

GC-MS analysis of bioactive compounds in the methanol extract of *Clerodendrum viscosum* leaves

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ABSTRACT

Background: *Clerodendrum viscosum* is commonly found in India and Bangladesh. Previously, various parts of this plant were reported for treatment of different types of diseases and there was no report on GC-MS analysis. **Objective:** To analyze and characterize the phytochemical compounds of methanol extract of *Clerodendrum viscosum* using GC-MS. **Materials and Methods:** The preliminary phytochemical screening of methanol extract was carried out according to standard procedures described in WHO guidelines. Various bioactive compounds of the extract were determined by GC-MS technique. **Results:** The presence of steroids, triterpenoids, alkaloids, saponins, flavonoids, tannins and carbohydrate was found on phytochemical screening of methanol extract of the leaves. The GC-MS analysis showed 16 peaks of different phytoconstituents namely acetamide, N,N-carboxylbis-, 4-Pyranone, 2,3-dihydro-, alpha-D-Galactofuranoside, methyl 2,3,5,6-tetra-O-methyl-, Glycerin, Xylitol, N,N-Dimethylglycine, 4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl-, Benzofuran, 2,3-dihydro-, 5-Hydroxymethylfurfural, 2(1H)Pyrimidinone, 1-methyl-, 2,4-Dihydroxy-5,6-dimethylpyrimidine, 3-Deoxy-d-mannonic lactone, 1,3-Methylene-d-arabitol, Orcinol, n-Hexadecanoic acid and Phenol, 4,4'-(1-methyl ethylidene) bis etc. **Conclusion:** The bioactive compounds present in the methanol extract of *Clerodendrum viscosum* suggest the application of this extract for the treatment of various diseases by the aborigine tribes.

Key words: Bioactive compounds, clerodendrum viscosum, GC-MS analysis

INTRODUCTION

Over the last few decades, use of herbal drugs has been emphasized due to their easy availability, therapeutic potential, least side effects and minimum cost. At present nearly 80% of the world population rely on plant based drugs for their health care need.^[1] Presently, phytoconstituents are playing pivotal role for development of novel compounds, which might be crucial for maintaining a healthy society. The human civilization has been maintaining an intimate relationship with the plants from time immemorial. They depend on plants and other natural sources for their well-being and survival.^[2] Various plants still available in the nature are yet to be explored for their medicinal potential.^[3]

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The informations regarding phytochemical compounds are not only supportive for discovery of therapeutic potential, but also have an active contribution towards discovery of new semi-synthetic and synthetic compounds.^[1] The novel molecules from plant sources have been instrumental in development of structurally modified compounds, which assist a lot in the development of modern therapeutic system.^[4]

The screening of plant extracts is an innovative strategy to find therapeutically active compounds in many plant species. Hence, Gas chromatography (GC) and Mass spectroscopy (MS) associated with particular detection techniques have become a sophisticated means for analysis of various compounds.^[5]

Clerodendrum viscosum Linn. (Family: Verbenaceae), is a shrub having quadrangular stem, large leaves of ovate shape, acuminate apex, entire or denticulate margin, cylindrical petiole and hairy leaves. The plant is of 0.9-2.4 meter in height and 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.^[6] This plant is common throughout India, Bangladesh, Myanmar, Thailand and Indonesia.

The leaf and root have been used in Indian traditional medicine for the treatment of asthma, fever, bronchitis, skin diseases, epilepsy, inflammation, tumors, worm infestation and snake bite.^[7,8] The fresh leaf juice is used as vermifuge, bitter tonic, febrifuge in malaria fever, especially in children. The leaves of this plant is also used by Munda tribes of Chota Nagpur for chest complaints and cough.^[6] The various parts of the plant are reported to have many biological activities like, antimicrobial,^[9] cytotoxic, anthelmintic,^[10] antioxidant and antinociceptive.^[11]

Literature survey revealed that till date, no work has been reported on GC-MS analysis of methanol extract of *Clerodendrum viscosum* leaves. Therefore, in our present study, it was thought worthwhile to isolate and characterize the bioactive phytochemical compounds from methanol extract of the plant with the help of GC-MS technique.

MATERIALS AND METHODS

Collection of plant material

The fully matured leaves were collected from Nayagarh district, Odisha, India. The plant was identified and authenticated by Dr. P. C. Panda, Senior Scientist, Taxonomy and Conservation Division, Regional Plant Resource Centre, Bhubaneswar, Odisha. The collected disease free leaves were washed to make free from dust and other plant materials. A voucher specimen was deposited in the herbarium of Department of Pharmacognosy, Siksha O Anusandhan University, Bhubaneswar, Odisha.

Preparation of plant extract

The air-dried leaves (50 g) were coarsely powdered and extracted with methanol (250 ml) in Soxhlet apparatus for 24 h. The extract was filtered and concentrated under reduced pressure at 40°C using rotary evaporator to obtain a viscous semi solid mass.

Phytochemical screening

The methanol extract was tested for various phytoconstituents such as steroids, triterpenoids, flavonoids, and tannins using standard method.^[12]

GC-MS analysis

The GC-MS analysis was carried out using a Clarus 500 Perkin-Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold - Perkin Elmer Turbomass 5.1 spectrometer with an

Elite - 1 (100% Dimethyl poly siloxane), 30 m × 0.25 mm ID × 1 µm of capillary column. The instrument was set to an initial temperature of 70°C, and maintained at this temperature for 3 min. At the end of this period the oven temperature was rose up to 300°C, at the rate of an increase of 10°C/min, and maintained for 9 min. Injection port temperature was ensured at 250°C and Helium flow rate at 1.5 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 40-700 (*m/z*). The ion source temperature was maintained at 230°C and Interface temperature was at 240°C. The MS start time was 3 min, and end time was 35 min with solvent cut time was of 3 min.

Using computer searches on a NIST Ver. 11 MS data library and comparing the spectrum obtained through GC-MS, compounds present in the plant samples were identified.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was 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 were confirmed.

RESULTS

Phytochemical investigation

The qualitative phytochemical screening of methanol extract of *Clerodendrum viscosum* showed the possession of steroids, triterpenoids, glycosides, flavonoids, polyphenolics and tannins.

GC-MS analysis

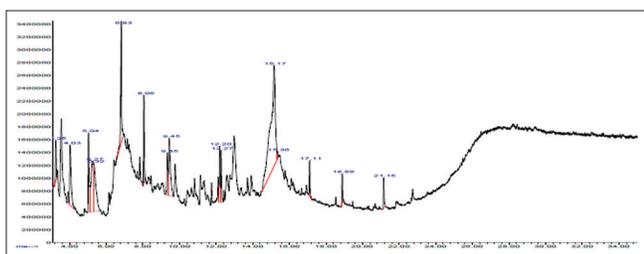
The results of GC-MS analysis of methanol extract revealed the presence of sixteen compounds. These identified compounds with their retention time (RT), molecular formula, molecular weight and concentration (peak area %) are presented in Table 1. The GC-MS chromatogram of sixteen compounds with their chemical structures is depicted in Figure 1. The GC-MS spectrum confirmed the presence of 16 components with the retention time; 3.25, 4.03, 5.04, 5.26, 5.32, 6.82, 8.064, 9.34, 9.45, 12.20, 12.27, 15.17, 15.35, 17.11, 18.89 and 21.15.

In term of % peak area, 4-Pyranone, 2,3-dihydro- (6.63%), alpha- D-Galactofuranoside, methyl 2,3,5,6-tetra-O-methyl- (5.28%), Glycerin (8.62%), Xylitol (8.46%), N, N-Dimethylglycine

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

Peak	RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)	Nature of the compound
1	3.25	Acetamide, N, N'-cabonylbis-	C ₅ H ₈ N ₂ O ₃	144.12	2.31	Amide
2	4.03	4-Pyranone, 2,3-dihydro-	C ₅ H ₆ O ₂	98.09	6.63	Ketone
3	5.04	alpha-D-Galactofuranoside, methyl 2,3,5,6-tetra-O-methyl-	C ₁₁ H ₂₂ O ₆	250.28	5.28	Glycoside
4	5.26	Glycerin	C ₃ H ₈ O ₃	92.09	8.62	Alcohol
5	5.32	Xylitol	C ₅ H ₁₂ O ₅	152.15	8.46	Alcohol
6	6.82	N, N-Dimethylglycine	C ₄ H ₉ NO ₂	103.4	7.98	Amino acid
7	8.064	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	144.12	4.19	Ketone
8	9.34	Benzofuran, 2,3-dihydro-	C ₈ H ₈ O	120.14	3.52	Heterocyclic aromatic
9	9.45	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126.11	7.11	Aldehyde
10	12.20	2 (1H) Pyrimidinone, 1-methyl-	C ₅ H ₆ N ₂ O	110.11	2.82	Ketone
11	12.27	2,4-Dihydroxy-5,6-dimethylpyrimidine	C ₆ H ₈ N ₂ O ₂	140.13	2.98	Alcohol
12	15.17	3-Deoxy-d-mannonic lactone	C ₆ H ₁₀ O ₅	162.14	35.69	Cyclic ester
13	15.35	1,3-Methylene-d-arabitol	C ₆ H ₁₂ O ₅	164.15	0.34	Carbohydrate
14	17.11	Orcinol	C ₇ H ₈ O ₂	124.13	1.57	Alcohol
15	18.89	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	0.89	Fatty acid
16	21.15	Phenol, 4,4'-(1-methyl ethylidene) bis-	C ₁₅ H ₁₆ O ₂	228.28	1.62	Alcohol

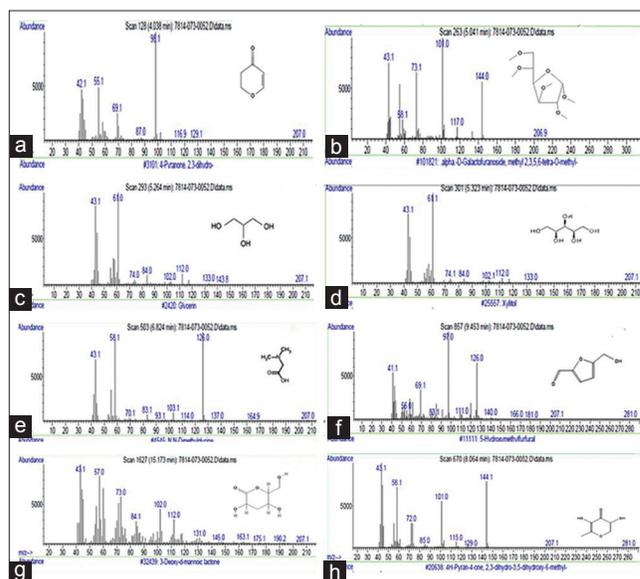
RT=Retention time

**Figure 1:** GC-MS chromatogram of methanol extract of *Clerodendrum viscosum* leaves

(7.98%), 5-Hydroxymethylfurfural (7.11%), 3-Deoxy-d-mannonic lactone (35.69%) were found as seven major compounds, where as acetamide, N, N'-cabonylbis-(2.31%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (4.19%), Benzofuran, 2,3-dihydro-(3.52%), 2 (1H) Pyrimidinone, 1-methyl-(2.82%), 2,4-Dihydroxy-5,6-dimethylpyrimidine (2.98%), 1,3-Methylene-d-arabitol (0.34%), Orcinol (1.57%), n-Hexadecanoic acid (0.89%) and Phenol, 4,4'-(1-methyl ethylidene) bis-(1.62%) are the nine minor compounds in the methanolic extract of *Clerodendrum viscosum*. The individual mass fragmentation of seven major compounds is illustrated in Figure 2a-g.

DISCUSSION

The identified major compounds possess some important biological potential for future drug development. The cyclic ester, 3-Deoxy-d-mannonic lactone (35.69%) is predominant followed by alcoholic compounds, glycerin (8.62%) and xylitol (8.46%). The compound, 3-Deoxy-d-mannonic lactone with highest % peak area (35.69) has previously been reported for antibacterial

**Figure 2:** (a) 4-Pyranone,2,3-dihydro (b) alpha-d-Galactofuranoside, methyl 2,3,5,6-tetra-O-methyl (c) Glycerin (d) Xylitol (e) N,N-Dimethylglycine (f) 5-Hydroxymethylfurfural (g) 3-Deoxy-d-mannonic lactone (h) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-

activity.^[13] Glycerin decreases intracranial pressure in numerous disease states, including Reye's syndrome, stroke, encephalitis, meningitis, pseudotumor cerebri, central nervous system tumor, and space occupying lesions. It is also effective in lowering intraocular pressure in glaucoma and shrinking the brain during neurosurgical procedures.^[14] The xylitol was reported for the treatment of systemic sclerosis caused by mutants streptococci.^[15] Xylitol found to be a lower-caloric alternative to table sugar, absorbed more slowly than sugar and it does not contribute to high blood sugar levels or hyperglycemia caused by insufficient insulin response. This characteristic profile of xylitol has been found to be beneficial for people

suffering from metabolic syndrome, which is a common disorder that includes insulin resistance, hypertension, hypercholesterolemia.^[16] Nasal spray of xylitol has also been reported to decrease the incidence of acute otitis, at the same time it is a very effective way of both assisting and stimulating the body's own natural nasopharyngeal washing and reducing both bacterial colonization and allergenic pollution.^[17] It has also been reported to possess antioxidant activity.^[18]

The amino acid, N, N-Dimethylglycine (7.98) is also found as major compound and is reported to have immune modulating properties.^[19] 5-Hydroxymethylfurfural is used as antioxidant and antiproliferative agent.^[20]

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

CONCLUSION

The correlation among the phytochemical constituents with their biological activities is now being the matter of innovative thought. *Clerodendrum viscosum* is a plant, traditionally used for the treatment of asthma, bronchitis, skin diseases, epilepsy and worm infestation. But till date, there are no reports on chromatographic analysis of methanolic extract of the plant. Here in, we first time report the presence of some important compounds in this plant isolated by GC-MS analysis. Thus, this type of study may give information on nature of active principles present in the medicinal plants and to identify the plants from their adulterants using isolated compounds as biomarker. These identified phytoconstituents presumed to be responsible for eliciting the traditional activity of this plant, *Clerodendrum viscosum*.

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