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RESEARCH ARTICLE

In-Vitro Anti-inflammatory Activity of Fractions of *Ailanthus excelsa* Roxb. by HRBC Membrane Stabilization

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ABSTRACT:
Present study deals with membrane stabilizing activity of petroleum ether, ethyl acetate and methanol fractions of leaves of *Ailanthus excelsa*. The phytochemical screening of the leaves of *Ailanthus excelsa* revealed the presence of alkaloids, sterols, saponins, flavonoids, phenolic compounds, carbohydrates and proteins. The inhibition of heat induced HRBC membrane lysis was taken as a measure of the anti inflammatory activity. The methanol fraction showed the most significant membrane stabilizing action on HRBC membrane as compare to other fractions. The maximum membrane stabilization of methanol fraction of A. *excelsa* was found to be 91.13% at a dose of 1000 µg/ml.

KEY WORDS: Anti inflammatory, *Ailanthus excelsa*, HRBC membrane stabilization

INTRODUCTION:
Inflammatory diseases are still one of the most important heath problems in the world. Inflammation is initiated as a healing process by the tissue in response to an injury by pathogens, irritants or cell damage. It is believed that current drugs available such as opoids and non –steroidal anti-inflammatory drugs (NSAIDS) are not useful in all cases of inflammatory disorders, because of their side effects and potency.[1] The screening and development of drugs for their anti-inflammatory activity is still in progress and there is hope for finding anti-inflammatory drugs from indigenous medicinal plants. [2]

Ailanthus excelsa Roxb. (Family- Simaroubaceae) is a large deciduous tree**.** It is commonly known as Tree of Heaven as well as Mahanimba. *Ailanthus excelsa* widely used in several indigenous systems of medicine for the treatment of various ailments viz. asthma, inflammatory diseases, ulcer and stomach problems, cancer, cardiac and hepatic disorders etc.[3-5] Traditional use in medicine of *A. excelsa* has instigated the investigations for possible anti-inflammatory activities.

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MATERIALS AND METHODS:
Plant material
The leaves of the plant *A. excelsa* were collected from Rajkot district of Gujarat, India. The plant material was identified by Faculty in botany, Biology Department, Gyanyagna College of Science and Management, Rajkot and a voucher specimen (Voucher No. AIP/14/01) has been retained in Department of Pharmacognosy, Atmiya Institute of Pharmacy, Rajkot, Gujarat.

Reagents and chemicals
All the reagents were of analytical grades. Indomethacin capsules (25 mg) standard non-steroidal anti-inflammatory drug was purchased from a pharmaceutical shop at Rajkot, Gujarat, India**.**

Preparation fractions of *A. excelsa*
The dried leaves of the plant *A. excelsa* were ground into powder using an electric grinder. Hundred gram of dried leaves powder was extracted by soxhlet extractor successively with petroleum ether, ethyl acetate and methanol. Thereafter, all fractions filtered and concentrated on water bath to a dry residue and kept in desiccator. The presence of different phytoconstituents like sterols, phenols, alkaloids, saponins, flavonoid, carbohydrates and proteins were detected as standard procedures given in the standard text. [6-9]

Anti-inflammatory activity
Membrane stabilizing activity assay
The reaction mixture (2ml) consisted of 1 ml test sample of concentrations (1000 µg/ml) and 1 ml of 10% human red

blood cells (HRBCs) suspension, instead of test sample only saline was added to the control test tube. Indomethacin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated 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: % membrane stabilization = [{Ac- At}/Ac] ×100.Where, Ac is the absorbance of control, At is the absorbance of test sample.[10]

Table 1: Effects of the fractions of *A. excels* leaves in membrane stabilizing activity assay

Test sample	Mean absorbance	% Membrane Stabilization (mean±S.E.M.)
Control	0.327	-
Indomethacine (100 µg/ml)	0.090*	72.48±0.14
Petroleum ether fraction (1000 µg/ml)	0.108*	66.97±0.12
Ethyl acetate fraction (1000 µg/ml)	0.146	55.35±0.17
Methanol fraction (1000 µg/ml)	0.029*	91.13±0.16

Values are expressed as mean± S.E.M.,(N=3). *Significantly different from control ($P < 0.01$)

RESULTS:

Petroleum ether, ethyl acetate and methanol fraction of leaves of *A. excels* were effective in inhibiting the heat induced hemolysis of HRBCs at 1000µg/ml as shown in Table 1. Methanol fraction showed the maximum RBC membrane stabilization 91.13% at 1000µg/ml.

The result of phytochemical screening of fractions of leaves of *A. excels* is given in Table 2. It reveals the presence of phenols, alkaloids, saponins, flavonoid, carbohydrates and proteins in methanol fraction. The ethyl acetate fraction shows the presence of phenols and sterols while petroleum ether fraction consists of sterols.

DISCUSSION:

Membrane stabilization is a process of maintaining the integrity of biological membrane such as erythrocyte and lysosomal membranes against heat induced lyses[11,12]. The erythrocyte membrane is analogous to the lysosomal membrane and as such, the effect of drug on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil. These neutrophil lysosomal constituents include bactericidal enzymes and protease, which upon extracellular release cause further tissue inflammation and damage.[13] The leaf fractions of *A. excelsa* may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation.

The phytochemical screening of the leaves of *A. excelsa* revealed the presence pharmacologically active constituents such as alkaloids, saponins, flavonoids and phenolic compounds in methanol fraction. The methanol fraction showed significant anti-inflammatory activity which may due to additive or synergistic effect of complex mixture of phytoconstituents.

Table 2: Results of phytochemical screening of various fractions of *A. excelsa*

Sr. No.	Chemical tests	Fractions		
		Petroleum ether	Ethyl acetate	Methanol
1.	Test for Alkaloids:			
	(a) Dragendorff's test	-ve	-ve	+ve
	(b) Wagner's test	-ve	-ve	+ve
	(c) Hager's test	-ve	-ve	+ve
	(d) Mayer's test	-ve	-ve	+ve
2.	Test for Saponin Glycosides:			
	(a) Foam test	-ve	-ve	+ve
	(b) Heamolytic test	-ve	-ve	+ve
3.	Test for Flavonoids:			
	(a) Shinoda test	-ve	-ve	+ve
4.	Test for Phenolic compounds and Tannins:			
	(a) Ferric chloride test	-ve	+ve	+ve
	(b) Lead acetate test	-ve	+ve	+ve
5.	Test for Phytosterols:			
	(a) Liebermann's test	+ve	+ve	-ve
	(b) Liebermann- Burchard's test	+ve	+ve	-ve
6.	Test for Fixed oils and Fats:			
	(a) Spot test	-ve	-ve	-ve
	(b) Tincture alkana test	-ve	-ve	-ve
7.	Test for Carbohydrates:			
	(a) Molish's test	-ve	-ve	+ve
	(b) Fehling's test	-ve	-ve	+ve
8.	Test for Proteins and Amino acids:			
	(a) Biuret test	-ve	-ve	+ve
	(b) Million's test	-ve	-ve	+ve

In conclusion, these findings rationalize the traditional usage of this plant as an anti-inflammatory activity. The plant contains many secondary metabolites such as alkaloids, saponins, flavonoids, phytosterols and phenolics which may be responsible for anti-inflammatory activity. Thus further studies would require for identification of the compounds responsible for this activity.

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