ORIGINAL ARTICLE

In Vitro, In Vivo Antiasthmatic Studies of *Talinum portulacifolium* F.

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Abstract

Aim: This investigation has been conducted to evaluate the antiasthmatic activity and phytochemical characterization using gas chromatography-mass spectrum (GC-MS) analysis of the leaf extracts of *Talinum portulacifolium*. **Materials and Methods:** Hydroalcoholic and acetone extracts of the plant were prepared. Preliminary phytochemical screening has been conducted. Antiasthmatic activity was determined by two experimental models. *In vivo* methods, histamine and acetylcholine (Ach)-induced bronchospasm in guinea pigs were studied. Pre convulsion time (PCT) was calculated. *In vitro,* experimental methods such as histamine and Ach-induced contractions in ileum were also studied. Percentage inhibition of contractions was calculated. Phytochemical characterization was studied using GC-MS analysis. **Results and Discussion:** In histamine and Ach-induced bronchospasm studies acetone extracts of the plant have significantly increased PCT 10.69 and 10.52 (**$P < 0.01$), one-way analysis of variance (ANOVA) Tukey's test compared with control. Histamine and Ach-induced ileum contraction studies also showed that the acetone extracts exhibited response 2.6 with 47% and 2.2 with 40% inhibition (*$P < 0.05$). The results were expressed by one-way ANOVA, Dunnett's test. The results of GC-MS analysis depicted following phytoconstituents with major peak area, namely, 79.29% methoxy-bis (cyclopentadiene), 2.83% - 5,10-dihexyl-5,10-dihydroindolo[3,2-b]indole-2,7-dicarbaldehyde, and 1.84% - 1,2-bis[3,4-dimethoxy benzyl]-1,2-bis (methoxymethyl) ethane. **Conclusion:** The results of this study clearly indicate that the hydroalcoholic and acetone extracts of *T. portulacifolium* can be used as promising antiasthmatic agents. The activity may be due to the presence of phytochemicals reported through GC-MS.

Key words: Asthma, bronchospasm, gas chromatography-mass spectrum, ileum contractions, phytochemicals, *Talinum portulacifolium*

INTRODUCTION

Bronchial asthma is a common chronic inflammatory disorder of airways characterized by chest tightness, wheezing, and breathlessness.[1] It is caused by various factors such as allergens, histamine, acetylcholine (Ach), and emotion. These factors activate immunoglobulin-E-mediated mast cells and release inflammatory mediators such as eosinophils, neutrophils, histamines, and bradykinesia.[2] Histamine participates in various cell physiological processes such as inflammation, gastric acid secretion, central, and peripheral neurotransmission,[3] hence the usage of antihistamines is a part of antiallergic therapy. Ach from parasympathetic nerves as neurotransmitter controls the symptoms and inflammation in allergic diseases through peripheral muscarinic receptors which are present on airway smooth muscle and secretory glands. Hence, muscarinic antagonists are one of the therapies of asthma.[4] Therefore, the drugs which block the effects of inflammatory mediators such as histamine and Ach are used in the treatment of asthma. Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids. This is a solid - liquid technique in which the stationary phase

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Received: 14-03-2017
Revised: 15-04-2017
Accepted: 03-05-2017

is a solid and mobile phase is a liquid. The rate at which the components of a mixture are separated depends on the activity of the adsorbent and polarity of the solvent.[5] In the past few years, gas chromatography-mass spectrum (GC-MS) analysis has become established as a key tool in secondary metabolite profiling.[6,7] GC separates volatile substances by percolating a gas stream over a stationary phase. MS is concerned with the electron ionization, subsequent fragmentation of molecules, determination of mass to charge ratio (m/e) for the relative abundance of ions produced. Knowing the fragmentation pattern, a possible structure of the original molecule can be suggested. MS have been successfully coupled to GC-MS, the most effective method for the identification of drug constituents. The drugs currently available for the treatment of asthma show poor response with few side effects. Hence, natural drugs which are immunomodulatory prove to be effective in combating the symptoms of asthma.[8] *Talinum portulacifolium,* native species of Andhra Pradesh, has been selected for this study. The genus *Talinum* comprises about 50 species.[9,10] Leaves of the plant possess anti-diabetic and aphrodisiac properties.[11] The plant was used to treat arthritis, backache, and diarrhea.[12] It is also used as a medicine for constipation and ulcer.[13] Previous chemical studies on this species reported that leaves and stem extracts contain quercetin, tannins, phosphates, urea, and various minerals with a larger amount of magnesium.[14] The objective of this study was to evaluate the antiasthmatic potential and phytochemical characterization on the leaf extracts of *T. portulacifolium.*

MATERIALS AND METHODS

Chemicals and reagents

Histamine hydrochloride, Ach, chlorpheniramine maleate, and atropine sulfate were purchased from Sigma-Aldrich chemical Co.

Experimental animals

Guinea pigs (400-600 g) of either sex were purchased from Mahaveer Enterprises, Hyderabad, Telangana, India, housed in standard conditions of temperature (22°C ± 2°C), relative humidity (55% ± 5%), and light (12 h light/dark cycles). They were fed with standard pellet diet and water *ad-libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Nirmala College of Pharmacy, Atmakur, Mangalagiri, Guntur District, Andhra Pradesh, India, approval no 012/IAEC/NCPA/PhD/2016-17, as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India.

Collection of plant material

The plant material was collected from local grounds of Prasadampadu, and Enikepadu coordinates 16°32′45″N 80°34′12″E of Vijayawada rural region, Krishna District, Andhra Pradesh, India. The plant specimen was identified and authenticated by Dr. P. Satya Narayana Raju, plant taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna University (ANU), Guntur (District), Andhra Pradesh, India. A voucher specimen 004/VIPW was deposited for future reference.

Preparation of the extract

The leaves were dried under shade, powdered coarsely using a mechanical grinder. Then, extraction was carried out using 50:50 methanol-water and acetone as solvents by Soxhlet apparatus (JSGW). The extracts obtained were dried using vacuum evaporator (Biotech). The percentage yield of extracts on air dried basis was obtained to be 33.33% w/w for hydroalcoholic extract and 29.21% w/w for acetone extract. The extracts were preserved in refrigerator till use.

Phytochemical screening

The preliminary phytochemical screening was carried out on the hydroalcoholic and acetone extracts to reveal the presence of phytochemicals present in the extracts.[15]

Acute toxicity testing

The animals were overnight fasted before the experiment. Different doses (50-2000 mg/kg, orally) of the hydroalcoholic and acetone extracts were administered to groups of guinea pigs. The animals were observed continuously for 1 h, next half-hourly intervals for 4 h for any gross changes in their behavior and then up to 24 h for any mortality as per the Organization for Economic Co-Operation and Development guidelines 425.[16]

Histamine-induced bronchospasm in guinea pigs

Guinea pigs of either sex were divided into four groups. Each group comprised four animals:

- Group 1: Control group animals received distilled water
- Group 2: Standard group animals received chlorpheniramine-maleate
- Group 3: Test 1 group animals received hydroalcoholic extract of *T. portulacifolium* (TPHA)
- Group 4: Test 2 group animals received acetone extract of *T. portulacifolium* (TPAE).

Animals were exposed to 0.1% w/v of histamine dihydrochloride aerosol in a histamine chamber (Sigma Scientific). Progressive dyspnea was observed in animals when exposed to histamine aerosol. Pre convulsion time (PCT) was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsions on day 0 (T_1). As soon as dyspnea commenced, the animals were removed from the chamber and placed in fresh air. Animals were given TPHA and TPAE at a dose of 400 mg/kg

Table 1: Effect of TPHA and TPAE on histamine-induced guinea pig bronchial contraction

Groups	Drug and dose	PCT (Mean±SEM)
Control	Distilled water p.o.	2.22±0.24
Standard	Chlorpheniramine 2 mg/kg	8.77±0.43**
Test 1	TPHA 400 mg/kg	4.38±0.7
Test 2	TPAE 400 mg/kg	10.69±1.45**

Each value was expressed as mean±SEM, where *n*=4 in each group at ***P*<0.01 compared with control by one-way ANOVA, Tukey's test. ANOVA: Analysis of variance, SEM: Standard error of the mean, TPHA: Hydroalcoholic extract of *Talinum portulacifolium*, TPAE: Acetone extract of *Talinum portulacifolium,* PCT: Pre convulsion time

Table 2: Effect of TPHA and TPAE on acetylcholine-induced guinea pig bronchial contraction

Groups	Drug and dose	PCT (Mean±SEM)
Control	Distilled water p.o.	3.22±0.60
Standard	Atropine sulfate 2 mg/kg	11.60±1.24**
Test 1	TPHA 400 mg/kg	7.22±1.05
Test 2	TPAE 400 mg/kg	10.52±1.58*

Each value was expressed as mean±SEM, where *n*=4 in each group at **P*<0.05, ***P*<0.01 compared with control by one-way ANOVA, Tukey's test. ANOVA: Analysis of variance, SEM: Standard error of the mean, TPHA: Hydroalcoholic extract of *Talinum portulacifolium*, TPAE: Acetone extract of *Talinum portulacifolium,* PCT: Pre convulsion time

orally (p.o.) once a day for 7 days. On the seventh day, 2 h after the last dose, PCT was recorded (T_2).

Ach-induced bronchospasm in guinea pigs

Guinea pigs of either sex were divided into four groups. Each group comprised four animals:

- Group 1: Control group animals received distilled water
- Group 2: Standard group animals received atropine sulfate
- Group 3: Test 1 group animals received TPHA
- Group 4: Test 2 group animals received TPAE.

Animals were exposed to 0.5% Ach chloride aerosol. The experimental procedure was followed as above.[17]

The percentage increase in time of PCT was calculated using the following formula:

Percentage increased in time of $\text{PCT} = \left(1 - \frac{T_1}{T_2}\right) \times 100$

where T_1 is PCT on day 0, and T_2 is PCT on day 7.

Statistical analysis

Results of the study were expressed as a mean ± standard error of the mean (SEM) and analyzed statistically using one-way analysis of variance (ANOVA), followed by Tukey test for multiple group comparison with a control to find out the level of significance. Data were considered statistically significant at $^*P < 0.05$ and $^{**}P < 0.01$.

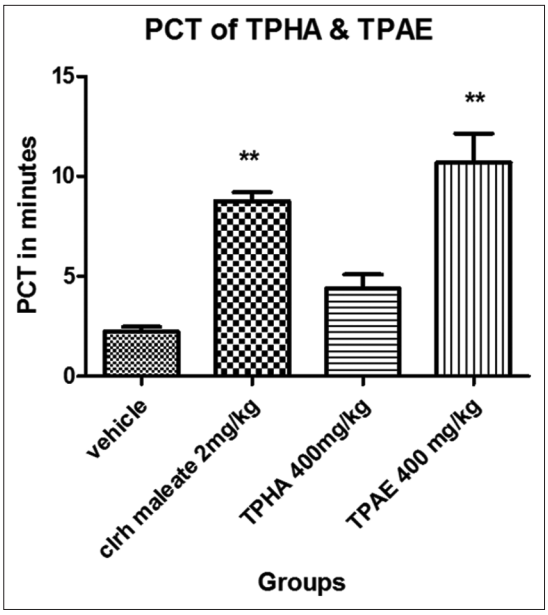

Figure 1: Effect of hydroalcoholic extract of *Talinum portulacifolium* and acetone extract of *T. portulacifolium* on histamine-induced guinea pig bronchial contraction

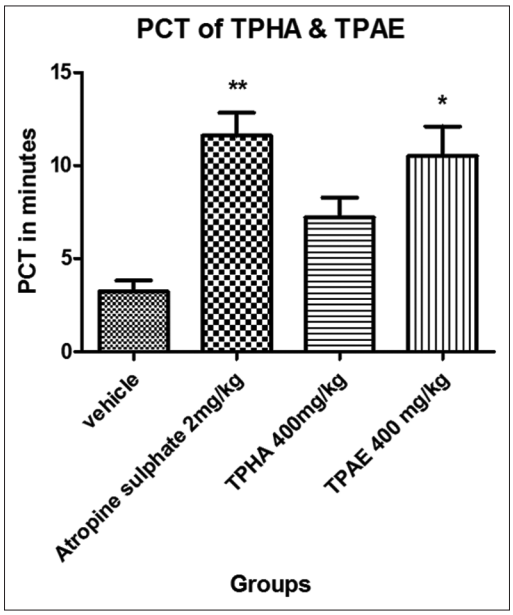

Figure 2: Effect of hydroalcoholic extract of *Talinum portulacifolium* and acetone extract of *T. portulacifolium* on Ach-induced guinea pig bronchial contraction

Histamine-induced guinea pig ileum contraction

Guinea pigs of body weight 200-500 g were selected and allowed to starve overnight with free access to water. The animals were killed by a blow on the head and exsanguinated. The ileum was isolated, cut into individual sections of 1 cm,

and then divided into four groups; each group consisted of four ileums.

- Group 1: Control group animals received histamine
- Group 2: Standard group animals received chlorpheniramine
- Group 3: Test 1 group animals received TPHA
- Group 4: Test 2 group animals received TPAE.

The isolated ileum was mounted in a 30 ml Organ bath (Lab Tree India) containing Tyrode solution, maintained at 37°C ± 1°C, and gassed with air. The tissue was equilibrated for 45 min during which the bath solution was replaced every 10 min. A drug tissue contact time of 1 min was maintained and 15 min time cycle was followed by recording the response of histamine. After obtaining a dose response curve of histamine on ileum, the extracts (0.5 mg) were added to the reservoir, and same doses of histamine were repeated in presences of extracts.

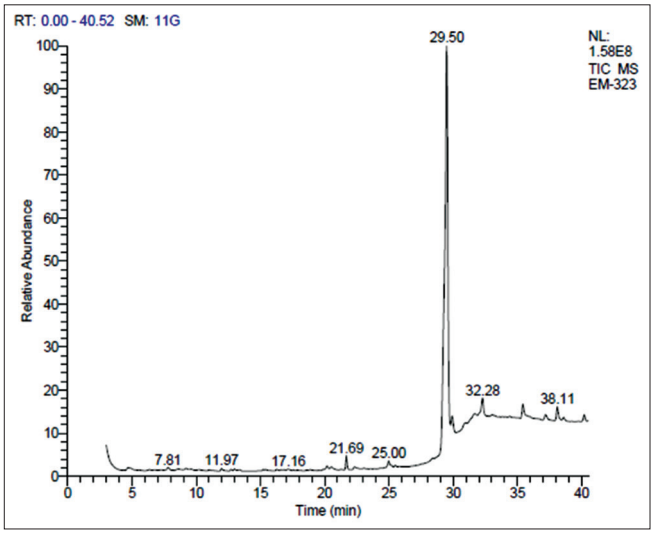

Figure 3: Gas chromatogram-mass spectrum of column fraction

Ach-induced guinea pig ileum contraction

- Group 1: Control group animals received Ach
- Group 2: Standard group animals received atropine sulfate
- Group 3: Test 1 group animals received TPHA
- Group 4: Test 2 group animals received TPAE.

The same above experimental procedure was carried out for the study.[18]

Statistical analysis

The results of the study were expressed as mean ± SEM and analyzed statistically using one-way ANOVA followed by Dunnett's test for individual comparison of groups with control. Data were considered statistically significant at $*P < 0.05$ and $**P < 0.01$.

Isolation by column chromatography

Column chromatography was performed on a classic 20 cm long × 2 cm diameter glass column packed with silica gel (Merck, Germany). TPAE (20 ml) was applied to the column by use of a pipette. It was eluted sequentially with n-hexane and chloroform. The chloroform fraction was studied by GC-MS.[5]

Table 3: Effect of TPHA and TPAE on histamine-induced guinea pig contractions on ileum

Groups	Drug and dose (mg)	Response (Mean±SEM)	% inhibition
Control	Histamine 0.5	4.9±0.08	0
Standard	Chlorpheniramine 0.5	1.8±0.91*	63.3
Test 1	TPHA 0.5	4.3±0.04*	12.3
Test 2	TPAE 0.5	2.6±0.07*	47

Each value was expressed as Mean±SEM, where n=4 in each group at $*P<0.05$ compared to control by one-way ANOVA, Dunnett's test. ANOVA: Analysis of variance, SEM: Standard error of the mean, TPHA: Hydroalcoholic extract of *Talinum portulacifolium,* TPAE: Acetone extract of *Talinum portulacifolium*

Table 4: Effect of TPHA and TPAE on acetylcholine-induced guinea pig contractions on ileum

Groups	Drug and dose (mg)	Response (Mean±SEM)	% inhibition
Control	Acetylcholine 0.1	5.5±0.27	0
Standard	Atropine sulfate 0.5	2.2±0.81*	60
Test 1	TPHA 0.5	3.6±0.28*	34.6
Test 2	TPAE 0.5	2.2±0.39*	40

Each value was expressed as mean±SEM, where n=4 in each group at $*P<0.05$ compared with control by one-way ANOVA, Dunnett's test. ANOVA: Analysis of variance, SEM: Standard error of the mean, TPHA: Hydroalcoholic extract of *Talinum portulacifolium*, TPAE: Acetone extract of *Talinum portulacifolium*

GC-MS analysis for the characterization of phytoconstituents

A Thermo GC-Trace Ultra Ver. 5.0, Thermo MS (Dual-Stage Quadrupole) DSQ II equipment (Thermo Scientific Co.) was used for carrying out the phytochemical investigation. Experimental conditions for GC-MS were DB 5-MS capillary standard non-polar column, dimension 30 min, ID 0.25 mm, film thickness 0.25 µm, carrier gas (mobile phase) helium was set at a flow rate of 1.0 ml/min, the oven temperature was 70°C raised to 260°C at 6°C/min, and injection volume was 1 µl. Samples were dissolved in chloroform, run fully at a range of 50-650 m/z. The results were compared using Wiley spectral library research program.

RESULTS

Phytochemical screening

Preliminary phytochemical screening of hydroalcoholic and acetone extracts of *T. portulacifolium* showed the presence of alkaloids, gum, mucilage, glycosides, terpenoids, tannins, flavonoids, steroids, amino acids, and proteins.

Acute toxicity testing

The hydroalcoholic and acetone extracts of the plant were administered orally to guinea pigs up to a dose of

Table 5: Major chemical constituents from column fraction

Retention time	Name of phytoconstituent	Mol. formula	Mol. weight	% peak area
7.81	Dodecane	$C_{12}H_{26}$	170	0.42
11.97	Tetradecamethyl cycloheptasiloxane	$C_{14}H_{42}O_7Si_7$	518	0.37
17.16	1-hydroxy-1-oxo-3,3-dimethyl-3H-2,1 benzoxaphosphole	$C_9H_{11}O_3P$	198	0.25
21.69	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.28
25.00	9,2-octadecadienoyl chloride	$C_{18}H_{31}ClO$	298	1.04
29.50	Methoxy-bis (cyclopentadiene)	$C_{11}H_{18}O$	166	79.29
32.28	5,10-dihexyl-5,10-dihydroindolo[3,2-b] indole-2,7-dicarbaldehyde	$C_{28}H_{34}N_2$	430	2.83
38.11	1,2-bis[3,4-dimethoxybenzyl]-1,2-bis (methoxymethyl) ethane	$C_{24}H_{34}O_6$	418	1.84

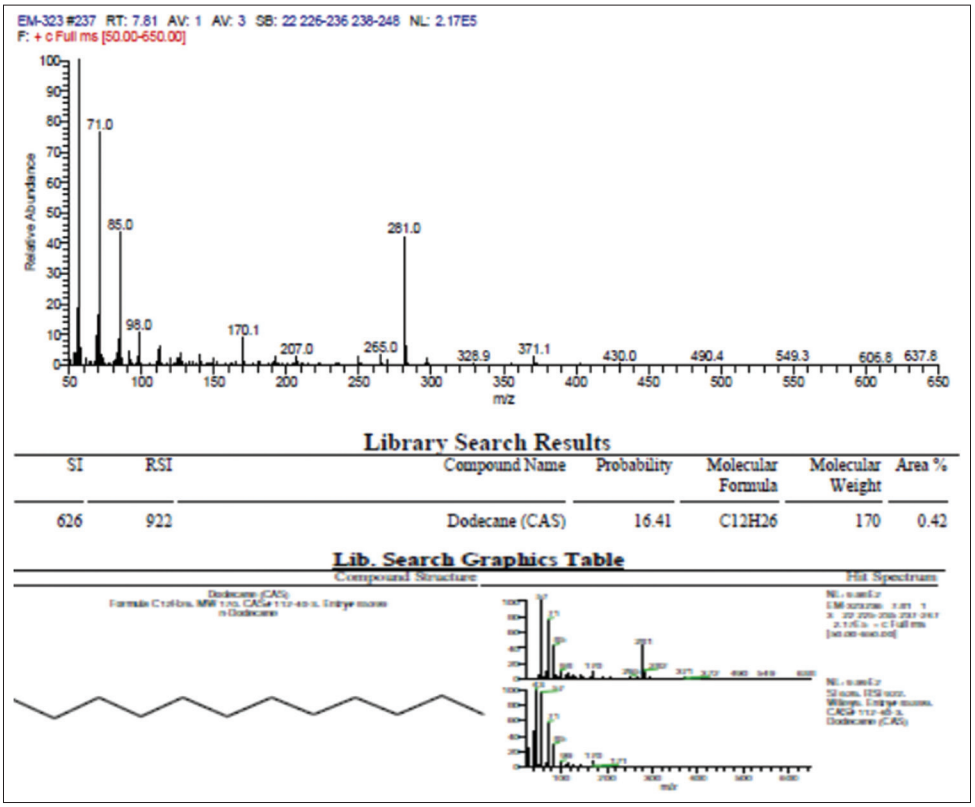

Figure 4: Mass spectrum at RT 7.61

2000 mg/kg body weight. After 24 h, the animals were found to be well-tolerated, safe with no signs of mortality and toxicity. Hence, a safe and therapeutically effective dose of 400 mg/kg of body weight was selected for this study.

Effect of TPHA and TPAE on histamine-induced bronchospasm in guinea pigs

The plant extracts offered protection against bronchospasm induced by histamine as compared to control. The increase in PCT at a dose of 400 mg/kg body weight of animals was found to be 4.38 and 10.69 for TPHA and TPAE, respectively. TPAE significantly (**$P < 0.01$) increased PCT following exposure to histamine when compared to TPHA [Table 1 and Figure 1].

Effect of TPHA and TPAE on Ach-induced bronchospasm in guinea pigs

The plant extracts offered protection against bronchospasm induced by Ach as compared to control. The increase in PCT at a dose of 400 mg/kg body weight of animals was found to be 7.22 and 10.52 for the hydroalcoholic and acetone extracts, respectively. TPAE significantly ($P < 0.01$) increased PCT following exposure to histamine when compared to TPHA [Table 2 and Figure 2].

Effect of TPHA and TPAE on histamine-induced guinea pig ileum contractions

The plant extracts inhibited the contraction induced by histamine as compared to control. The percentage of inhibition was found to be 12.3% and 47% for TPHA and TPAE, respectively. In isolated guinea pig ileum studies, the TPAE significantly ($P < 0.01$) inhibited the contraction of ileum when compared to TPHA [Table 3].

Effect of TPHA and TPAE on Ach-induced guinea pig ileum contractions

The plant extracts inhibited the contraction induced by histamine as compared to control. The percentage of inhibition was found to be 12.3% and 47% for TPHA and TPAE, respectively. In isolated guinea pig ileum studies, the TPAE significantly ($P < 0.01$) inhibited the contraction of ileum when compared to TPHA [Table 4].

Identification of chemical constituents

The compounds were identified by MS attached through GC. The MS analyses the compounds eluted at different times to identify the nature and structure of them. The large compound fragments into small compounds giving rise to the appearance

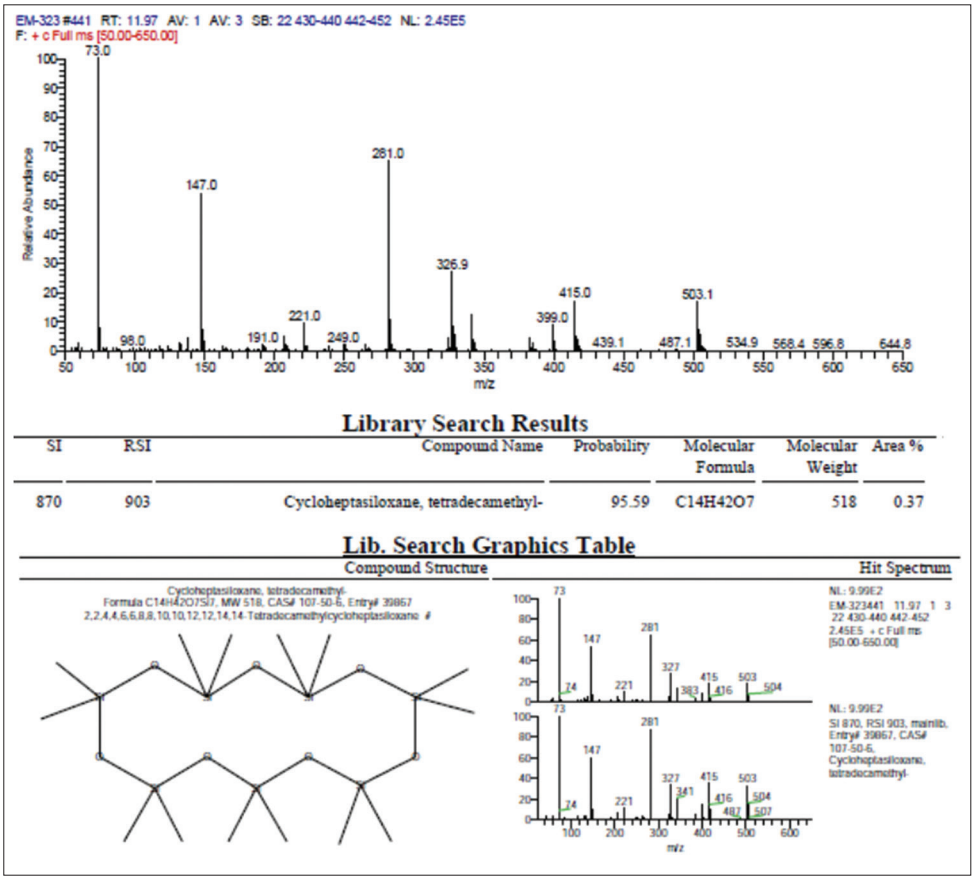

Figure 5: Mass spectrum at RT 11.97

of peaks at different m/z ratios. Identification of the chemical constituents was done on the basis of retention index using a library search and by comparing the MS and retention data with literature. These MS are fingerprints of those compounds which can be identified from the library data. The GC-MS confirmed the presence of various components with different retention times as illustrated in Figure 3. The identified components by GC-MS from the isolated fraction of TPAE were shown [Tables 5 and 6, Figures 4-11]. The phytoconstituents with major % peak area include methoxy-bis (cyclopentadiene) (79.29%), 5,10-dihexyl-5,10-dihydroindolo[3,2-b]indole-2,7-dicarbaldehyde (2.83%), and 1,2-bis[3,4-dimethoxybenzyl]-1,2-bis(methoxymethyl) ethane (1.84%) [Figures 9-11].

DISCUSSION

The study presents indications that hydroalcoholic and acetone extracts of the plant *T. portulacifolium* can relieve bronchoconstriction. This presumption is based on the examination that the extracts of the plant inhibited the contractions produced by histamine and Ach in guinea pig bronchi and ilei. Thus, these extracts may relieve airway constriction by opposing excessive stimulation of the H1 histaminergic and/or the M3 muscarinic receptors on the smooth muscle cells of the airways.

Histamine was released from mast cells and basophils by antigenic stimulation causing smooth muscle contraction, increased vascular permeability, and mucus formation. Histamine can provoke bronchoconstriction; it may also be responsible for bronchial hypersensitivity which is a common feature of asthma. Mast cells with their mediator can be regarded as a center for initiation of the chronic allergic reaction. The guinea pig bronchial and ileum smooth muscles have H1 receptors. The stimulation of H1 receptors causes contraction of bronchi and ileum.[19]

Dyspnea, asphyxic convulsions like symptoms resembling bronchial asthma can be instigated by inhalation of spasmogens, namely, histamine and Ach in guinea pigs. This is because histamine H1 sensitive excitatory receptors and Ach muscarinic receptors in the airway smooth muscle of man and animals have been established.[20] They are the most sensitive animals for the study of asthma and allergic disease.[21] The end point pre-convulsive dyspnea was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of asphyctic convulsions, i.e., PCT.[22] Prolongation of PCT indicates spasmolytic and anticholinergic activity. In this study, the TPAE at 400 mg/kg significantly (**$P < 0.01$) inhibited histamine and acetylcholine-induced bronchoconstriction. Ach causes bronchoconstriction by activating M3 (Gq IP3/DAG pathway)

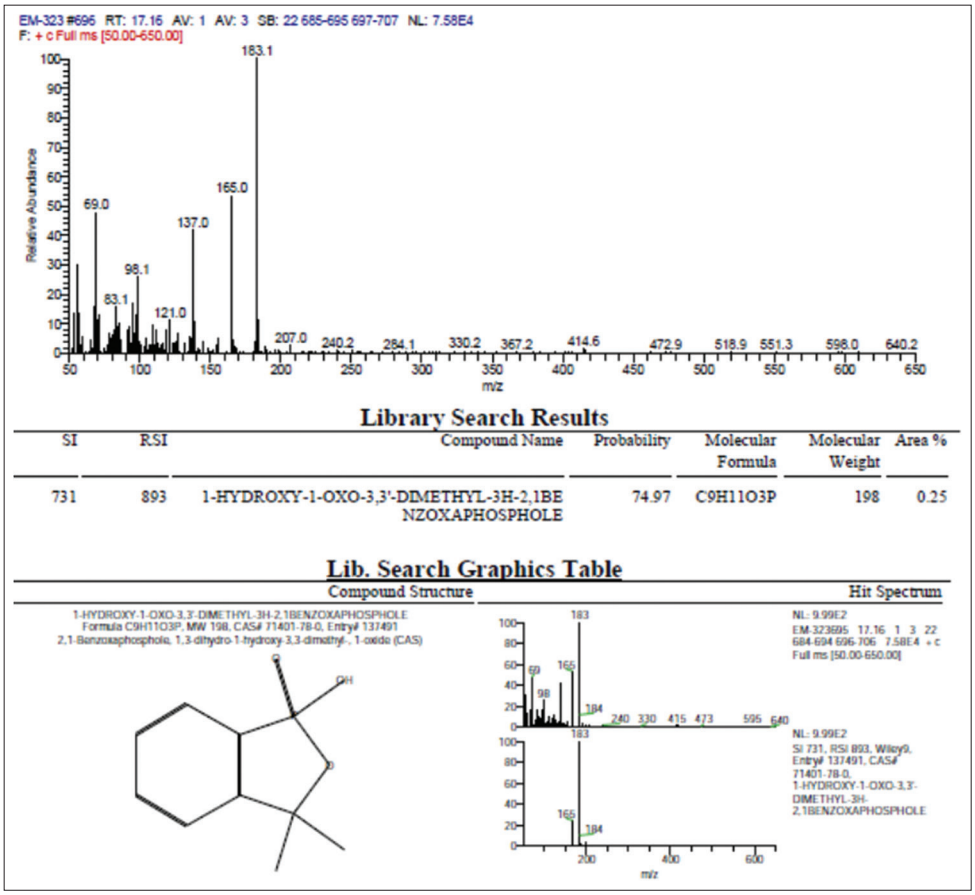

Figure 6: Mass spectrum at RT 17.16

Table 6: Other chemical constituents identified through GC-MS

Retention time	Name of phytoconstituent	Mol. formula	Mol. weight	% peak area
4.69	Phenol	C_6H_6O	94	0.66
6.36	Benzoic acid methyl ester	$C_8H_8O_2$	136	0.17
8.60	2-piperidone	C_5H_9NO	99	0.25
9.22	1,3-bis(4-chlorobenzyl)-5,6-dihydrobenzo[f] quinazoline	$C_{26}H_{20}Cl_2N_2$	430	0.24
9.62	Trimethylsilyl9-à-hydroxy-3-methoxyimino-23,24-bisnorchol-4-enoate	$C_{26}H_{43}NO_4Si$	461	0.19
12.70	2-cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	$C_{13}H_{22}O_2$	210	0.22
12.98	4-methyl-7-methoxyisatin	$C_{10}H_9NO_3$	191	0.35
13.43	Ethyl 4-(chloromethylene)-2,2-diphenyl-3-oxazoline-5-car boxylate	$C_{19}H_{16}ClNO_3$	341	0.25
15.23	Tetradecanal	$C_{14}H_{28}O$	212	0.39
18.87	2-[4,5-bis (methylthio)-1,3-thiole-2-ylidene]-N-tosyl-[1,3]-dithiolo[4,5-c] pyrrole	$C_{17}H_{15}NO_2S_7$	489	0.25
20.18	Cyclohexanecarboxaldehyde, 3,3-dimethyl-5-oxo-	$C_9H_{14}O_2$	154	0.79
20.52	2-nonenal, 2-pentyl-	$C_{14}H_{26}O$	210	0.57
22.32	3,9-diazatricyclo[7.3.0.0 (3,7)]dodecan-2,8-dione	$C_{10}H_{14}N_2O_2$	194	0.41
28.36	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyloctasiloxane	$C_{16}H_{50}O_7Si_8$	578	0.16
29.92	1,2-bis[1-(2-hydroxyethyl)-3,6-diazahomoadamantantydene-9]hydrazine	$C_{22}H_{36}N_6O_2$	416	1.38
33.06	Stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	410	0.42
35.41	13-docosenamide, (Z)-	$C_{22}H_{43}NO$	337	2.67
37.19	Stigmasta-5,22-dien-3-ol, (3á,22e)-	$C_{29}H_{48}O$	412	0.94
38.60	Spiro[isoquinoline-1,2'-indene],1,2,3,4,2',3'-tetrahydro-6'-hydroxy-6,7,3',7'-tetramethoxy-2-methyl-1'-oxo-	$C_{22}H_{25}NO_6$	399	0.41

GC-MS: Gas chromatography-mass spectrum

(guanine nucleotide binding protein q polypeptide inositol triphosphate/diacylglycerol) receptors. The reduction in contraction of ileum indicates that the plant extracts possess M3 antagonistic activity. M3 receptors are present at smooth muscle such as GIT, bronchi, and uterus. Smooth muscles in most organs are contracted. Contractility of bronchi increases by Ach action on M3 receptors.[23]

The plant extracts were tested for their capability of inhibiting ileum contraction induced by histamine and Ach. The study was used to detect antihistaminic and anticholinergic properties of plant extracts. The reduction in contraction indicated that reversible anticholinergic (maybe an M3 antagonist), the spasmolytic activity of the plant extracts. In histamine and acetylcholine-induced guinea pig ileum contraction studies the extracts at 0.5 mg exhibited significant (*$P < 0.05$) antihistaminic and anticholinergic activities, respectively. It is comparable to standard drugs atropine and chlorpheniramine-maleate. The possible involvement of the muscarinic receptor in the apparent bronchospasmolytic effect of the plant extracts is in accordance with the decreasing effect of such a preparation on guinea pig tracheal ring contractions caused by carbachol.[24] Additional support for an antimuscarinic effect of the plant extracts was provided by its relaxation of isolated guinea pig ilei and porcine bladder strips precontracted with Ach or carbachol.[25,26] The apparent anti-histaminergic effect of the plant extracts noted in this study is in accordance with its ability to reduce the force of contraction of isolated pig ilei caused by histamine.[25,27]

The H1 receptor antagonistic activity of the extracts has been supported by antiulcer activity using an ethanolic extract of the leaves of the plant showed significant ($P < 0.001$) activity at 400 mg/kg against histamine-induced ulcerations.[28] The possible mechanism of action for ulcerogenic may be mediated by the parietal cells by the transmitter substances histamines and Ach through a common pathway involving cyclic adenosine monophosphate and calcium ions, interact with the gastric proton pump 9H+/K+-ATPase.[29]

The methanolic extract of *T. portulacifolium* was studied *in vivo* for analgesic activity by acetic acid induced a writhing response in albino mice. The extract produced significant ($P < 0.05$) analgesic property at 2000 mg. This seems to provide a rationale for the use of this plant in pain and inflammation.[30]

Phytochemical screening showed the presence of flavonoids,[31] steroids,[32] and terpenoids[33,34] which were reported to possess smooth muscle relaxant, bronchodilator, and spasmolytic properties. Flavonoids possess potential pharmacological activities such as antiallergic, anti-inflammatory, and antioxidant activities.[35,36]

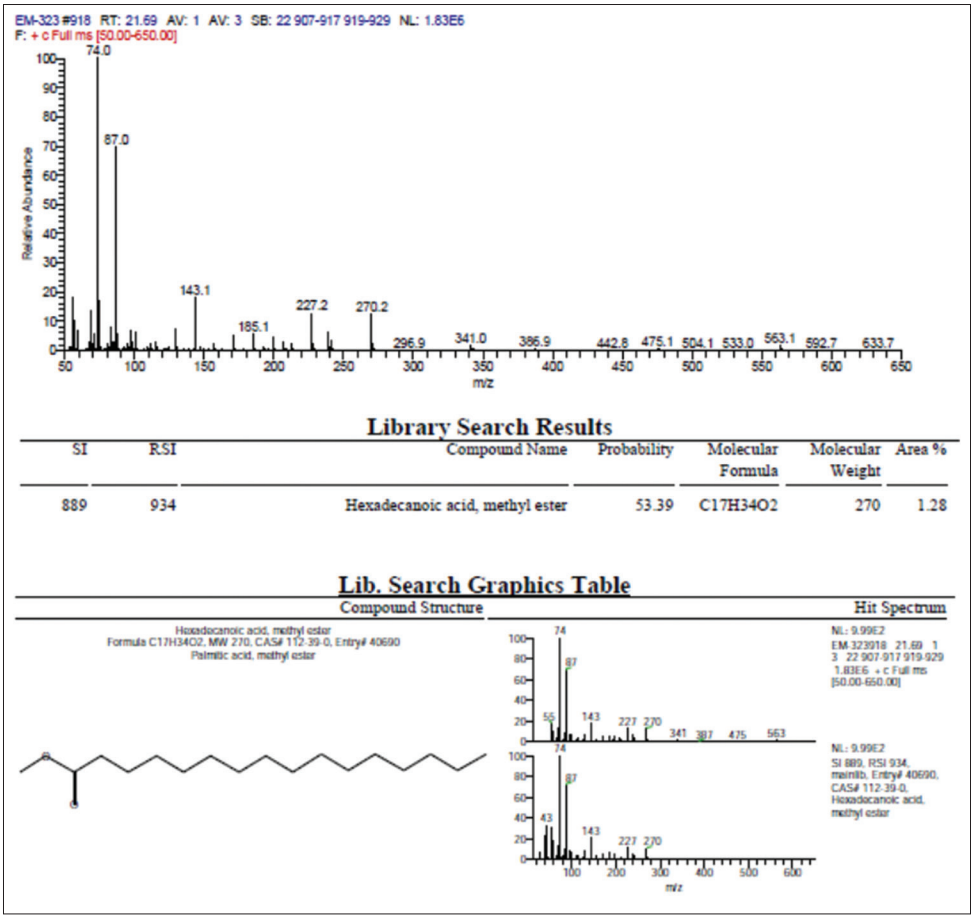

Figure 7: Mass spectrum at RT 21.69

The apparent anti-histaminergic and anticholinergic properties of extracts are further supported by the presence of the highest amount of flavonoids, viz., quercetin, luteolin, and kaempferol. The flavonoid quercetin was identified by HPLC from the leaf and stem extracts of the plant. The plant contains minerals such as calcium, magnesium, sodium, potassium, and phosphorus in good amount.[14] The possible involvement of the histaminergic and muscarinic receptors is in line with the data available for this study. These observations have been attributed to the role of quercetin in allergy and inflammation,[37] as an antihistaminic[38] and an anticholinergic agent.[39]

Luteolin and kaempferol have been isolated from the leaves of the plant.[40] The antiallergic activity of the plant extracts can be further attributed to the antiallergic and antihistaminic properties of luteolin.[41] The scientific data on kaempferol were antiallergic through mast cell stabilization[42] is in accordance with the results of the study.

The extracts increased PCT through dilatation of the bronchial smooth muscles more against histamine than Ach. Therefore, the extracts may possess bronchodilator activity. Further, there was more inhibition of histamine-induced ileum contractions supporting H1 receptor antagonistic activity than the antimuscarinic property of plant extracts.[43]

GC-MS analysis reported the presence of major constituents, namely, methoxy-bis (cyclopentadiene), 5,10-dihexyl-5,10- dihydro-indolo [3,2-b]indole-2,7-dicarbaldehyde, and 1,2-bis[3,4-dimethoxy benzyl]-1,2-bis (methoxymethyl) ethane [Table 5] and other constituents [Table 6] which may be responsible for the therapeutic activity.

CONCLUSION

From above experimentation, we can conclude that the extracts of *T. portulacifolium* have antihistaminic anticholinergic and spasmolytic activities, it can be used in the asthmatic condition. Further study is required to isolate, elucidate and study the pharmacological behavior of potent phytochemicals eluted through GC-MS toward their antiallergic nature by antihistaminic, anticholinergic studies and also by various other mechanisms.

ACKNOWLEDGMENT

The authors are thankful to Jawaharlal Nehru Technological University, Kakinada, East Godavari (District), Andhra Pradesh, India; Vijaya Institute of Pharmaceutical

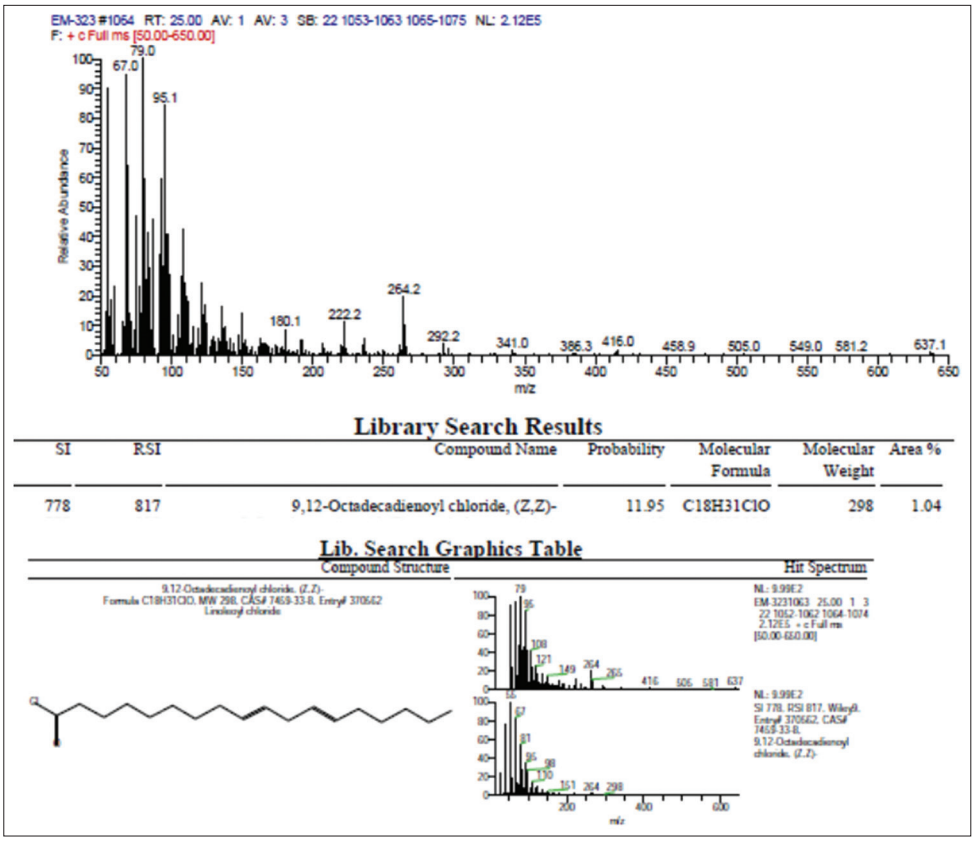

Figure 8: Mass spectrum at RT 25.00

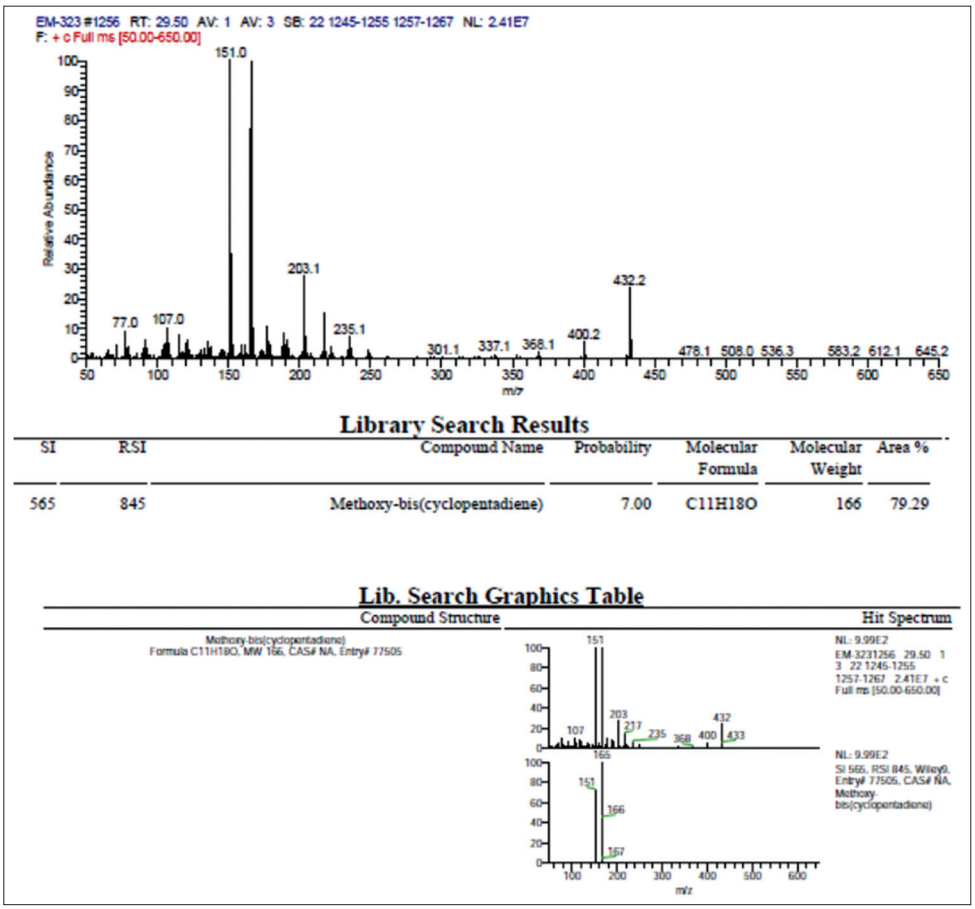

Figure 9: Mass spectrum at RT 29.50

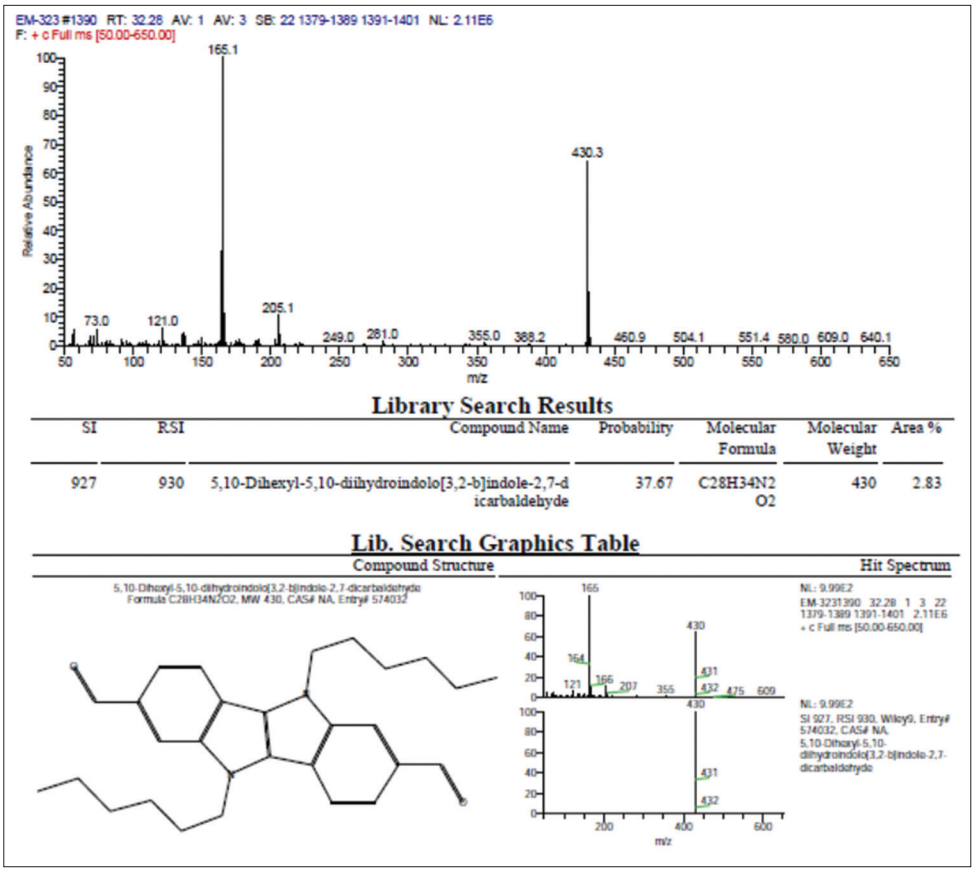

Figure 10: Mass spectrum at RT 32.28

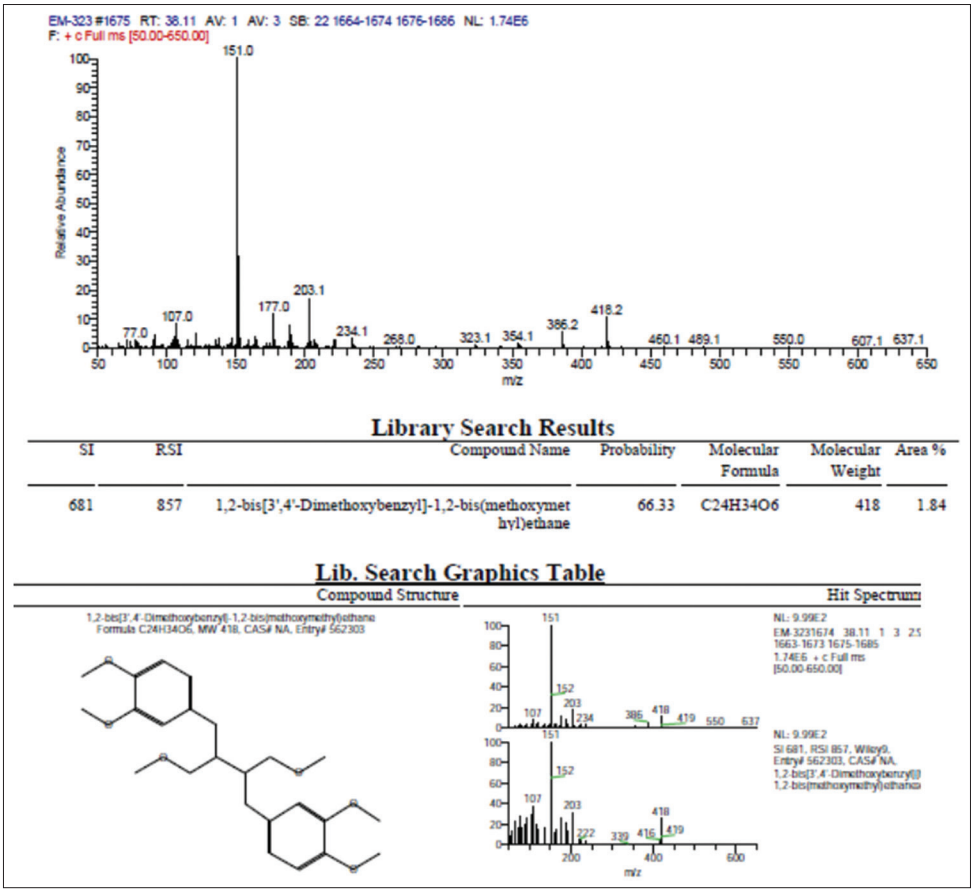

Figure 11: Mass spectrum at RT 38.11

Sciences for Women, Enikepadu, Vijayawada, Krishna (District), Andhra Pradesh, India; Nirmala College of Pharmacy, Atmakur, Mangalagiri, Guntur (District), Andhra Pradesh, India; Department of Pharmaceutics, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur (District), Andhra Pradesh, India, for their kind encouragement and support.

REFERENCES

1. Holgate ST, Polosa R. Treatment strategies for allergy and asthma. Nat Rev Immunol 2008;8:218-30.
2. Sagar R, Sahoo HB, Kar B, Mishra NK, Mohapatra R, Sarangi SP. Pharmacological evaluation of *Calendula officinalis* L. On bronchial asthma in various experimental animals. Int J Nutr Pharmacol Neurol Dis 2014;4:95-103.
3. Goyal RK. Practical in Pharmacology. 3rd ed. Ahmedabad: B.S. Shah Prakashan; 2003. p. 103.
4. Kubo N, Minami T, Hori Y, Yamashita T, Kumazawa T. Enhanced parasympathetic nerve activities in experimentally-induced nasal hypersensitivity. Acta Otolaryngol Suppl 1989;463:14-20.
5. Dinan L, Harmatha J, Lafont R. Chromatographic procedures for the isolation of plant steroids. J Chromatogr A 2001;935:105-23.
6. Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L. Metabolite profiling: From diagnostics to systems biology. Nat Rev Mol Cell Biol 2004;5:763-9.
7. Kell DB, Brown M, Davey HM, Dunn WB, Spasic I, Oliver SG. Metabolic footprinting and systems biology: The medium is the message. Nat Rev Microbiol 2005;3:557-65.
8. Thomas E, Anees TP, Thomas DG, Anandan RR. GC-MS analysis of phytochemical compounds present in the rhizomes of *Nervilia aragoana* Gaud. Asian J Pharm Clin Res 2013;6:68-74.
9. Grubben GJ, Denton OA. Plant Resources of Tropical Africa: Vegetables. Wageningen: PROTA Foundation; 2004. p. 519.
10. Pullaiah T. Encyclopedia of World Medicinal Plants. Vol. 5. New Delhi: Regency Publications; 2006. p. 2358.
11. Babu KR, Vinay K, Sameena SK, Prasad SV, Swapna S, Rao AC. Antihyperglycemic and antioxidant effects of *Talinum portulacifolium* leaf extracts in streptozotocin diabetic rats: A dose-dependent study. Pharmacogn Mag 2009;5:1-10.
12. Kiring JW. A survey of traditional health remedies used by the Maasai of Southern Kaijiado district, Kenya. Ethnobot Res Appl 2006;4:61-73.
13. Thangavel K, Maridass M, Sasikala M, Ganesan V. *In vitro* micropropagation of *Talinum portulacifolium* L. Through axillary bud culture. Ethnobot Lealf 2008;12:413-8.
14. Adithya ES, Sasikumar JM, Kumar K, Lakshmi MS, Christabel PH. *In vitro* antioxidant activity, mineral content and HPLC analysis of *Talinum portulacifolium* (Forssk.) Asch. ex Schweinf. Leaf and stem. Int J Pharm Pharm Sci 2012;4:423-9.
15. Evans WC. Trease and Evans Pharmacognosy. 15th ed. London: W. B. Sunders Company Ltd.; 2005.
16. OECD. OECD Guidelines for the Testing of Chemicals-425, Acute Oral Toxicity-Up and Down Procedure (UDP). Paris: OECD; 2008.
17. Nimgulkar CC, Patil SD, Kumar BD. Anti-asthmatic and anti-anaphylactic activities of *Blatta orientalis* mother tincture. Homeopathy 2011;100:138-43.
18. Patil SD, Ahale SV, Surana SJ. Evaluation of antiasthmatic activity and anaphylactic activity of *Balanites aegyptica* (Delile), balanitaceae. Asian J Pharm Clin Res 2011;4:52-5.
19. Goodman LS, Gilman A. The Pharmaceutical Basis of Therapeutics. 10th ed. London: Macmillan Press; 2001.
20. Busse WW, Calhoun WF, Sedgwick JD. Mechanism of airway inflammation in asthma. Am Rev Respir Dis 1993;147:S20-4.
21. Vogel HG. Drug Discovery and Evaluation: Pharmacological Assays; Including a CD-ROM. Berlin, Heidelberg: Springer Science & Business Media; 2002.
22. Mitra SK, Gopumadhavan S, Venketaranganna MV, Antulikar SD. Antiasthmatic and antianaphylaxis effect of E-721 B, a herbal formulation. Indian J Pharmacol 1999;31:133-7.
23. Rang HP, Dale MM, Ritter JM, Moore PK. How drugs act: Molecular aspects. In: Pharmacology. 5th ed. Ch. 3. Philadelphia, PA: Churchill Livingstone; 2003.
24. Ozolua RI, Eboka CJ, Duru CN, Uwaya DO. Effects of aqueous leaf extract of *Bryophyllum pinnatum* on guinea pig tracheal ring contractility. Niger J Physiol Sci 2010;25:149-57.
25. Mans DR, Toelsie J, Mohan S, Jurgens S, Muhringen M, Illes S, *et al.* Spasmogenic effect of a *Solanum melongena* leaf extract on guinea pig tracheal chains and its possible mechanism(s). J Ethnopharmacol 2004;95:329-33.
26. Schuler V, Suter K, Fürer K, Eberli D, Horst M, Betschart C, *et al. Bryophyllum pinnatum* inhibits detrusor contractility in porcine bladder strips - Apharmacological study towards a new treatment option of overactive bladder. Phytomedicine 2012;19:947-51.
27. Nassis CZ, Haebisch EM, Giesbrecht AM. Antihistamine activity of *Bryophyllum calcium*. Braz J Med Biol Res 1991;25:929-36.
28. Gundamaraju R, Maheedhar K, Hwi KK. Exploiting the phenomenal anti-ulcerogenic potential of *Talinum portulacifolium* ethanolic extract whole plant on albino rats: The therapeutic potential of Chinese herb machi xi Anke (*Portulaceae*). Pharmacogn Res 2014;6:227-33.
29. Goel RK, Sairam K. Antiulcer drugs from indigenous sources with emphasis on *Musa sapientum, Tamra bhasma, Asparagus racemosus* and *Zingiber officinale*. Indian J Pharmacol 2010;34:100-10.
30. Satyanarayana K, Krishnaveni K, Mishra R, Kumar KS. Evaluation of analgesic activity of *Talinum*

portulacifolium root extract on the experimental animal model. J Pharm Res 2012;5:4100-2.
31. Srinivas KV, Koteswara Rao Y, Mahender I, Das B, Rama Krishna KV, Hara Kishore K, *et al.* Flavanoids from *Caesalpinia pulcherrima*. Phytochemistry 2003;63:789-93.
32. Hazekamp A, Verpoorte R, Panthong A. Isolation of a bronchodilator flavonoid from the Thai medicinal plant *Clerodendrum petasites*. J Ethnopharmacol 2001;78:45-9.
33. Taur DJ, Patil RY. Evaluation of antiasthmatic activity of *Clitoria ternatea* L. roots. J Ethnopharmacol 2011;136:374-6.
34. Ezike AC, Akah PA, Okoli CO. Broncho spasmolytic activity of the extract and fractions of *Asystasia gangetica* leaves. Int J Appl Res Nat Prod 2008;1:8-12.
35. Terao J, Piskula M, Yao Q. Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. Arch Biochem Biophys 1994;308:278-84.
36. Ferrándiz ML, Alcaraz MJ. Anti-inflammatory activity and inhibition of arachidonic acid metabolism by flavonoids. Agents Actions 1991;32:283-8.
37. Shaik YB, Castellani ML, Perrella A, Conti F, Salini V, Tete S, *et al.* Role of quercetin (a natural herbal compound) in allergy and inflammation. J Biol Regul Homeost Agents 2006;20:47-52.
38. Kahraman A, Erkasap N, Köken T, Serteser M, Aktepe F, Erkasap S. The antioxidative and antihistaminic properties of quercetin in ethanol-induced gastric lesions. Toxicology 2003;183:133-42.
39. Datta B, Datta S, Khan T, Kundu J, Rashid M, Nahar L, *et al*. Anticholinergic, cytotoxic and anti-HIV-1 activities of sesquiterpenes and a flavonoid glycoside from the aerial parts of *Polygonum viscosum*. Pharm Biol 2004;42:18-23.
40. Sunil J, Nath MS, Raja S, Vinatha B. Phytochemical studies on *Talinum portulacifolium* (Forsk). Sci J Pharm 2010;1:1-4.
41. Jeon IH, Kim HS, Kang HJ, Lee HS, Jeong SI, Kim SJ, *et al.* Anti-inflammatory and antipruritic effects of luteolin from *Perilla* (*P. frutescens* L.) leaves. Molecules 2014;19:6941-51.
42. Singh B, Nadkarni JR, Vishwakarma RA, Bharate SB, Nivsarkar M, Anandjiwala S. The hydroalcoholic extract of *Cassia alata* (Linn.) Leaves and its major compound rhein exhibits antiallergic activity *via* mast cell stabilization and lipoxygenase inhibition. J Ethnopharmacol 2012;141:469-73.
43. Sagar R, Sahoo HB. Evaluation of antiasthmatic activity of ethanolic extract of *Elephantopus scaber* L. leaves. Indian J Pharmacol 2012;44:398-401.

Source of Support: Nil. **Conflict of Interest:** None declared.