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GC-MS analysis of bioactive compounds in methanolic extract of bhat (*Clerodendrum viscosum*) leaves in Bangladesh

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Abstract

Clerodendrum viscosum (local name bhat), a medicinal plant, is widely found in Bangladesh and India. This species is enriched with various medicinal compounds reported in previous studies. However, there is a scarcity of information about the bioactive compounds of this plant on GC-MS analysis in Bangladesh. For instance, this study aims to determine the bioactive compounds of methanol extract of *Clerodendrum viscosum* leaves obtained from the Soxhlet extraction method. The preliminary phytochemical screening was carried out according to standard procedures described in WHO guidelines. Various bioactive compounds of the extract were determined by the GC-MS technique. In addition, the GC-MS analysis showed nineteen phytoconstituents; among them, six compounds possess some important biological activity. From this result, it is evident that *Clerodendrum viscosum* has possible application in the phytopharmaceutical sector.

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Introduction

The use of herbal medicine has become increasingly popular worldwide and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Estakhr *et al.*, 2011). In developing countries, it is estimated that about 80% of the population really depends on traditional medicine for their primary healthcare (Saha MR *et al.*, 2008). Traditional medicinal plants are often cheaper, locally available and easily consumable, raw, or as simple medicinal preparations (Srinivasan *et al.,* 2013). Due to the toxicity of chemical products, the high cost of chemical drugs, the remoteness and /or insufficiency of health centers, especially in rural settings, which limits the genuine handling of public health problems, have favored the use of plant-based drugs (Egbe B Besong *et al.*, 2021). So there arises a need to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies. Hence, Gas Chromatography (GC) and Mass Spectroscopy (MS) associated with particular detection techniques have become a sophisticated means for analysis of various compounds (Vinodh KS *et al.*, 2013).

Bhat or Hill glory bower (*Clerodendrum viscosum*, family: Verbenaceae) is a shrub of about 1–2m in height but may grow beyond this length.

The plant contains a quadrangular stem and large leaves of ovate shape. Flowers are whitish-pink in color with long pubescent pedicels in stalked cymes and the fruits are four-lobed drupe of 8 mm in diameter (Kiritikar KR and Basu BD., 1971). The plant is often experienced with a widely noxious odor.

The plant is common throughout Bangladesh, India, Myanmar, Thailand, and Indonesia. In Bangladesh, the leaf juice is used as a strong anthelmintic, emetic, mild laxative and cholagogue, and is externally used for tumours, skin diseases, snakebite and scorpion sting (Ghani Abdul, 2003). In India, various roots, stems and leaves of the plant are reported to be used as medicine for the treatment of asthma, cataract, malaria, diseases of blood, skin and lung by the tribals of Chotanagpur plateau of eastern India (Kiritikar KR and Basu BD., 1991). Similarly, in Thailand, leaves and roots are used as medicine for the treatment of intestinal infections and kidney dysfunctions (Islam Md S *et al.*, 2013). Therefore, biological activities like antimicrobial (Oly WT, 2011), cytotoxic, anthelmintic (Rahman MM, 2013), antioxidant and antinociceptive (Rahman MM, 2011) are found in various parts of the plant.

As various parts of the Bhat plant contain biologically active compounds, in this paper, we have focused on isolating and characterize the bioactive phytochemical compounds from the methanol extract of the plant with the help of GC-MS technique.

Materials and methods

Collection, authentication and processing of plant materials

Fresh leaves were collected from roadside of Lalmonirhat district, Bangladesh. The plant sample was identified to be *Clerodendrum viscosum* by Applied Botany Research Division, BCSIR Laboratories, Rajshahi, Bangladesh. The Collected leaves were washed thoroughly with running water to remove the dirt from the sample. Then leaves were dried in the oven at 50°C for 3 days; after drying, sorting was done to separate the damaged parts, then mashed using a blender to produce bhat leaves powder. The coarse powder was stored in an air-tight container with marking for identification and kept in a cool, dark and dry place for future use.

Extraction procedures

About 100 gm of dried powdered leaves were extracted with methanol in a Soxhlet extractor for 36 hours. The extract was filtered and concentrated under reduced pressure at 40°C using a rotary evaporator to obtain a viscus semi-solid mass.

Phytochemical screening

The methanol extract was tested for various phytoconstituents such as steroids, phenolics, triterpenoids, flavonoids and tannins using standard methods (WHO, 1998).

GC-MS conditions

GC-MS technique was used in this study to identify the phytocomponents present in the extracts. This was carried out at BCSIR Laboratories, Rajshahi, Bangladesh. The analysis was carried out using Shimadzu 2010 Plus Gas Chromatograph equipped and coupled to a mass detector QP2020 Shimadzu with column SH-Rxi-5Sil MS and the length of the column was 30 m (L) × 0.25 mm (ID) × 0.25 μm (DF). The instrument was set to an initial temperature of 80°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was rose up to 280°C and maintained for 5 min. Injection port temperature was ensured at 220°C and Helium flow rate at 1.72 ml/min. The ionization voltage was 70 eV. The samples were injected in splitless mode. The mass spectral scan range was set at 45-350 (m/z). The ion source temperature was maintained at 280°C and the interface temperature was at 230°C. The MS start time was 3.20 min, and the end time was 50 min with a solvent cut time of 3.20 min. Identification of the peaks was based on computer matching of the mass spectra with the National Institute of Standards and Technology (NIST 08, NIST 08s and NIST 14) library. The name, molecular weight and structure of the components of the test materials were also confirmed by NIST library.

Results

Phytochemical investigation

The qualitative phytochemical screening of methanol extract of bhat leaves showed the possession of phenolics, flavonoids, diterpenoids and tannins.

Table 1. Phytochemical compounds identified in methanol extract of *Clerodendrum viscosum* leaves.

SL No.	RT	Name of the Compound	Molecular formula	MW	Peak area (%)
1	3.254	Methyl pyruvate dimethyl acetal	$C_6H_{12}O_4$	148.15	0.237
2	8.134	o-Anisic acid, 3-chloroprop-2-enyl ester	$C_{11}H_{11}ClO_3$	226.65	22.570
3	8.325	Tridecane	$C_{13}H_{28}$	184.36	1.033
4	8.503	Silane, ethyldimethylphenyl-	$C_{10}H_{16}Si$	164.31	33.032
5	10.167	Pentadecane	$C_{15}H_{32}$	212.41	2.744
6	10.208	Dodecane, 4,6-dimethyl-	$C_{14}H_{30}$	198.39	2.678
7	11.236	Phenol, 4-(1-methylpropyl)-	$C_{10}H_{14}O$	150.22	1.492
8	11.264	Azacyclohexane, 3-methylamino-1-methyl-	$C_7H_{16}N_2$	128.22	0.160
9	11.423	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	212.41	1.199
10	15.608	Heptadecane	$C_{17}H_{36}$	240.5	1.541
11	15.706	Octadecane, 1-iodo-	$C_{18}H_{37}I$	380.4	3.592
12	16.228	Phenol,3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.32	3.789
13	22.436	Methyl 6,6,8,8-tetramethyl-3-oxo-2,5,7,9-tetraoxa-6,8-disilaundecan-11-oate	$C_{10}H_{22}O_7Si$	282.08	6.135
14	28.187	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270.45	2.073
15	32.095	Phytol	$C_{20}H_{40}O$	296.5	0.646
16	46.211	Retinoic acid	$C_{20}H_{28}O_2$	300.43	1.109
17	48.131	2-Propenoic acid, 2-methyl-, 1,2-ethanediylbis(oxy-2,1-ethanediyl)ester	$C_{14}H_{22}O_6$	286.32	4.263
18	49.013	4a(2H)-Naphthalenol, 2-bromo-4,4-dichloro	$C_{10}H_{15}BrCl_2$	286.03	7.614
19	49.596	1-Cyclohexanol, 2-[1-(phenylsulfonyl)methylidene	$C_{13}H_{16}O_3S$	252.26	4.093

RT = Retention Time, MW = Molecular Weight.

GC-MS analysis

The results of GC-MS analysis of methanol extract revealed the presence of nineteen compounds (Table 1, Fig. 1). The GC-MS spectrum confirmed the presence of 19 compounds with the retention time; 3.254, 8.314, 8.325, 8.503, 10.167, 10.208, 11.236, 11.264, 11.423, 15.608, 15.706, 16.228, 22.436, 28.187, 32.095, 46.211, 48.131, 49.013 and 49.596.

In term of % peak area, Methyl pyruvate dimethyl acetal (0.237%), o-Anisic acid, 3-chloroprop-2-enyl ester (22.570%), Tridecane (1.033%), Silane,

ethyldimethylphenyl- (33.032%), Pentadecane (2.744%), Dodecane, 4,6-dimethyl- (2.678%), Phenol, 4-(1-methylpropyl)- (1.492%), Azacyclohexane, 3-methylamino-1-methyl- (0.160%), Dodecane, 2, 6, 11-trimethyl- (1.199%), Heptadecane (1.541%), Octadecane, 1-iodo- (3.592%), Phenol, 3,5-bis(1, 1-dimethylethyl)- (3.789%), Methyl 6, 6, 8, 8-tetramethyl-3oxo-2,5,7,9-tetra (6.135%), Hexadecanoic acid, methyl ester (2.073%), Phytol (0.646%), Retionic acid (1.109%), 2-propenoic acid, 2-methyl-1,2-ethanediylbis (4.263%), 4a(2H)-Naphthalenol, 2-bromo-4,4-dichloro (7.614%), 1-Cyclohexanol, 2-[1-(phenylsulfonyl)methyl- (4.093%). Among these, some compounds show biological activity although these are found in minor amount.

The individual mass fragmentation of some biological active compounds is illustrated in Fig. 2.

Discussion

The identified six compounds possess some important biological potential for future drug development. Among identified compounds Silane, ethyldimethylphenyl- has the highest percent peak area. Organo-silane compound is a justified and popular adhesion promotor used in dentistry (Christie and Jukka, 2012).

The compound, Pentadecane, is classified as an alkane that exerts antimicrobial and anti-inflammatory effects (Xin Qi Chuah *et al.*, 2018). 3,5-bis(1,1-dimethylethyl)-phenol is a phenolic compound considered to be a very important plant constituents responsible for free radical scavenging ability owing to the presence of hydroxyl groups (Sougata Ghosh *et al.*, 2013). The hexadecanoic acid methyl ester is also a bioactive compound that has antibacterial and antifungal activity (Mustapha N. *et al.*, 2016).

The compound phytol has previously been reported for antioxidant, antimicrobial, immune-modulating, anxiolytic, metabolism-modulating, autophagy and apoptosis, antinociceptive activity (Gharari Zahra *et al.*, 2016). Retinoic acid is used in the treatment of acute promyelocytic leukemia. It has also been reported as an active metabolite of vitamin A (Flynn PJ *et al.*, 1983).

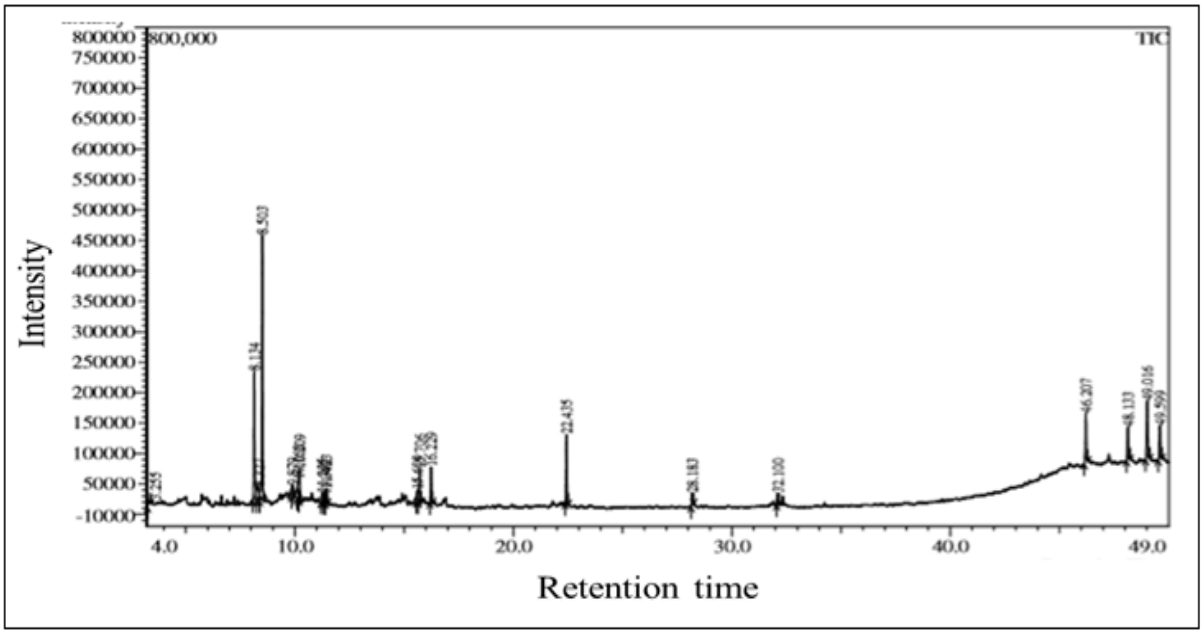

Fig. 1. GC-MS Chromatogram of methanol extract of bhat (*Clerodendrum viscosum*) leaves.

The above-mentioned compounds isolated from the methanolic extract of bhat leaves seem to possess the reported biological activity and further study of these phytoconstituents may prove the medicinal importance in the future.

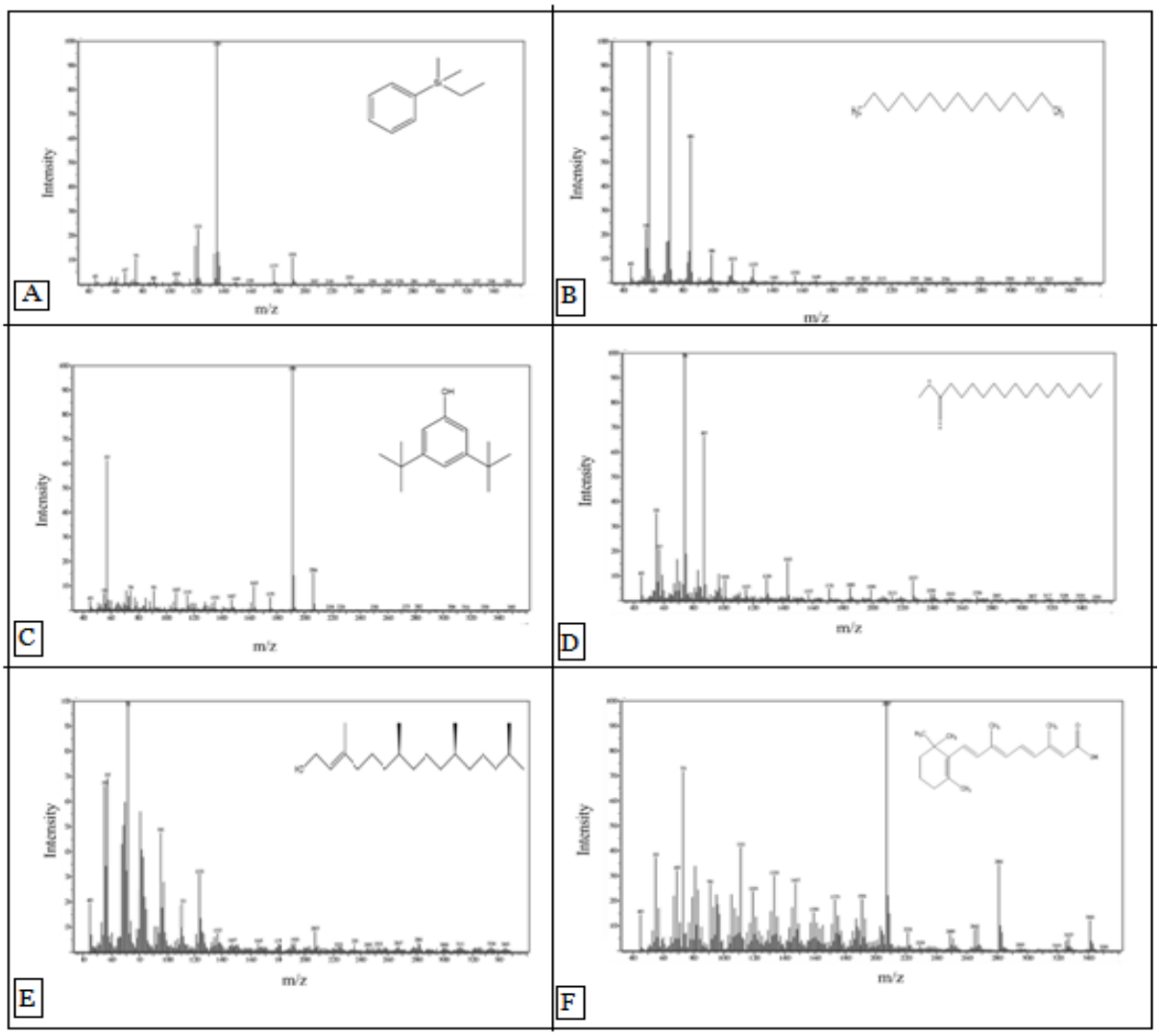

Fig. 2. Mass Spectrum of (a) Silane, ethyldimethylphenyl- (b) Pentadecane (c) Phenol, 3,5-bis(1,1-dimethylethyl)- (d)Hexadecanoic acid, methyl ester (e) Phytol (f) Retinoic acid.

Conclusion

In Bangladesh, we first time reported the presence of some important compounds in Bhat plant identified by GC-MS analysis. The identified compounds after GC-MS analysis of *Clerodendrum viscosum* leaves extract justify the use of bhat leaves by the traditional practitioner. Therefore, separation of every single compound and subjecting it to biological activity will definitely give fruitful outcomes as the identified compounds were previously reported with important bio-activity. Therefore, this plant is recommended as a source of phytopharmaceutical value.

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