EVALUATION OF ANTI-ASTHMATIC POTENTIAL OF ETHYL ACETATE FRACTION OF *PLUCHEA LANCEOLATA*

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Summary

The present study was to evaluate the anti-asthmatic potential of *Pluchea lanceolata* (PL) family Asteraceae ethyl acetate fraction. The ant-asthmatic potential of ethyl acetate fraction was evaluated by *in vitro* animal model in isolated guinea pig tracheal chain preparation. The following study was carried out using dose 100 µg /ml. of ethyl acetate fraction that showed significant relaxant action against histamine induced contraction. The ethyl acetate fraction showed significant anti-asthmatic activity of 57.81±1.22 at the dose of 100µg and can be used for its anti-asthmatic properties.

Key words: *Pluchea lanceolata,* Histamine, Guinea pig trachea, Anti-Asthmatic.

Introduction

Humans have relied on plant based drugs since the beginning of civilisation and still plant species are being used for the development or discovery of new drugs [1]. The therapeutic activity of plants is due to the presence of active constituents in them that may vary depending upon the environmental conditions and age [2]. There is a worldwide increase in the consumption of plant based drugs [3].

Herbal drugs are not only used as medicine but are also being used as dietary supplement and also as natural cosmetics [4]. Plants are still a major source for the discovery on new drug molecules [5]. When compared with synthetic drugs, natural herbal products are safe and have few or no side effects [6].

On the bases of literature survey and traditional uses of Asteraceae family, it provides a great amount of information to the researchers to explore the plants of this family for various pharmacological activities. Also known as the daisy or sun flower family, Asteraceae family is the largest family of flowering plants. It contains more then 24000-3000 species and 1600-1700 genera [7]. It can inhabit almost in every environment. The largest genera of Asteraceae family are *Senecio* with 1500 species, *Vernonia* with 1000 species *Cousinia* with 600 and *Centaurea* also with 600 species.

Asthma can be characterised by hyper response of trachea bronchial smooth muscles to various stimuli, which results in the narrowing of the air tubules along with increase secretion from mucosal membrane, accompanied by mucosal edema and mucus plugging [8]. Almost 200 million people worldwide suffer from asthma with the mortality rate of 0.2 million per year. In India alone 15 million people suffer from asthma. The cause of bronchial asthma includes food, allergens, season, viral infection, air pollutants, various irritants, genetics etc. The present line of treatment mainly includes like mast cell stabilizers, corticosteroids, anti-cholenergics, methylxanthines and sympathomimetics. But this line of treatment is accompanied by various side effects, therefore plant based drugs are now being considered as an alternative approach to control this disease because it is considered safe and without any side effects. With these objectives in mind the present study is designed to evaluate the antioxidant and anti asthmatic activity of *Pluchea lanceolata* leaves.

Pluchea lanceolata fam. Asteraceae is a small shrub usually found in the hot climatic regions of India i.e. Punjab, Rajasthan, upper West Bengal and Uttar Pradesh [9]. It has been found that Pluchea has been used as antipyretic, analgesic, bitter, laxative, nervine tonic and is also recommended for dyspepsia and rheumatoid arthritis. Pluchea lanceolata has been found to inhibit carrageenan, histamine, serotonin, hyaluronidase and sodium urate induced pedal

inflammation [10]. The extract of *Pluchea lanceolata* has also been found to inhibit protein exudation and leucocyte migration, along with turpentine- induced joint oedema, adjuvant-induced polyarthritis, carrageenan and cotton pellet-induced granuloma formation [11]. *Pluchea lanceolata* also attenuates cadmium induced genotoxicity and oxidative stress [12].

Materials and methods

Plant material

The leaves of *Pluchea lanceolata* were purchased from the local market of sector-45, Chandigarh (India) in the month of august 2010. It was authenticated and the voucher specimen no. - 1518/116 has been deposited in the National Institute of Science Communication and Information Resources (New Delhi). The leaves were cleaned, dried and carefully powdered in the grinder at room temperature and were kept in a properly protected container.

Extraction

The powdered leaves of *Pluchea lanceolata* were extracted with ethanol: water (70:30) as solvent system for 72 hr via cold maceration [13]. The solvent was completely removed by rotary evaporator to obtain the crude extract. The crude extract obtained was diluted with water and then fractionated successively with n-butanol and ethyl acetate [14]. The ethyl acetate fraction was then further used for the evaluation of anti-asthmatic activity.

Phytochemical screening

Phytochemical screening of *Pluchea lanceolata* was carried out for various constituent such as: flavonoid, tannins, alkaloid, terpenoids, carbohydrates, anthroquinone glycoside, coumarin glycoside, proteins according to standard procedures [15].

Drugs and chemicals

Histamine was obtained from John Baker INC. The other solvents like hexane, ethyl acetate, n-butanol, chloroform, and ethanol were of analytical grade and purchased from merk, Qualigen and loba chemicals.

Animals

Guinea pig of either sex (400-500gm) procured from Rayat Institute of Pharmacy were used for the study. The animals were maintained in polypropylene cages of standard dimensions at a temperature of 28 ± 1°C and standard 12 hour day/night rhythm. The animals were fed with standard pellet diet *ad-libitum* and water. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animal) registration no. 874/ac/05/CPCSEA.

Anti-asthmatic activity of Pluchea lanceolata ethyl acetate fraction via Guinea pig tracheal chain preparation.

Overnight fasted guinea pig was sacrificed and trachea was mounted in an organ bath containing Krebs solution. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Kreb's solution of the composition: NaCl (5.9), KCl (0.35), $CaCl_2$ (0.28), $MgSO_4$ (0.11), $NaHCO_3$ (2.1), KH_2PO_4 (0.16) and glucose 2.0 g/L, which was continuously aerated and maintained at 37 ± 0.5ºC. One end of the tracheal chain was attached to an S-shaped aerator tube and other attached to an isotonic frontal writing lever to smoked drum. Tissue was allowed to equilibrate for 45 min under a load of 400 mg. A dose response curve for histamine was taken in variant molar concentrations, by maintaining 15 min time cycle. After obtaining a dose response curve of histamine on trachea, the ethyl acetate fraction of *Pluchea lanceolata* was added to the reservoir and same doses of histamine were repeated. The fraction was dissolved in PEG 400 and water. (PEG 400 used alone was without any contractile effect.) Graph of percentage of maximum contractile response on ordinate and negative logarithm of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and in presence of the crude extracts and the three fractions [16].

Results and Discussion

Phytochemical screening

Preliminary phytochemical screening of *Pluchea lanceolata* leaves revealed the presence of chemical constituents such as triterpenes, carbohydrates, sterols, flavonoids, tertiary and quaternary bases. Flavonoids have been reported in various Asteraceae plants and have shown various pharmacological including anti-asthmatic activities. (Table. 1)

Table 1: Phytochemical chemical screening of PLEAF

Chemical constituents	Result
Alkaloids	-
Flavonoids	+ + +
Protein and amino acid	+
Triterpenoids	++
Saponin	-
Carbohydrates	-
Phytosterols	+
Tannins	-

Where:

+ + +: indicates very high presence of the corresponding constituent.

+ +: indicates high presence of the corresponding constituent.

+: indicates minor presence of the corresponding constituent.

- : indicates absence presence of the corresponding constituent.

Pharmacological evaluation of Pluchea lanceolata ethyl acetate fraction

Effect of PL ethyl acetate fraction (PLEAF) (100 µg/ml) on Histamine induced contraction of isolated guinea pig tracheal chain preparation.

In the present study, histamine produced dose dependent contraction of guinea pig tracheal chain preparation as indicated in Table 2. The modified physiological salt solution containing Ethyl acetate extract of *Pluchea lanceolata* (100µg/ml) significantly inhibited (*p<0.05, **p<0.01), ***p<0.001) the contractile effect of histamine. The results were as summarized in Table. 2.

Table No.2 Effect of PLEAF on Histamine induced contraction of isolated guinea pig tracheal chain preparation.

Sr.No.	Histamine Concn. Dose (10µg/ml)	negative log molar concn. of Histamine	% maximum contraction	
			control	PLEAF 100µg/ml
1	0.1	7.08	18.13±1.55	10.66±0.69
2	0.2	6.79	27.21±2.99	13.33±0.86***
3	0.4	6.48	39.69±0.63	24.96±076***
4	0.8	6.18	48.93±1.72	36.99±1.34**
5	1.6	5.88	64.11±3.22	41.70±2.17***
6	3.2	5.58	86.99±3.90	49.61±1.20***
7	6.4	5.23	100.00±4.11	57.81±1.22***

Values are expressed in Mean ± SEM (n=6). Statistical analysis done by using Bonferroni post test. (*p<0.05, **p<0.01), ***p<0.001), significantly different from control.

Discussion

The ethyl acetate fraction of *Pluchea lanceolata* showed maximum anti-asthmatic potential. It showed anti-asthmatic activity in a dose dependent manner and can be used as anti-asthmatic agent for human welfare.

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Rashmi Arora developed and designed the protocol of experiment. Veneet Kumar Dhingra conducted the phytochemical screening under the supervision of N.S. Gill. Anti-asthmatic activity was performed by Veneet Kumar Dhingra and A. C. Rana.

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