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PHYTOCHEMICAL SCREENING AND ASSESSMENT OF *ADHATODA VASICA* (LEAF) FOR ANTIASTHMATIC ACTIVITY

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Abstract:

In present study, the bronchodilating effect was evaluated by observing the effect of ethanolic extract of *Adhatoda vasica* on acetylcholine and histamine aerosol induced broncho-constriction in guinea pigs. Significant increase in preconvulsion time was observed the due to pretreatment with *Adhatoda vasica* when the guinea pigs were exposed to either Ach or histamine aerosol. The bronchodilating effect of *Adhatoda vasica* was comparable to ketotifen. It has been reported that *Albizzia lebbeck* and *Ocimum sanctum*, which are well known anti-asthmatic herbal drugs have similar mechanism of action. Spasmolytic effect of *Adhatoda vasica* was also evaluated by observing the effect of their ethanolic extract on histamine and Ach induced contractions of guinea pig ileum. *Adhatoda vasica* was found to be dose dependently inhibited ileum contractions induced by histamine and Ach. These effects of *Adhatoda vasica* support the improvement in the symptoms and lung function parameters of asthmatic subjects. The possible mechanism of action may be blocked of H_2 and Ach receptors leading to inability of smooth muscle to respond to respond to histamine and Ach induced spasm leading to inhibition of bronco-constriction.

On the basis of all the studies we conclude that this plant has potential activity in petroleum extracts for anti-microbial and ethanolic extracts showed higher activity for analgesic, anti-inflammatory and anti-asthmatic activity.

Key words: Adhatoda Vasica, Antiasthamiatic, Asthama

INTRODUCTION

Plants have played an important role as various medicinal agents since ages. The knowledge of Indian medicinal plants and their uses in the Ayurvedic and Unani system of medicine have led to many Scientific Investigations and Researches throughout the world. Researches on the Indian medicinal plants have been going on far more than half a century. Various active principles have been isolated from the plants and many of them play a dominating role in the modern therapy. Many early workers have done considerable investigation on the medicinal flora of India, which ultimately contributed a wide variety of active constituents and plant drug to modern therapy[1].

Natural product drug materials are a diverse group of product ranging from parts of plants, through simple extract, to isolated active constituents. The definition encompasses a wide range of natural materials, which are important for their therapeutic activity or as pharmaceutical adjuvant.

India is sitting on a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicine. This country is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world. There are very few medicinal herbs of commercial importance, which are not found in this country. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the Third World countries[2].

Medicinal herbs have been use in one form or another, under indigenous systems of medicine like Ayurveda, Siddha and Unani. India, with its traditional background, needs to increase its share in the world market. But unlike China, India has not been able to capitalize on this herbal wealth by promoting its use in the developed world, despite their renewed interest in herbal medicines. Such herbal medicines will find speedy access into those countries. India is a land of immense biodiversity in which two out of eighteen hot spots of the world are located. India is also one of the twelve mega biodiversity countries in the world. It is well known that traditional herbal medicines existed before the application of the modern scientific methods to health care; and even today, majority of the world population depends on herbal health care practices. Exploring traditional herbal medicines in the context of modern science is the need for optimum and proper utilization of traditional plant drugs. In the last decade, WHO recognizing the importance of herbal medicine has passed many resolutions vis-a-vis improving the quality and efficacy of plants drug[3]. It is true that many scientific studies have been done on a large number of medicinal plants in India.

There are two approaches to develop successful drugs from medicinal plants. One is a phytochemical approach which emphasizes the development of pure phytochemicals as drugs. Although there are success stories of ancient insights on medicinal plants leading to the discovery of chemical entities as drugs, this type of drug discovery is expensive and time consuming. In India, The Central Drug Research Institute, Lucknow, studied approximately 2500 plants over a period of more than two decades.

ADHATODA VASICA

Adhatoda vasica (AV) belong to the family *Acanthaceae*. AV has been used in traditional Indian medicine for thousands of years to treat respiratory disorders. The plant is used extensively in the treatment of asthma, cough, bronchitis and tuberculosis, joint pain, lumber pain, sprains, eczema, malaria, rheumatism, swellings, venereal diseases, as an anti-hyperglycemic, anti-diarrhoeal, anticonvulsant and cytotoxic. Quinazoline alkaloids present in the leaves are established as active principles. In the indigenous food preparations, AV leaves were made into a decoction with pepper and dried ginger. But the modern medicine searched its active ingredients and found out that vasicine, oxyvasicine and vasicinone are the alkaloids present in vasaka, the active ingredients for expelling sputum from the body. Bromhexine, a synthetic derivative of the alkaloid vasicine found the market in the treatment of respiratory disorders. Some of the herbal preparations containing AV leaves used in the treatment of asthma disorder worldwide are kada, fermiforate, salustuss, and kanjangand spirote.

The leaves, roots and young plants of AV contain the quinazoline alkaloids vasicine, 7-hydroxyvasicine, vasicinolone, 3-deoxyvasicine, vasicol, vasicoline, vasicolinone, adhatodine, anisotine) betaine, steroids carbohydrate and alkanes. In the flowers triterpines (a-amirine), and flavonoids (Apigenin, astragalin, kaempferol, quercetin, vitexin) have been found. *Adhatodavasica*, also known as Malabar nut tree is part of the Acanthaceae plant family[4,5].

Table 1: Vernacular name of *A. Vasica* worldwide

S.No.	Language	Vernacular names
1	Hindi	Arusha
2	Sanskrit	Vasaka
3	English	Malabar Nut
4	Kannada	Adusoge
5	Bombay	Adulsa
6	Gujarati	Alduso
7	Telugu	Addasaramu
8	Tamil	Adadodai
9	Malayalam	Atalotakam
10	Oriya	Basongo
11	Bengali	Bakas
12	Persian & Urdu	Arusa
13	Punjab	Bhekar

ENTAL PROCEDURE AND METHODOLOGY

COLLECTION AND AUTHENTICATION

Leaves of *Adhatoda vasica* were collected from Hathiyakheda, District-Sehore, and Madhya Pradesh, India. In April 2015, it was authenticated by Dr**.** J. R. Patel (Professor) at Department of Pharmacognosy, RKDF College of Pharmacy, RKDF University, Bhopal, Madhya Pradesh, India.

MACROSCOPIC CHARACTERIZATION

Leaf is pale green, fruity in odor, bitter taste, oblong shape, 8.0-14.2 cm length and 4-5 cm width.



1 2 3

1: Whole plant 2-Fresh leaves 3- Dried leaves

MICROSCOPIC CHARACTERIZATION

Transverse section of leave showed xylem, phloem, Lamina, collenchymas, epidermis, Palisade, Spongy, Mesophyll, abaxial surface (figure 1) and Prismatic form of calcium

oxalate crystal present in Mesophyll, Palisade ratio 5-6, 5-8.5,Stomatal index 10.8- 14.2-18.1 for lower surface.

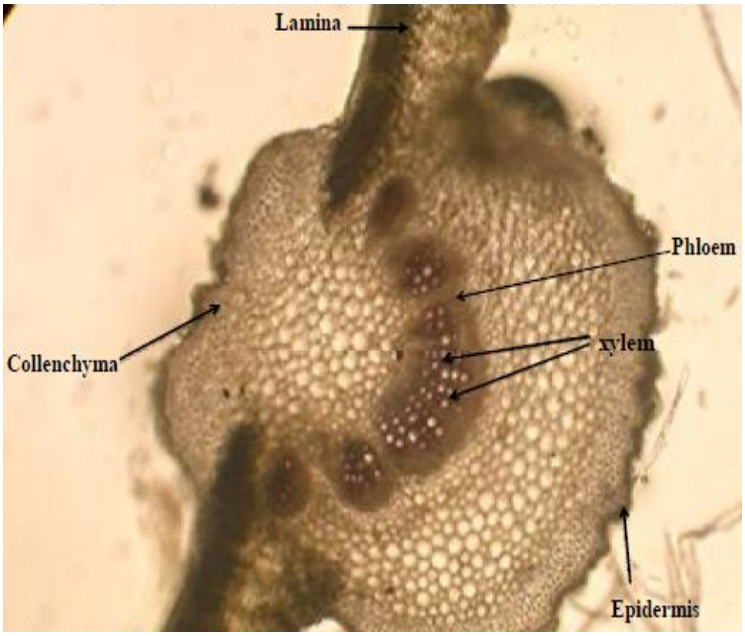

Figure 1: Microscopic Character: Transverse section of Adhatoda vasica leaf

EXTRACTION

The leaves of Adhatoda vasica were extracted after drying and grinding in proper manner by adopting the following procedure:

Drying and Grinding

The collected plant materials were washed with water to make free from any dust or foreign matter and dried in open shade. After air-drying, the plant materials were packed in polythene bags and bags were closed tightly. Whenever required, the plant materials were taken from these stocks. Dried drugs were powdered in the grinder until the drug was finally coarse powdered. After grinding of drug was stored in the airtight container at room temperature and coarsely powdered drug material was used for extraction.

Ethanolic Extraction

The drugs was subjected to the particle size reduction by suitable

Method (mills)

↓

Then the drugs is packed in the soxhlet extractor

↓

Extracted with ethanol for 48 hrs.

↓

Then the ethanol extract was subjected for concentration by using band tube to recover the solvent.

↓

Ethanol extract is kept in freezer for crystallization

↓

Concentrated ehanolic extract was use for further phytochemical screening

MOISTURE CONTENT (LOSS ON DRYING)

The moisture content of a drug should be minimized in order to prevent decomposition of crude drug either due to chemical changes or microbial contamination. Excess moisture also indicates that the purchaser is paying a high price for unwanted water. Loss on drying or heating to constant weight can be determined for material, which do not contain compounds, which are volatile at the temperature of drying[17].

Approximately 2 gm of sample was accurately weighed and transferred in a previously weighed weighing bottle. The bottle was stoppered loosely and placed in an oven at 105°C for 30 minutes. After drying the bottle was cooled at room temperature in a dessicator and weighed till a constant weight was obtained. The loss on drying was calculated with reference to air dried sample.

The extracts obtained were then subjected to various qualitative tests for the identification of various plant constituents.

PHYOTOCHEMICAL SCREENING

It is the process to know the presence or absence of number of chemical.

Plant material is subject to preliminary phytochemical screening for the detection of various plant constituents.

Screening:- screening is the process of separation and isolation of active principle from of plant sources.

Screening is helpful in :-

To get lead for "discovery of new therapeutic agents"

To find "new sources" for economic material.

To help expand "Chemotaxonomy"

To produce "Semi synthetic "derivatives.

Tests for detection of Alkaloids

- **Mayer reagent (potassium Mercuric iodide Solution)**: Test solution produce cream colour precipitate with Mayer reagent which indicates the presence of alkaloids.
- **Wagner reagent (Iodine Potassium Iodide solution) :** Test solution produce reddish brown Precipitate with Wagner reagent which indicates the presence of alkaloids.
- **Hager's reagent (Saturated solution of Picric acid):** Test solution produce yellow precipitate with hager reagent which indicates the presence of alkaloids.

Tests for detection of Carbohydrates

- ***Molish's Test:*** To prepare this reagent, 10 gm of α-napthol was dissolved in 100 ml of 95% ethanol[18]. The reagent was added to aqueous and alcoholic extract as such as to hydrolyzed extract (heated with dil HCl on a water bath). Purple colour was obtained indicating the presence of carbohydrates.

Test for Proteins and Amino acids

- **Millon's test :** To the test solution add about 2ml of million reagents white precipitate was not observe it indicates absence of amino acid.
- **Biuret test :** To the alcoholic extract of the powdered drug 1 ml of dilute sodium hydroxide was added. Followed by this one drop of very dilute copper sulphate solution was added. Violet color was not obtained indicating the absence of proteins.

Test for Glycosides

General test :

✦ Test A

200 mg of the powdered drug was extracted with 5 ml of dilute sulphuric acid by warming on a water bath, filtered and neutralised with 5% sodium hydroxide solution. Then 0.1 ml of Fehlings solution A and B were added, until it becomes alkaline and heated on a water bath for 2 minutes.

✦ Test B

200 mg of the powdered drug was extracted with 5 ml of water instead of sulphuric acid. Boiled and equal amount of water was added instead of sodium hydroxide solution. Then 0.1 ml of Fehlings solution A and B were added, until it becomes alkaline and heated on a water bath for 2 minutes.

The quantity of red precipitate formed in test A is greater than in test B indicating the presence of glycosides.

Test for Anthraquinones glycosides

- **Borntrager's test:**

The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The inorganic layer was separated and ammonia solution was added slowly.

No red color is observe in ammonical layer indicating the absence of anthracene derived glycosides.

- **Modified Borntrager's test :**

About 0.1 gm of the powdered leaf was boiled for two minutes with dilute hydrochloric acid and few drops of ferric chloride solution was added, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the benzene extract and shaken well.

Colour was not observed in ammonical layer indicating the not of anthracene derived glycosides.

Test for cyanogenetic glycosides

Small quantity of the powdered leaf was placed in a stoppered conical flask with just sufficient water to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place[19].

No Change in the colour of the sodium picrate paper was observed indicating the absence of cyanogenetic glycosides.

Test for cardiac glycosides

- ***Keller Killiani test***

About 1 gm of the powdered leaf was boiled with 10 ml of 70% alcohol for two minutes, cooled and filtered. To the filtrate 10 ml of water and 5 drops of solution of lead sub acetate were added and filtered. The filtrate was then extracted with chloroform and the chloroform layer was separated and evaporated to dryness. The residue was dissolved in 3 ml of glacial acetic acid containing a trace of ferric chloride. To this 3 ml of concentrated sulphuric acid was added to the sides of the test tube carefully.

Reddish brown layer acquiring bluish green colour after standing was observed indicating the presence of deoxy sugars of cardiac glycosides.

- ***Raymond Test***

  To the alcoholic extract of the leaf, hot methanolic alkali was added.

  Violet color was produced indicating the presence of cardiac glycosides.

- ***Legal's Test***

To the alcoholic extract of the powdered drug, pyridine and alkaline sodium nitro prusside solution were added.

Red colour was formed indicating the presence of cardiac glycosides.

Coumarin glycosides

A small amount of powdered drug was placed in test tube and covered with a filter paper moistened with dilute sodium hydroxide solution. The covered test tube was placed on water bath for several minutes. Then the paper was removed and exposed to UV light.

Green fluorescence was not observed indicating the absence of coumarin glycosides.

Test for Steroid and Triterpenoids

- ***Salkowski Test***

Few drops of concentrated sulphuric acid were added to the above solution, shaken well and set aside.

The chloroform layer of the solution was not turned red in color indicating the absence of sterols.

Libermann-Burchard's Test

To the chloroform solution few drops of acetic anhydride was added and mixed well. 1 ml of concentrated suiphuric acid was added through the sides of the test tube and set aside for a while.

A brown ring was not formed at the junction indicating the absence of sterols.

- ***Test for Saponins***

About 0.5 gm of the powdered drug was boiled gently for 2 minute with 20 ml of water and filtered while hot and allowed to cool. 5 ml of the filtrate was then diluted with water and shaken vigorously.

Frothing occurred indicating the presence of saponins.

- **Test for Tannins**

To the aqueous of the powdered drug, few drops of ferric chloride solution were added. Bluish black color was produced, indicating the presence of tannins.

Test for Flavonoids

- ***Shinoda Test***

A little amount of the powdered drug was heated with alcohol and filtered. To the alcoholic solution a few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for 5 minutes.

Purple color was obtained indicating the presence of flavonoids.

- ***Alkaline reagent test***

To the alcoholic extract of the powdered drug, few drop of sodium hydroxide solution was added.

Yellow color formed, turning to colorless on addition of few drops of dilute acid indicating the presence of flavonoids.

- ***Zinc Hydrochloride Test***

To the alcoholic extract, mixture of zinc dust and concentrated hydrochloric acid was added. Formation of red color indicating the presence of flavonoid.

PHYTOCHEMICAL/CHROMATOGRAPHIC STUDIES

Thin layer chromatography

Out of the many chromatographic methods presently available, thin layer chromatography is widely used for the rapid analysis of drug and drug preparations. Thin layer chromatographic study is at last beginning to overcome the poor image, which it has suffered in the past with the development of high performance TLC plates. The introduction of new stationary phases and the instrumentalization of virtually all the aspects of the technique. TLC has been transformed into a modern sensitive and high performance analytical methods.

There are number of factors that make TLC a very suitable analytical technique.

- The time required for the demonstration of most of the characteristic constituents of a drug by TLC is very short.
- In addition to quantitative detection, TLC also provides semi-quantitative information on the major active constituents of the drug and drug preparation, thus enabling an assessment of drug quality.
- TLC provides a chromatographic drug fingerprint. It is therefore, suitable for monitoring the identity and purity of the drugs and for detecting adulterations and substitution.
- With the aid of appropriate separation procedures, TLC can be used to analyze the drug combinations and phyto-chemical preparations.

Thin layer chromatography is a separation technique in which a stationary phase consisting of an appropriate material is spread in a uniform thin layer on a support layer or support plate of glass, metal or plastic. Solutions of analytes are deposited on the plate prior to development. The separation is based on the adsorption, partition, ion exchange or on combination of these mechanisms and is carried out by migration (development) of solutes (solution of analytes) in a solvent or suitable mixture of solvent through the thin layer (stationary phase).

TLC Plates: Traditionally TLC has been performed using a limited number of stationary phases (silica gel G, alumina, cellulose, kieselghur and polyamide) coated onto glass, plastic or aluminum foil. The chromatography is carried out using plates either pre-coated or prepared using following methods:

1. Spraying procedure
2. Spreading procedure
3. Immersion procedure

TLC is performed in various steps, which are as follows:

Preconditioning of Plates

It may be necessary to wash plates prior to separation. This can be done by migration of appropriate solvents; the plate may also be impregnated. At the time of use the plates may be activated if necessary by heating at 100-105^{o}C for 1 hour.

Chromatographic Chamber

A chromatographic chamber with a flat bottom and twin trough of inert, transparent material of a size suitable for the size of plates to be used and provided with a tightly fitting lid. For horizontal development chamber is provided with a trough for the mobile phase and it additionally contains a device for directing the mobile phase to stationary phase.

Sample Applicators

Micro-pipettes, micro syringes, calibrated disposable or linomat applicator system (HPTLC) capillary tube is some of the systems that are used for the proper application of the samples or solutions onto the plates and thus ensure a better resolution.

Development

In the beginning, TLC was performed by simply placing the plate in a glass chamber containing an appropriate amount of solvent, which was then allowed to migrate the required distance needed to obtain the desired separation. There is currently in existence a large variety of development techniques derived in order to improve upon the type of separation afforded by the basic TLC technique. These methods include continuous development, multiple development and its instrumentalized variants of programmed multiple development (PMD) and automated multiple development (AMD), continuous multiple development (which combines the continuous and multiple development techniques), circular or radial development and anti-circular development, over pressurized TLC, two dimensional TLC, triangular development, and centrifugal development.

Figure 2: Development of TLC plate

Separation of chemical constituents by thin layer chromatography

Prepare the TLC plate by using distilled water

↓

Keep the plate in oven at 105^{o}c for 30 minutes

↓

Developed the developing tank by using the different ratio of the mobile phase

↓

Ratio of the mobile phase used(ethyl acetae: methanol: ammonia (8: 0.5: 0.2))

↓

spot the sample on TLC plate using capillary tube

↓

Keep the plate in developing tank

↓

When mobile phase run 3/4th of the TLC plate, Plate is removed from the developing tank

↓

Keep the TLC plate for air dry for to two minutes

↓

Spray the Dragendorff's reagent on TLC plate

↓

Two spot was observed

Figure 3: Prepared Thin layer chromatograph plate

Detection and Quantification

In the basic TLC technique, detection is based on the human eye aided by a vast array of selective spray reagents and the use of plates impregnated with fluorescent indicators, which allow compounds to be detected by fluorescence quenching. For quantitative evaluation, especially in HPTLC, a range of TLC, UV/visible scanners is available which are capable of operating in one any of several modes. Thus, scanners are available which can measure absorbance, fluorescence and fluorescence quenching and many are capable of obtaining spectra of individual spots *in situ*. Apart from this type of detector, many are described including the use of video camera and computer based image-processing system as an alternative to scanning densitometry.

Densitometry is *in situ* instrumental measurement of visible, UV absorbance, fluorescence quenching directly. The scanner converts the spot band on the layer into a chromatogram consisting of peaks similar in appearance to that of HPLC chromatogram. The portion of the scanned peaks on the recorder chart is related to R_f values of the spots on the layer and the peak height or area is related to the concentration of the substance on the spot.

R_f value determination

R_f value of each separated component was calculated using the following formula.

$$R_f = \frac{\text{Distance travelled by the component}}{\text{Distance travelled by the solvent front}}$$

The following solvent systems were prepared for various plant materials.

Solvent Systems

Toluene: methanol: dioxane: ammonia

Ethyl acetae: methanol: ammonia

Detecting Reagent

- ✓ UV 254 nm
- ✓ Spraying with perchloric acid
- ✓ Spraying with Dragendorff's reagent
- ✓ Spraying with antimony trichloride in HCl
- ✓ Spraying with sulphuric acid

DETERMINATION OF ANTI-ASTHMATIC ACTIVITY

Asthma is a chronic condition involving the respiratory system in which the airways occasionally constrict, become inflamed, and are lined with excessive amount of mucus, often in response to one or more triggers. These episodes may be triggered by such things as exposure to an environmental stimulant such as an allergen, environmental tobacco smoke, cold or warm air, perfume, pet dander, moist air, exercise or exertion or emotional stress. In children, the most common triggers are viral illness such as those that cause the common cold. This airway constriction responds to bronchodilators. Most patients feel well but can have mild symptoms and they may remain short of breath after exercise for longer periods of time than the unaffected

individual. The symptoms of asthma, which can range from mild to life threatening, can usually, be controlled with a combination of drugs and environmental changes.

MATERIAL

ANIMAL: - Guinea pigs

1. **SEX**: - Mixed sex.
2. **BODY WEIGHT**: - 350-500gm.

Guinea pigs of either sex (350-500gm) were selected for present study. The animals were grouped and housed in polyacrylic cages, with not more than six animals per cage and maintained under standard laboratory conditions. They were allowed free access to standard dry pellet diet and water *ad libitum* during the experiment. All experimental procedures were followed in strict accordance with the guideline prescribed by the Committee for the Purpose of Control and Supervision on Experimental on Animals (CPCSEA) and were approved by the Institutional Animal Ethical Committee.

The guinea pigs were housed under standardized animal house condition (12 hrs light and dark cycles, at 25±27°c and 35-60% humidity) and feed with standard rodent diet. All animals had free access of water. The study was approved by institutional animal Ethical Committee (IAEC).

CHEMICALS

- Ketotifen fumarate
- Ethanolic extract of *adhatoda vasica*

Method screening of anti-asthmatic activity

In vivo study

Studies on acetylcholine and Histamine induced branchospasm in guinea pigs

Guinea pigs of either sex weighing between 350 to 500 g were selected and randomly divided into four groups each containing six animals. The drugs were administered orally in 0.5% in CMC. The single dose treatment was given one and half an hour before the study. The following schedule of treatment was administered.

Later the animals were exposed to an aerosol of 0.25% Histamine and time for preconvulsion state was noted for each animal as described by Sheth *et. al* (1972). After about 15 days of washout period, the same animals were given the above treatment and time for preconvulsion state was noted for 0.5% acetylcholine bromide aerosol spray.

In vitro study

Studies on isolated guinea pig ileum

Overnight fasted guinea pigs of either sex weighing 400-600 g were sacrificed using cervical dislocation method. Ileum was quickly dissected out and mounted in an organ bath maintained at 30±0.5°C and containing 20 ml Tyrode's solution under basal tension of 500 mg. the composition of solution mM was NaCl, 0.137; $CaCl_2$, 1.8; KCl, 2.70; Glucose, 5.55; $NaHCO_3$, 11.9; $MgCl_2$, 1.0; NaH_2PO_4. The solution was continuously bubbled with air. The responses to drug were recorded

on student physiograph using isotonic transducer, which exerted a vessel tension equivalent to 500 mg load on tissue. The tissue was allowed to equilibrate for 30 minutes, during which, the bathing solution was changed at every 10 minutes. The contractile responses of ileum to various agonists (Acetylcholine, Histamine and $BaCl_2$) were recorded in presence and absence of ethanolic extracts of *adhatoda vasica.*

RESULTS AND DISCUSSION

MACROSCOPIC CHARACTERIZATION

Leaf is pale green, fruity in odor, bitter in taste, oblong shaped, 8.0-14.2 cm length and 4-5 cm width.

MICROSCOPIC CHARACTERIZATION

Transverse section of leave showed xylem, phloem, Lamina, collenchymas, epidermis, Palisade, Spongy, Mesophyll, abaxial surface (figure 1) and Prismatic form of calcium oxalate crystal present in Mesophyll, Palisade ratio 5-6, 5-8.5,Stomatal index 10.8- 14.2-18.1 for lower surface.

MOISTURE CONTENT DETERMINATION

2 gm of sample was accurately weighed and transferred in a previously weighed weighing bottle. The bottle was closed loosely by stopper and placed in an oven at 105°C for 30 minutes. After drying the bottle was cooled at room temperature in a dessicator and weighed till a constant weight was obtained. The loss on drying was calculated with reference to air dried sample. The results are given in table 2.

Table 2: Loss on drying of crude drugs

Parameter	Loss on drying %w/w
Loss on drying	10.2

QUALITATIVE ANALYSIS

Plant material is subject to preliminary phytochemical screening for the detection of various plant constituents. It is the process to know the presence or absence of number of chemical.

Table 3: Chemical constituents present in ethanolic extract of *Adhatoda vasica*

S.NO.	PLANT CONSTITUENT	RESULT
1.	Alkaloids	+ve
2.	Carbohydrates	+ve
3.	Glycosides	+ve
4.	Saponins	+ve
5.	Phytosterols	-ve
6.	Proteins	-ve
7.	Flavonoids	+ve
8.	Fat & oils	-ve
9.	Tannins & phenolic compound	+ve
10.	Gum and Mucilage	-ve

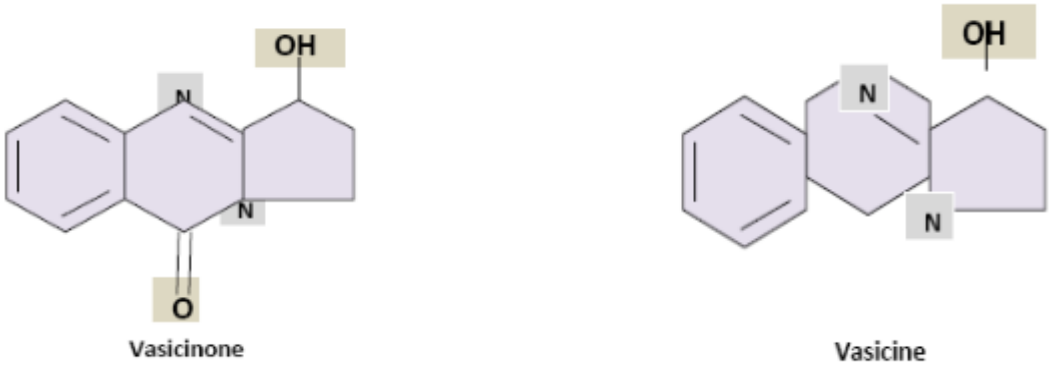

Figure 4 (a): Structure of alkaloid Vasicinone and Vasicine

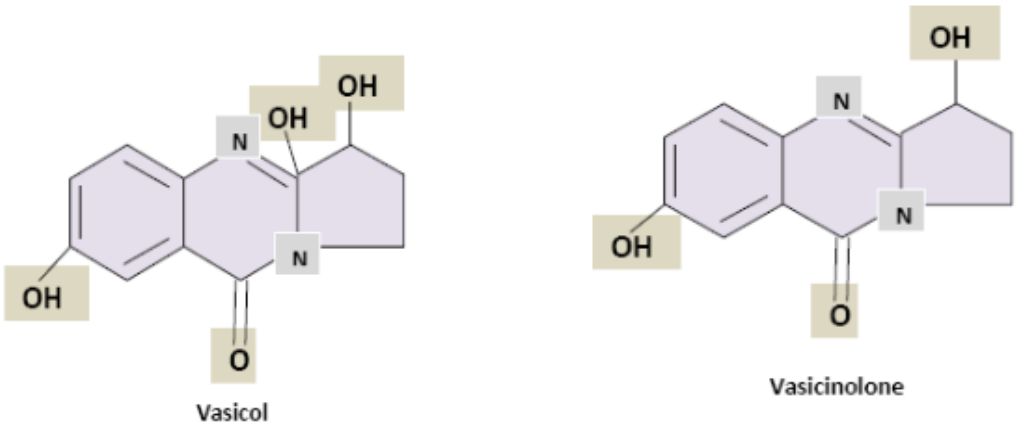

Figure 4 (b): Structure of alkaloid Vasicol and Vasicinolone

5.5 CHROMATOGRAPHIC STUDIES (Thin layer chromatography):

TLC has been performed using a limited number of stationary phases (silica gel G, alumina, cellulose, kieselghur and polyamide) coated onto glass, plastic or aluminum foil.

Table 4: Data represents various solvent systems, R_f values of the ethanolic extract of *Adhatoda Vasica*.

Drug	Solvent systems	Ratio of Solvents	Rf values of the spots	No.of Spots
Ethanolic extract of Adhatoda Vasica	Toluene: methanol: dioxane: ammonia	(2 : 2 : 5 :1)	0.54 0.62	02
	Ethyl acetae: methanol: ammonia)	8: 0.5: 0.2	0.56	01

DETERMINATION OF ANTI-ASTHMATIC ACTIVITY

In vivo study

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Table 5: Effect of ethanolic extract of *adhatoda vasica* on Ach and Histamine induced branchospasm in guinea pigs

Group	Dose	% increase in preconvulsion	
		Acetylcholine	Histamine
Group I	1mg/kg	27.64±2.14	34.38±2.11
Group II	250 mg/kg, p. o.	32.25± 2.54	17.36±1.85
Group III	500 mg/kg, p. o.	47.58±3.98	34.33±2.11
Group IV	750 mg/kg, p. o.	54.91±4.42	39.67±3.88

n=6, values are expressed in Mean ± SD, P <0.05, using student 't' Test

Group I - Standard-treated with 1mg/kg

Group II - Treated with 250 mg/kg p.o. of ethanolic extract of *Adhatoda vasica* (in 0.5% CMC)

Group III - Treated with 500 mg/kg p.o. of ethanolic extract of *Adhatoda vasica* (in 0.5% CMC)

Group IV - Treated with 750 mg/kg p.o. of ethanolic extract of *Adhatoda vasica* (in 0.5% CMC)

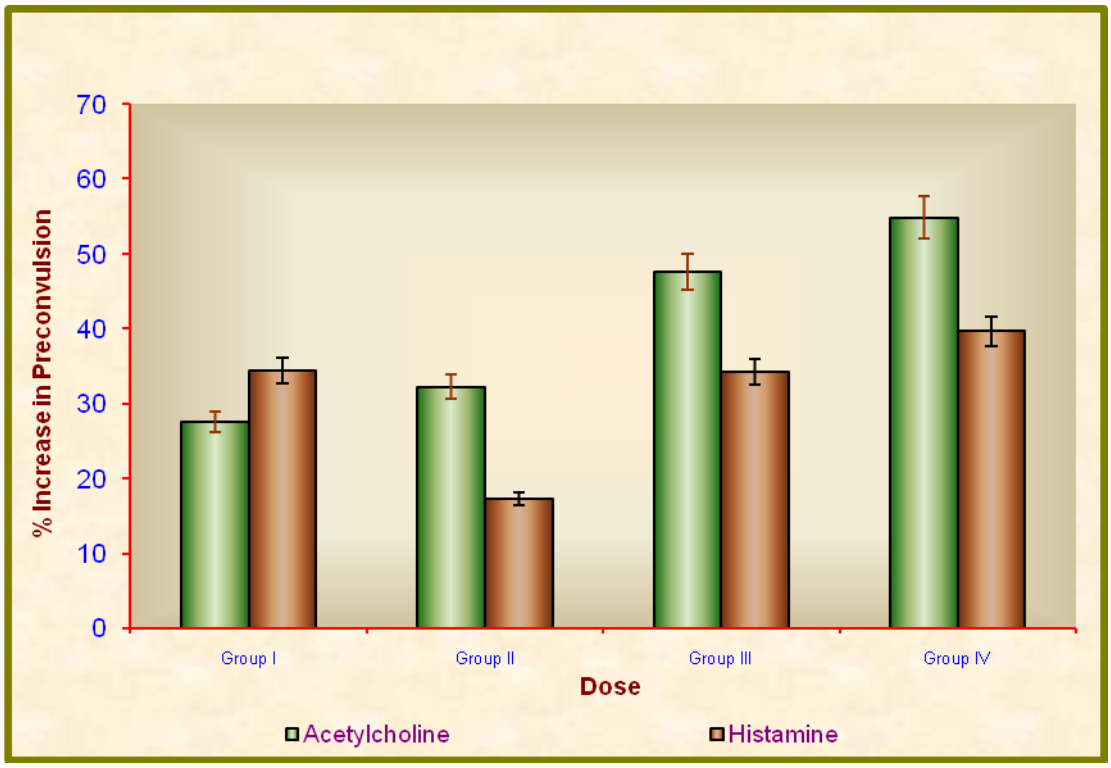

Figure 5: Effect of ethanolic extract of *Adhatoda vasica* on Ach and Histamine induced branchospasm in guinea pigs

Table 6: Effect of ethanolic extract of Adhatoda vasica (250µg/ml) on histamine induced contraction of isolated guinea pig ileum preparation

S.No.	Dose of Histamine (10µg/ml)	% Maximum response	
		Control	Test
1.	0.3	21.22±1.23	14.47±1.04
2.	0.6	39.22±4.57	26.67±3.66
3.	0.9	52.19±3.54	34.14±4.41
4.	1.2	65.21±4.17	48.15±2.41
5.	1.5	73.14±5.24	51.44±2.57

n=6, values are expressed in Mean ± SD, P <0.05, using student 't' Test

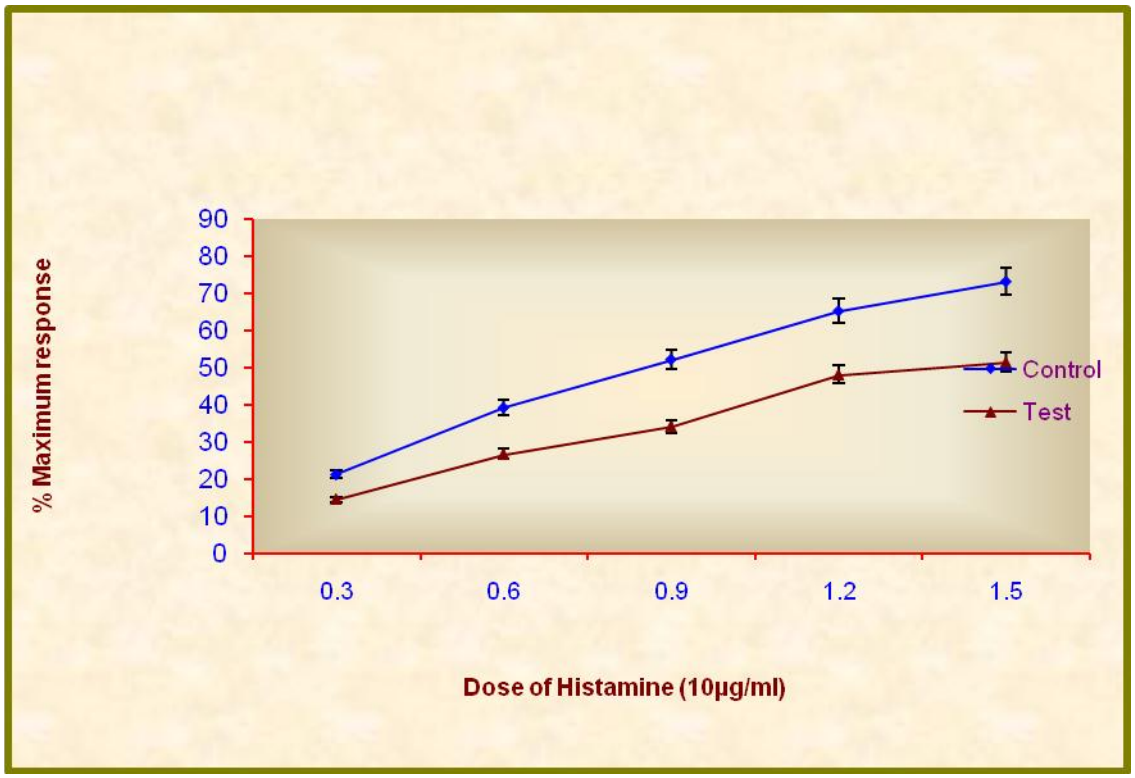

Figure 6: Effect of ethanolic extract of *Adhatoda vasica* (250µg/ml) on histamine induced contraction of isolated guinea pig ileum preparation

Table 7: Effect of ethanolic extract of *Adhatoda vasica* (500µg/ml) on histamine induced contraction of isolated guinea pig ileum preparation

S. No.	Dose of Histamine (10µg/ml)	% Maximum response	
		(Control)	Test
1	0.3	31.25±2.54	24.54±2.74
2	0.6	59.64±4.57	35.27±3.66
3	0.9	81.94±4.87	51.22±4.55
4	1.2	89.36±5.01	59.05±3.74
5	1.5	97.29±6.11	62.55±3.47

n=6, values are expressed in Mean ± SD, P <0.05, using student 't' Test

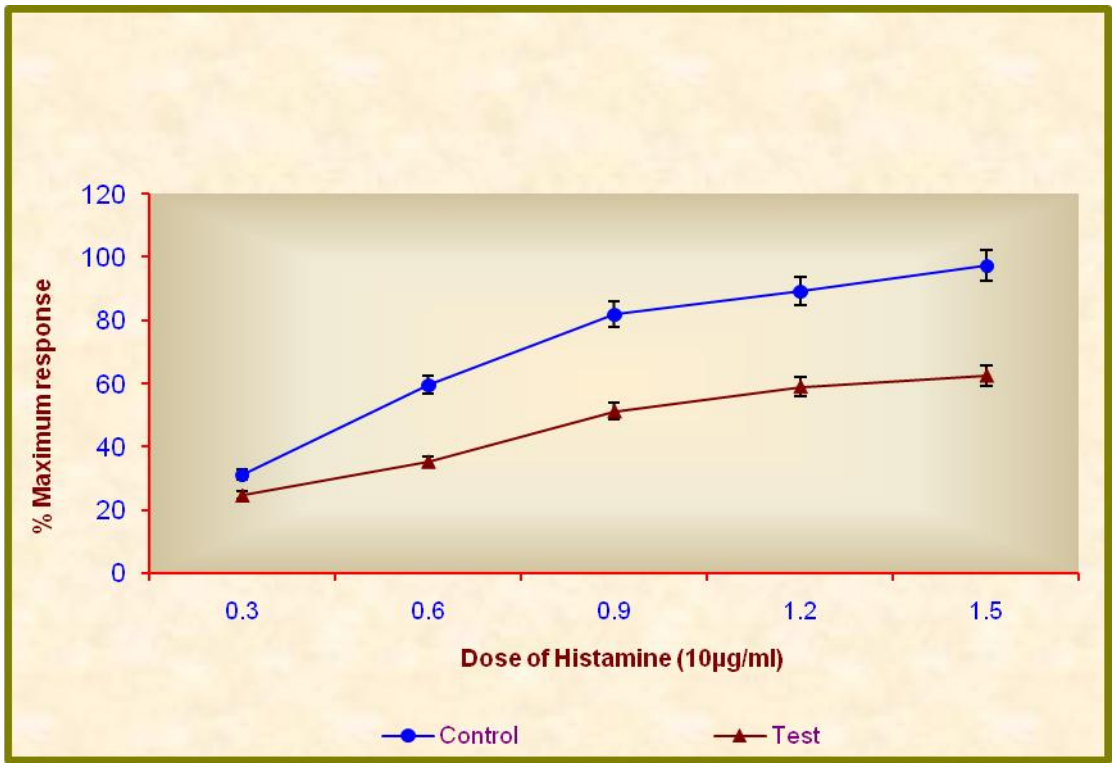

Figure 7: Effect of ethanolic extract of*Adhatoda vasica* (500µg/ml) on histamine induced contraction of isolated guinea pig ileum preparation

Table 8: Effect of ethanolic extract of Adhatoda vasica (750µg/ml) on histamine induced contraction of isolated guinea pig ileum preparation

S. No.	Dose of Histamine (10µg/ml) (ml)	% Maximum response	
		(Control)	Test
1	0.3	36.55±3.61	25.78±1.24
2	0.6	65.33±3.57	37.28±3.46
3	0.9	84.99±3.47	61.31±1.82
4	1.2	90.24±4.11	71.41±4.64
5	1.5	99.05±4.24	65.59±4.79

n=6, values are expressed in Mean ± SD, P <0.05, using student 't' Test

The results from our clinical study on *Adhatoda vasica* suggested that it is effective in reducing the symptoms of bronchial asthma and also improve the lung function parameters of asthmatic subjects, several experimental studies were done on the ethanolic extract of *Adhatoda vasica* to reveal the possible mechanism of action of anti-asthmatic activity.

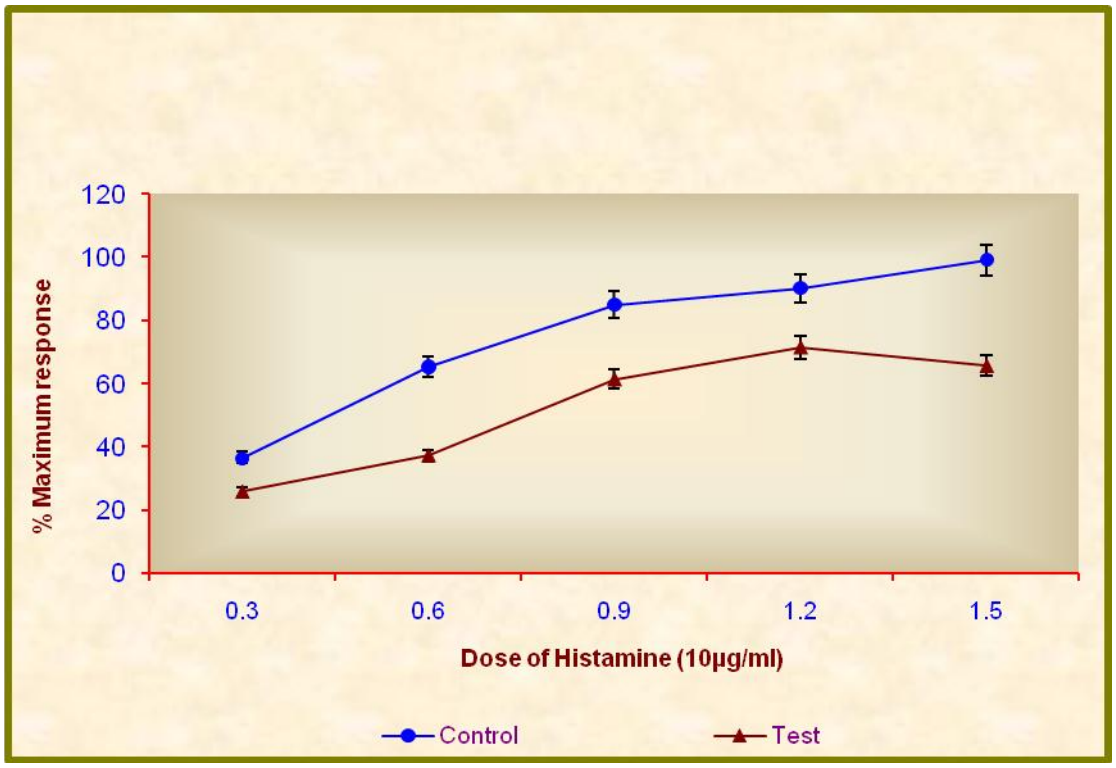

Figure 8: Effect of ethanolic extract of *Adhatoda vasica* (750µg/ml) on histamine induced contraction of isolated guinea pig ileum preparation

Significant increase in preconvulsion time was observed due pretreatment with ethanolic extract of *Adhatoda vasica* 250, 500, 750 mg/kg p.o. when the guinea pigs were exposed to either acetylcholine (0.5%) or histamine (0.25%) aerosole. The spasmolytic effect of ethanolic extract of *Adhatoda vasica* was comparable to ketotifen (1mg/kg). Histamine as well as acetylcholine produced dose-dependent contractions of isolated guinea pig ileum. Pretreatment with ethanolic extract of ethanolic extract of *Adhatoda vasica* 250, 500, 750 µg/ml significantly inhibited the contractions to histamine as well as acetylcholine.

SUMMARY AND CONCLUSION

In present study, the bronchodilating effect was evaluated by observing the effect of ethanolic extract of *Adhatoda vasica* on acetylcholine and histamine aerosol induced broncho-constriction in guinea pigs. Significant increase in preconvulsion time was observed the due to pretreatment with *Adhatoda vasica* when the guinea pigs were exposed to either Ach or histamine aerosol. The bronchodilating effect of *Adhatoda vasica* was comparable to ketotifen. It has been reported that *Albizzia lebbeck* and *Ocimum sanctum*, which are well known anti-asthmatic herbal drugs have similar mechanism of action. Spasmolytic effect of *Adhatoda vasica* was also evaluated by observing the effect of their ethanolic extract on histamine and Ach induced contractions of guinea pig ileum. *Adhatoda vasica* was found to be dose dependently inhibited ileum contractions induced by histamine and Ach. These effects of *Adhatoda vasica* support the improvement in the symptoms and lung function parameters of asthmatic subjects. The possible mechanism of action may be blocked of H_2 and Ach receptors leading to inability of smooth muscle to respond to respond to histamine and Ach induced spasm leading to inhibition of bronco-constriction.

Ketotifen fumarate, a well-known mast cell stabilizer, reduces synthesis of prostaglandins E_2, leukotriene C_2, and B_4. It also inhibits release of histamine, serotonin and other inflammatory mediators from mast cell. Simultaneously it blocks H_1 receptors. Adhatoda vasica, Albizzia lebbeck, Coleus forskohlii, Tylophora asthmatica etc. are several all known drugs from indigenous plant sources used in asthma and have been reported to have mast cell stabilizing activity.

Thus inflammatory mediators have been implicated in the pathogenesis of allergic an inflammatory disorders like bronchitis. Anti-inflammatory drugs suppress the inflammation response by inhibiting infiltration and activation of inflammatory cells as well as their synthesis. Over lease of mediators and the effects of inflammatory mediators, the carrageenan induced paw edema model rats is known to be sensitive to cycloxygenase inhibitors. Since, serotonin, histamine and prostaglandins are the common mediators of both bronchial inhibitors. Since, serotonin, histamine and prostaglandins are the common mediators of both bronchial asthma and inflammation, the beneficial effect of ethanolic extract of *Adhatoda vasica* could be due to inhibition of their release possibly due to inhibition of the enzyme cycloxygenase leading to inhibition of prostaglandin synthesis.

In India, the patients with bronchial asthma commonly prescribed with antibiotics. It has been reported that these patients are resistant to man antibiotics prescribed. It is possible that these patients are suffering from bronchial infection but have been diagnosed as asthmatic patients because of their symptoms like breathless. Use of this medicinal plants can substitute antibiotics to treat associated infection present study, water extract and ethanolic extract *adhatoda vasica* was found to posses good antimicrobial activity when tested against various respiratory pathogens that can be used to control respiratory complication.

Results of the experimental studies of *adhatoda vasica* suggest that anti-asthmatic activity could be due to its bronchodilator, mast cell stabilizing and antimicrobial property. The possible mechanism of action may be blockade of H_1 and Ach receptors leading to inhibitory of smooth muscle to respond to histamine and Ach induced spasm leading to inhibition of bronchoconstriction.

Various active principles have been isolated from the plants and many of them play a dominating role in the modern therapy. Many early workers have done considerable investigation on the medicinal flora of India, which ultimately contributed a wide variety of active constituents and plant drug to modern therapy.

In the current search for new drugs having, for example, antitumour or hypotensive activity the plants involve, unlike many of the more traditional medicaments, very often show no immediate indication of pharmacological activity. Investigators are thus faced with the problem of making a systematic investigation from among the thousands of species still unexamined. One obvious line of approach is to start with folk medicine of the world on the assumption that these materials have already been subjective to some human screening. For many areas of the world, the plants used in folklore have been adequately recorded.

The plant *Adhatoda vasica* is employed for variety of purposes in indigenous as well as folk medicine, it was therefore considered worth to take up this plant for the present study.

Adhatoda vasica Plant is described in Ayurveda and other system of medicines for anthelmintic, purgative, anti-fertility activity, snake-bite, cough and skin diseases. The plant exhibited anti-inflammatory activity in preliminary screening. Looking to the availability of the drug in India as common weed and its manifold uses. It is considered worthwhile to investigate anti-asthmatic activity, antimicrobial activity, anti-inflammatory and analgesic activity.

The he literature has been surveyed from the different sources like, Jawahar Lal Nehru library, Dr. H.S. Gour University Sagar (M.P.); National Medical Library, New Delhi; National Institute of Pharmaceutical Education and Research, SAS Nagar Chandigarh and Library of Shri Ramnath Singh Mahavidyalaya (Pharmacy), Gormi, Bhind, (M.P.). It has also taken from the internet like Sciencedirect.com and Pubmed, Google, Herbal grounds etc.

Adhatoda vasica have been recognized in Ayurvedic literature and in other literature used in snake-bite that potentiate immune system and which is interrelate with cellular component of the human circulatory system. It has direct relation to delayed and acute type of humoral responses i.e. inflammation that is why this drug could be successfully used as anti-asthmatic activity, antimicrobial activity, anti-inflammatory activity and analgesic activity.

Although very less pharmacological and phytochemical studies have been undertaken on *Adhatoda vasica*, very little attention has been paid to biological activity.

Various Chemical tests have been done on both the extracts by different reagents and chemicals for the determination of the type of chemical class of compounds. The number of reagents were used i.e. Mayer's Reagent, Dragendorff's Reagen, Wagner's Reagent, Hager's reagent, Molish's Test, Hasch's Test, Fehling's Test, Selivanoff's Test, Lieberman Burchard's Test, Filter Paper Test, Saponification test, Foam Test, Heamolytic Test, Ferric Chloride Test, Gelatin Solution Test, Magnesium Ribbon Test, Shinoda test, Dragendroff's Test, Mayer's test, Hager's test, Wagner's test, Milon's Test, Biuret Reaction and reported in table .

Thin layer chromatography and column chromatography has been performed in the different solvent system of different polarity retardation factor and their detecting reagents were reported.

Ethanolic extract of *Adhatoda vasica* at 250 mg/kg body weight per day (EEBR-I) when given orally as a suspension the paw volume were reduced by 55.03 % whereas in case of ethanolic extract of *Adhatoda vasica* at 500 mg/kg body weight per day (EEBR-II) shows 65.54 % inhibition after 3 hr which indicate that effect of ethanolic extract of *Adhatoda vasica* is reflect in dose dependent manner. Both EEBR-I and EEBR-II showed inhibitory effect on carrageenan-induced paw edema thus, exhibiting anti-inflammatory effect against acute inflammation.

In case of petroleum ether extract of *Adhatoda vasica*at 250 mg/kg body weight per day (PEEBR-I) reduced the paw volume 04.73 % and petroleum ether extract of *Adhatoda vasica*at 500 mg/kg body weight per day (PEEBR-II) exhibited 07.43% reduction in paw volume after 3 hr so petroleum ether extract of *Adhatoda vasica* don't possess significant anti-inflammatory activity when compared with control and Indomethacin treated animals. It may be due to absence of flavonoid in the petroleum ether extract.

Thus, it is concluded that the ethanolic extract of bark of *Adhatoda vasica* produces significant analgesic and anti-inflammatory activities in dose dependent manner.

The results from our earlier clinical study on *adhatoda vasica* suggested that it is effective in reducing the symptoms of bronchial asthma and also improve the lung function parameters of asthmatic subjects, several experimental studies were done on the alcoholic and aqueous extracts of *adhatoda vasica* to reveal the possible mechanism of action of anti-asthmatic activity.

Bronchial asthma is characterized by increased airway reactivity to spasmogens. An initial event in asthma appears to be the release of inflammatory mediators (e.g. Histamine, Tryptase, Leukotrienes and prostaglandin). Some of these mediators directly cause acute bronco constriction, airway hyper responsiveness and bronchial airway inflammation. In present study, the bronchodilating effect was evaluated by observing the effect of alcoholic extract of *adhatoda vasica* on acetylcholine and histamine aerosol induced broncho-constriction in guinea pigs. Significant increase in preconvulsion time was observed the due to pretreatment with *adhatoda vasica* when the guinea pigs were exposed to either Ach or histamine aerosol. The bronchodilating effect of *adhatoda vasica* was comparable to ketotifen. It has been reported that *Albizzia lebbeck* and *Ocimum sanctum*, which are well known anti-asthmatic herbal drugs have similar mechanism of action. Spasmolytic effect of *adhatoda vasica* was also evaluated by observing the effect of their alcoholic extract on histamine. Ach, 5-HT and $BaCl_2$ induced contractions of guinea pig ileum. *adhatoda vasica* was found to be dose dependently inhibited ileum contractions induced by histamine and Ach. These effects of *adhatoda vasica* support the improvement in the symptoms and lung function parameters of asthmatic subjects. The possible mechanism of action may be blocked of H_2 and Ach receptors leading to inability of smooth muscle to respond to respond to histamine and Ach induced spasm leading to inhibition of bronco-constriction.

In addition to bronchodilators, a significant number of therapeutic approaches for bronchial asthma have been designed based on antagonizing specific mediators released from mast cells. Mast cell degranulation is important in the initiation of immediate response following exposure to allergens. Mast cells found throughout the walls of the respiratory tract, and increased numbers of these cells have been described in the airways of asthmatic with an allergic component. Compound 48/80 is one of the most potent mast cell degranulators, which causes liberations of mediators of inflammation such as histamine, leukotrienes, platelet activating factors, chemotactic factors for eosinophils neutrophils etc. from mast cells. They play a significant role in airway inflammatory response such as airway eosinophilia, late asthmatic response and airway hyper responsiveness as well as in immediate hypersensitivity reaction like bronchial contraction. Degranulation of mast cells has been taken as the criteria of positive anaphylaxis. Ketotifen fumarate, a well-known mast cell stabilizer, reduces synthesis of prostaglandins E_2, leukotriene C_2, and B_4. It also inhibits release of histamine, serotonin and other inflammatory mediators from mast cell. Simultaneously it blocks H_1 receptors. Khellin is a compound isolated from Ammi visnaga and its structural analogue furanochromone khellin. Cromolyn sodium, which is developed from the structural modification of Khllin is the mast cell stabilizer used in the treatment of mild to moderate asthma. Adhatoda vasica.Albizzia lebbeck, Coleus forskohlii, Tylophora asthmatica etc. are several all known drugs from indigenous plant sources used in asthma and have been reported to have mast cell stabilizing activity. A significant protection of rat peritoneal mast cells from disruption by antigen and compound 48/80 by

anxious extract of *adhatoda vasica* points towards its ability to interfere the release and/or synthesis of mediates of inflammation, indicating its mast cell stabilizing activity.

Airway inflammation has been demonstrated all form of asthma. Even in mild asthma, there is an inflammatory response involving infiltration, peculiarly with activated eosinophils and lymphocytes, with neutrophils and mast cells. The degree of banchial hyperresponsiveness and airway obstruction is closely linked to the extent of inflammation. Thus inflammatory mediators have been implicated in the pathogenesis of allergic an inflammatory disorders like bronchitis. Anti-inflammatory drugs suppress the inflammation response by inhibiting infiltration and activation of inflammatory cells as well as their synthesis. Over lease of mediators and the effects of inflammatory mediators, the carrageenan induced paw edema model rats are known to be sensitive to cycloxygenase inhibitors. Since, serotonin, histamine and prostaglandins are the common mediators of both bronchial inhibitors. Since, serotonin, histamine and prostaglandins are the common mediators of both bronchial asthma and inflammation, the beneficial effect of alcoholic extract of *adhatoda vasica* could be due to inhibition of their release possibly due to inhibition of the enzyme cycloxygenase leading to inhibition of prostaglandin synthesis.

In India, the patients with bronchial asthma commonly prescribed with antibiotics. It has been reported that these patients are resistant to man antibiotics prescribed. It is possible that these patients are suffering from bronchial infection but have been diagnosed as asthmatic patients because of their symptoms like breathless. In allopathy, multidrug approach is there where patients receive bronchodilators, corticosteroids along with antibiotics. Sometimes, nonpathogenic bacteria accumulate due to the bronchial obstruction and plugging, causing serious infection. Plants produce a range of chemical substance to prompt themselves from the attack of various pathogenic microorganisms. The substances that can either ambit the growth of microorganisms or kill them are considered for developing new drugs for various infectious diseases. Use of these medicinal plants can substitute antibiotics to treat associated infection present study, water extract and alcoholic extract *adhatoda vasica* was found to possess good antimicrobial activity when tested against various respiratory pathogens that can be used to control respiratory complication. Water extract was found to be more active as compared to alcoholic extract.

Results of the experimental studies of *adhatoda vasica* suggest that anti-asthmatic activity could be due to its bronchodilator, mast cell stabilizing and antimicrobial property. The possible mechanism of action may be blockade of H_1 and Ach receptors leading to inhibitory of smooth muscle to respond to histamine and Ach induced spasm leading to inhibition of bronchoconstriction.

On the basis of all the studies we conclude that this plant has potential activity in petroleum extracts for anti-microbial and ethanolic extracts showed higher activity for analgesic, anti-inflammatory and anti-asthmatic activity.

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