Evaluation of *Viburnum punctatum* Buch.-Ham.ex D. Don for preliminary phytochemical constituents and GC-MS analysis on ethanolic stem extract

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Abstract: *Viburnum punctatum* Buch.- Ham.ex D.Don (*Viburnum acuminatum* Wall) is a therapeutic plant belonging to Caprifoliaceae family, under the order Dipsacales. It is a monotypic sort Viburnum, local to India, Bhutan, Nepal, Thailand, Cambodia, Vietnam, Indonesia and China. It is ordinarily called "Konakaram" in Tamil. It is a little evergreen tree, regularly found in sholas and damp woods, over 1200m in South East Asia. A significant number of these species are perceived for their restorative properties from early circumstances of this century. The leaves, stem, barks and roots of huge numbers of these species have been researched recently and have been appeared to have different cytotoxic, antimicrobial, antinociceptive, antispasmodic, and uterine sedative properties. In the present investigation, the preliminary phytochemical analysis was carried out on four different solvents and the ethanolic extract of *Viburnum punctatum* stems has been subjected to GC-MS examination. GC-MS investigation of the ethanolic extract eluted the presence of eight peaks exhibited and the six major chemical constituents were found. They were identified as 12-Phenyl-2,3,7,8- tetramethoxy - 5H - (1)- benzapyrano [4,3-c] isoquinoline, Tetradecamethyl cycloheptasiloxane, Hexadecamethylcyclooctasiloxane, 5- (Hydroxymethyl)-2-(1-methyl-2-2-imidazolyl)-1H-benzamidazole, 1H-Purin-6-amine[(2-fluorophenyl]-(CAS) and 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester.

Keywords: *Viburnum punctatum* stems, Phytochemical analysis, Ethanol extract, GC-MS analysis, Phytocompound.

I. INTRODUCTION

India has a rich legacy of utilizing Ayurvedic arrangement of drug which goes back to 5000 years or increasingly and facilitating a few great many therapeutically profitable plants having a place with the several families (Prabhu *et al*., 2011). Assorted scopes of bioactive particles are delivered by the plant which makes them an enhanced wellspring of various assortments of meds. The antiquated educational works incorporated into the Atharva, Veda, Charaka and Sushruta includes a rich legacy of learning to preventive and therapeutic solutions. Characteristic items assumes essential part in tranquilize advancement programs in the pharmaceutical business (Baker *et al*., 1995) and right around half of every single present day sedate are of common beginning (Stuffiness *et al*., 1985).

As indicated by an investigation by World Health Organization, almost 80% of the total populace is relying upon natural drugs for their medicinal service issues (Jayasheela *et al*., 2014; Farnsworth *et al*., 1985). Various long standing and essential drug are acquired from plants; they require superimposed to the economy. The real supply of crude materials required for substance, pharmaceutical, agrochemical, beautifiers and nourishment businesses is from the plants. For the disengagement of optional metabolites, endeavors are made to utilize biotechnological instruments and in addition plant cell tissue and organ culture methods for the past numerous years.

Viburnum punctatum (Viburnum acuminatum Wall) belongs to the family Caprifoliaceae. It is a bush or little tree, evergreen, to 9mm tall. It has a place with monotypic sort Viburnum, local to India, Indonesia, Bhutan, Cambodia, Nepal, Thailand, Vietnam and China. *Viburnum punctatum* leaves were customarily utilized for the treatment of fever, stomach issue and specified to have hostile to intermittent impact (Renjith Alex *et al*., 2014). One of the genera of Caprifoliaceae in particular, *Viburnum Linn.* species contains around 200 species all through the world and around 17 species accessible in the slopes of India (Prabhu *et al*., 2009; The Wealth of India, 2003). In the late 1960s and mid-1980s, the logical investigations on the sort *Viburnum Linn*. species

were voluminous. In any case, the quantity of species subjected for ponders and the territories of examinations were strikingly limited (Prabhu *et al*., 2009). A couple of them have been explored for their phytochemicals particularly on the leaves, stem bark and roots.

A few scientists have examined the distinctive parts of the plant phytochemically and found to contain sugars, glycosides, sterols, terpenoids and phenolic constituents. The plant additionally answered to contain triterpenes, saponins in root, adhesive, tannin and lignin in leaf, saponins, starch grains and tannins in stem and glycoside, terpenoid and sterols in leaves (Renjith Alex *et al*., 2014).

Among the above recorded synthetic constituents, phenolic compounds, terpenoids and their glycosides might be the reason for organic reactions. Also, a subjective synthetic screening and spectrophotometric examination of concentrates were performed to uncover that some portion of these species contains an apparent sum and an extensive variety of phenolic compounds (Prabhu *et al*., 2009; Nadkarni, 2002). These phytochemicals are responsible for various pharmacological actions like antimicrobial and antioxidant activities (Fukuyama *et al.,* 2005). The natural phenolic atoms, for example, flavonoids, anthocyanins, bioflavones and other phenolic glycosides have, just been investigated and known for their applications against a few human illnesses, cardiovascular disarranges, interminable aggravation and GIT related inconveniences (Prabhu *et al*., 2011).

II. RESEARCH METHODOLOGY

2.1. Plant material: Collection, authentication and processing

The fresh healthy plant stems of *Viburnum punctatum* Buch.-Ham. ex D. Don were collected from the Nilgiri Hills, Tamil Nadu, India during the month of June - July. The fruits of *Viburnum* greatly enhance the identification of the species. The collected plant material was identified and authenticated by Dr. M. Palanisamy, Scientist, Botanical Survey of India, Southern Regional Centre, Coimbatore, India (Voucher No. BSI/ SRC/ 5/ 23/ 2014-15/ Tech/ 513) and a voucher specimen has been reserved in the Department of Biotechnology, Sri Ramakrishna College of Arts and Science (Autonomous), Coimbatore, Tamil Nadu, India. Freshly collected stems of *Viburnum punctatum* were washed thoroughly with tap water, shade dried and then homogenized to a fine powder using a mechanical grinder and stored in airtight bottles. The powdered stems were seived through a No. 40 sieve for powder analysis. The coarse stem powder was used for further extraction process.

2.2. Solvents, Chemicals and Instruments

Analytical/ laboratory reagent grade chemicals were used for the studies, which were purchased from Sigma Aldrich, Bangalore, India. The solvents were selected on the basis of polarity for the extraction of bioactive compounds. The solvents include ethanol, chloroform and petroleum ether which were used for further studies. The GC - MS analysis was carried out on a Thermo GC - Trace Ultra Version: 5.0, Thermo MS DSQ II interfaced to the mass spectrometer (GC-MS) instrument.

2.3. Phytochemical extraction and analysis of extracts

2.3.1. Phytochemical extraction

10 grams of pulverized stem material were soaked separately in 250 ml aqueous, ethanol, chloroform, petroleum ether and kept on a rotary shaker for 24 hours. The extracted material from solvents was filtered through a Whatmann No. 1 filter paper in separate flasks and the process was repeated until all the soluble compounds had been extracted. The extract was then concentrated under reduced pressure in a rotary

evaporator, weighed and stored at 4°C until further analysis. The dried aqueous, ethanol, chloroform and petroleum ether extracts were finally dissolved in their respective solvents in a proportion of 10 mg/ml.

2.3.2. Preliminary Phytochemical analysis

The stem extracts were analyzed for alkaloids, flavanoids, pholobatannins, glycosides, phenols, saponins, lipids and fat, tannins, quinines, cardiac glycosides, coumarins, acids, steroids, phytosterols, proteins and carbohydrates.

Detection of Alkaloids (Yusuf *et al*., 2014) - About 50 mg of solvent free extract was stirred with 3 ml of dilute hydrochloric acid and then filtered thoroughly. The filtrate was tested carefully with various alkaloid reagents as follows:

Mayer's test - To 1 ml of filtrate, few drops of Mayer's reagent were added by the side of the test tube. The white or creamy precipitate indicate test as positive.

Dragendorff's test - To 1ml of filtrate, 2ml of Dragendorff's reagent are added and the result was observed carefully. A prominent yellow precipitate confirms the test as positive.

Detection of Carbohydrates (Pandey and Tripathi, 2014) - Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's test - Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Molisch's test - Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Benedict's test - Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Detection of Glycosides (Pandey and Tripathi, 2014)

Legal's test - Chloroform (3 ml) and ammonia solution (10 %) was added to 2 ml plant extract. Formation of pink colour indicates presence of glycosides.

Detection of Proteins (Pandey and Tripathi, 2014) - The extract was dissolved in 10 ml of distilled water and filtered through Whatmann No 1 filtrate is subjected to test for proteins and amino acids.

Millon's test - To 2 ml of filtrate, few drops of Millon's reagent are added. The result was observed . A white precipitates indicated presence of proteins.

Biuret test - An aliquot of 2 ml of filtrate was treated with drop of 2% copper sulphate solution. To this, 1 ml of ethanol (95 %) was added, followed by excess of potassium hydroxide pellets. The pink colour in ethanol layer indicates the presence of proteins.

Detection of Amino acid (Pandey and Tripathi, 2014)

Ninhydrin test - Two drops of ninhydrin solution (5 mg of ninhydrin in 200 ml of acetone) are added to two ml of aqueous filtrate. The colour change was observed. A characteristic purple colour indicates the presence of amino acid.

Detection of Tannins (Pandey and Tripathi, 2014)

Gelatin Test - The extract (5 mg) was dissolved in 5 ml of distilled water and 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of Phenols (Pandey and Tripathi, 2014)

Ferric Chloride Test - The extract (5 mg) was treated with 3 - 4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of Flavanoids (Pandey and Tripathi, 2014) - An aqueous solution of the extract was treated with ammonium hydroxide solution. The yellow fluorescence indicates the presence of flavonoids.

Detection of Coumarins (Morsy, 2014) - 10 % NaOH (1ml) was added to 1 ml of the plant extract, formation of yellow colour indicates the presence of coumarins.

Detection of Saponins (Yusuf *et al*., 2014) - Distilled water 2 ml was added of each plant extract and shaken in a graduated cylinder for 15 mins lengthwise. Formation of 1 cm foam indicates the presence of saponins.

Detection of Quinine (Morsy, 2014) - Concentrated sulphuric acid (1 ml) was added to 1 ml of each of the plant extract. Formation of red colour indicates the presence of quinines.
Detection of Cardiac glycosides (Morsy, 2014) - Glacial acetic acid (2 ml) and few drops of 5% ferric chloride were added to 0.5 % of the extract. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicated the presence of cardiac glycosides.
Detection of Terpenoids (Morsy, 2014) - Chloroform (2 ml) and Concentrated sulphuric acid was added carefully to 0.5 ml of extract. Formation of red - brown colour at the interface indicated the presence of terpenoids.
Detection of Acids (Morsy, 2014) - Plant extract 0.5 ml was treated with sodium bicarbonate solution. Formation of effervescence indicates the presence of acids.
Detection of Phlobatannins (Pandey and Tripathi, 2014) - Few drops of 10 % ammonia solution were added to 0.5 ml of plant extract. Appearance of pink colour precipitate indicates the presence of phlobatannins.
Detection of Steroids and Phytosteroids (Pandey and Tripathi, 2014) - To 0.5 ml of the plant extract, equal volume of chloroform was added and subjected with few drops of concentrated sulphuric acid. Appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

2.3.3. Identification of phytocompounds using GC-MS analysis

The plant ethanolic extract was used for the GC-MS analysis. 1µl of the ethanolic stem extract of *Viburnum punctatum* have been employed for GC-MS analysis (Gopalakrishnan *et al*., 2011; Muthuchelian *et al.*, 2011). The phytochemical investigation of ethanolic extract was performed on a GC equipment Thermo GC - Trace Ultra Version: 5.0, Thermo MS DSQ II interfaced to the mass spectrometer (GC-MS) instrument and employing the following conditions: column DB 5 - MS capillary standard Non - polar column, in a RD operator at the dimension of 30 minutes, ID: 0.25 mm and Film: 0.25 µm. Helium (99%) was used as the carrier gas at a constant flow of 1ml/min and an injection volume of 1 microliter was employed. The temperature of oven was programmed from 70°C and raised to 260°C at 6 C/ min. The Mass spectrum was taken from low mass (m/z): 50 to high mass (m/z): 650.

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on the GC-MS mass spectrum was done using the database of National Institute Standard and Technology (NIST). The NIST have more than 62,000 patterns. Mass spectrum of the unknown component was compared with the spectrum of the known components and stored in the NIST library. The compound name, molecular weight and structure of the components of the test materials were ascertained. This is done in order to determine whether this plant species contains any individual compound or group of compounds, which may substantiate its current commercial and traditional use as an herbal medicine. Further it helps to determine the most appropriate methods of extraction for these compounds (Muthuchelian *et al.*, 2011 and Abirami *et al.*, 2012).

III. RESULTS AND DISCUSSION

3.1. Phytochemical analysis

In the present study, the investigation of phytochemical screening was carried out in different solvent extracts like; Aqueous, Ethanol, Petroleum ether and Chloroform extract of stem of *Viburnum punctatum.* The result revealed that the ethanolic extract of *Viburnum punctatum* recorded the presence of alkaloid, flavonoid, phenol, tannins, phytosterol and glycosides followed by other extract (Table.1). The presence of active constituents was found more in ethanolic extract when compared to other organic solvents. It was concluded that the ethanolic extract of *Viburnum punctatum* stems contain more important constituents for pharmacological activity. The plant also reported to contain triterpenes, saponins in root, mucilage, tannin and lignin in leaf, saponins, starch

grains and tannins in stem and glycoside, terpenoid and sterols in leaves (Renjith Alex *et al*., 2014; Jayasheela *et al*., 2015). The presence of these phytochemical constituents will show the reason behind the antimicrobial property of the plant extracts.

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloid, flavonoid, tannins, phenolic compounds, saponins and phytosterols (Britto and Sebastian, 2012; Nithyadevi and Sivakumar, 2015). The presence of alkaloids, saponins, flavonoids, phenolic compounds, tannins, phytosterol and terpenoids are used in analgesic, antiplasmodic and bacteriocidal activities (Stary, 1998). The folkloric data close by likewise asserts about the foundation of *Viburnum* species that the plant root may have antimicrobial, antidiabetic, cell reinforcement, cytotoxicity, antiulcer and antispasmodic properties because of the nearness of triterpenoids, phenolic mixes, flavonoids, saponins, tannins and anthocyanins. The nearness of these phytochemical constituents will demonstrate the purpose for the antimicrobial property of the plant separates, since all these auxiliary metabolites has been now revealed for their antimicrobial property which can be utilized as a part of the treatment of irresistible ailments caused by normal human bacterial pathogens. Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development (Prabhu *et al*., 2009; Kathiresan Prabhu *et al*., 2011).

3.2. GC-MS analysis

The results pertaining to GC-MS analysis of the ethanolic stem extract of *Viburnum punctatum* lead to the identification of a number of compounds. These compounds were identified through mass spectrometry attached with GC. The mass spectrometer analyses the compounds eluted at different times to identify the nature and structure of the compounds. GC-MS chromatogram of the ethanolic stem extract of *Viburnum punctatum* (Fig 1) showed 8 peaks indicating the presence of eight phytochemical constituents. The eight phytoconstituents were characterized and identified on comparison of mass spectra of the constituents with the NIST library.

Six major phytochemical constituents in mass spectra analysis were identified as 12-Phenyl-2,3,7,8-tetramethoxy-5H-(1)-benzapyrano[4,3- c]isoquinoline, Tetradecamethyl cycloheptasiloxane, Hexadecamethylcyclooctasiloxane, 5- (Hydroxymethyl)-2-(1-methyl-2-2-imidazolyl)-1H-benzamidazole, 1H-Purin-6-amine[(2- fluorophenyl]-(CAS) and 1,4-Benzenedicarboxylic acid,bis (2-ethylhexyl)ester. These compounds belong to a phenolic group. Radical scavenging activities of phenolic compounds play a key role in ameliorating healing and even preventing several ailments in living being (Jayasheela *et al*., 2016). It is a well-known fact that the plants synthesize phenolic compounds for diverse purposes, which may be of protective, functional or as metabolic end products in nature (Yadhav *et al.,* 2005; Prabhu *et al.,* 2011). But, human exploit them as valuable medicines/ phytopharmaceuticals by focusing on their anti-oxidant potential with or without modification.

IV. CONCLUSION

The present study was carried out for analysing the phytoconstituents present in the stems of *Viburnum punctatum.* Six phytochemical constituents were identified by performing GC-MS analysis. The isolation of these individual compounds will have a tremendous contribution in the drug delivery systems for various human diseases. So it is recommended as a plant of phytopharmaceutical importance. This examination can be valuable as a reference to advance with some further propelled examinations exploiting the species for their natural bioactivity, toxicity profile, effect on the ecosystem and agricultural products. The herbal phenolic molecules such as flavonoids, anthocyanins, bioflavones and other phenolic glycosides have already been explored and known for their applications against several human ailments - cardiovascular disorders, chronic inflammation and GIT related troubles. A quest for a search of herbal phenolic compounds is still a renewed interest in the science of natural products as a source of valuable medicines.

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Table 1: Preliminary Phytochemical screening of *Viburnum punctatum* stems

Phytochemical	Test	AQ	EH	PE	CH
Alkaloids	Mayers Test	+	+	-	+
	Dradendroffs Test	+	+	-	+
Carbohydrates	Fehlings test	-	-	-	-
	Molisch's test	-	-	-	-
	Benedict's test	-	-	-	-
Glycosides	Legals test	+	++	-	-
Proteins	Millon's test	+	+	-	+
	Biuret test	+	+	-	+
Aminoacids	Ninhydrin test	-	-	-	-
Tannins	Gelatin test	+	+	+	-
Phenols	Ferric chloride test	+	++	-	-
Flavonoids	NaoH test	+	+	-	-
Coumarins	NaOH test	+	+	-	-
Quinine	Conc. H_2SO_4 test	-	-	-	-
Saponins	Foam test	-	-	-	-
Cardiac glycosides	Glacial Acetic Acid + $FeCl_3$ + Conc. H_2SO_4	-	-	-	-
Terpenoids	Chloroform + Conc. H_2SO_4	-	++	+	-
Acids	Sodium Bicarbonate test	-	-	-	-
Phlobatannins	10% ammonia Solution	-	-	-	-
Steroids & phytosteroids	Chloroform + Conc. H_2SO_4	+	+	-	-

[Note: (+) Positive; (-) Negative; AQ - Aqueous, EH – Ethanol, PE - Petroleum Ether, CH Chloroform]

Table 2: GC-MS analysis of phytocompounds identified from *Viburnum punctatum* ethanolic stem extract

S. No	Retention time	Compound Name	Molecular formula	Peak area (%)
1	7.04	12-Phenyl-2,3,7,8-tetramethoxy-5H-(1)-benzopyrano[4,3-c]isoquinoline	$C_{26}H_{23}NO_5$	7.11
2	9.14	Tetradecamethylcycloheptasiloxane	$C_{14}H_{42}O_7Si_7$	2.76
3	9.65	Tetradecene	$C_{14}H_{28}$	1.04
4	10.20	Phenol,2-methyl-5-(1-methylethyl-(CAS)	$C_{10}H_{14}O$	0.53
5	11.50	Hexadecamethylcyclooctasiloxane	$C_{16}H_{48}O_8Si_8$	1.26
6	13.36	1-Hexadecanol(CAS)	$C_{16}H_{34}O$	1.07
7	14.40	12-Phenyl-2,3,7,8-tetramethoxy-5H-(1)-benzopyrano[4,3-c]isoquinoline	$C_{26}H_{23}NO_5$	0.81
8	15.25	1,4-Butylene glycol dimethacrylate	$C_{12}H_{18}O_4$	0.22
9	17.17	Cyclodecasiloxane	$C_{20}H_{60}O_{10}Si_{10}$	1.31
10	17.64	1-Octadecene(CAS)	$C_{18}H_{36}$	1.35
11	18.25	Butanal,2-methyl-(CAS)	$C_5H_{10}O$	0.69
12	18.92	Tetradecanoic acid	$C_{16}H_{32}O_2$	0.33
13	19.42	5-(Hydroxymethyl)-2-(1-methyl-2-imidazolyl)-1H-benzimidazole	$C_{12}H_{12}N_4O$	64.26
14	19.73	1H-Purin-6-amine,[(2-fluorophenyl)methyl]-(CAS)	$C_{12}H_{10}FN_5$	0.30
15	20.26	Methyl (E)-2-(3-cyclopropyl-7-norcaranyl)acetate	$C_{13}H_{20}O_2$	0.63
16	21.73	3-Eicosene, (E)	$C_{20}H_{40}$	1.06
17	22.07	1H-Purin-6-amine,[(2-fluorophenyl)methyl]-(CAS)	$C_{12}H_{10}FN_5$	0.64
18	22.89	Hexadecanoic acid	$C_{18}H_{36}O_2$	0.52
19	23.38	1-(1-Methoxymethoxyethyl)cyclohexene	$C_{10}H_{18}O_2$	0.26
20	24.23	1H-Purin-6-amine,[(2-fluorophenyl)methyl]-(CAS)	$C_{12}H_{10}FN_5$	0.44
21	25.48	1-Docosene	$C_{22}H_{44}$	0.31
22	26.35	1H-Purin-6-amine,[(2-fluorophenyl)methyl]-(CAS)	$C_{12}H_{10}FN_5$	0.38
23	28.27	1H-Purin-6-amine,[(2-fluorophenyl)methyl]-(CAS)	$C_{12}H_{10}FN_5$	0.37
24	28.90	1-Eicosanol(CAS)	$C_{20}H_{42}O$	0.29

25	29.69	1H-Purin-6-amine,[(2-fluorophenyl)methyl]-(CAS)	$C_{12}H_{10}FN_5$	0.27
26	30.89	1H-Purin-6-amine,[(2-fluorophenyl)methyl]-(CAS)	$C_{12}H_{10}FN_5$	0.29
27	34.09	Cis-11-Eicosenamide	$C_{20}H_{39}NO$	0.39
28	36.19	1,4-Benzenedicarboxylic acid, Bis(2-ethylhexyl)ester	$C_{24}H_{38}O_4$	0.46
29	37.21	Eleutherolic acid	$C_{12}H_{10}O_4$	0.45
30	38.96	13-Docosenamide,(Z)	$C_{22}H_{43}NO$	10.20

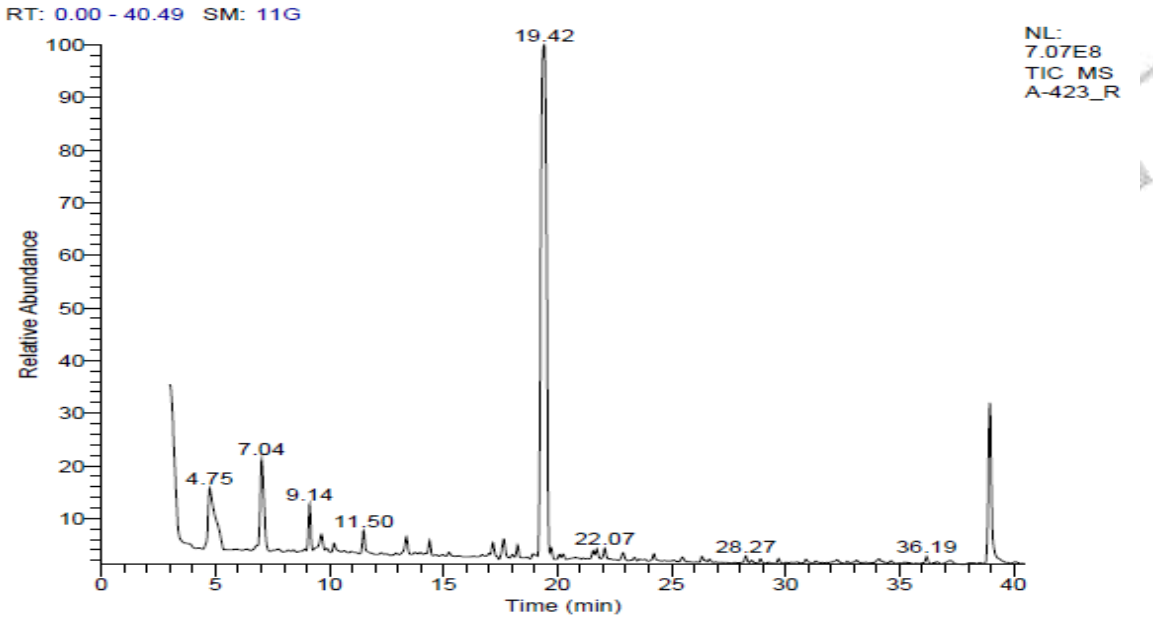

Fig 1: GC-MS analysis of *Viburnum punctatum* ethanolic stem extract