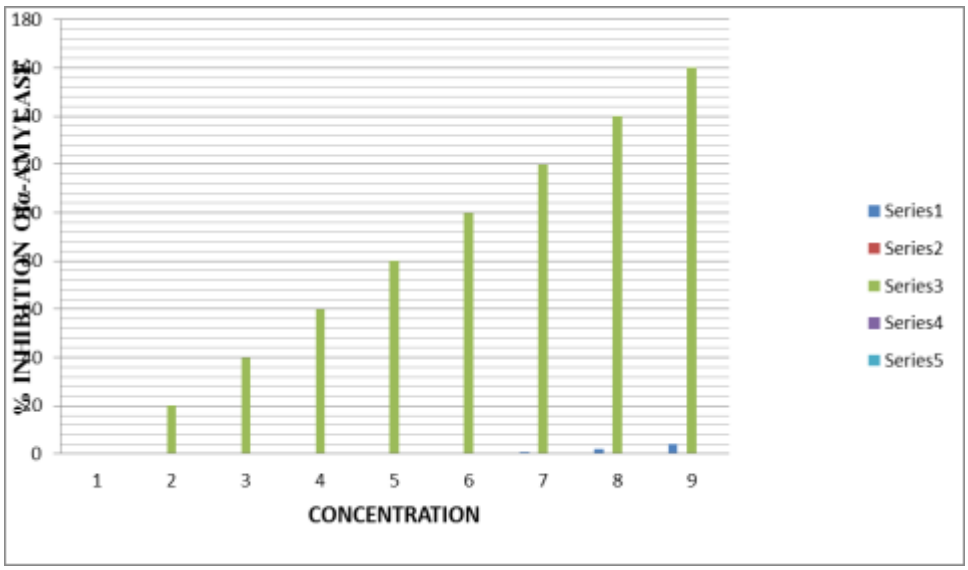
Anti-Inflammtory Activity

In the present study, results indicate that the chloroform extracts of abutilon indicum seed 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 abutilon indicum fruit 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

Abutilon indicum fruit 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 abutilon indicum fruit 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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