Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(2): 1160-1171

Received: 01-01-2017
Accepted: 02-02-2017

Mamillapalli Vani
(1). Research Scholar Jawaharlal Nehru Technological University, Kakinada, East Godavari District, Andhra Pradesh, India
(2). Department of Pharmacognosy & Phytochemistry, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada, Pin: 521108, Krishna District, Andhra Pradesh, India

SK Abdul Rahaman
Department of Medicinal Chemistry, Nirmala college of Pharmacy, Atmakur, Mangalagiri, Guntur (Dt.), Andhra Pradesh, India

Avula Prameela Rani
Department of Pharmaceutics, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur (Dt.). Andhra Pradesh, India

Correspondence
Mamillapalli Vani
(1). Research Scholar Jawaharlal Nehru Technological University, Kakinada, East Godavari District, Andhra Pradesh, India
(2). Department of Pharmacognosy & Phytochemistry, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada, Pin: 521108, Krishna District, Andhra Pradesh, India

In vivo antiasthmatic studies & phytochemical characterization on the stem extracts of *Alternanthera sessilis* L. using Guinea pigs model

Mamillapalli Vani, SK Abdul Rahaman and Avula Prameela Rani

Abstract
The present investigation has been carried out to evaluate the antiasthmatic activity by *in vitro* and *in vivo* models *in* guinea pigs followed by phytochemical characterization using Gas chromatography Mass spectrum analysis of the hydroalcoholic and acetone extracts of the stem extracts of *Alternanthera sessilis.* In histamine and acetylcholine-induced bronchospasm studies acetone extracts of the plant have significantly increased PCT 10.52 and 11.36 by Tukey's test (**p<0.01), compared with control. Histamine and acetylcholine-induced ileum contraction studies also showed that the acetone extracts exhibited response was1.8 with 63.3% and 2.7 with 51% inhibition by Dunnett's test (p<*0.05). 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 respectively. The results of present study suggest the usage of plant extracts as antiasthmatic agents due to the phytochemicals reported through GC-MS.

Keywords: *Alternanthera sessilis*, asthma, bronchospasm, ileum contractions, GC-MS

1. Introduction
Asthma is a complex inflammatory disease. It causes airway narrowing associated with changes in the levels of eosinophils, mast cells, lymphocytes, cytokines and other inflammatory cell mediators [1]. Asthma patients have high levels of specific IgE that bind to receptors on mast cells and other inflammatory cell regulators [2]. The interaction between IgE antibody and antigen results in the activation of a series of inflammatory cellular reactions, including the release of mediators such as histamines, prostaglandins, and leukotrienes, which subsequently lead to contraction of airway smooth muscle and bronchoconstriction [3].The medicinal plant used for the treatment of asthma should have anti-inflammatory, immunomodulatory, antihistaminic, anticholinergic, smooth-muscle relaxant and antiallergic activities [4]. Current asthma therapy lacks satisfactory success due to adverse effect; hence patients are seeking complementary and alternative medicine to treat their asthma [4]. Asthma affects about 300 million people worldwide and it has been estimated that a further 100 million will be affected by 2025 [5]. *Alternanthera sessilis* is used as a vegetable in Asia, traditionally used in skin diseases, to cure wounds and as an antidote. The plant is reported for various pharmacological activities like haematinic [6], antioxidant [7], anti-inflammatory [8], hepatoprotective [9], antiulcer [10], antimicrobial [11], and wound healing [12]. It is reported to contain β- carotene [13], lupeol [14], α and β spinasterol [15], β-sitosterol, stigmasterol [16], and campesterol [13]. The literature survey indicates there are no reports available on antiasthmatic studies on *A.sessilis*. Therefore the present study was planned to conduct *in-vitro* and *in vivo* animal model studies for antiasthmatic activity and characterization of phytochemicals using Gas chromatography-mass spectrum (GC-MS) analysis.

2. Materials and Methods
2.1 Chemicals and Reagents
Histamine hydrochloride, acetylcholine, chlorpheniraminemaleate, atropine sulfate were purchased from Sigma- Aldrich chemical Co.

2.2 Experimental
Guinea pigs (400–600 g) of either sex were purchased from Mahaveer enterprises, Hyderabad,Telangana, India, housed in standard conditions of temperature (22 ± 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 (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.3 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, Dept. of Botany & Microbiology, Acharya Nagarjuna University (ANU), Guntur (Dt), Andhra Pradesh, India. A voucher specimen 002/VIPW was deposited for future reference in the department of Pharmacognosy, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada.

2.4 Preparation of the extract
The stems 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) and preserved in refrigerator till use. The yield of hydroalcoholic extract was found to be 20% w/w on air-dried basis. The yield of acetone extract was found to be 21% w/w on air dried basis respectively.

2.5 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 [17].

2.6 Acute toxicity testing
The animals were overnight fasted prior to 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 hr, next half-hourly intervals for 4 hours for any gross changes in their behavior and then up to 24 hours for any mortality as per the Organization for Economic Co-Operation and Development (OECD) guidelines 425 [18].

2.7 Histamine- induced bronchospasm in guinea pigs
Guinea pigs of either sex were divided into four groups. Each group comprised of 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 *Alternanthera sessilis* (ASHA)
Group-4: Test-2 group animals received acetone extract of *Alternanthera sessilis* (ASAE)
Animals were exposed to 0.1% w/v of histamine dihydrochloride aerosol in a histamine chamber (Sigma Scientific). Progressive dyspnoea 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 dyspnoea leading to the appearance of convulsions on day 0 (T_1). As soon as dyspnoea commenced, the animals were removed from the chamber and placed in fresh air. Animals were given ASHA and ASAE at a dose of 400 mg/kg orally (*p.o.*) once a day for 7 days. On the seventh day, 2 hours after the last dose, PCT was recorded (T_2).

2.8 Acetylcholine- induced bronchospasm in guinea pigs
Guinea pigs of either sex were divided into four groups. Each group comprised of 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 ASHA
Group-4: Test-2 group animals received ASAE
Animals were exposed to 0.5% acetylcholine chloride aerosol. The experimental procedure was followed as above. [19]
The percentage increase in time of PCT was calculated using the following formula:

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

Where T_1 is PCT on day 0 and T_2 is PCT on day 7.

2.9 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 1cm, 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 ASHA
Group 4: Test-2 group animals received ASAE
The isolated ileum was mounted in a 30 ml Organ bath (Lab Tree India) containing a tyrode solution, maintained at 37 ± 1 ^{0}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.

2.10 Acetylcholine- induced guinea pig ileum contraction
Group 1: Control group animals received acetylcholine
Group 2: Standard group animals received atropine sulfate
Group 3: Test-1 group animals received ASHA
Group 4: Test-2 group animals received acetone extract of ASAE
The same above experimental procedure was carried out for the study [20].

2.11 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). ASAE (20 ml) was applied to the column by use of a pipette. It was eluted sequentially with solvents in the increasing order of polarity. The chloroform fraction was collected, found spots with the solvent system dichloromethane: n-hexane at 1:1 ratio on thin layer chromatography (TLC), and sent for GC-MS analysis [21].

2.12 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 30mts, ID 0.25mm, film thickness 0.25µm, carrier gas (mobile phase) helium was set at a flow rate of 1.0 ml/min, the oven temperature was 70 ^{0}C raised to 260 ^{0}C at 6 ^{0}C/min and injection volume was 1µl. 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 [22].

2.13 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, followed by Tukey's 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 $^{**}p<0.01$ respectively.

3. Results

3.1 Phytochemical screening

Preliminary phytochemical screening of ASHA and ASAE showed the presence of alkaloids, glycosides, terpenoids, tannins, flavonoids, steroids, amino acids, and proteins.

3.2 Acute toxicity testing

The hydroalcoholic and acetone extracts of the plant were administered orally to guinea pigs up to a dose of 2000 mg/kg body weight. After 24 hrs, 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 the present study.

3.3 Effect of ASHA and ASAE 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 4.22 and 10.52 for ASHA and ASAE respectively. ASAE significantly ($^{**}P<0.01$) increased PCT following exposure to histamine than ASHA (Table 1; Fig 1).

Table 1: Effect of ASHA and ASAE on histamine-induced guinea pig bronchial contraction

S.no	Groups	Drug and Dose	PCT Mean ± SEM
1	Control	Distilled water p.o.	2.22±0.24
2	Standard	Chlorpheniramine 2mg/kg	8.77±0.43**
3	Test-1	ASHA 400mg/Kg p.o.	4.22±1.04
4	Test-2	ASAE 400mg/Kg p.o.	10.52±1.58**

Note: Each value was expressed as Mean ± SEM, where n=4 in each group;$^*p<0.05$,
$^{**}p<0.01$ compared with control by one-way ANOVA, Tukey's test.

3.4 Effect of ASHA and ASAE on acetylcholine- induced bronchospasm in guinea pigs

The plant extracts offered protection against bronchospasm induced by acetylcholine as compared to control. The increase in PCT at a dose of 400 mg/kg body weight of animals was found to be 7.38 and 11.36 for ASHA and ASAE respectively. ASAE significantly ($^{**}P<0.01$) increased PCT following exposure to acetylcholine when compared to ASHA (Table 2 & Fig. 2).

Table 2: Effect of ASHA and ASAE on acetylcholine-induced guinea pig bronchial contraction

S.no	Groups	Drug and Dose	PCT Mean ± SEM
1	Control	Distilled water p.o.	3.22±0.60
2	Standard	Atropine sulphate 2mg/kg	11.60±1.24**
3	Test-1	ASHA 400mg/Kg p.o.	7.38± 0.96
4	Test-2	ASAE 400mg/Kg p.o.	11.36±1.44**

Note: 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.

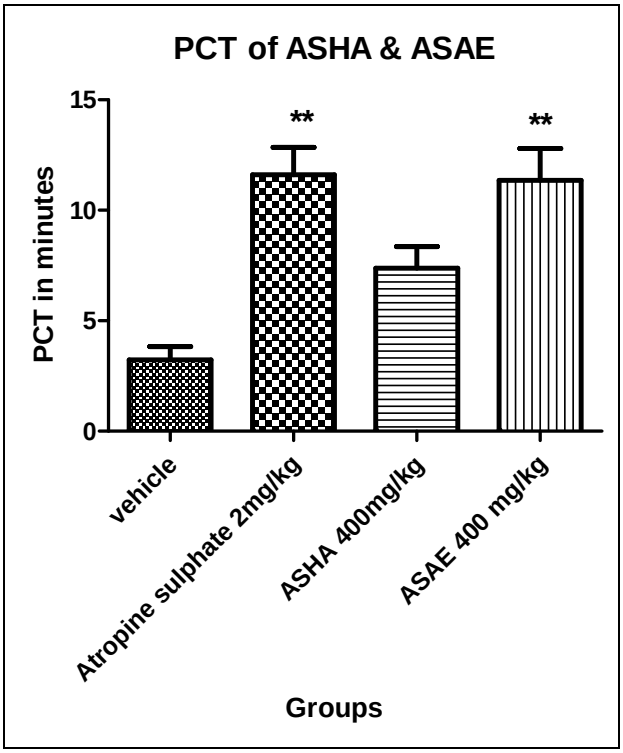


Fig 2: Effect of ASHA and ASAE on acetylcholine-induced guinea pig bronchial contraction

3.5 Effect of ASHA and ASAE 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 61.3% at the response of 1.9 and 63.3% at the response of 1.8 for ASHA and ASAE respectively. In isolated guinea pig ileum studies both ASHA and ASAE significantly ($^*p<0.05$) inhibited the contraction of ileum when compared to control (Table 3).

Table 3: Effect of ASHA and ASAE on histamine-induced guinea pig contractions on ileum

S.no	Groups	Drug and Dose	Response Mean ± SEM	% Inhibition
1	Control	Histamine 0.5 mg	4.9±0.08	0%
2	Standard	Chlorpheniramine 0.5mg	1.8±0.91*	63.3%
3	Test-1	ASHA 0.5 mg	1.9 ± 0.09*	61.3%
4	Test-2	ASAE 0.5 mg	1.8 ± 0.34*	63.3%

Note: 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.

3.6 Effect of ASHA and ASAE on acetylcholine-induced guinea pig ileum contractions

The plant extracts inhibited the contraction induced by acetylcholine as compared to control. The percentage of inhibition was found to be 6% at the response of 5.2 and 51% at the response of 2.7 for ASHA and ASAE respectively. In isolated guinea pig ileum studies, the ASAE significantly (*p<0.01) inhibited the contraction of ileum when compared to ASHA (Table 4).

Table 4: Effect of ASHA and ASAE on acetylcholine-induced guinea pig contractions on ileum

S.no	Groups	Drug and Dose	Response Mean ± SEM	% Inhibition
1	Control	Acetylcholine 0.1mg	5.5±0.27	0%
2	Standard	Atropine sulphate 0.5mg	2.2±0.81*	60%
3	Test-1	ASHA 0.5 mg	5.2 ± 0.65*	6%
4	Test-2	ASAE 0.5 mg	2.7 ± 0.06*	51%

Note: 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.

3.7 Identification of chemical constituents

The compounds were identified by mass spectrometry attached through GC. The mass spectrometer 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 of peaks at different m/z ratios[21]. Identification of the chemical constituents was done on the basis of retention index (RI) using a library search and by comparing the mass spectral and retention data with literature. These mass spectra are fingerprints of those compounds which can be identified from the library data [22]. The GC-MS confirmed the presence of various components with different retention times (Fig. 3).The identified components by GC-MS from the isolated fraction of ASAE were shown (Table 5- 6; Fig. 4-11).

The phytoconstituents with major % peak area were 9-Methoxy-5-methyl-6-phenyl-3, 4, 5, 6-tetrahydro-2H-1, 5-benzoxazocinium iodide monohydrate (2.00%), cyclohexasiloxane, dodecamethyl (0.40%), 2,6-Naphthalenedione, octahydro-1,1,8a-trimethyl-,trans(4.16%), 2-(4'-methoxyphenyl)-5-(4"- ethoxy naphthyl) thiophene (2.19%), hexadecanoic acid, methyl ester (3.89%), 9-octadecenoic acid (Z)-, methyl ester (3.89%), 5-(1-bromo-1-methyl-ethyl)-2-methyl-cyclohexanol(5.61%), 2,2-dimethylcholest-4-en-3-one (4.95%) respectively.

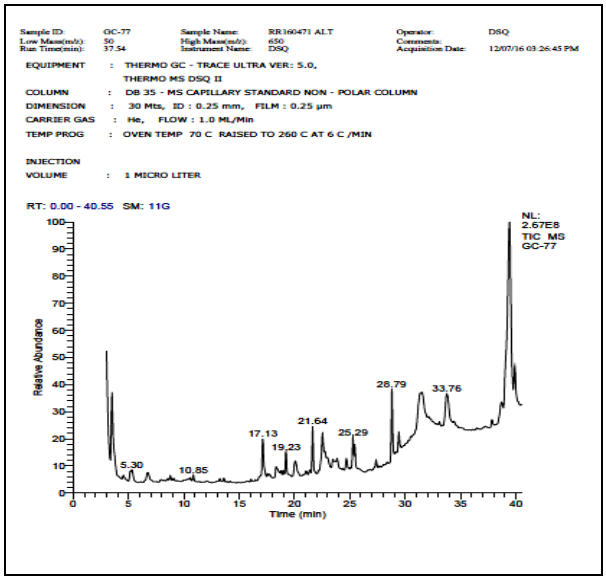


Fig 3: GC-MS spectrum of ASAE column fraction

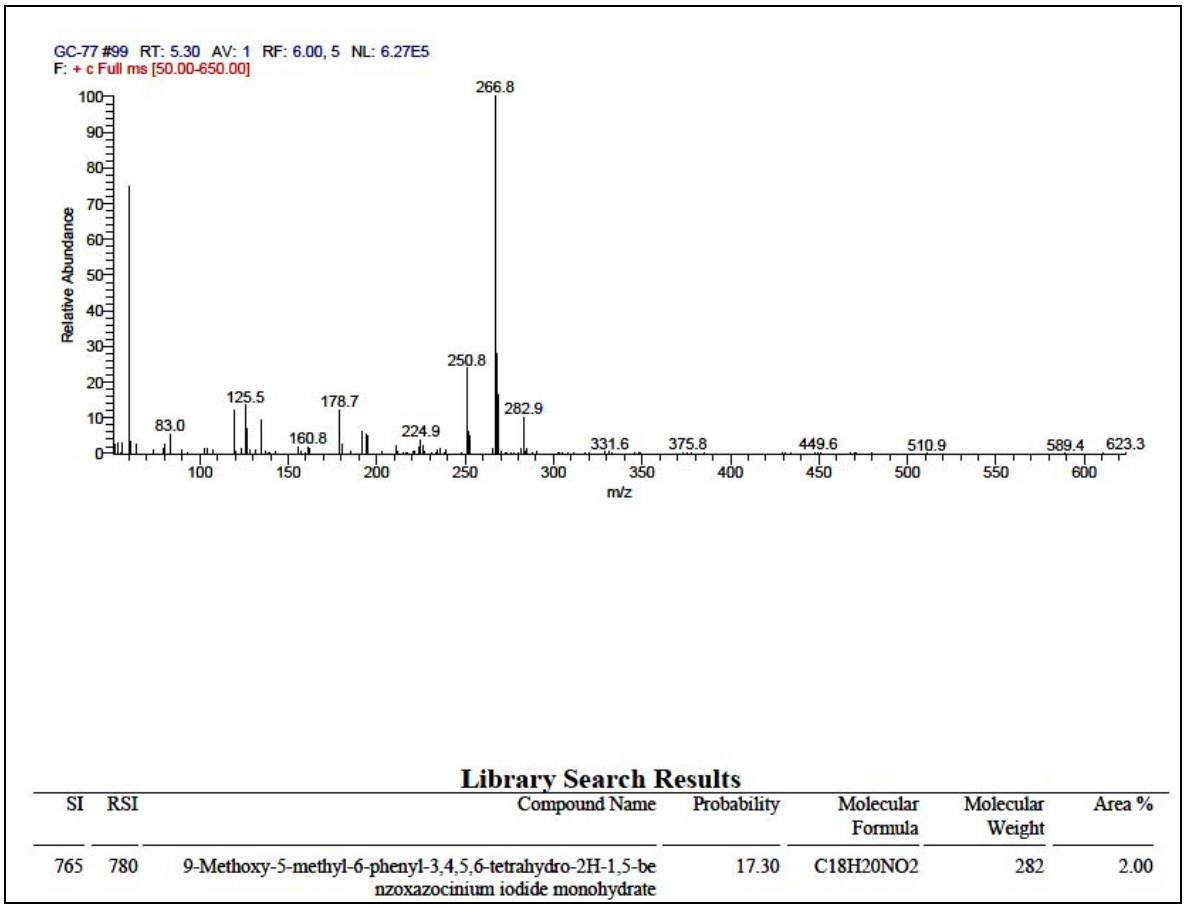
Table 5: Major chemical constituents of ASHA from column fraction by GC-MS analysis

S. no	Retention time	Name of Phyto constituent	Mol. Formula	Mol. weight	% peak area
1.	5.30	9-Methoxy-5-methyl-6-phenyl-3,4,5,6-tetrahydro-2H-1,5-benzoxazocinium iodide monohydrate	C18H20NO2	282	2.00
2.	10.85	Cyclohexasiloxane, dodecamethyl	C12H36O6Si6	444	0.40
3.	17.13	2,6-Naphthalenedione, octahydro-1,1,8a-trimethyl-, trans	C13H20O2	208	4.16
4.	19.23	2-(4'-Methoxyphenyl)-5-(4"-ethoxynaphthyl)thiophene	C22H18O2S	346	2.19
5.	21.64	Hexadecanoic acid, methyl ester	C17H34O2	270	3.89
6.	25.29	9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	296	3.89
7.	28.79	5-(1-Bromo-1-methyl-ethyl)-2-methyl-cyclohexanol	C10H19BrO	234	5.61
8.	33.76	2,2-dimethylcholest-4-en-3-one	C29H48O	412	4.95

Table 6: Other chemical constituents of ASHA from column fraction by GC-MS analysis

S. no.	Retention time	Name of Phyto constituent	Mol. Formula	Mol. weight	% peak area
1.	3.50	2,2-Dimethyl-3,3-(1',2'-ethylidene)-5-chloro-1,2-dihydrobenzofuran	C12H13ClO	208	7.52
2.	4.53	2,5,5-Trimethyl-2-(butylthio)cycloheptatriene	C14H22S	222	0.46
3.	6.73	Ethyl4-(chloromethylene)-2,2-diphenyl-3-oxazoline-5-carboxylate	C19H16ClNO3	341	1.67
4.	8.77	3-[4'-(2"-Chlorophenyl)-2'-thiazolyl]-2,4-dioxo-1.2.3.4-tetrahydroquinazoline	C17H10ClN3O2S	355	0.62
5.	10.56	1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-heptasiloxane	C14H44O6Si7	504	0.50
6.	13.25	1-Methyl-3-(3,4-dimethoxyphenyl)-6,7-imethoxyisochromene	C20H22O5	342	0.47
7.	17.74	6-(4'-Chlorophenyl)-2-methyl-4-phenylpyridine	C18H14ClN	279	0.45
8.	18. 34	6-Nitro-cylohexadecane-1,3-dione	C16H27NO4	297	1.74
9.	20.07	1,3,5-Trisilacyclohexane, 1,1-dimethyl-	C5H16Si3	160	2.28

10.	21.05	9H-Purin-6-ol, 8-(3,3,3-trifluoro-1-propenyl)thio-	C8H5F3N4OS	262	0.46
11.	22.54	Sodwanone O	C30H48O5	488	6.58
12.	23.47	2-Cyclopentene-1-undecanoic acid, methyl ester	C17H30O2	266	0.59
13.	23.84	1-Methyl-trans-decahydroquinol-4-ol (equat.)	C10H19NO	169	1.71
14.	24.69	9-Methyl-11-oxo-1,6-iazatricyclo[7.2.0.0(6,8)]undecane	C10H16N2O	180	1.02
15.	27. 36	2-Cyclopentene-1-tridecanoic acid, methyl ester, (S)-	C19H34O2	294	0.87
16.	29.42	(2s,4r)-4-hydroxy-2-benzylprolin-halbhydrat	C14H22O2	222	1.60
17.	31. 34	Di-t-butyl 2-bromophenylphosphate	C14H23BrO4P	365	7.21
18.	37.27	cis-5,5-Dimethyl-1,3-dithiane 1,3-dioxide	C6H12O2S2	180	0.42
19.	37.84	cis-trans-cis-2,4,6,8-tetramethyltetraphenylcyclotetrasiloxane	C28H32O4Si4	544	0.71
20.	38.67	7-(3-(4-Bromophenyl)-4,6-dimethoxy-2-methylindolyl)ethanone	C19H18BrNO3	387	1.47
21.	39.43	Carotene	C40H56	536	32.43
22.	39.91	8,12-Diethyl-1,9-dioxo-2,3,7,13,17,18-hexamethyl-1,19,22,24-tetrahydro-21H-bilin	C29H34N4O2	470	2.15



Library Search Results

SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
765	780	9-Methoxy-5-methyl-6-phenyl-3,4,5,6-tetrahydro-2H-1,5-benzoxazocinium iodide monohydrate	17.30	C18H20NO2	282	2.00

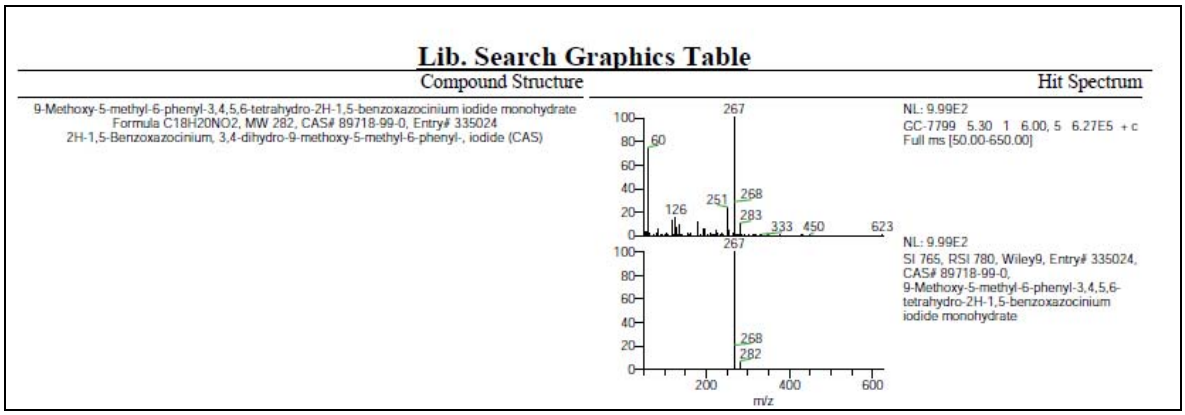
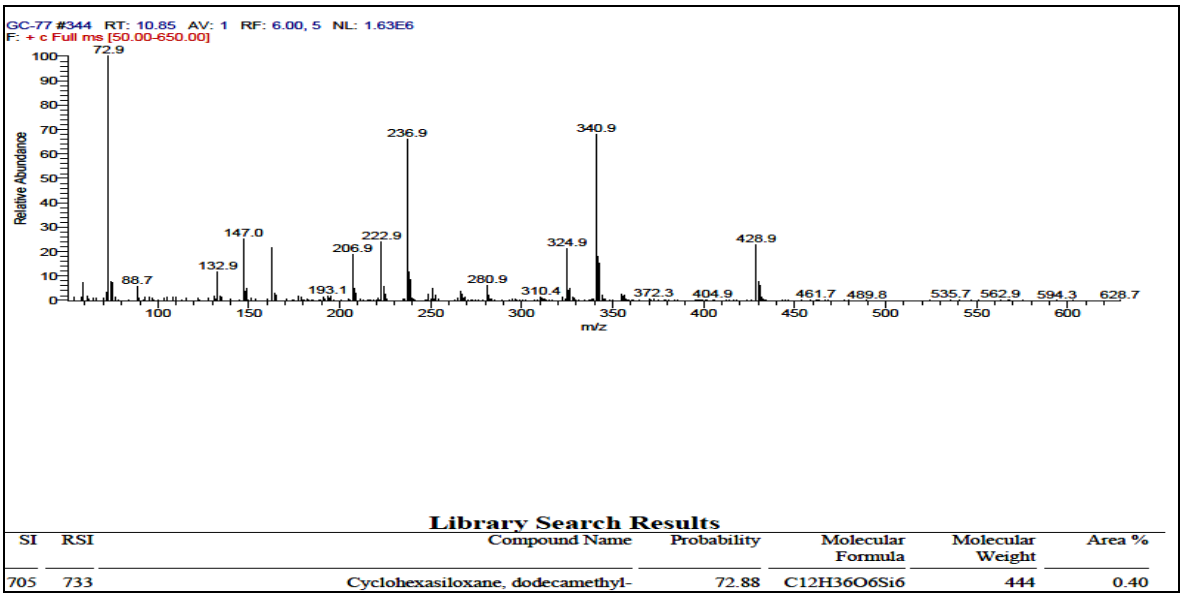


Fig 4: Mass spectrum at RT 5.30



Library Search Results

SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
705	733	Cyclohexasiloxane, dodecamethyl-	72.88	C12H36O6Si6	444	0.40

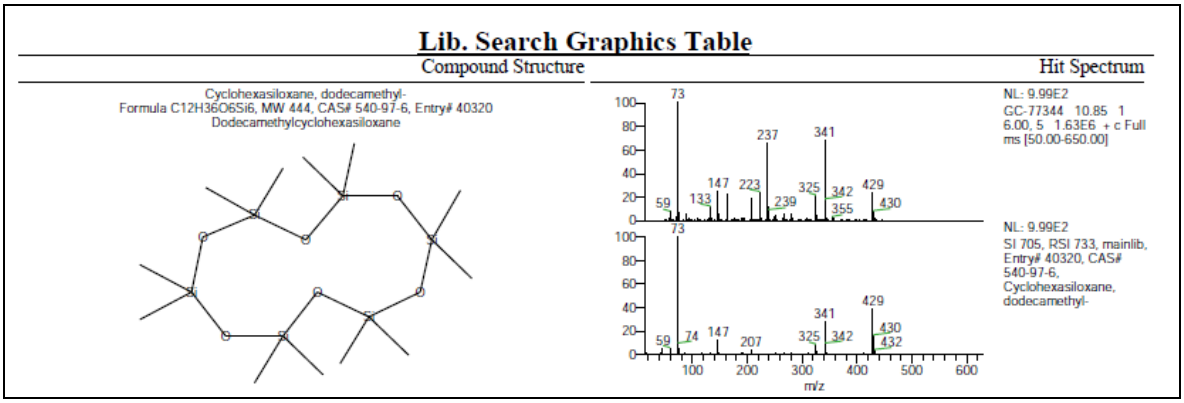
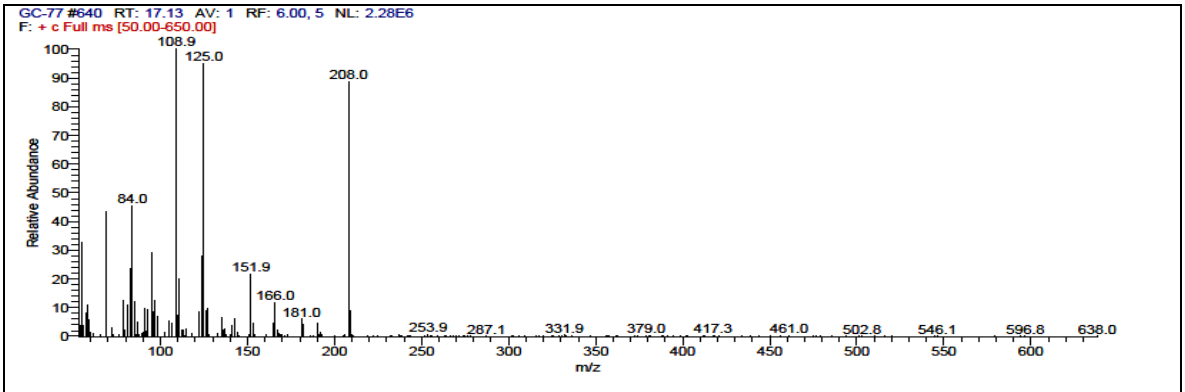


Fig 5: Mass spectrum at RT 10.85



Library Search Results

SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
625	660	2,6-Naphthalenedione, octahydro-1,1,8a-trimethyl-, trans- (CAS)	23.90	C13H20O2	208	4.16

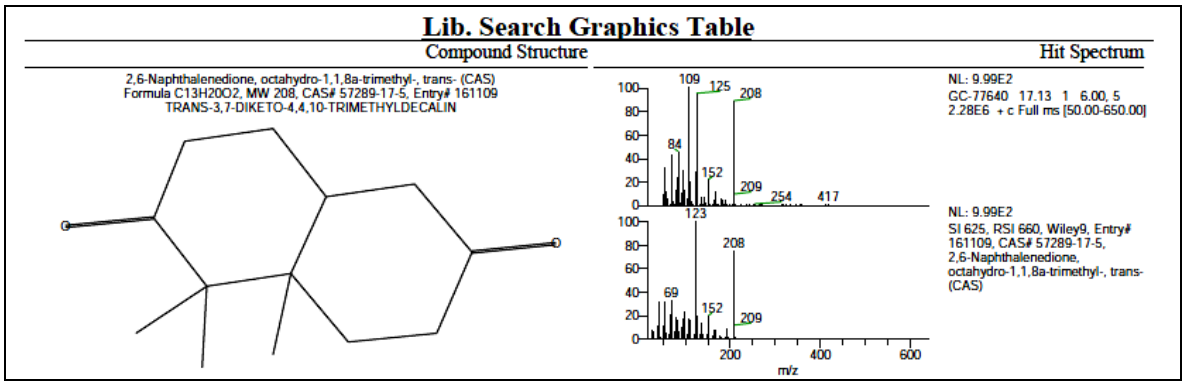
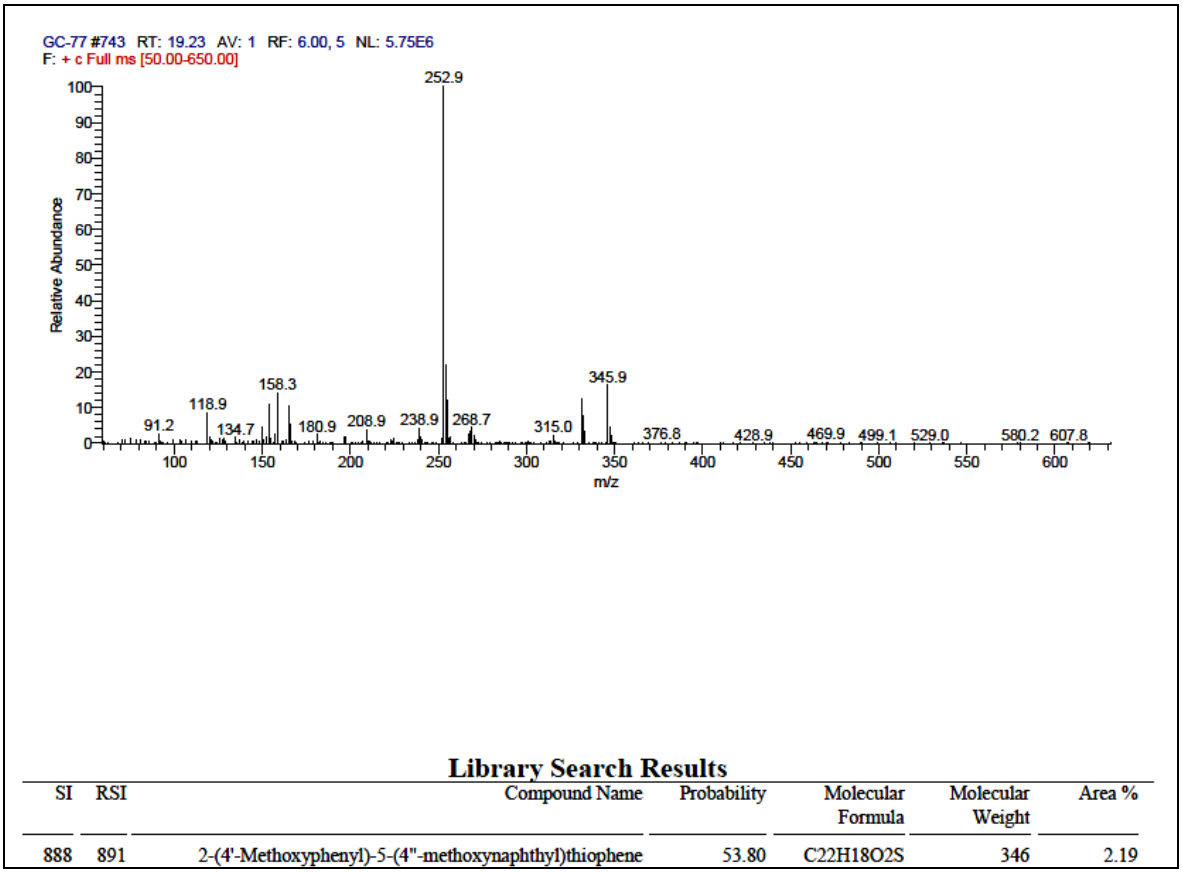


Fig 6: Mass spectrum at RT 17.13



Library Search Results

SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
888	891	2-(4'-Methoxyphenyl)-5-(4"-methoxynaphthyl)thiophene	53.80	C22H18O2S	346	2.19

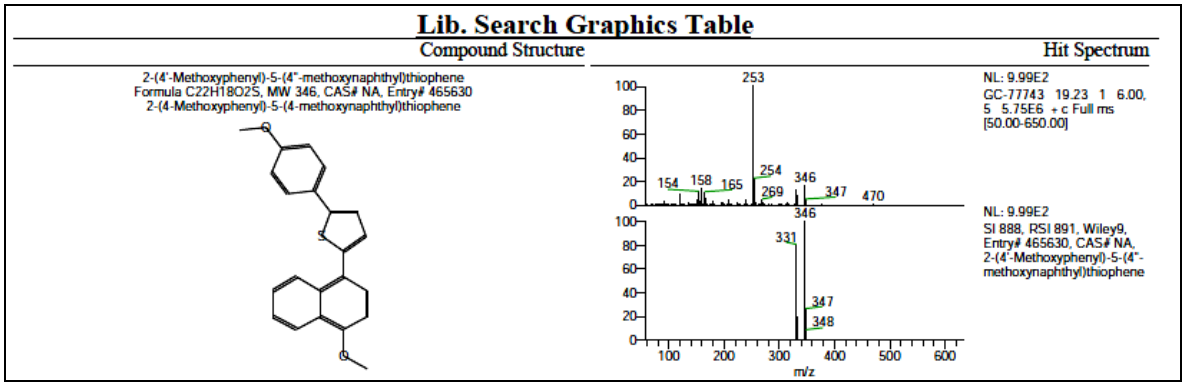
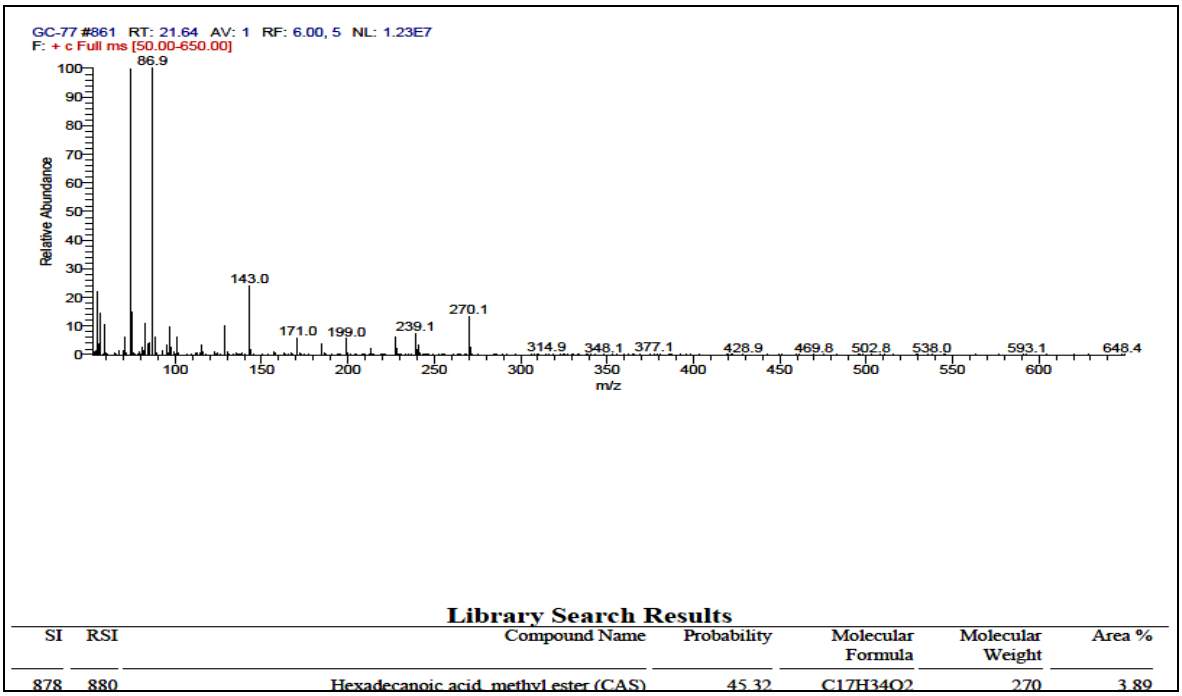


Fig 7: Mass spectrum at RT 19.23



Library Search Results

SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
878	880	Hexadecanoic acid, methyl ester (CAS)	45.32	C17H34O2	270	3.89

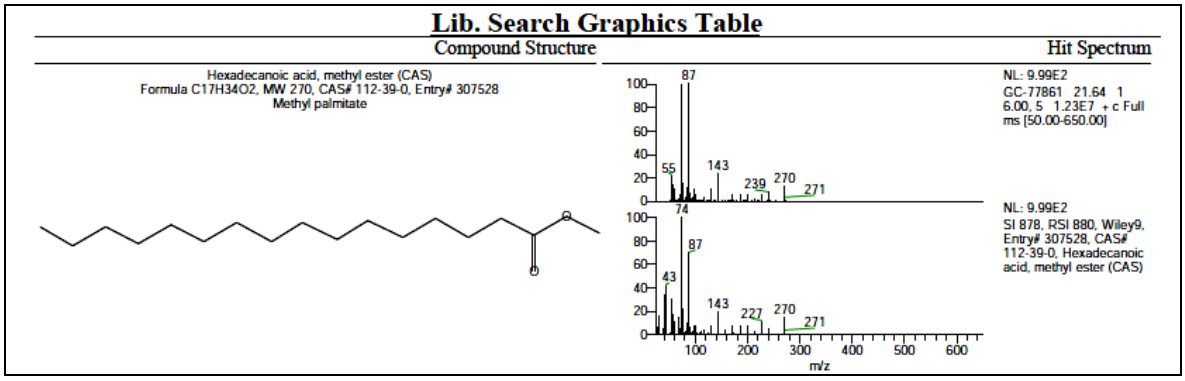
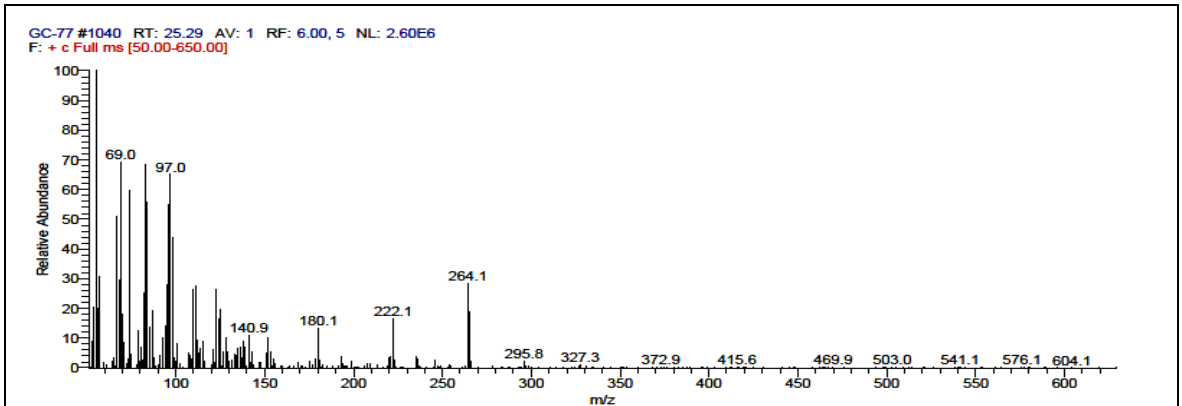


Fig 8: Mass spectrum at RT 21.64.



Library Search Results

SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
849	867	9-Octadecenoic acid (Z)-, methyl ester (CAS)	7.80	C19H36O2	296	3.89

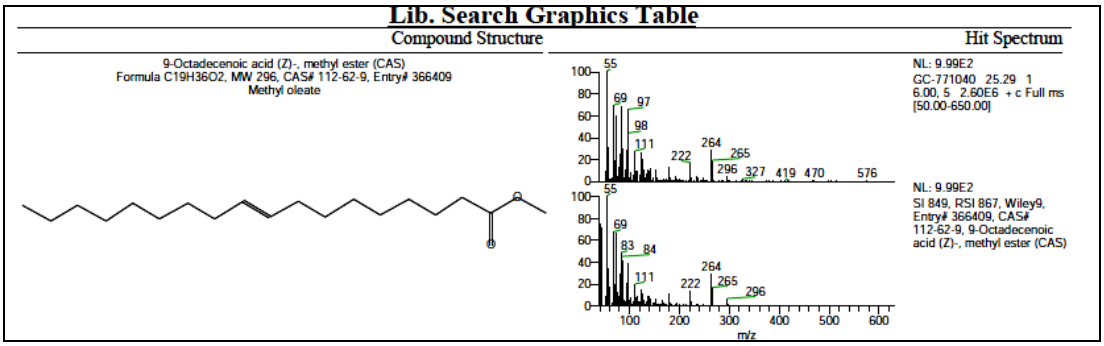
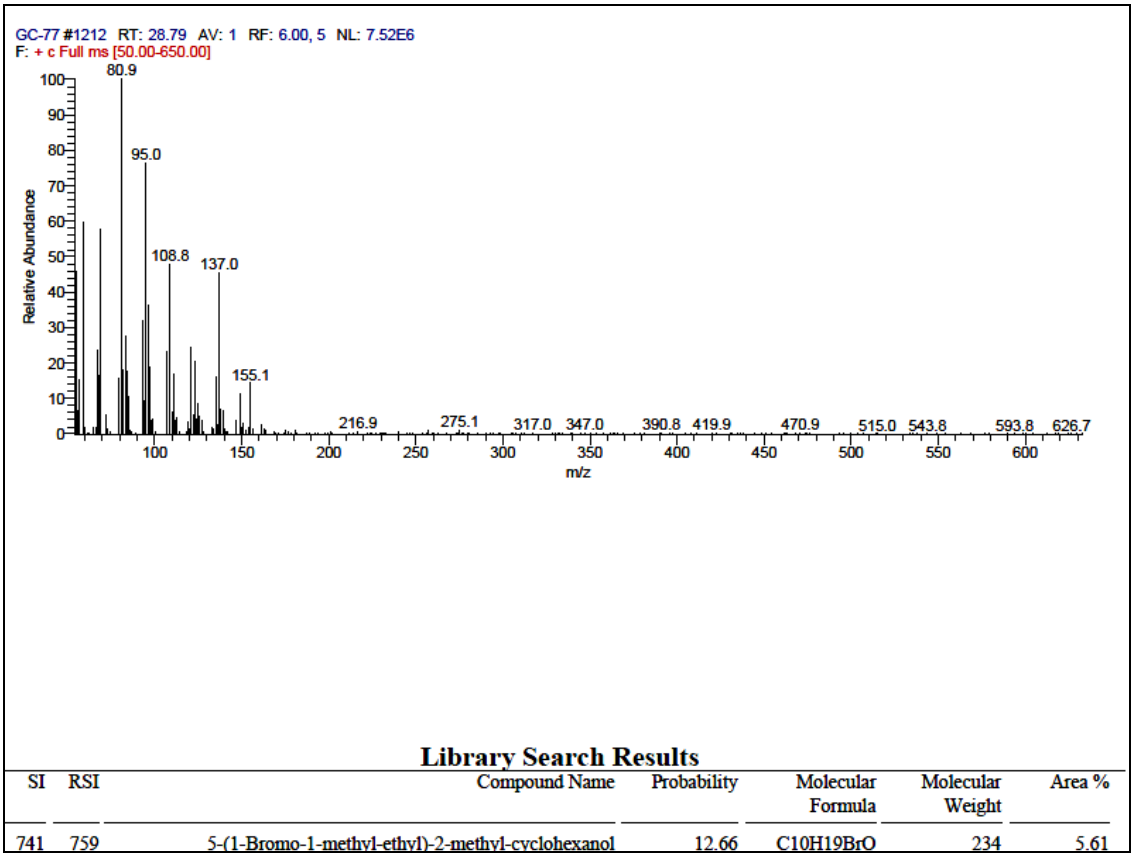


Fig 9: Mass spectrum at RT 25.29



Library Search Results

SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
741	759	5-(1-Bromo-1-methyl-ethyl)-2-methyl-cyclohexanol	12.66	C10H19BrO	234	5.61

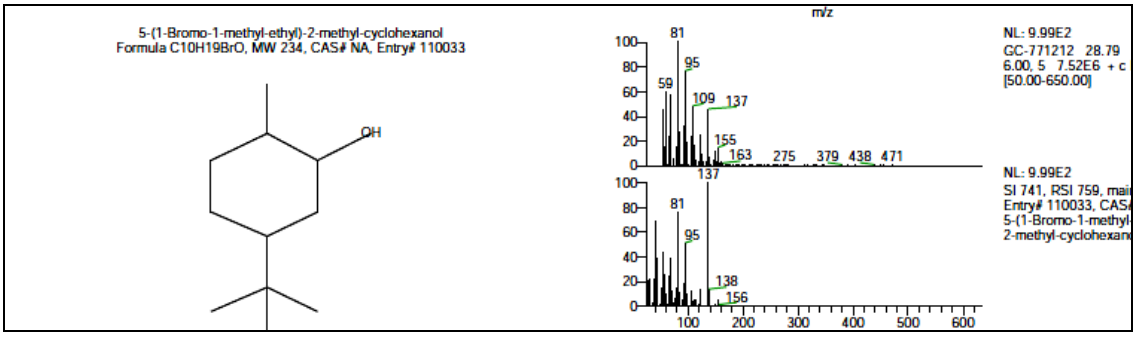
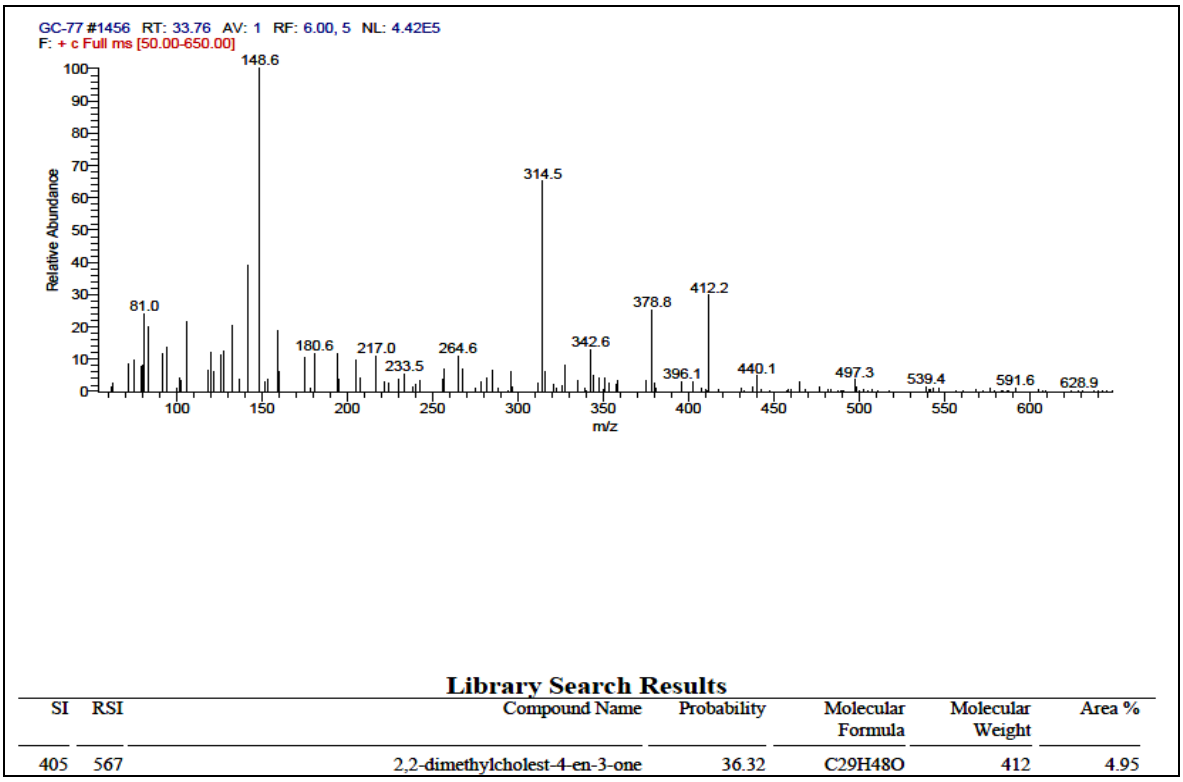


Fig 10: Mass spectrum at RT 28.79



Library Search Results

SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
405	567	2,2-dimethylcholest-4-en-3-one	36.32	C29H48O	412	4.95

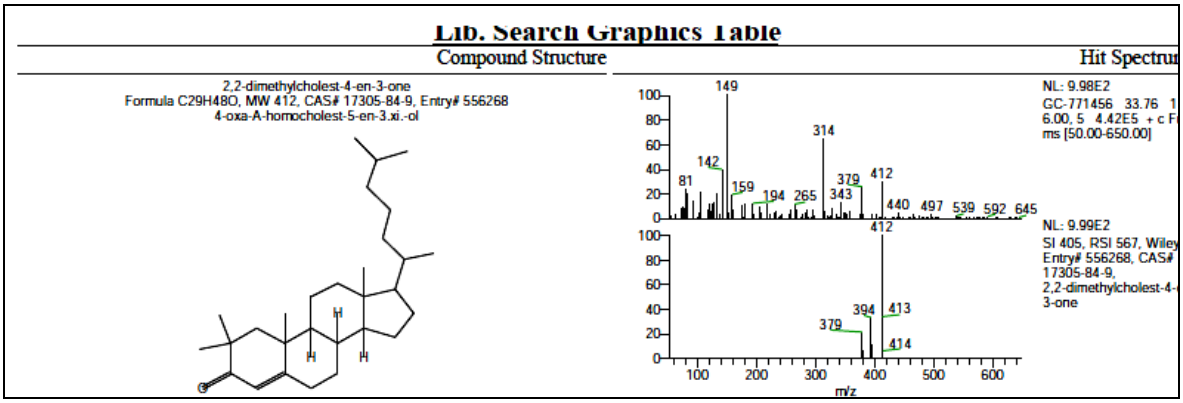


Fig 11: Mass spectrum at RT 33.76

4. Discussion

The study presents indications that hydroalcoholic and acetone extracts of the plant *A.sessilis* can relieve bronchoconstriction. This presumption is based on the examination that the extracts of the plant inhibited the contractions produced by histamine and acetylcholine in guinea pig bronchi and ilei [29, 31]. 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 [19]. 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 [23].

Dyspnoea, asphyxic convulsions like symptoms resembling bronchial asthma can be instigated by inhalation of spasmogens namely histamine and acetylcholine in guinea pigs. This is because histamine H1 sensitive excitatory receptors and acetylcholine muscarinic receptors in the airway smooth muscle of man and animals have been found [24]. They are the most sensitive animals for the study of asthma and allergic disease [25]. The end point pre-convulsive dyspnoea (PCD) was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of asphyctic convulsions *i.e.* pre-convulsion time (PCT) [26]. Prolongation of PCT indicates spasmolytic and anticholinergic activity. In the present study, the ASAE at 400mg/kg significantly (**p<0.01) inhibited histamine (PCT 10.52) and acetylcholine (PCT 11.36) induced bronchoconstriction. Ach (Acetylcholine) causes bronchoconstriction by activating M3 (Gq IP3/DAG pathway) (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 like GIT, bronchi, uterus. Smooth muscles in most organs are contracted. Contractility of bronchi increases by Ach action on M3 receptors [27].

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 [27]. In histamine (ASAE 1.8 response with 63.3% inhibition) and acetylcholine (ASAE 2.7 response with 51% inhibition) induced guinea pig ileum contraction studies the extracts at 0.5mg exhibited significant (*p<0.05) antihistaminic and anticholinergic activities respectively. It is comparable to standard drugs atropine and chlorpheniraminemaleate. The possible involvement of the muscarinic receptor in the apparent broncho spasmolytic 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 [28]. 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 acetylcholine or carbachol [29, 30]. The apparent anti-histaminergic effect of the plant extracts noted in the current study is in accordance with its ability to reduce the force of contraction of isolated pig ilei caused by histamine [31, 29].

ASHA and ASAE exhibited antihistaminic and anticholinergic activities. ASAE exhibited PCT 10.52 (**p<0.01) in the histamine-induced bronchospasm study when compared to control. In acetylcholine-induced bronchospasm study, ASAE exhibited PCT 11.36 (**p<0.01) when compared to control. These studies suggest that ASAE can inhibit H1 receptors as well as muscarinic receptors. The results of histamine-induced ileum contraction study suggest that ASAE exhibited 63.3% inhibition at response 1.8 (*p<0.05). In acetylcholine-induced ileum contraction study ASAE exhibited 51% inhibition at response 2.7 (*p<0.05) where ASHA could inhibit 6%. These results indicate H1receptor antagonistic activity of ASAE. We can assume that ASAE can act as a potential antiasthmatic agent [28, 29, 30].

The support for the antihistaminic and anticholinergic properties exhibited by the plant extracts in the current study has been attributed to the scientific reports on the plant. The leaf extracts of the plant inhibited inflammation mediated through release of histamines and prostaglandins [32]. The plant contains sterols like β-Sitosterol, stigmasterol *etc*. [16]. The anti-inflammatory activity of plant sterols already had been established [33, 34]. Further, the plant is reported to contain lupeol [35, 16]. In a recent study, lupeol showed analgesic activity in an inflammatory model of pain through inhibition of interleukins [36]. In addition, polyphenolic compounds catechin, ellagic acid, and rutin identified in the HPLC analysis, which showed analgesic activity in previous studies [37, 38, 39]. The extracts of the plant had been shown to possess central stimulating analgesic effects [40]. However, the plant extracts showed antiulcer activity indicating antihistaminic activity [10, 41]. The plant possesses wound healing activities [12]. Wound healing involves complex series of interactions between different cell types and cytokine mediators [42].

The extracts increased PCT through dilatation of the bronchial smooth muscles more against histamine than acetylcholine. 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]. The plant extract showed the presence of carotene (Table 6) through GC-MS analysis. Carotene was reported to exhibit antihistaminic, anticholinergic [44] and antiasthmatic properties [45]. These observations indicate the involvement of histaminergic and muscarinic receptors and valid use of plant extracts for the current study. Further GC-MS analysis reported the presence of major constituents 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 (methoxy methyl) ethane respectively (Table 5) and other constituents (Table 6) which may be responsible for the therapeutic activity [22].

5. Conclusion

From abone experimentation we can conclude that the extracts of *Alternanthera sessilis* 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 towards their antiallergic nature by antihistaminic, anticholinergic studies and also by various other mechanisms.

6. Acknowledgements

Authors are thankful to Jawaharlal Nehru Technological University, Kakinada, East Godavari (Dt.), A.P., India, Vijaya Institute of Pharmaceutical Sciences for Women,, Enikepadu, Vijayawada, Krishna (Dt.), A.P., India, Nirmala college of Pharmacy, Atmakur, Mangalagiri, Guntur (Dt.), A.P., India, Department of Pharmaceutics, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur (Dt.), A.P., India for their kind encouragement and support.

7. References

1. Holgate ST, Polosa R. Treatment strategies for allergy and asthma. Nature Reviews Immunology. 2008; 8:218-230.
2. Donno DM, Bittesnich D, Chetta A, Olivieri D, Lopez-Vidriero MT. The effect of inflammation on mucociliary clearance in asthma. Chest. 2000; 118(4):1142-1149.
3. Tattersfield AE, Knox AJ, Britton JR, Hall IP. Asthma. Lancet. 2002; 360:1313-1322.
4. Greenberger PA. Therapy in the management of rhinitis asthma complex. *Allergy* and *Asthma* Proceedings. 2003; 24:403-407.
5. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy. 2004; 59:469-478.
6. Erna C Arollado, Marina O Osi. Hematinic activity of *Alternanthera Sessilis* (L.) *R. Br*. (*Amaranthaceae*) in mice and rats. E-International Scientific Research Journal. 2010; 2:2094-1749.
7. Yadav Sanjay Kumar, Das Sanjib. Evaluation of Anti-Diarrhoeal Property of Crude aqueous Extract of *Alternanthera sessilis Linn.* International Journal of Pharmaceutical Innovations. 2013; 3(3):110-115.
8. Sahithi B, Rajani GP, Sowjanya K, Gupta D. Anti-inflammatory activity of ethanolic and aqueous extracts of *Alternanthera sessilis Linn*. Pharmacologyonline. 2011; 1:1039-1043.
9. Song-Chow Lin, Yun-Ho Lin, Shyh-Jong Shyuu, Chung-Ching Lin. Hepatoprotective effects of Taiwan folk medicine: *Alternanthera sessilis* on liver damage induced by various hepatotoxins. Phytotherapy Research. 2006; 8(7):391-398.
10. Roy amit, Saraf S. Antioxidant and Antiulcer activities of an Ethnomedicine: *Alternanthera Sessilis*, Research

Journal of Pharmacy and Technology. 2008; 5:369-372.
11. Johnson M, Wesely EG, Selvan N, Kavitha MS. *In vivo* and *invitro* Antibacterial efficacy of *Alternanthera sessilis* (*Linn*.). International Journal of Pharma Research and development. 2010; 2(10):72-79.
12. Sunil S Jalalpure, Nitin Agrawal MB, Patil R Chimkode, Ashish Tripathi. Antimicrobial and wound healing activities of leaves of *Alternanthera sessilis Linn*, International Journal of Green Pharmacy. 2008; 2:141-144.
13. Pullaiah T. Encyclopedia of World Medicinal Plants, Vol 1, Published by Regency Publication, New Delhi, 2011.
14. Gupta AK. Indian medicinal plants, ICMR, New Delhi, 2004, 151-7.
15. Rastogi RP, Compendium of Indian medicinal plant, 2nd ed., CDRI, Lucknow, 1993, 1970-9.
16. Sinha P, Arora VK, Wahi SP. Chemical investigation on *Alternanthera sessilis*. Indian Drugs. 1984; 1:139-40.
17. Evans WC. Trease and Evans' pharmacognosy. 15th ed., W.B. Sounders Company Ltd, London, 2005.
18. OECD guidelines for the testing of chemicals-425. Acute oral toxicity-up and down procedure (UDP), 2008.
19. Chandrakant Nimgulkar C, Dattatray Patil S, Dinesh Kumar B. Anti-asthmatic and anti-anaphylactic activities of *Blatta orientalis* mother tincture. Homeopathy. 2011; 100:138-143.
20. Savita D Patil, Sameer V Ahale, Sanjay J Surana. Evaluation of antiasthmatic activity and anaphylactic activity of *Balanites aegyptica (Delile), Balanitaceae*. Asian Journal of Pharmaceutical and Clinical Research. 2011; 4:52-55
21. Elizabeth Thomas, Anees TP, Della Grace Thomas, RR Anandan. GC-MS Analysis of phytochemical compounds present in the rhizomes of *Nervilia aragoana Gaud*. Asian Journal of Pharmaceutical and Clinical Research. 2013; 6(3):68-74.
22. Manjamalai A, Yasaswini Narala, Aiswarya Haridas, Berlin Grace VM. Antifungal, anti-inflammatory and GC-MS analysis of a methanolic extract of Plectranthus amboinicus. International Journal of Current Pharmaceutical Research. 2011; 3:129-132.
23. Goodman LS, Gilman A. The Pharmaceutical basis of therapeutics, 10th ed., Macmillan Press, London, 2001.
24. Busse WW, Calhoun WJ, Sedgwick JD. Mechanism of airway inflammation in asthma. American Revised Respiratory Disease. 1993; 147:S20-S24.
25. Vogel HG. Drug Discovery and Evaluation: Pharmacological Assays; Including a CD-ROM. Springer Science & Business Media. Berlin Heidelberg, 2002.
26. Mitra SK, Gopumadhavan S, Venketaranganna MV, Antulikar SD. Antiasthmatic and antianaphylaxis effect of E-721 B, a herbal formulation. Indian J Pharmacol. 1999; 31:133-137.
27. Rang HP, Dale MM, Ritter JM, Moore PK. Chapter 3: How drugs act: molecular aspects. In: Pharmacology, Fifth ed: Churchill Livingstone, 2003.
28. Ozolua RI, Eboka CJ, Duru CN, Uwaya DO. Effects of aqueous leaf extract of *Bryophyllum pinnatum* on guinea pig tracheal ring contractility. Nigerian Journal of Physiological Sciences. 2010; 25:149-157.
29. Mans DRA, Toelsie JS, Mohan S, Jurgens S, Muhringen M, Llles S *et al*. Spasmogenic effect of a *Solanum melongena* leaf extract on guinea pig tracheal chains and its possible mechanism (s). Journal of Ethnopharmacology. 2004b; 95:329-333.
30. Schuler V, Suter K, Furer D Eberli, Horst M, Betschart C, Brenneisen R e al. *Bryophyllum pinnatum* inhibits detrusor contractility in porcine bladder strips: A pharmacological study towards a new treatment option of overactive bladder. Phytomedicine. 2012; 19:947-951.
31. Nassis CZ, Haebisch EM, Giesbrecht AM. Antihistamine activity of *Bryophyllum calcium*. Brazilian Journal of Medical and Biological Research. 1991; 25:929-936.
32. Subhashini T, Krishnaveni B, Srinivas Reddy C. Anti-Inflammatory activity of leaf extracts of *Alternanthera sessilis*. Hygeia-Journal for Drugs and Medicines. 2010; 2(1):54-56.
33. Pegel KH. The importance of sitosterol and sitosterol in human and animal nutrition. South African Journal of Science. 1997; 93:263-268.
34. Gupta MB, Nath R, Srivastava N, Shanker K, Kishor K, Bhargava KP. Anti-inflammatory and antipyretic activities of ß-sitosterol. Planta Medica. 1980; 39:157-163.
35. Jou HJ, Lin YM, Lin YC, Chen FC. Constituents of *Alternanthera sessilis*. *R. Br* Huaxue.1979; 1:22-25.
36. Gallo MBC, Sarachine MJ. Biological activities of lupeol. International Journal of Biomedical and Pharmaceutical Sciences. 2009; 3:46-66.
37. Guruvayoorappan C, Kuttan G. (+)-Catechin inhibits tumor angiogenesis and regulates the production of nitric oxide and TNF-alpha in LPS stimulated macrophages. Innate Immunity. 2008; 14:160-174.
38. Gainok J, Daniels R, Golembiowski D. Investigation of the anti-inflammatory, antinociceptive effect of ellagic acid as measured by digital paw pressure via the Randall-Selitto meter in male Sprague–Dawley rats. American Association of Nurse Anesthetists Journal. 2011; 79:S28-S34.
39. Gene RM, Cartana C, Adzet T, Marin E, Parella T, Canigueral S. Antiinflammatory and analgesic activity of *Baccharis trimera*: identification of its active constituents. Planta Medica. 1996; 62:232-235.
40. Himangsu Mondal, Sanjib Saha, Khalijah Awang, Hemayet Hossain, Abdulwali Ablat, Md Khirul Islam, *et al*. Central-stimulating and analgesic activity of the ethanolic extract of *Alternanthera sessilis* in mice. BMC Complementary and Alternative Medicine. 2014; 14:398.
41. Praveen Nayak, Shweta Nayak, DM Kar, Prabhat Das. Pharmacological evaluation of ethanolic extracts of the plant *Alternanthera sessilis* against temperature regulation, Journal of Pharmacy Research. 2010; 3(6):1381-1383
42. Nayak BS, Anderson M, Pereria Pinto LM. Evaluation of wound healing potential of *Catharanthus roseus* leaf extract in rats. Fitoterapia. 2007; 78:540-4.
43. Sagar R, Sahoo HB. Evaluation of the antiasthmatic activity of ethanolic extract of *Elephantopus scaber* L. leaves. Indian J Pharmacol 2012; 44:398-401.
44. Balakhovskii SD, Ryvkina DE, Fedorova VN. Physiological effects of substances obtained by oxidation of carotene; antihistaminic and anti-acetyl cholinic effect of retinene. Doklady Akademii Nauk SSSR. 1953; 93(5):869-70.
45. Uteshev DB, Prokof'ev PS, Kovaleva VL, Uteshev BS, Storozhanov GI. The effect of anti-asthmatic preparations on the proliferation of human peripheral mononuclear cells. Eksperimental' naia Klincheskaia Farmakologiia. 1999; 62(2):48-50.