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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS AND IN VITRO ANTI-INFLAMMATORY ACTIVITY OF ABUTILON INDICUM

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ABSTRACT

The present study was carried out to evaluate the anti-inflammatory property of the ethanolic extracts of the whole plants *in vitro* method and it was estimated by Human Red Blood Cell membrane (HRBC) stabilization method. This method showed significant anti- inflammatory property of the different concentrations tested. The ethanolic extract at a concentration of 200µg/ml showed potent activity on comparing with the standard drug diclofenac sodium. The extracts exhibited the % of protection in a dose dependent manner. The physicochemical characters of *Abutilon indicum* Linn. was studied and it was observed that moisture content (3.2 %), total ash (40 %), water soluble ash (26.56 %), acid insoluble ash (25 %), alcohol soluble extractive value (6.89 %), water soluble extractive value (38.68 %) and fluorescence analysis were determined. The phytochemical screening was also carried out to find various phyto constituents present in the *Abutilon indicum* Linn. The qualitative phytochemical analysis of whole plants reveals the presence of alkaloids, glycosides, saponins, triterpenes, carbohydrate, steroid, protein, phytosterol, flavonoid, phenol, fixed oil and fats.

INTRODUCTION

Inflammation

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents. There are mainly two types of inflammation which are as follows

Acute inflammation

It is associated with increased vascular permeability, capillary infiltration and emigration of leukocytes.

Chronic inflammation

It is associated with infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, fibrosis and proliferation (angiogenesis). Inflammation is a common clinical conditions and rheumatoid arthiritis (RA) is a chronic debilitating autoimmune disorder (Nadkarni, 2000), that affects about 1% of the population in developed countries (Cardinali and Esquifino, 2003).

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The classic signs of inflammation are local redness, swelling, pain, heat and loss of function (Pervical, 1999). Nitric oxide (NO) is a gaseous short lived free radical has been implicated as a mediator of inflammation and modulation of biosynthesis or activity of NO results in amelioration of acute inflammation and experimental arthiritis model (Daniel 1998; Zumora and Billar 2000). NO is generated via the oxidation of the terminal guanidine nitrogen atom of L-arginine by the enzyme Nitric Oxide Synthase (NOS). Three major isoforms of Nitric Oxide Synthase (NOS) have been identified. Two expressed constitutively, are calcium/calmodulin dependent and are classified together as constitutive NOS isoforms (cNOS). The third is cytokine-inducible, calcium/calmodulin-independent isoform of NOS (iNOS) is regulatedin the gene by a variety of inflammatory mediators (Corbett 1991). Increased NOS activity or NO release have been demonstrated in both acute and chronic models of inflammation (Mederos, 1995). Further, administration of Larginine a precursor for NO synthesis increased the paw swelling in adjuvant arthiritis (Corbett 1991). NSAIDS are among the most commonly used drugs worldwide. They are prescribed for orthopaedic conditions such as osteoarthritis, soft-tissue injuries and fractures etc (Malizos, 2009). NSAIDS e.g Ibuprofen and naproxen etc. are used in the above said conditions. The other class of drugs is glucocorticoids e.g cortisone and prednisone etc. However, besides their high costs, severe adverse reactions and toxicity, including some risk of infections in subsets of patients being

treated with biological response modifiers e.g Tumour necrosis factor, alpha blocking agents (Barnes 2002). The side-effects with currently used drugs are G.I ulceration and bleeding, Renal damage, Hypertension, Hyperglycemia. Besides the above side-effects, the greatest disadvantage in presently available potent aynthetic drugs lies in their toxicity and reappearance of symptoms after discontinuation (Srinivasan *et al*., 2011). *Abutilon indicum* belonging to family Malvaceae, *Abutilon indicum* is a perennial shrub, softly tomentose and upto 3 m in height. The flowers are yellow in color, peduncle jointed above the middle. The petioles are 3.8-7.5 cm long; stipules 9 mm long; pedicels often 2.5-5 mm long, axillary solitary, jointed very near to top and the seeds are 3-5 mm, kidney shaped, reniform, tubercled or minutely stellate hairy, black or dark brown (Kirtikar and Basu, 1994; Prajapati *et al*., 2003; Nadkarni, 1995). *Abutilon indicum* has been used as anthelmentic, antiemetic,anti-inflammatory, in urinary or uterine discharge, piles, antidote. It is used in treatment of fever, dry cough, bronchitis, gonorrhea and leprosy. Hence the present investigation is to screen the whole plants of *Abutilon indicum* Linn. for *in vitro* anti-inflammatory activity.

MATERIALS AND METHODS

Plant Collection and Identification

The plant species namely *Abutilon indicum* Linn. plants were collected in and around Mannargudi, Thiruvarur (Dt), Tamil Nadu.

Preparation of plant powder

The plants were air dried under shade for 10-15 days. Then the dried materials was grinded to fine powder using an electric grinder and stored in air tight bottles. The powder matter was used further phytochemical, physico-chemical analysis, *in vitro* anti-inflammatory, antimicrobial activities.

Extraction of Plant Material

Ethanol and aqueous extracts were prepared according to the methodology of Indian pharmacopoeia (Anonymous, 1955). The coarse powder material was subjected to soxhlet extraction separately and successively with ethanol and distilled water. These extract were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (40°C- 50°C) the ethanol and aqueous extracts put in air tight container stored in refrigerator.

Physicochemical Characters

The different ash values and the different physiochemical parameters were screened.

Preliminary Phytochemical Screening (Anonymous, 1955)

Qualitative phytochemical analysis was carried out for all the extracts as per the standard methods.

In vitro anti- inflammatory activity

Human red blood cell (HRBC) membrane stabilization method: Fresh blood was collected and centrifuged at 3,000 rpm. The packed cells were washed with isosaline (0.90% Nacl) and a 10% suspension was made. The reaction mixture (4.5ml) consists of 2ml of hyposaline (0.25% w/v Nacl), 1 ml of 0.15 M phosphate buffer (P^H 7.4) and 1ml of test solution (25,50,75,100,200 mg/ml) in isosaline, 0.5 ml of 10% HRBC in isosaline was added. For test control, 1ml of distilled water was used instead of hyposaline (to produce 100% hemolysis), while product control lacked red blood cells. The mixtures were incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min. Diclofenac sodium was used as the reference drug. The hemoglobin content in the suspension was estimated using a spectrophotometer at 560nm (Franzotti *et al.,* 2000). Percentage of membrane stabilizing activity was calculated as follows:

% of Membrane stabilization = 100 - [(OD of Test sample / OD of control) × 100]

RESULTS AND DISCUSSION

In recent years, although technology and medicine have developed extensively, some countries have made it obligatory to use natural products for many different purpose due to decrease in natural richness and draw-backs. Like in many other countries, the plants known by people with health benefits are picked up and used for the treatment of various diseases. The present study to evaluate the screening of phytochemical compounds, physico-chemical analysis, *in vitro* anti inflammatory and antimicrobial activity of the ethanolic extracts of *Abutilon indicum* Linn.

Table 1. Physico-chemical characters of *Abutilon indicum* Linn

S. No.	Parameters tested	Percentage (%) Yield
1.	Moisture content	3.2
	Ash value	
2.	Total ash	40
3.	Acid insoluble ash	25
4.	Water soluble ash	26.56
	Extractive value	
5.	Alcohol soluble extractive	6.89
6.	Water soluble extractive	38.68

Table 2. Fluorescence analysis of *Abutilon indicum* Linn

S. No.	Particulars of the Treatment	Under Ordinary Light
1.	Powder as such	Brownish green
2.	Powder + Water	Pale green
3.	Powder + conc.H_2SO_4	Light reddish brown
4.	Powder + Acetone	Dark green
5.	Powder + Acetic acid	Greenish brown
6.	Powder + $Fecl_3$	Pale brownish green
7.	Powder + NaoH	Green
8.	Powder + $CHCL_3$	Dark green
9.	Powder + Sodium Nitrite	Light green
10.	Powder + NaCl	Light green
11.	Powder + Ammonium Hydroxide	Light reddish brown
12.	Powder + Picric acid	Dark green
13.	Powder + Iodide	Pale green
14.	Powder + conc.HCL	Light brown
15.	Powder + 1N HCL	Light brown
16.	Powder + 1N KOH	Dark green

Physico-chemical analysis of *Abutilon indicum*

Physico-chemical characteristic such as moisture content, ash content, acid insoluble ash content, water soluble ash content, alcohol soluble extractive and water soluble extractive were represented in Table 1. The moisture content of the *Abutilon indicum* was found to be 3.2%. The ash content of *Abutilon*

indicum was found to be 40%. Since the accepted range was 50%, which implies that the plant has normal complexes of inorganic and organic components. The result suggested a high deposit of mineral elements in the leaves (Anonymous, 1955).

Table 3. Phytochemical Screening of *Abutilon indicum* Linn

S. No.	Names of the test	Ethanol extract	Aqueous extract
1.	Alkaloid	+	+
2.	Carbohydrate	+	+
3.	Glycosides	+	+
4.	Saponin	+	+
5.	Phytosterols	+	+
6.	Fixed oil and fats	+	+
7.	Resin	+	+
8.	Phenols	+	+
9.	Tannins	+	+
10.	Flavonoid	+	+
11.	Protein and amino acid	+	-
12.	Steroid	+	+
13.	Triterpenes	+	+
14.	Gums and mucilage	+	+
15.	Coumarins	-	-
16.	Coumarins	-	-

(+) = Indicates Presence (-) = Indicates Absence

Table 3. Phytochemical Screening of *Abutilon indicum* Linn

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3.	Glycosides	+	+
4.	Saponin	+	+
5.	Phytosterols	+	+
6.	Fixed oil and fats	+	+
7.	Resin	+	+
8.	Phenols	+	+
9.	Tannins	+	+
10.	Flavonoid	+	+
11.	Protein and amino acid	+	-
12.	Steroid	+	+
13.	Triterpenes	+	+
14.	Gums and mucilage	+	+
15.	Coumarins	-	-
16.	Coumarins	-	-

(+) = Indicates Presence (-) = Indicates Absence

The water soluble ash value was found to be 26.56% and acid insoluble ash value was found to be 25% (Rajurkar *et al*., 2009). Alcohol soluble and water soluble extractive were found to be 6.89% and 38.68% respectively.

Fluorescence analysis of *Abutilon indicum*

Table 2 showed the behavior of *Abutilon indicum* Linn plant powder on treatment with different reagents. Powder of *Abutilon indicum* appeared to be brownish green in colour. On treatment with concentrated HCl and 1N HCl it gives light brown colour. When treated with concentrated H_2SO_4 and ammonium hydroxide gives light reddish brown colour. When powders of *Abutilon indicum* treated with water, iodine and $FeCl_3$ to give pale green and pale brownish green colour is obtained. Treated with 1N KOH, picric acid, acetone and $CHCl_3$ gives dark green colour. When powders of *Abutilon indicum* was treated with acetic acid gives greenish brown colour. Treated with NaOH gives green colour and treated with sodium nitrite and NaCl gives light green colour.

Qualitative Phytochemical analysis

Qualitative phytochemical analysis of ethanolic and aqueous extract of *Abutilon indicum* was represented in Table 3. The preliminary phytochemical analysis revealed the presence of alkaloids, Carbohydrates, Glycosides, Saponins, phytosterols, Fixed oil and fat, resins, phenol, tannins, flavonoids, proteins and amino acids, steroids, triterpenoids, gums and mucilage in ethanolic and aqueous extracts of *Abutilon indicum*. Chlorogenic acids, coumarins are absent in ethanolic extract of *Abutilon indicum* and proteins, chlorogenic acids and coumarins are absent in aqueous extract of *Abutilon indicum*.

Anti-inflammatory activity

The HRBC membrane stabilization method was used for the *in vitro* anti-inflammatory activity of the ethanolic extract of the *Abutilon indicum* Linn. was presented in Table 4. The HRBC membrane stabilization activity of the ethanolic extract of the *Abutilon indicum*. At different concentrations 25mg/ml, 50mg/ml, 75mg/ml, 100mg/ml, 200mg/ml showed 60.83%, 65.58%, 70.33%, 75.08% and 80.83% inhibition of denaturation in hypotonic solution while standard diclofenac sodium 25mg/ml showed 75.08% inhibition of denaturation. On the basis of the results obtained in the present study, it is concluded that ethanol extract of *Abutilon indicum* Linn. whole plants has potent anti-inflammatory and anti microbial activities. Thus the ethanolic whole plants of *Abutilon indicum* extracts may be attributed to the presence of phenolic compounds and flavonoids etc., Therefore, further investigation is needed to isolate and identify the active compounds present in the plant extract and its efficacy.

Table 4. *In vitro* anti-inflammatory activity of ethanolic extracts of *Abutilon indicum* Linn

S. No.	Concentration (mg /ml)	Ethanolic Extract		Standard (Diclofenac sodium)	
		Optical density at 560 nm	% of Protection	Optical density at 560 nm	% of Protection
1.	Control	0.00	-	0.00	-
2.	25	0.43	60.83	0.49	75.08
3.	50	0.46	65.58	0.52	80.83
4.	75	0.49	70.33	0.55	85.89
5.	100	0.52	75.08	0.58	90.95
6.	200	0.55	80.83	0.61	95.90

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