Original Article

Anti-microbial and Anti-Inflammatory Activity of Bioactive Components of *Pavonia odorata* Wild

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ABSTRACT

Root extracts of *Pavonia odorata* has to investigate the bioactive compounds using GC-MS analysis. It revealed the existence of 9 compounds. The present investigation was undertaken by comparative study of antimicrobial and anti-inflammatory activity between chloroform, ethyl acetate and methanolic extracts. The chloroform and ethyl acetate extract has excellent activity against *Staphylococcus aureus* (24 ± 0.69 & 22 ± 1.38 mm) and *Candida albicans* (19 ± 0.24 & 16 ± 1.02 mm). The anti-inflammatory activity of root extracts, Carrageenan-induced rat paw edema has frequently used to assess the anti-inflammatory effects of natural products. Chloroform extract has higher activity than ethyl acetate extract. It may be due to the presence of bioactive components.

Keywords: GC-MS Analysis, anti-microbial activity, anti-inflammatory, *Pavonia odorata*.

INTRODUCTION

Nature has provided a complete store-house of remedies to cure all ailments of mankind. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. A number of plants have been documented for their medicinal potential, which is in use by the traditional healers, herbal folklorists and in Indian system of medicine namely Siddha, Ayurveda and Unani[1]. The use of herbal medicine has become increasingly worldwide popular and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. WHO has considered phytotherapy in its health programme; because these drugs are safe, cost effective and most importantly people have faith in them. The demand for crude drugs has undergone a considerable change in recent years due to aggressive marketing of the crude drugs. Standardized extracts from them or pure phyto-pharmaceuticals need to

be studied extensively for their quality, purity, potency, safety and efficacy[2].

Pavonia odorata wild belongs to the family Malvaceae. It is known as sugandhabala in native Indian sub-tropical areas, scientifically known as *Pavonia odorata*[3]. The roots and shoots of this plant are extremely aromatic. Ayurveda, the oldest of all healing sciences has recorded the use of Sugandhabala herb and its extract as cooling, demulcent, carminative, diaphoretic, and diuretic, fever[4]. Previously reported from this plant, the presence of sesquiterpene alcohol called as pavonenol ($C_{15}H_{24}O$; m.p 52-55˚C). The roots yield an essential oil that contains isovaleric acid, isovaleraldehyde,aromadendrene, pavonene, α-terpinene, azulene and pavonenol[5,6]. It has anti-bacterial and anti-inflammatory activity and also used in a number of ayurvedic formulations. Hence the present investigation was undertaken by comparative study of anti-microbial and anti-inflammatory activity between chloroform and ethyl acetate extract and more than 9 bioactive components were identified by GC-MS analysis.

MATERIALS AND METHODS

Plant material

The plants have selected on their wide medicinal uses in the traditional literature. The root part of the *Pavonia odorata* was used as the test plant which has collected from Sannanallur near Kumbakonam, Thanjavur District in Tamilnadu in the month of October and authenticated by Prof. N.Ramakrishnan, Head and Associate Professor and voucher specimens (Department of Botany) and voucher specimens (GACBOT-232) were deposited at the Herbarium of the Department of Botany, Government Arts College (Autonomous), Kumbakonam, Bharathidasan University, India.

Extraction and fractionation

The dried roots of *Pavonia odorata* extracted with 90% methanol (MeOH) (4 X 500 ml) under reflux. The methanol extract (84 g) has subjected to column chromatography with silica gel (60-120 mesh) as the stationary phase. The charged column has then eluted with different solvents of chloroform (4 x 250 ml) and ethyl acetate (4 x 250 ml) to yield several sub fractions. The fractions have collected and the solvent recovered by simple distillation and has concentrated *in vacuo* and left in an ice-chest for a week. The residue from chloroform and ethyl acetate fractions (24.3 & 18.6 g) have taken up in Me_2CO and left in an ice-chest for two days when a brown solid separated and recrystallized from hot methanol.

GC-MS Analysis

GC-MS analysis of $CHCl_3$ and EtOAc fractions have performed on a Hewlett Packard HP 6890 Gas Chromatography with Hewlett Packard 5973 mass spectrometer system equipped with a DB-5 capillary column (30 m x 0.25 mm id, film thickness 0.25 µm). The oven temperature has programmed from 70-240°C at the rate of 5°C/min. The ion source has set at 240°C and electron ionization at 70 eV. Helium used as the carrier gas at a flow rate of 1 mL/min. Scanning range was 35 to 425 amu. Interpretation on mass spectrum of the unknown part has compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials have ascertained.

Anti-microbial activity

Anti-microbial activity test has carried out in the following variation of the method originally described by Bauer et al[7]. Muller Hinton ager has prepared and

autoclaved at 15 lbs pressure for 20 minutes and cooled to 45˚C.The cooled media has poured on to sterile petriplates and allowed for solidification. The plates with media have seeded with the respective microbial suspension using sterile swab. The plant extracts has prepared at different dose individually placed on the each petriplates discs and placed control and standard (Ciprofloxacin and Amphotericin) discs. The plates have incubated at 37˚C for 24 hrs. After incubation period, the zone of inhibition surrounding the discs has measured using a transparent ruler and the diameter recorded in mm.

Anti-inflammatory activity by carrageenan induced paw edema

The anti-inflammatory activity of the test compounds has evaluated in male albino rats employing the method. Animals have fasted overnight and have divided into control, standard and different test groups each consisting of six animals. The different test concentration at the dose of 300 mg / kg of petroleum ether, chloroform and methanolic extract and 100 mg / kg Diclofenac sodium has administrated to the animals by oral route. Control group animals have received 1% DMSO at the dose of 10 ml / kg body weight. They housed in cages and kept under standard conditions at 26 ± 2°C and relative humidity 60 - 65% and 12 h light and 14 h dark cycles each day for one week before and during the experiments. The acute inflammation has induced by the sub-plantar administration of 0.1 ml of 1% carrageenan in the right paw. Paw volume has measured by using digital plethysmometer (Ugo Basile-Italy) before administration of carrageenan and after 1, 2, 3, 4 and 5 hrs intervals. The efficacy of different drug has tested on its ability to inhibit paw edema compared to control group.

Volume of edema = Final Paw Volume - Initial Paw Volume

The Percentage inhibition of paw edema has calculated by the formula as below.

% Inhibition of Paw edema = [(VC – VT) / VC] x 100

Where, VC = Paw edema of control group and VT = Paw edema of treated group

Statistical analysis

The experimental results has expressed as statistical comparisons of Mean ± SEM carried out by one way analysis of variance (ANOVA) followed by Dunnet Multiple Comparisons Test. P values less than 0.05 has considered as statistically significant.

RESULTS AND DISCUSSION

Identification of Bioactive compounds by GC-MS

The components present in the chloroform and ethyl acetate fractions of *Pavonia odorata* roots have identified by GC-MS analysis (Figure 1 & 2). The active principles with their retention time (RT), molecular formula, molecular weight (MW), peak area has presented in the Table 1. The GC-MS spectrums in chloroform extract showed a molecular ion peak at m/e 388.224, 436.318, 355.106, 194.130, 238.193, 122.073, 496.340, 460.286 and 166.062 have identified namely (1) 5aH, 3a, 12-Methano-1H- cyclopropa [5′, 6′] cyclodeca [1′,2′,1,5] cyclopenta [1, 2-d][1,3]dioxal-13-one, (2) ethyl iso-allocholate, (3) 2,7-Dipheny l,1,6-dioxopyridazino [4, 5, 2′, 3′] pyrrolo [4′, 5′-d] pyridazine, (4) bicyclo [4, 3, 0] nonan-7-one,1- (2-methoxyvinyl), (5) cedran diol,8S,13 and four major compounds has identified in the ethyl acetate extract namely (6) Phenyl alcohol, (7) 9,12,15-

octadecatrienoic acid 2,3-bis [(trimethyl silyl)oxy] propyl ester [Z,Z,Z], (8) 1,5-bis (3-cyclopentylpropoxy)-1, 13, 3, 5, 5-hexamethyltrisiloxane, (9) benzoic acid-2-hydroxy,ethylester.

Anti-microbial activity

In the present study the *in vitro* anti-microbial activity of the root extracts of *Pavonia odorata* has observed against five tested micro organisms presented in Table 2. Hence the study suggests that the extracts especially chloroform extract was suitable to screen for the antibacterial activity. The chloroform extracts posse's excellent activity against *Staphylococcus aureus* (24 ± 0.69 mm) and *Candida albicans* (19 ± 0.24 mm). The data indicates that the ethyl acetate extract also exhibited good anti-bacterial activity against the tested bacterial organisms. The activity of the ethyl acetate extract against *S. aureus* (22 ± 1.38 mm) and *C. albicans* (18 ± 1.02 mm) has found as well known anti-bacterial agent against Ciprofloxacin (23±1.38 mm) and *C. albicans* (20 ± 1.20 mm) while the methanol extracts showed anti-bacterial activity against all bacterial pathogens, maximum zone of inhibition was showed against *S. aureus* (18 ± 0.43 mm). This showed the root extract of plant can be used for medicinal purposes. In the present study the maximum activity may be due to the presence of bioactive components present in the both extracts.

Anti- inflammatory activity

Inflammation is a response of living tissue to injuries that involve activation of various enzyme, mediators release, cell migration, tissue breakdown and repair[8]. Carrageenan induced hind paw edema is the standard experimental model of acute inflammation[9]. Carrageenan induced paw edema takes place in three phases, in the first phase (1 hr after carrageenan induce) involves the release of serotonin and histamine from mast cells, in second phase (2 hrs) has provided by kinins and the third phase (3 hrs) has mediated by prostaglandins, the cycloxygenase and lipoxygenase products[10]. The effect of methanol, chloroform and ethyl acetate extracts of *Pavonia odorata* on carrageenan-induced rat paw oedema at one hour interval study was compared to that control for the evaluation of anti-inflammatory activity on the basis of mean ± standard deviation (M±SD) inhibition of paw oedema volume (Table 3). The experiment showed the extracts exhibited statistically significant ($p<0.001$) inhibition of paw volume in a dose dependent manner. Significant inhibition of paw oedema has observed with chloroform, ethyl acetate and methanol extract doses tested till the fifth hour. However, maximum inhibition of paw oedema has found to 3.78 ± 0.04, 3.76 ± 0.08 and 3.85 ± 0.04 at first hour at doses of 200 mg/kg body weight, respectively. As shown in the results, restraint of paw edema (at 5th hrs) for *P. odorata* extracts in, chloroform, ethyl acetate and methanol extracts with 3.01 ± 0.02, 2.86 ± 0.05 and 3.28 ± 0.02 respectively. It showed the plant extract have significant ($P <0.01$; $P< 0.001$) anti-inflammatory effect and the results have compared with Diclofenac sodium 100 mg / kg and showed the paw volume reduction of 2.72 ± 0.05. Although the inhibition of paw oedema with the ethyl acetate extract has much less than that found with the standard drug Diclofenac sodium at a dose of 100 mg / kg body weight, the duration of action has found to comparable to that of Diclofenac sodium till the fifth hour during investigation. All extracts and standard groups decreased the thickness of edema of the hind paw in different percentages compared to the control group. Methanolic extract has shown to inhibit the induced inflammatory response to carrageenan to a

lesser extent than ethyl acetate and chloroform extracts. Ethyl acetate extract posse's higher activity than chloroform extract and the higher activity may due to bioactive components present in the plant extract and this can used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals. Thus further work can be carried on the isolation procedure for finding out the exact moiety responsible for the biological activity.

CONCLUSION

The present investigation, $CHCl_3$ root extract of *Pavonia odorata* has better anti-microbial activity compare to ethyl acetate and methanol extracts. The anti-inflammatory effect of ethyl acetate and chloroform fractions may have related to different chemical composition presented in the plant nature which has proved as anti-inflammatory activity compared with standard drug. Methanolic extract has shown to inhibit the induced inflammatory response to carrageenan to a lesser extent than ethyl acetate and chloroform extracts. Bioactive substances from this plant have employed to develop drugs for treats inflammation and microbial diseases.

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Table 1: GCMS data for chloroform and ethyl acetate extract of *Pavonia odorata*

S. No.	Compound Name	MF	MW	RT	Peak Area	% of Peak Area
1	Chloroform extract 5aH-3a, 12-Methano-1H- cyclopropa [5', 6'] cyclodeca [1',2',1,5] cyclopenta [1, 2-d][1,3]dioxal-13-one	$C_{23}H_{32}O_5$	388	12.964	225397	54.40
2	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	13.675	24645	5.95
3	2,7-Dipheny l,1,6-dioxopyridazino [4, 5, 2', 3'] pyrrolo [4', 5'-d] pyridazine	$C_{20}H_{13}N_5O_2$	355	10.919	63863	15.41
4	Bicyclo [4, 3, 0] nonan-7-one,1- (2-methoxyvinyl)	$C_{12}H_{18}O_2$	194	7.842	94927	22.91
5	Cedran diol,8S,13	$C_{15}H_{26}O_2$	238	8.875	5472	1.32
6	Ethyl acetate extract Phenyl alcohol	$C_8H_{10}O$	122	5.042	2303681	24.25
7	9,12,15-octadecatrienoic acid 2,3-bis [(trimethyl silyl)oxy] propyl ester [Z,Z,Z]	$C_{27}H_{52}O_4Si_2$	496	12.452	115274	1.21
8	1,5-bis (3-cyclopentylpropoxy)-1, 13, 3, 5, 5-hexamethyltrisiloxane	$C_{22}H_{48}O_4Si_3$	460	7.442	387580	4.08
9	Benzoic acid-2-hydroxy, ethylester	$C_9H_{10}O_3$	166	6.031	6691780	70.45

Table 2: Inhibition zones formed by *Pavonia odorata* root extracts and Standard antibiotics

S. No.	Microorganisms	Diameter of inhibition zones (mm / 50 µL) (M±SD)			
		Chloroform Extract	Ethyl acetate Extract	Methanol Extract	Standard (10 µg)
1	*Escherichia coli*	9 ± 1.02	8 ± 0.84	6 ± 0.62	14±1.02*
2	*Staphylococcus* aureus	24 ± 0.69	22 ± 1.38	18 ± 0.43	23±1.38*
3	*Aspergillus Niger*	11 ± 0.20	10 ± 0.48	07 ± 0.74	14±1.02**
4	*Aspergillus flavus*	9 ± 0.31	7 ± 0.88	05 ± 1.02	14±1.05**
5	*Candida albicans*	19 ± 0.24	18±1.02	10 ± 0.69	20±1.20**

Bacteria Standard* - Ciprofloxacin (10 µg); Fungal Standard** - Amphotericin - B (10 µg)
Values are expressed in Mean ± Standard Deviation (M±SD) (n=3)

Table 3: Anti-inflammatory activity of *Pavonia odorata* root extracts by carrageenan induced paw edema

S. No.	Treatment	Anti-inflammatory activity (cm) (M±SD)				
		1 h	2 h	3 h	4 h	5 h
1	Normal Control	2.46±0.01	2.44±0.01	2.42±0.01	2.41±0.05	2.41±0.01
2	Inflammatory Control (1% carragenan)	3.89±0.04	3.67±0.06	3.58±0.05	3.57±0.04	3.56±0.02
3	Standard (1% carragenan + Diclofenac sodium(100 mg / kg)	3.90±0.01	3.45±0.10	3.24±0.15	2.91±0.08	2.72±0.05
4	1% carragenan + Chloroform extract (200 mg / kg)	3.78±0.04	3.66±0.05	3.41±0.08	3.40±0.03	3.01±0.02
5	1% carragenan + Ethyl acetate extract+(200 mg / kg)	3.76±0.08	3.56±0.03	3.35±0.10	3.14±0.04	2.86±0.05
6	1% carragenan + Methanolic extract(200 mg / kg)	3.85±0.04	3.77±0.05	3.64±0.08	3.59±0.03	3.28±0.02

Data presented above are mean ± standard deviation (M±SD).

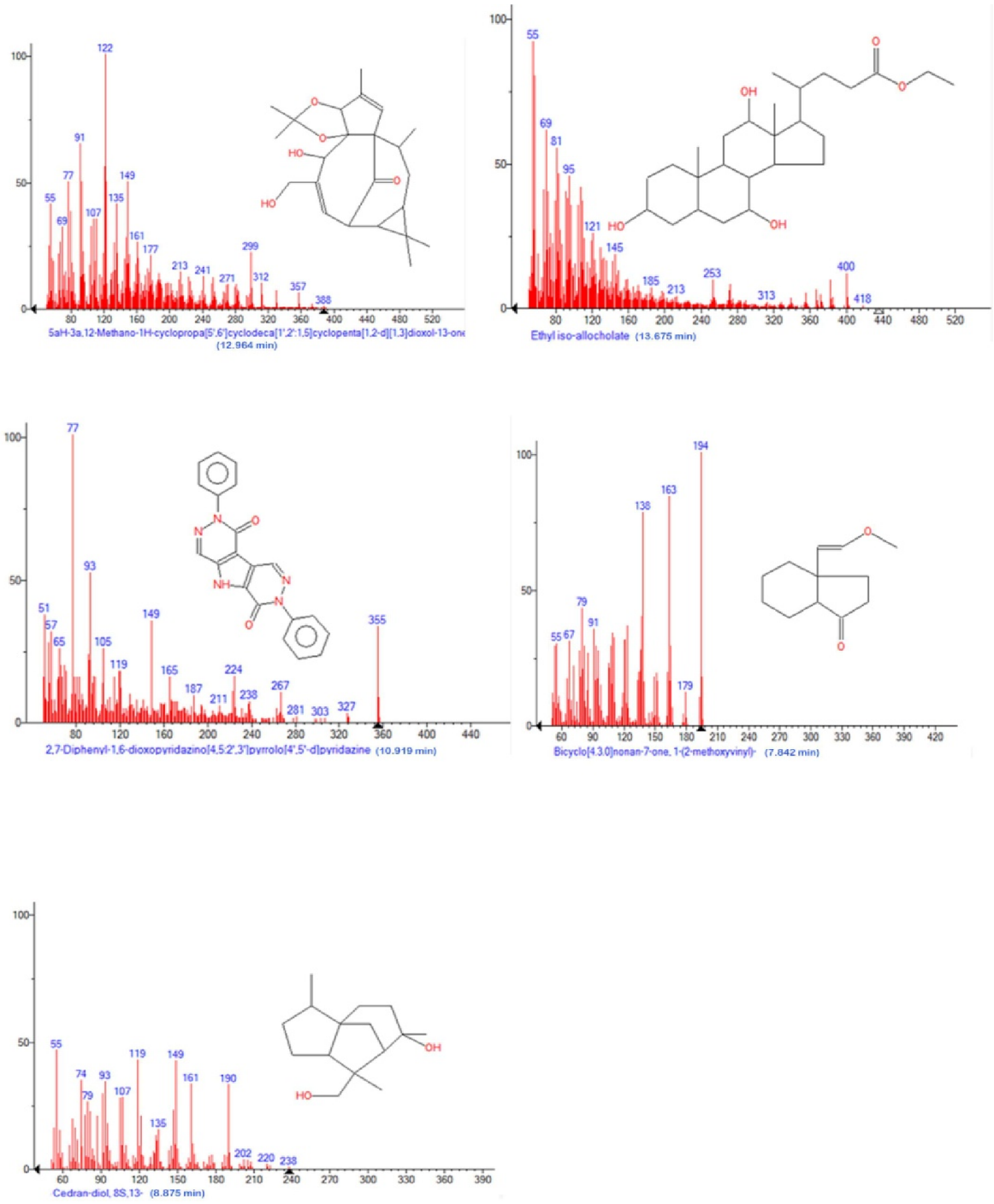

Figure 1: GC-MS identification of bioactive compounds from Chloroform extract of *Pavonia odorata.*

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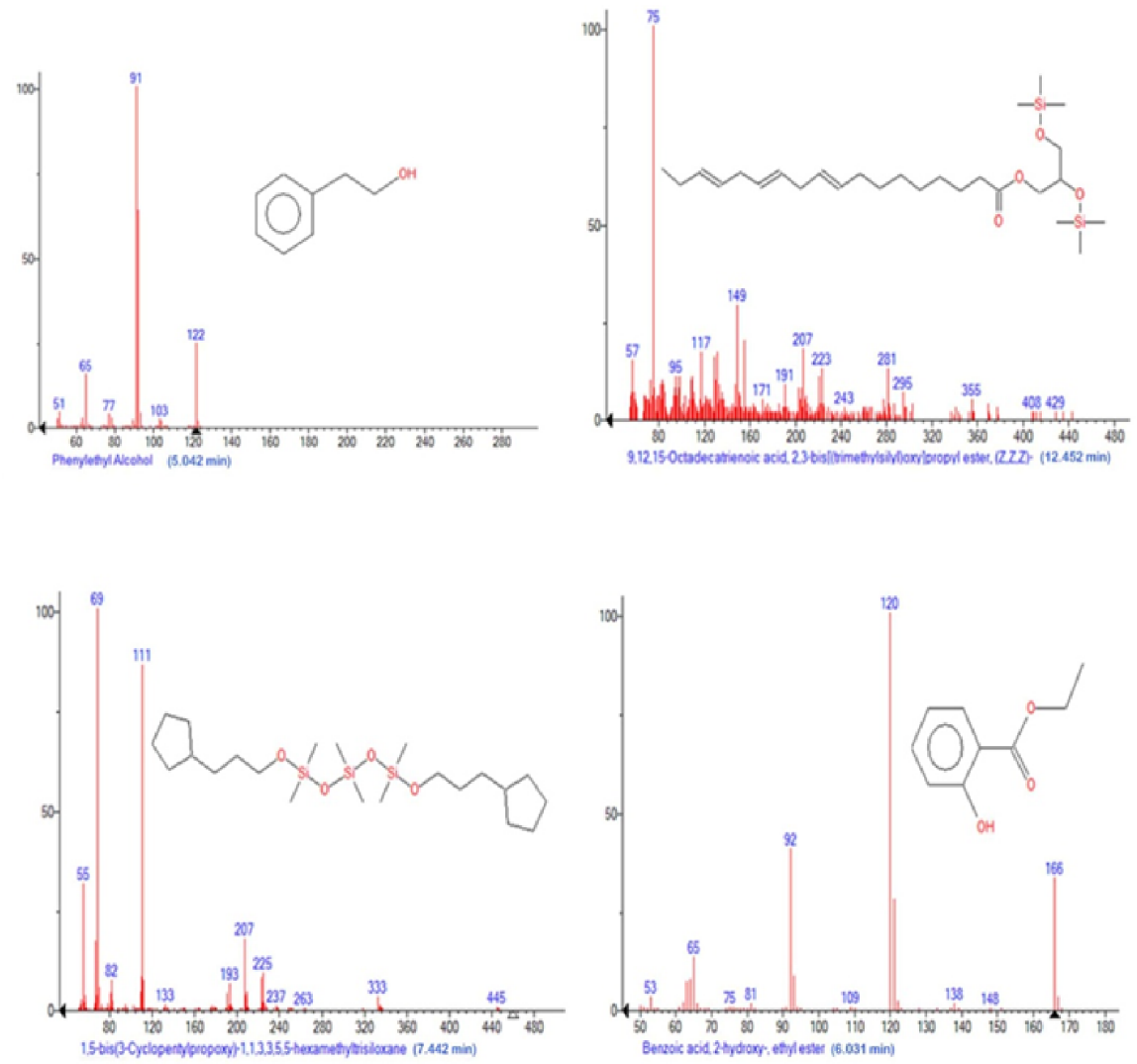

Figure 2: GC-MS identification of bioactive compounds from ethyl acetate extract of *Pavonia odorata.*