WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL RESEARCH

Review Article

2024
Volume: 01
Issue: 02
Page: 25-48

REVIEW STUDY OVER NEURACANTHUS SPHAEROSTACHYUS DALZ.

Vimal Raikwar* and Dr. Vijay Nigam

Daksh Institute of Pharmaceutical Science Narayanpura Road, Chhatarpur Madhya Pradesh (India).

Received: 25 October 2024 **Revised: 15 November 2024** **Accepted: 05 December 2024**

Corresponding Author: Vimal Raikwar

Address: Daksh Institute of Pharmaceutical Science Narayanpura Road, Chhatarpur Madhya Pradesh (India).

ABSTRACT

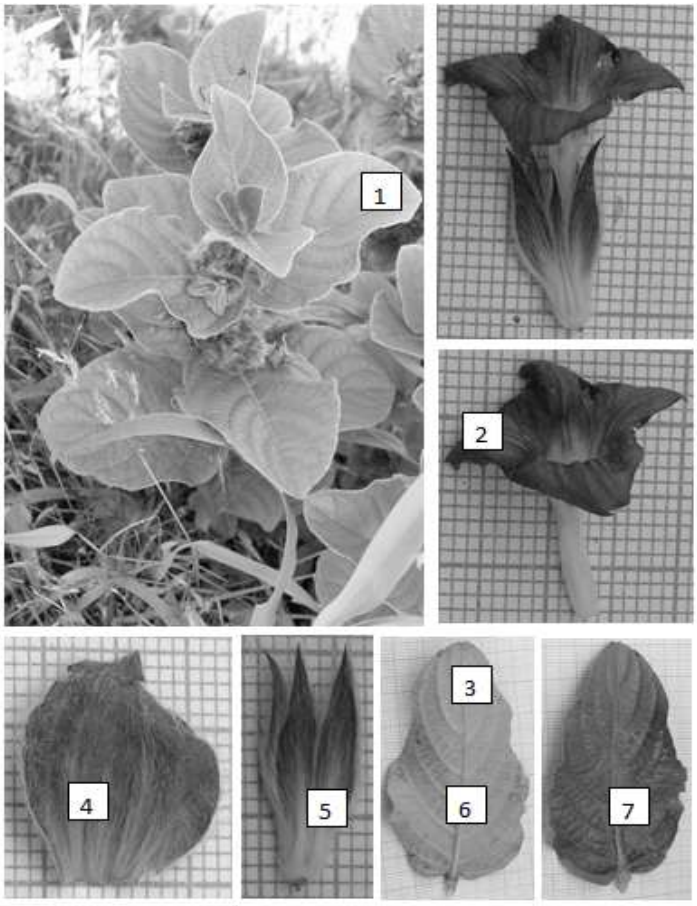
Neuracanthus sphaerostachyus Dalz. species shows the spherically arranged flowering head. Mostly flowers are observed in purple colour. It is an annual herb with 30-60 cm high.Flowers arranged in rounded clusters without a stalk. Leaves are 5-10 cm in length, and are almost without a stalk. *Neuracanthus sphaerostachyus* has been traditionally used to treat skin diseases,cough and asthma.The genus *Neuracanthus Sphaerostachyus Dalz* 113 species distributed across Asia, Southeast Asia, and Australia. *Neuracanthus Sphaerostachyus Dalz* plants grow in tropical and subtropical forests with humid lowlands.Featur ingtheir large greenheart-shaped or arrow-shape dear leaves and occasionally red-orange fruit, they are very popular ornamental plants and are widely used as traditional medicines to treat various diseases such as jaundice, snake bite, boils, and diabetes. This manuscript critically analysed the distribution, traditional uses, and phytochemical contents of 96 species of *Neuracanthus Sphaerostachyus Dalz.* The numerous biological activities of *Neuracanthus Sphaerostachyus Dalz* species were alsopresented, which include anti-cancer, antidiabetic and antihyperglycaemic, antioxidant, antidiarrhoea, antimicrobial and antifungal, antiparasitic (antiprotozoal and anthelminthic), antinociceptive and anti-inflammatory, brine shrimp lethality, hepatoprotective, anti- hemagglutinin, anti-constipation and diuretic, and radioprotective activities as well as acute toxicity studies. Research articles were acquired by the accessing three scientific databases comprising PubMed, Scopus, and Google Scholar. For this review, specific information was obtained

using the general search term "*Neuracanthus Sphaerostachyus Dalz*", followed by the "plant species names" and "phytochemical" or "bioactivity" or "pharmacological activity".The accepted authority of the plant species was referred from the plant list.org . Scientific studies have revealed that thegenus is mainly scattered throughout Asia. It has broad traditional benefits, which have been associated with various biological properties such as cytotoxic, antihyperglycaemic, antimicrobial, and anti-inflammatory. *Neuracanthus Sphaerostachyus Dalz* species exhibit diverse biological activities that are very useful for medical treatment. The genus *Neuracanthus Sphaerostachyus Dalz* was reported to be able to produce a strong and high-quality anti-cancer compound, namely alocasgenoside B, although information onthis compound is currently limited. Therefore,it is strongly recommended to further explore the relevant use of natural compounds present in the genus *Neuracanthus Sphaerostachyus Dalz*, particularly as an anti- cancer agent. With only a few *Neuracanthus Sphaerostachyus Dalz* species that have been scientifically studied so far, more attention and effortis required toestablish the linkbetween traditionaluses, active compounds, and pharmacological activities of various species of this genus.

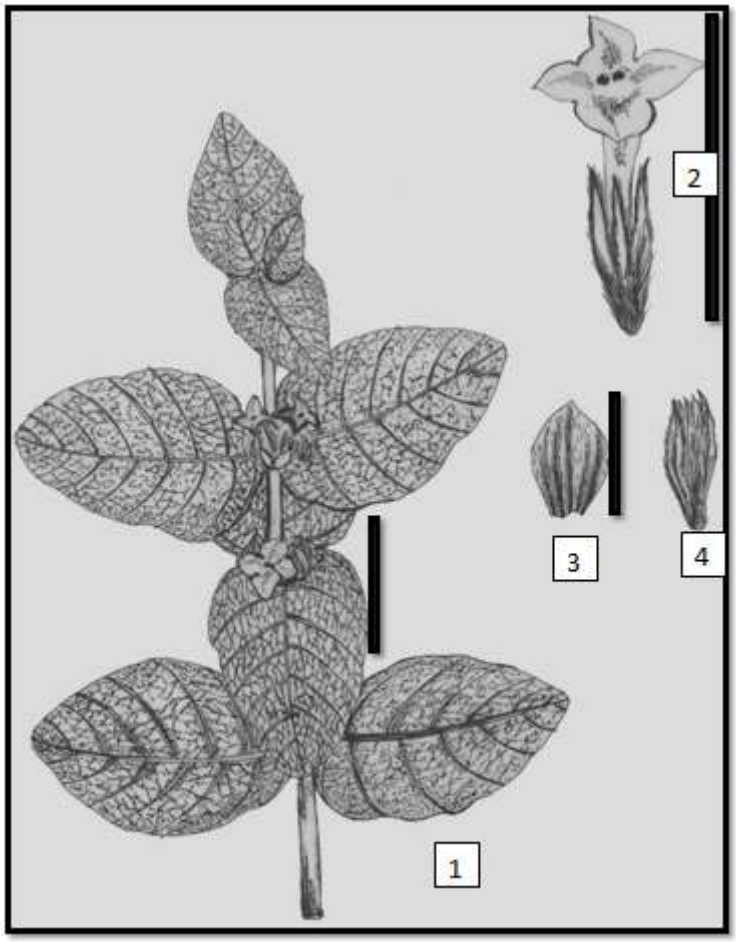
1. INTRODUCTION

Neuracanthu ssphaerostachyus Dalz. belongs to the family of Acanthaceae (Ruelliafamily),known as Pin cushion plant due toits floral structure and commonly known as Putliyo (Hindi) , Golgonda (Marathi) and Gantherae Gandharo (Gujarati). It is nativeto Indian regions and widely distributed in Western Ghats (Goa),Deccan and throughout the Gujarat. This plant is traditionallyused in different areas of Western Ghats. The mixture of ash ofwhole plant with jaggery or honey is used for 2-3 times a dayorally to cure cough and asthma.Root paste is applied onringworm *Neuracanthus sphaerostachyus* shows presence ofVanillic acid, Syringic acid, Melilotic acid and 6-OH Luteolin

Detail of *Neuracanthus sphaerostachyus Dalz* are mentioned below.



1.Habit,2.&3.Flower4.Bract.,5.Calyx,6.Leaf-dorsalview, 7. Leaf-Ventralview.



1.Habit,2.Flower,3.Bract,4:Twolippedcalyx.

Botanical name: **Neuracanthus spaerostachyus** (Nees)

Dalzell (=*Ledpidagathis spaerostachyus* Nees, = *N. lawii*)

Common name: Pin Cushion Plant

Hindi: Putliyo

Marathi: गोलगोडा Golgonda

Gujarati: Ganthera

Botanical name: ***Neuracanthus sphaerostachyus***

Family: ***Acanthaceae*** (Acanthus family)

Key characters: Stems not winged, Flowers in dense sphericalor globose spikes. Bracts grandular hairy, Bractoles shorter than calyx, Calyx entire. Seeds glabrous, Bands on pollen grains not wavy.

Flowering and fruiting: September-June.

Distribution: India–Endemic to Peninsular India.

Localities:-Through out state-Dhule,Kolhapur,Mumbai,Nasik,Pune,Raigad, Ratnagiri, Satara, Sindhudurg, districts.

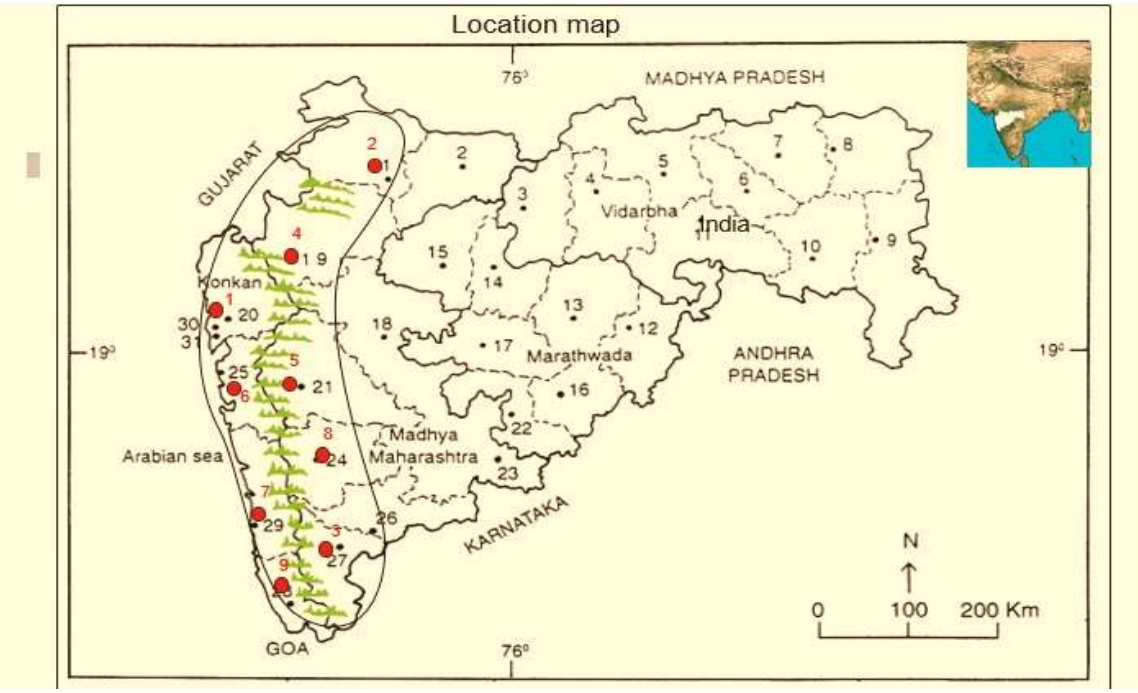
Ecology: Common on gentle hills lopes, open grass land sand fringes of forests. Altitude ranges between 600- 1200 m above sea level,

Traditional use of *neuracanthus sphaerostachyus*

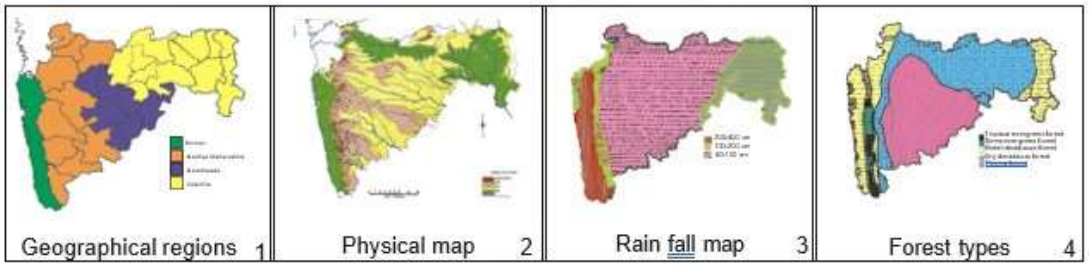
Neuracanthus sphaerostachyus is a plant that has been traditionally used in the treatment skin disorder, asthma and cough

- **Cough and asthma**: A mixture of the plant's ash with jaggery or honey is taken orally 2–3 times a day.
- **Skin diseases**: A paste of the plant's root is applied to treat ringworm.

Neuracanthus sphaerostachyus is also known as the pin cushion plant due to its floral structure. It is native to India and is commonly found in the Western Ghats, Deccan, and Gujarat. The plant is a perennial that grows in the seasonally dry tropical biome.



1.Dhule 2.Jalgaon 3. Buldhana 4.Akola 5.Amaravati 6.Wardha 7.Nagpur 8. Bhandara 9. Gadchiroli 10. Chandrapur 11. Yavatmal 12. Nanded 13. Parbhani 14. Jalna 15. Aurangabad 16.Latur 17. Beed 18. Ahmadnagar 19 Nashik 20. Thane 21. Pune 22. Osmanabad 23. Solapur 24.Satara 25. Raigad 26. Sangli 27. Kolhapur 28. Sindhudurg 29. Ratnagiri 30.Mumbai 31.Greater Mumbai.



Localities-

1. Bombay, 2. Dhule, 3. Kolhapur, 4. Nasik, 5. Pune, 6. Raigad, 7. Ratnagiri, 8. Satara, 9. Sindhudurg

Geographical distribution:

Latitude (DMS): Between 18 ° 31'- 16° N & Longitude (DMS): 73 ° 50'-74°.5' E;

2. MATERIALS AND METHODS

2.1Collection of plant

Neuracanthus sphaerostachyus Dalz leaves were collected from Girnar forest region of Gujarat.

2.2 Sectioning

Freeh and sections of leaves were taken and cleared with chloral hydrate. The sections of plant materials were treated with phlor-oglucinol and a drop of concentrate dhydrochloric acid to stain thelignified elements. The sections were also

stained with dilute so-lution of iodine to study the starch grains. For study of the powder drug, the dried leaves were ground andpassed through a sieve 60 mesh.

2.3 Quantitative microscopy

Plant leaves were washed thoroughly and followed by stainingwith safranin for quantitative microscopic studies.

2.3.1 Stomatal number

It is the average number of stomata per square mm of the epidermis of the leaf. A minimum often readings were taken from different locations of the leaf and the average value was counted.

2.3.2 Stomatal Index

It is the percentage inwhich the number of stomata to the total number of epidermal cells, each stoma being counted as one cell. Stomatal Index was counted by using the following equation,

$S.I=(S/E+S)\times 100$

where, S.I=Stomatal Index,

S=Number of stomata per unit area,

E = Number of epidermal cells in the same unit area.

2.3.3Vein-Islet and vein termination nnumber

Counted the number of vein-islets in 4 sq.mm area in the central part between the mid rib and the margin on leaf surface. Counted the number of vein termination sin4sq.mm area of the central part between the midrib and the margin on leaf surface.

2.3.4 Palisade ratio

It is the average number of palisade cells beneath eachepidermal cells of a leaf. It is determined by counting the palisadecells beneath four continuous epidermal cells.

2.4 Powder microscopy

The dark brown fine powder was treated with different chemical reagents for the detection of the phytoconstituents with colour changes and observed under ordinary daylight by the standard method.

2.5Loss on drying (gravimetric determination)

It is the amountof volatile matterof any kind (including water)that can be driven off. One gram of each of the samples was weighed accurately, spread on shallow Petri dish and heated at a Regulated temperature of 105±1°C to constant weight. The samples were weighed immediately after removing from the oven.Loss on drying is expressed as the loss in weight in percent w/w.

2.6 Determination of pH

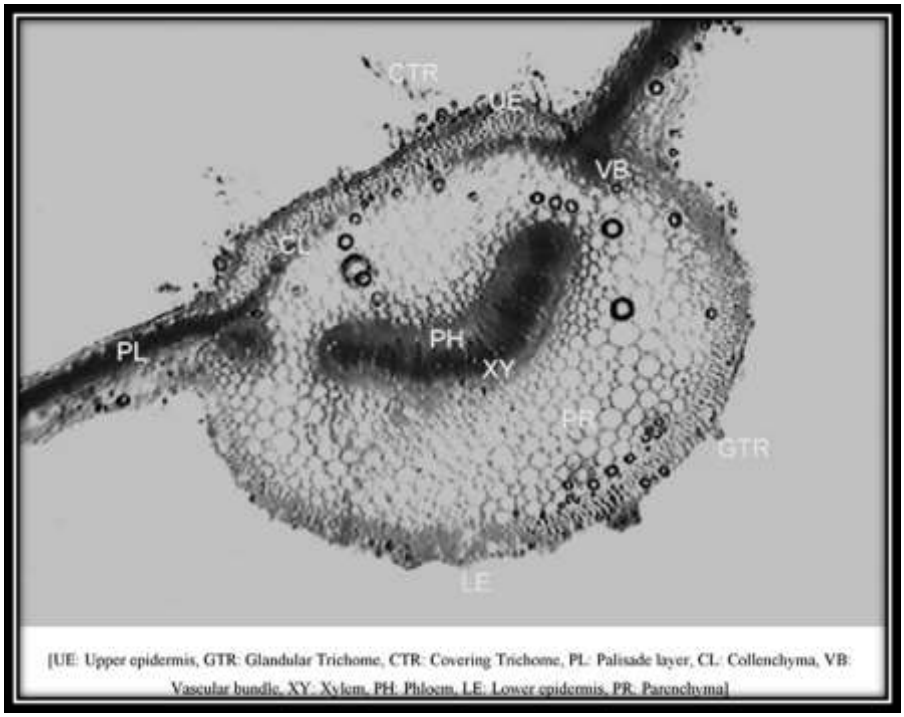
The pH value represents the acidity or alkalinity of an aqueous solution.

2.6.1 pH of 1% solution

Accurately weighed 1g of air-dried coarsed powdered material dissolved in 100 ml of distilled water. The pH of the water solubleportion was measured with calibrated pH meter at 25 °C.

2.6.2 pH of 10%solution

Accurately weighed 10 g of air-dried; coarsely powdered ma-terial was dissolved in 100 ml of distilled water. The pH of thewater soluble portion was measured with standardized pH meterat 25 °C.



[UE: Upper epidermis, GTR: Glandular Trichome, CTR: Covering Trichome, PL: Palisade layer, CL: Collenchyma, VB: Vascular bundle, XY: Xylem, PH: Phloem, LE: Lower epidermis, PR: Parenchyma]

[MCT: Multicellular Covering Trichome, SGT: Sessile glandular Trichome]

2.7 Determination of ash values

2.7.1 Total ash

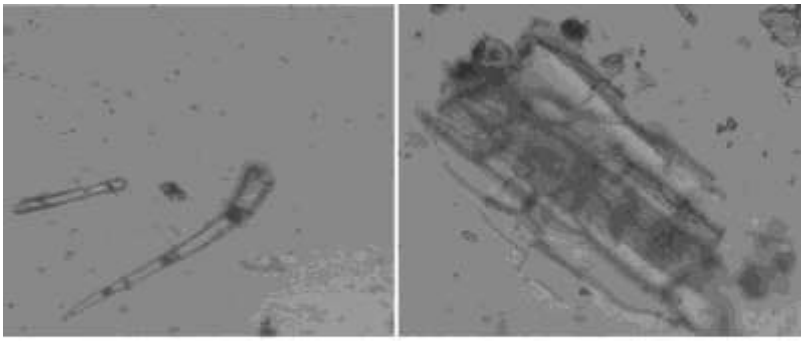
About 8 g of the ground air-dried material, accurately weighed, inaignited and tared crucible and incinerated in a muffle furnace at a temperature not exceeding 450 °C

until the formation of ash. The sample was then cooled and transferred to desiccator and then weighed. The percentage of total ash was determined with reference to air dried sample.

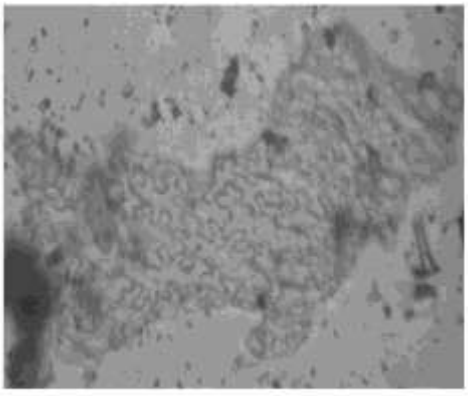
2.7.2 Acid in soluble ash

To the crucible containing the total ash added 25 ml of 2N hydrochloric acid and boiled for 5min. The insoluble matter collected on filter paper (ash less). Filter paper was washed with hot wateruntil the filtrate became neutral. It was ignited to constant weight and allowed the residue to cool in a desiccator, then weighed immediately. The acid insoluble (percentage) ash was calculated in reference to air dried material.

2.7.3 Water soluble ash



Trichomes of Neurocanthus Fibres with parenchymatous cells



Fragment of Epidermis showing wavy walled cells of Neuracanthus

To the crucible containing the total ash, added 25ml of distilled water and boiled for 5 min. The insoluble matter was collected onash less filter paper and washed with hot water and ignited toconstant weight. The residue was allowed to cool in a desiccatorand then weighed immediately. The weight of insoluble ash wassubtracted from the weight of total ash and the difference inweight indicated the weight of

water-soluble ash. The percentageof water-soluble ash was calculated with reference to air-driedmaterial.

Table1: Determination of leaf constant of *Neuracanthus sphaerostachyus.*

Sr.no.	Parameter	Range	Average
1.	Stomatalnumber	7-11	9
2.	Stomatal index	2-5	4
3.	Vein Islet number	16-19	17
4.	Vein Termination number	9-13	11
5.	Palisade Ratio	3-5	4

Table 2: Histo chemical colour reactions of *Neuracanthus sphaerostachyus*.

Reagent	Constituent	Colour
Conc. H_2SO_4	Saponins or Lipids	Bluishblack
Weak Iodine solution	Starch	e
Millonsreagent	Protein	Red
Dragendroffregent	Alkaloids	e
Phloroglucinol+ HCl	Lignin	Faintpink
Mg+HCl	Flavonoids	Yellow
IodineSolution	Cellulose	e
Causticalkali+HCl	Calciumoxalate	Green

Table 3: Behaviour of the powder with different chemical reagents on leaves powder of *Neuracanthus sphaerostachyus.*

Reagent	Colour/Precipitate	Constituent
Aq. $FeCl_3$	No change	Tanninsabsent
Ammonia solution	No change	Anthraquinone glycosides absent
5% Aq. KOH	No change	Anthraquinoneglycosidesabsent
Aq. $HgCl_2$	No precipitate	Alkaloids absent
Aq. $AgNO_3$	White precipitate	Protein present
Conc. H_2SO_4	Reddish-pink	Lipids present
MgeHCl	Yellow	Flavonoidspresent
PicricAcid	Nochange	Alkaloidsabsent
Iodine Solution	No change	Starch absent

2.8 Chemicals and drugs

All the used chemicals were of analytical grade and were pro-cured from Chemdyes Corporation, Rajkot, India.

2.9 Statistical analysis

A result of pH values and Ash values has been expressed as results ± Standard Deviation(S.D.).

Evaluation of anti-asthmatic activity of*Neuracanthus sphaerostachyus*Dalz. leaveextract

Neuracanthus sphaerostachyus has been used traditionally to cure skin diseases , cough , and asthma western ghat. Extensive review of literature and evidences indicating the utility of this plant in the treatment of asthma prompted us to investigate the anti asthmatic activity of the plant in different experimental screening method

In vitro & in-vivo anti-asthmatic activity of the methanolic and aqueous extract *Neuracanthus sphaerostachyus* leaves was investigated using various experimental models. In-vivo studies compound 48/80 induced systemic anaphylaxis and In vitro studies like compound 48/80 induced mastcell degranulation, Milk induced leucocytosis & eosinophilia was evaluated at 125, 250, and 500 mg/kg, orally dose. Methanolic and aqueous extract of the drug showed a significant bronchodilation, anti-histaminic, mast cstabilizing, and anti-cholinergic activity in their respective evaluation parameters dose-dependently. Ketotiffumarate (10µg/ml),Dexamethasone (50mg/kg)and Disodium chromo glycate (10mg/kg)were used asastand control The present Study concluded That Methaholic and Aqueous Extract of *Neuracanthus sphaerostachyus* has Significant anti-asthmatic Potential Benefit.

Keywords: Neuracanthus Sphaerostachyus compound 48/80, mast cell degranulation, leukocytosis, eosinophilia

Introduction

Asthma is a chronic inflammatory disorder with narrowing of bronchial airways (Limbasiya, 2012). World health organization stated asthma as a chronic disease with episodes of the airways and can be viewed as a syndrome (WHO, 1991).Asthma involves bronchial tubes and show sweezing, shortness of breath and coughing. It is an allergic reaction which induces inflammation leading to narrowing of the airways and causes spasm and difficulty in breathing.Asthma can be seen in both children and adults in developed as well as developing countries. Allergic patients are more prone to develop asthma. Asthma may involve various inflammatory cells like eosinophils, macrophages, mast cells, epithelial cells, and activated lymphocytes.

Inflammatory cells go on recruit various cytokines, adhesion molecules, and other mediators. Inflammation may progress to an acute, sub-acute or chronic phase which produces airway remodeling, an increase in vascular permeability as well as increases in mucus secretion. It

alters airway structure reversibly or permanently.

Herbal medicines are the backbone of about 75 to 80% of the world, especially in developing countries. It has better access to primary health care with good acceptability, more compatible with the human body and, less side effects. While focusing on the biological activities of plants during the last decade, it shows the presence of plenty of compounds with anti-asthmatic potential (Singh et al., 2014).

Extraction of plant material

Extractive values of crude drugs were used to determine the number of active constituents extracted with solvents from a given amount of medicinal plant material. The successive extraction was carried out in soxhlet apparatus with a known quantity of powder in different organic solvents like Hexane, chloroform, methanol and then water.After exhaustive extraction, the solvent was filtered and concentrated under reduced pressure at 50-55°C (Indian Pharmacopoeia, 1966).

Animals

Female Wistar rats (150-200 g) and Swiss albino mice (25-30 g) were used for the study. The animals were kept in polypropylene cages and maintained at a temperature of 26 ± 2 C. Animals were ° fed with diet a guide for the care and use of laboratory animals. The animals were acclimatized for 10 d under standard husbandry conditions as Relative humidity 45 - 55%, and 12 h light and dark cycle (CPCSEA, 2003).

Acute toxicity study

Female Wistar rats of 150 – 200 g and Swiss albino mice of 25- 30 g body weight were selected to find out the acute toxicity study of methanolic and aqueous extract of Neuracanthus sphaerostachyus leaves.

The dose of 2000 mg/kg was selected on the basis of Up and Down Procedure (UDP) as per OECD Guideline No.425. All animals were observed for 24 h to detect autonomic or behave oral changes in responses to the extracts. Then the Mortality in each group was observed for 14 d (OECD, 2001).

The methanolic and aqueous extracts of leaves of Neuracanthus sphaerostachyus were found to be nontoxic at a dose of 1500 mg/kg, orally. Hence, LD cut off value of methanolic and aqueous extract were fixed as 1500 mg/kg. Therefore1/10 , 1/6 , 1/3 of the LD cut off value

that was th th rd 50 approximately 150, 250 and 500 mg/kg were selected as screening dose for anti-asthmatic activity. Assessment of anti-asthmatic activity in vitro Compound 48/80 induced degranulation of rat mesenteric mast cells

Normal saline containing 5 units/ml of heparin was injected in the peritoneal cavity of male rats lightly anesthetized with ether. After a gentle abdominal massage, peritoneal fluid containing mast cell was collected in centrifuge tubes placed over ice. Peritoneal fluid of 5 rats was collected and centrifuged at 2000 rpm for 5 min. The supernatant solution was discarded and the cells were washed twice with saline and re-suspended in 1 ml of saline. (MENS- Methanolic extract of leaves, AENS – Neuracanthus sphaerostachyus Aqueous extract of leaves) Neuracanthus sphaerostachyus

Test tube I: Normal control, 0.1 ml peritoneal fluid
Test tube II: Disease control, 0.1 ml peritoneal fluid + 0.1 ml compound 48/80.
Test tube III: Standard control, 0.1 ml peritoneal fluid + 0.1 ml compound 48/80 + 0.1 ml Ketotifen fumarate (10 g/ml)µ
Test tube IV: 0.1 ml peritoneal fluid + 0.1 ml compound 48/80 + MENS (0.5 mg/ml)
Test tube V: 0.1 ml peritoneal fluid + 0.1 ml compound 48/80 + MENS (1 mg/ml)
Test tube VI: 0.1 ml peritoneal fluid + 0.1 ml compound 48/80 + MENS (2 mg/ml)
Test tube VII: 0.1 ml peritoneal fluid + 0.1 ml compound 48/80 + AENS (0.5 mg/ml)
Test tube VIII: 0.1 ml peritoneal fluid + 0.1 ml compound 48/80 + AENS (1 mg/ml)
Test tube IX: 0.1 ml peritoneal fluid + 0.1 ml compound 48/80 + AENS (2 mg/ml) Each test tube was incubated for 15 min at 37°C with peritoneal fluid and respective drug treatment. Compound 48/80 (0.1 µg/ml) was added to each test tube except test

Tube no. I. After further incubation for 10 min at 37°C, the cells were stained with 0.1 % toluidine blue solution made in distilled water and examined under the high power of light microscope. Same procedure was repeated in triplicate manner. % degranulation of the mast cells in the different test tubes treated with different doses of methanolic and aqueous extracts was calculated by counting the number of degranulated mast cells from total of at least 100 mast cells counted under microscope and from it percentage protection against degranulation was calculated. Each piece was observed under high power light microscope (Vogel, 2008).

% Protection of mast cell from total of at least 100 mast cells will be counted.
% Protection of mast cells=Total mast cells-Degranulated mast cells

Milk induced leucocytosis and eosinophilia

Swiss albino mice (female) of 25-30 gm were selected and randomly divided into nine groups of six each. All samples including suspensions, solutions of drugs and plant extracts were freshly prepared. The extracts were used as a suspension in 0.5% v/v Tween 80 in normal saline (0.9%) and administered orally. Blood samples were collected from retro orbital plexus under light ether anesthesia and different groups were assigned as described below:

Group I: Normal control, Distilled water
Group II: Disease control, Boiled cooled milk (4 ml/kg, s.c.)
Group III: Standard control, Dexamethasone (50 mg/kg i.p.)
Group IV: Boiled, cooled milk + MENS (150 mg/kg)
Group V: Boiled, cooled milk + MENS (250 mg/kg)
Group VI: Boiled, cooled milk + MENS (500 mg/kg)
Group VII: Boiled, cooled milk + AENS (150 mg/kg)
Group VIII: Boiled, cooled milk + AENS (250 mg/kg)
Group IX: Boiled, cooled milk + AENS (500 mg/kg)

Treatment drugs were administered orally 1 h before milk injection. Total leucocyte and eosinophil count was performed in each group before and after 24 hours of milk injection. Total and differential count were determine Total leucocyte and eosinophil count was measured before and 24 hours after milk injection. Assessment of anti-asthmatic activity in vivo Compound 48/80 induced systemic anaphylaxis The study was conducted by using swiss albino mice. Compound 48/80 was administered by intraperitoneal route to the albino

Mice which causes mortality. The mice of different 9 groups were pretreated with respective different doses of various plant extract by oral route and the standard drug, Disodium chromoglycate by intraperitoneal route. After one hour of the treatment compound 48/80 was given by intraperitoneal route
Group I: Normal control, saline (1 ml/kg)
Group II: Disease control, Compound 48/80 (8 mg/kg)
Group III: Standard control, Disodium chromoglycate (10 mg/kg)
Group IV: Methanolic extract of leaves of Neuracanthus sphaerostachyus (150 mg/kg)
Group V: Methanolic extract of leaves of Neuracanthus sphaerostachyus (250 mg/kg)

Group VI: Methanolic extract of leaves of Neuracanthus sphaerostachyus (500 mg/kg)
GroupVII: Aqueous extract of leaves of Neuracanthus sphaerostachyus (150 mg/kg)
Group VIII: Aqueous extract of leaves of Neuracanthus sphaerostachyus (250 mg/kg)
Group IX: Aqueous extract of leaves of Neuracanthus sphaerostachyus (500 mg/kg)

The mortality was observed after 1 hour of anaphylactic shock. Percentage of mortality was calculated by using the following formula (Chitme, 2010, Subhashini et al., 2013, Mehta, 2008, Das and Chauhan 2013).

Percentage of mortality= (No.of dead mice/Total No. of mice) ×100

Statistical analysis

All values are presented as mean ± SEM of six animal Differences between means were assessed by one-w analysis of variance (ANOVA) followed by Dunnett's teP<0.05wasconsidered significant.

3 RESULTS

Macroscopic evaluation of *N.sphaerostachyus* leaves revealed that the leaves are simple,green,oppositeandsessileabout10-12 cm in length and 5-7 cm wide with elliptic oblong Shape The apex is acute, base is cordate. The leaves are having reticulate venation. The margin is wavy and Surface is pubescent. Odour is slight and Taste is acrid and mucilaginous.

Microscopic studies of *N. sphaerostachyus* leaves shows Lamina with upper epidermis which is single layered, wavy walled cells covered with cuticle, containing 3-4 celled multicellular covering trichomes and sessile glandular trichomes. Mesophyll is dorsi ventral and Palisade is single layered, compact and radially elongated cells. Spongy parenchyma in 2-3 layered, loosely arranged with intercellular spaces and lower epidermis resembles the upper epidermis but numbers of trichomes were less. Midrib with collenchym, thick walled cellulosic cells arranged in 3-4 layers below the upper epidermis and above the lower epidermis .Vascular bundles with arc shaped, collateral and 3 in number with two lateral and one median, phloem were well developed and xylem consists of vessel elements with tracheid es and fibres. Powder microscopy of *N.sphaerostachyus* leaves revealed trichomes, fibres and epidermal cells. Trichomes were multicellular covering

trichomes and sessile glandular trichomes. Fibres passing through parenchymatous cells were present and epidermal cells found as a polygonal fragment with wavy walled epidermal cells.

Leaf constant of *N. sphaerostachyus* were determined with different parameters and presented in. Transverse sections of leaves of *N.sphaerostachyus* using standard method were treated with different reagents and the results are given in. Behaviour with different chemical reagents was performed on the crude powder of Leaves of *N. sphaerostachyus* using different re-agents to detect the phytoconstituents with colour changes under ordinary daylights by the standard method and characteristic changes observed are summarized in. Fluorescence characteristic of the powder as such and after treating with some chemical reagents were observed in daylight as well as under ul-traviolet radiations. The results are recorded in. The standard physico chemical protocols developed from the Indian Pharmacopoeia were followed by calculating moister content, Ph and ash values. The values obtained are presented in.

Compound 48/80 induced degranulation of rat mesenteric mast cells

MENS was significantly effective in inhibiting compoud 48/80 induced degranulation of rat mesenteric mast cells 2 mg/ml dose with 76.26 % inhibition of degranulation Standard anti-asthmatic drug ketotifenfumarate showed maximum inhibition of degranulation with82.46%acomparedwithdiseasecontrol

Milk induced leucocytosis and eosinophilia

The effects of extracts of MENS and AENS on milk induce cytosis and eosinophilia are shown in table 2 and The methanolic extractact 500 mg /kg dose showed

Table 1: Effect of MENS and AENS onc ompound 48/80 induced degranulation of ratmesenteric mast cells.

Treatment	Concentration(mg/ml)	%Mastcelldegranulation	%Inhibitionofdegranulation
Normalcontrol	-	2.03±0.256	-
Diseasecontrol	-	72.83±0.546	-
Standardcontrol	0.01	12.362±0.245***	82.46
MENS	0.5	28.25±1.023*	61.23
MENS	1	22.56±0.356*	64.43
MENS	2	17.03±0.589**	76.26
AENS	0.5	33.56±0.128*	56.74
AENS	1	26.28±0.652*	61.29
AENS	2	20.46±0.458**	72.56

Values were expressed as mean ± SEM. N = 6 in a group ;* p <0.05,**p<0.01,***p<0.001; Vs Disease control. Data areanalysed by using one wayANOVfollowedby Dunnett's test.

Table 2: Effect of MENS and AEN Sonmil kinducedleu kocytosisinmice.

Treatment	Concentration (mg/kg)	Differenceintotalleucocyte count(cu/mm)
Normalcontrol	-	78±11.25
Diseasecontrol	-	**4**56±218.12
Standardcontrol	50	2108±158.06****
MENS	150	4123±128.24
MENS	250	3624±246.42*
MENS	500	2621±214.24**
AENS	150	4223±198.26
AENS	250	3841±126.84*
AENS	500	2832±196.08**

Valueswereexpressedasmean±SEM. N=6inagroup;*p<0.05,**p<0.01, ***p<0.001;VsDiseasecontrol.DataareanalysedbyusingonewayANOVAfollowedby Dunnett's test

Treatment	Concentration (mg/kg)	Differenceineosinophi count(cu/mm)
Normalcontrol	-	34±3.21
Diseasecontrol	-	148±8.36
Standardcontrol	50	56±2.36***
MENS	150	110±5.34
MENS	250	91±6.32*
MENS	500	62±4.28**
AENS	150	118±6.26
AENS	250	96±4.48*
AENS	500	64±2.64**

Valueswereexpressedasmean±SEM. N=6inagroup;*p<0.0 **p<0.01,***p<0.001;VsDiseasecontrol.DataareanalysedbyusionewayANOVAfollowedbyDunnett'stest

Maximum anti-asthmatic effect .The anti-asthmatic effect of the MENS was more potent and significant,comparedtoAENS.

Compound 48/80 induced systemic anaphylaxis

Compound 48/80 showed 100% mortality in mice. Standard control–DSCG showed 83% protection from mortality. Treatment with MENS 250, 500 mg/kg and AENS 250, 500mg/kg showed protection dose dependently against compound48/80 induced mortality. A significant protection (67%) was observed at 500mg / kg of MENS treatment

4 DISCUSSION

All medicinal systems have developed quality control and for herbal medicines, standardization is also required to assure the quality of the drug. These parameters help in the proper identification of plant with their substitute herbal materials which often found in the market. Human usage can be permitted after ensuring the iridentity, quality, purity and safety of drug .Various studies like microscopic analysis, macroscopic analysis and fluorescence

Table 4: Fluores cencecharacteristics of powdered leaves of *Neuracanthus sphaerostachyus*.

Sr.no.	Treatment	UnderOrdinarylight	UVlight	
			Long	Short
1	Powder	Greenish	DarkGreenish	LightBrownish
2	Drypowder+ Nitrocellulose	BlackGreenish	Darkgreenishbrown	Greenish brown
3	Powder+1MNaOHinMethanol	Yellowishgreen	Yellowishgreen	Darkblackishgreen
4	Powder+1NNaOHinMethanol,mountedwithNitrocellulose	DarkYellowishgreen	DarkYellowishgreen	Brown
	inAmylacetate			
5	Powder+1MHCl	Lightgreen	Darkgreen	Brownish
6	Powder+1MHCl,mounted with Nitrocellulosein Amyl acetate	Lightgreen	Darkgreen	Brownish
7	Powder+1MNaOH	Lightyellowishgreen	Yellowishgreen	Lightbrown
8	Powder+1MNaOH,mounted with Nitrocellulose in Amyl acetate	Greenish brown	Black	Brightgreenishbrown
9	Powder+50%HNO_3	Greenish	Darkgreen	Darkbrown
10	Powder+50%H_2SO_4	Green	Darkgreen	Greenishblack

Analysis identify the particular drug and provide authenticity of raw material . Morphological and microscopical studies of leaves will be helpful in the identification of *N.sphaerostachyus Dalz.* Quantitative analysis of pharmacognostic characters may be helpful to establish quality standards of the plant. These simple, inexpensive, reliable standards can help society whenever using the drug as folk medi-cine. These studies will also be helpful for manufacturer for assessing the purity of raw material. The methods mentioned here can be considered as characteristics to identify and auth enticate this drug.

Table 5: Physicochemical values of *Neuracanthus sphaerostachyus* leave powder.

Parameters	Results±S.D.
Organolepticcharacteristics Appearance	Powder
Colour	Greenish
Odour	Characteristics odour
Taste	Acrid
Lossondrying	7.8±0.48
pHvalue pHof1%aqueoussolution	5.43±0.01
pH of10% aqueoussolution	5.92±0.01
Ashvalues(%) Totalash	4.72±0.11
Water solubleash	1.35±0.11
Acid in solubleash	2.47±0.11

Mast cell degranulation is an important parameter into initiation of many immediate responses following exposure with kinds of allergens. Degranulated mast cel all owto release mediators of inflammation like histamine leukotrienes ,platelet activating factors (PAFs) wiche motactic factors for eosinophils, neutrophilse (Limbasiya, 2012). Compound 48/80 (10 µg/ml) produc signific ant disruption of mast cells which was significant inhibited in a dose-dependent manner by pretreatment with emethanolic and aqueous extracts of *Neuracanth*

Table 4: Compound48/80inducedsystemicanaphylaxis.

Sr.No.	**Treatment**	**Dose(mg/kg)**	**%Mortality**
III	NormalcontrolDiseasecontrol	-8	-100
III	Standardcontrol	10	17***
IV	MENS	150	100
V	MENS	250	50**
VI	MENS	500	33***
VII	AENS	150	100
VIII	AENS	250	83
IX	AENS	500	50**

Values are expressed as mean ± SEM. N=6inagroup;*p<0.05,**p<0.01,***p<0.001 Vs Disease control. Data are analysed by using one way ANOVA.Mortality (%) within 1 h following compound 48/80 injection was representedas the numberofdeadmice ÷totalnumber ofmice×100.

Sphaerostachyus in 0.5, 1 and 2 mg/kg doses. The results were comparable to the reference standard ketotifen (10µg/ml). Thus it indicates that MENS has significant mast cell stabilizing activity.

Leukocyte recruitment releases the inflammatory mediators such as cytokines, histamine and other asthmatic inflammatory cells promoting ongoing inflammation. Biopsies taken from asthmatics exhibits eosinophil as the most characteristic inflammatory cell in the respiratory tract and reside in submucosal and epithelial layers.Eosinophil count to more than 4 % of total leukocyteisanab normal increase and termed as eosinophilia.

Most cases of asthma involve increase in the eosinophil count. Eosinophilisa critical contributing parameter involving bronchial mucosa with allergic inflammation to the late asthmatic reaction producing hyper-secretion of mucus and bronchial congestion. Eosinophilplaysrole as an inflammatorycellduring laterphasesin the development of allergic asthma which secrete mediators such as tumor necrosis factor (TNF-α),eosinophil cationic protein, neurotoxins derived from eosinophil and prostaglandins resulting in epithelial shedding, Bronchospasm ,and allergic inflammation in the respiratory tract.

Milkinducedleukocytosisandeosinophiliainmicemimicsthecondition of stress induced asthma and it helps to evaluate such clinical findings (MaliandDhake,2011). It has been exhibited that parental administration of milk leading to increasing leukocyte and eosinophil count significantly after 24 hours of administration. Among both these extracts, 250 and 500 mg /kg dose has shown significant activity as compared to standard Dexamethasone (50mg /kg)in a dose-dependent manner.

Systemic anaphylaxis involves allergic reactions to occur and may exhibit fatal consequences .The process can be demonstrated by challenging mice with compound 48/8Untreated mice may show 100 % mortality. It involve.

Stchain of reaction with decreased cyclic AMP production histamine release due to the influx of Ca^{+2}(Kimataeta2000).However ,pre-treatment with both them ethanolic a aqueous extracts of leaves of *Neuracanthus sphaerostachy* showed significant protection up to 67% with a low mortalrateat500mg/kgdose.

CONCLUSION

The present study establishes acute toxicity study,invitro invivo anti-asthmatic screening for methanolic and aqueous extracts of leaves of *Neuracanthus sphaerostachyus.* Tanti-asthmatic activity of *Neuracanthus sphaerostachyus* might be due to the presence of Flavonoid (6-OH Luteoliand other various potential phytoconstituents like

Phenocompounds /Tann ins, Steroids and,Triterpenoids Methanolic extract shows significant an asthmatic activity at a dose of the 500mg /kg .Furth investigations are required to extrapolate active comp one of the extract and to establish the mechanism of action.

REFERENCES

1. Khare CP. Indian medicinal plants, an illustrated dictionary. Springer Publication, 2007; 372.
2. Punjani BL, Kumar V. Traditional medicinal plant remedies to treat cough andasthmatic disorders in the Aravalli ranges in North Gujarat, India. J Nat Rem, 2002; 2/2: 173e8.
3. Daniel M, Sabnis SD. Chemosystematics of some Indian members of the A canthaceae. Proc Indian Acad Sci (Plant Sci), 1987; 97(4): 315e32.
4. WallisTE.Textbookofpharmacognosy,vol.4.CBSPublications, 1958; 139e40.
5. Trease GE, Evans NC. Pharmacognosy. London: Crowell-Collier and Macmillan Publishers Ltd., 1972; 140e7.
6. Kay LA. The microscopical studies of drugs. 1st ed. London: Bailliere and Cox, 1938; 138e42.
7. Johanson DA. Plantmicrotechnique. NewYork: McGrawHillandCo, 1940; 183.
8. WallisTE.Textbookofpharmacognosy.London:J&AChurchillLtd.;1967.5.
9. Chase CR, Pratt RJ. Fluorescence of powdered vegetable drugs with particularreference to development of a system of identification. J Am Pharm Assoc1949;38:324e31.
10. WHO. Quality control methods for medicinal plants, vol. I. Geneva: World Health Originations; 1998. p. 30e3.
11. Anonymous. Indianpharmacopoeia. 2nded. New Delhi: Government of India; 1966. p. 33e4.
12. Cooke.T(1904):Flora of Presidency of Bombay.Vol.2:465.(rep.Ed.1967)
13. Hooker, J.D. *et al.*(1885):The flora of British India. London. Vol.4: 491. Santapau, H. & P.V. Bole.(1952).A note on*Neuracanthus sphaerostachyus*
14. Dalz.*J.Bombaynat.Hist.Soc*.52:897-900.
15. Singh N.P. & S. Karthikeyan (2000):(eds), Flora of Maharashtra state, Dicotyledones.Vol. 2: . B.S.I., Calcutta:652.

16. Tetali, *et al*. (2000): Endemic Plants of India (A Status Report ofMaharashtra State). Naoroji Godrej Centre for Plant Research, Shirwal: 42.
17. Anonymous. Indian Pharmacopoeia. 1966. Government of India, 2nd ed., 33-4.
18. Chitme HR, Malipatil M, Chandrashekhar VM, Prashant PM. 2010. Antiallergic activity of aristolochia bracteolate Lank in animal model. Indian Journal of Experimental Biology 48: 46-52.
19. Committee for the Purpose of Control and Supervision on Experiments on Animals. 2003. CPCSEA guidelines for laboratory animal facility. Indian Journal of Pharmacology 35:257-74.
20. Daniel M, Sabnis SD. 1987. Chemosystematics of some Indian members of the acanthaceae. Indian Academy of Sciences (Plant Sci) 97(4):315-32.
21. Das B, Chauhan R. 2013. Anti-histaminic and mast cell stabilizing Activity of a fern-lygodium flexuosum. International Journal of Life Sciences Biotechnology & Pharma Research 2(3): 1-13.
22. Khare CP. 2007. Indian medicinal plants, An illustrated dictionary, Springer publication 372-3. Limbasiya KK, Modi VR, Tirgar PR, Desai TR, Bhalodia PN
23. 2012. Evaluation of anti-asthmatic activity of dried whole plant extract of using various experimental Leucas aspera animal models. International Journal of Phytopharmacology 3(3): 291-98.
24. Mali RG, Dhake AS. 2011. Evaluation of effects of Bauhinia variegata stem bark extracts against milk-induced eosinophilia in mice. Journal of Advances in Pharmceutical Technology and Research. 2(2): 132–34.
25. Kimata M, Shichijo M, Miura T, Serizawa I, Inagaki N, Nagai H. 2000. Effects of luteolin, quercetin and baicalein on immunoglobulin E-mediated mediator release from human cultured mast cells. Clinical and Experimental Allergy 30(4): 501-8.
26. Mehta AA, Agrawal B. 2008. Investigation into the mechanism of action of Moringa oleifera for its anti-asthmatic activity. Oriental Pharmacy and Experimental Medicine 8(1): 24-31.
27. Punjani BL, Kumar V. 2002.Traditional medicinal plant remedies to treat cough and asthmatic disorders in the Aravalli ranges in North Gujarat, India. Journal of Natural Remedies 2(2): 173-8.
28. Singh SK, Patel JR, Dubey PK, Thakur S. 2014. A review on antiasthmatic activity of traditional medicinal plants. International journal of pharmaceutical sciences and research 5(10): 1000-07.

29. Subhashini, Chauhan PS, Sharda K , Dash D, Singh R. 2013. Curcumin Inhibits Compound 48/80 Induced SystemicAnaphylaxis American Journal of Life Sciences 1(4): 165-170. .
30. Taur DJ, Patil RY. 2012. Effect of leaves on Abrus precatorius milk induced leukocytosis and eosinophilia in the management of asthma. Asian Pacific Journal of Tropical Biomedicine S40-S42.
31. Vogel HG. 2008. Drug discovery and evaluation, Pharmacological Assays, 3rd ed. 511 - 547.
32. WHO 1991. Chronic respiratory diseases. Available at. http:// www.who.int/ respiratory/ asthma/ en.