



RESEARCH ARTICLE

NATURAL PRODUCT CHEMISTRY

CHEMICAL INVESTIGATION OF THE SIMPLE ASCIDIAN *PHALLUSIA NIGRA* SAVIGNY, 1816 OF TUTICORIN COAST BY GC-MS**S. GOPALAKRISHNAN^{a*}, V. K. MEENAKSHI^b AND D. SHANMUGA PRIYA^a**^a Dept of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli-627 012, Tamilnadu, India.^b Dept of Zoology, A.P.C. Mahalaxmi college for women, Tuticorin-627 012, Tamilnadu, India.**S. GOPALAKRISHNAN**

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ABSTRACT

Ascidians is a rich source of bioactive secondary metabolites. *Phallusia nigra*, a simple ascidian belonging to the family Ascidiidae found in plenty throughout the year along the Tuticorin coast of India. The methanolic extract of *Phallusia nigra* was subjected to GC-MS analysis to determine the possible bioactive components. The analysis revealed the presence of eleven chemical constituents of which the major are: Methyl 3-bromo-1-adamantaneacetate (24.65%), n-Hexadecanoic acid (24.45%), 11-Hexadecen-1-ol,(Z)- (17.64%) and 2,6-Dimethyl-6-trifluoroacetoxyoctane (9.82%).



KEYWORDS

Phallusia nigra, GC-MS analysis

INTRODUCTION

Ascidians are marine sedentary organisms. *Phallusia nigra* is a simple ascidian belonging to the family Ascidiidae¹. Hundreds of new compounds have been isolated from ascidians, the majority of which are amino acid derivatives. It is the biological activity associated with many of these natural products that are responsible for research focus on these marine organisms. To date, the most notable examples of bioactive ascidian compounds include didemnin B^{2,3}, dehydrodidemnin B, ecteinascidin-743^{4,5}, sulcatin⁶, stolonin acids A&B⁷, bistramidin A,B,C,D & K⁸. Ascidians are renowned for their overwhelming bias towards the production of nitrogenous secondary metabolites. However, with the continued chemical interest in this group of animals, an increasing number of non-nitrogen containing metabolites are being isolated. Hence the objective of the present investigation is to identify the possible chemical constituents with the aid of GC-MS analysis.

EXPERIMENTAL

Collection of animal material

Phallusia nigra (Family : Ascidiidae) was collected from Tuticorin coast in the month of October 2010 by SCUBA diving. Molluscan shell, calcareous rock fragments attached to the foot of the animal was carefully removed. They were identified using key to identification of Indian ascidians⁹. A voucher specimen No. 2083 has been submitted in the ascidian collection of museum of the Department of Zoology, A. P. C. Mahalaxmi College for women, Tuticorin – 628002, Tamilnadu, India.

Preparation of extract

The whole animal was dried in shade and homogenized to get a coarse powder. The powder was successively extracted with various solvents such as petroleum ether (40°-60°C),

benzene, chloroform, methanol and water. The extracts were concentrated in a rotary evaporator under reduced pressure. 2 µl of the methanolic extract of *Phallusia nigra* was employed for GC-MS analysis¹⁰.

Instruments and chromatographic conditions

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite -1 fused silica capillary column (30 × 0.25 mm 1D × 1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature 250°C; ion source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

Identification of components

Interpretation on mass spectrum of GC-MS was conducted using the data base of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components were 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 ascertained.

RESULTS AND DISCUSSION

GC-MS analysis

GC-MS chromatogram of the methanolic extract of *Phallusia nigra* (Figure - 1) showed 11 peaks indicating the presence of 11 chemical constituents. On comparison of the mass spectra of the constituents with the NIST library the 11 constituents were characterized

and identified (Table 1). The various chemical constituents which contributes to the medicinal activity to the animal is given in Table 2. It can be stated that these eleven chemical constituents have not been reported elsewhere from ascidians especially from *Phallusia nigra* and hence can be considered as first report to the world.

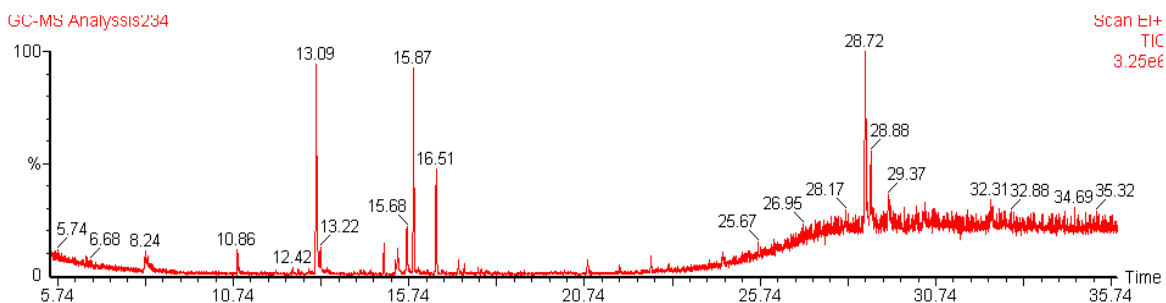


Figure. 1
GC-MS Chromatogram of methanolic extract of *Phallusia nigra*

Table 1
Chemical components identified in the methanolic extract of *Phallusia nigra* by GC-MS

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area (%)
1.	8.24	Propanoic acid, 2-methyl-, 2-phenylethyl ester	C ₁₂ H ₁₆ O ₂	192	2.00
2.	10.86	DL-3,4-Dimethyl-3,4-hexanediol	C ₈ H ₁₈ O ₂	146	2.61
3.	13.09	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	24.45
4.	15.03	dl-6-Methyl-5-hepten-2-ol	C ₈ H ₁₆ O	128	2.20
5.	15.68	2-Methyl-3-decanol	C ₁₁ H ₂₄ O	172	5.21
6.	15.87	11-Hexadecen-1-ol, (Z)-	C ₁₆ H ₃₂ O	240	17.64
7.	16.51	2,6-Dimethyl-6-trifluoroacetoxyoctane	C ₁₂ H ₂₁ F ₃ O ₂	254	9.82
8.	20.82	Didodecyl phthalate	C ₃₂ H ₅₄ O ₄	502	1.80
9.	22.62	Cyclohexane, 1,1'-(2-methyl-1,3-propanediyl)bis-	C ₁₆ H ₃₀	222	1.20
10.	28.72	Methyl 3-bromo-1-adamantaneacetate	C ₁₃ H ₁₉ BrO ₂	286	24.65
11.	28.88	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	C ₁₃ H ₂₂ OSi ₂	250	8.42

Table 2
Activity of the chemical components identified in the methanolic extract of *Phallusia nigra* by GC-MS

No.	RT	Name of the compound	Molecular Formula	M W	Peak Area %	Nature of the compound	#Activity
1.	8.24	Propanoic acid, 2-methyl-, 2-phenyl ethyl ester	C ₁₂ H ₁₆ O ₂	192	2.00	Ester	Antimicrobial
2.	10.86	DL-3,4-Dimethyl-3,4-hexanediol	C ₈ H ₁₈ O ₂	146	2.61	Alcohol	Antimicrobial
3.	13.09	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	24.45	Palmitic acid	Antioxidant Hypocholesterolemic Nematicide Pesticide Lubricant Antiandrogenic Flavor Hemolytic
4.	15.03	dl-6-Methyl-5-hepten-2-ol	C ₈ H ₁₆ O	128	2.20	Alcohol	Antimicrobial
5.	15.68	2-Methyl-3-decanol	C ₁₁ H ₂₄ O	172	5.21	Alcohol	Antimicrobial
6.	15.87	11-Hexadecen-1-ol, (Z)-	C ₁₆ H ₃₂ O	240	17.64	Unsaturated alcohol	No activity reported
7.	16.51	2,6-Dimethyl-6-trifluoroacetoxyoctane	C ₁₂ H ₂₁ F ₃ O ₂	254	9.82	Fluro compound	Antimicrobial
8.	20.82	Didodecyl phthalate	C ₃₂ H ₅₄ O ₄	502	1.80	Plasticizer	Antimicrobial Antifouling
9.	22.62	Cyclohexane, 1,1'-(2-methyl-1,3-propane diyl) bis-	C ₁₆ H ₃₀	222	1.20	Alicyclic compound	No activity reported
10.	28.72	Methyl 3-bromo-1-adamantaneacetate	C ₁₃ H ₁₉ BrO ₂	286	24.65	Bromo compound	Antimicrobial
11.	28.88	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	C ₁₃ H ₂₂ OSi ₂	250	8.42	Ketone	No activity reported

**Source : Dr. Duke's phytochemical and ethnobotanical databases [online database]. The spectrum and chemical structure of the four major chemical constituents are presented in Figure 2 to Figure 5. They were identified as Methyl 3-bromo-1-adamantaneacetate (24.65%), n-Hexadecanoic acid (24.45%), 11-Hexadecen-1-ol, (Z)- (17.64%), 2,6-Dimethyl-6-trifluoroacetoxyoctane (9.82%) respectively.*

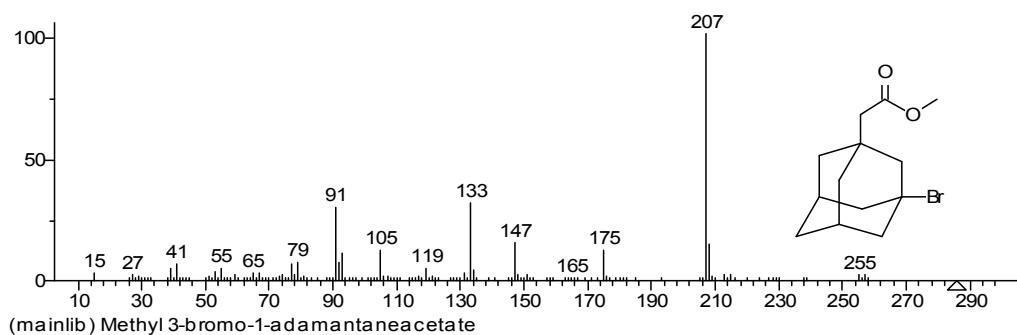


Figure. 2

Mass_spectrum of Methyl 3-bromo-1-adamantaneacetate (RT: 28.72)



Figure. 3

Mass_spectrum of n-Hexadecanoic acid (RT:13.09)

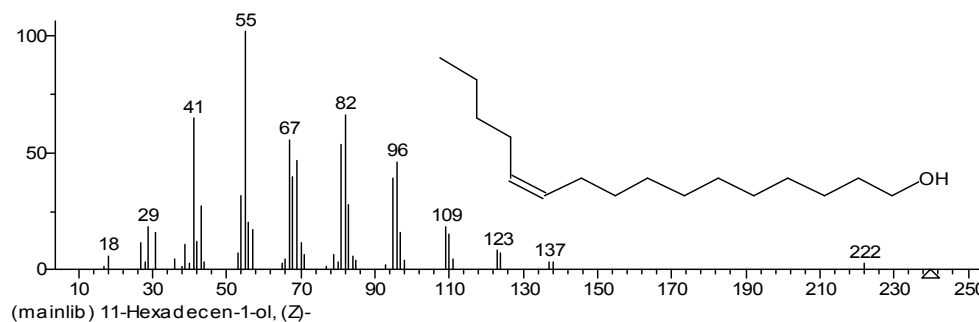


Figure. 4

Mass_spectrum of 11-Hexadecen-1-ol, (Z)- (RT: 15.87)

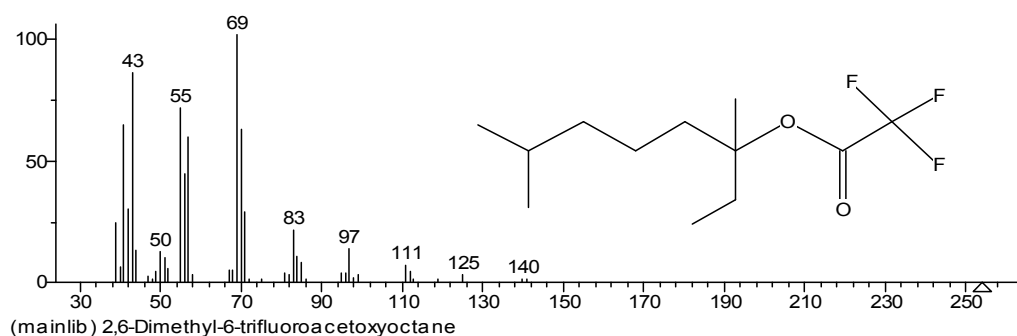


Figure. 5

Mass_spectrum of 2,6-Dimethyl-6-trifluoroacetoxyoctane (16.51)

CONCLUSION

The study clearly indicates that the methanolic extract of *Phallusia nigra* rich in many phytochemicals like Methyl 3-bromo-1-adamantaneacetate, n-Hexadecanoic acid, 11-Hexadecen-1-ol, (Z)-, 2,6-Dimethyl-6-trifluoroacetoxyoctane which contributes the activities like antimicrobial, antioxidants, hypocholesterolemic, nematicide, pesticide, antiandrogenic, hemolytic and antifouling. However further detailed study is required to

target the isolation of the active principle responsible for the activities.

ACKNOWLEDGEMENT

The authors thank Dr.K.Alagusundaram, Director and Dr.S.Kumaravel, Senior Scientist, Indian Institute of Crop Processing Technology, Thanjavur-613005, Tamilnadu, India for providing all the facilities and support to carry out the work.

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