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Original Article

GC-MS ANALYSIS OF BIOACTIVE CONSTITUENTS OF METHANOLIC EXTRACT OF LEAVES OF ACTINODAPHNE BOURDILLONII GAMBLE

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ABSTRACT

Objective: The investigation was carried out to characterize the chemical constituents present in the methanolic extracts of leaves of Actinodaphne *bourdillonii* Gamble using GC-MS.

Methods: The chemical constituents of methanolic extract of A. *bourdillonii* were studied by using Perkin Elmer Gas Chromatography- Mass Spectroscopy.

Results: The GC-MS analysis of the methanolic extract revealed the presence of 18 compounds. The major chemical constituents are Tobacco compounds- 4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)- (33.47%), Terpene alcohol compounds - 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (10.78%), Myirstic acid - Tetradecanoic acid (9.89%) and Sugar moiety compounds -1,6-Anhydro-2,4-dideoxy-á-D-ribo-hexopyranose (7.93%). The analysis of bioactive principles of methanolic extract of leaves of *A. bourdillonii* has not been reported previously.

Conclusion: *A. bourdillonii* is a valuable tree with numerous medicinal properties which contains various bioactive principles. Such studies will be very much help full in designing a new drugs for the therapeutic values.

Keywords: *Actinodaphne bourdillonii*, Terpene alcohol, Myirstic acid, Sugar moiety.

INTRODUTION

Herbal medicine has its origin in ancient cultures including those of the Egyptians, Americans, Indians and Chinese. It involves the medicinal use of plants to treat disease and enhance general health and wellbeing. Ayurveda is the world's oldest science of health care. The written tradition dated back around 5,000 years, but the oral tradition in India is timeless (www. hengleys. com). The plants serve as a very good source of secondary metabolites with potential biological activites. As an alternative to microbial sources, screening of plants for the biologically active principles has now increased significantly [1].

Even today many tribal communities used the medicines from natural sources extensively to cure sick. Today the medicinal world is posed with complex challenges [2]. Because recently, problems with drug resistant microorganisms, side effects of modern drugs, and emerging disease like viral hepatitis, cancer, arthritis, diabetes, AIDS for which no medicines are available, have encouraged an interest in plants again as a significant source of new medicines. Among 2, 50,000 to 5, 00,000 species of higher plants only small percentage has been investigated for the presence of bioactive compounds [3].

Hence the present investigation was carried out to determine the possible chemical constituents in *A. bourdillonii* by GC – MS studies. *A. bourdillonii* belongs to the family Lauraceae and it is called Eeyoli or Malavirinj in Malayalam. The family Lauraceae contains green medium sized trees found in the tropics especially in India, China, East Africa and South East Asian countries like Malaysia, Indonesia and the Philippines. It is a big family consisting of 62 genera and over 2000 to 2500 species [4].

MATERIALS AND METHODS

Plant material

The leaf samples were collected from Munnar, Idukki District Kerala, (10°1'22"N 77°2'22"E) India. The taxonomic identity of the plant was confirmed from the Rapinat Herbarium and Centre for Molecular Systematic, St. Joseph's College (Campus) Tiruchirappalli 620020, TamilNadu, India. The voucher number is RD 001.

Preparations of plant extract [5]

Fresh plant materials washed under running tap water were shade dried, homogenized to fine powder and stored in airtight bottles. 10g of the powder was macerated in 100 ml of methanol, placed on a mechanical shaker for 48 hours. The extract was filtered using No.1 Watt man filter paper. The filtrate was concentrated using the rotary evaporator. The extract was stored in the refrigerator for further analysis.

Gas Chromatography- Mass Spectroscopy (GC-MS) analysis

The GC/MS analysis of the methanolic extract of *A. bourdillonii* was conducted in a Perkin Elmer GC Clarus 500 system, which was interfaced with a Mass Spectrometer. The GC/MS instrument had a Elite-1 fused silica capillary column with a dimension of 30 × 0.25 mm ×0.25 µm df, composed of 5% Dipheny 95% Dimethyl poly siloxane, operated in electron impact mode at 70 eV. The carrier gas used was helium, which had a constant flow rate of 1 ml/min and with an injection volume of 2 µl was employed (split ratio of 10:1). The injector was operated at 250°C and the oven temperature was programmed as follows: 110°C for 2 min, gradually increased to 200°C at 10°C/min, then to 280°C at 5°C/min which was maintained as a isothermal temperature for 9 min. The Mass spectrum of the A. *bourdillonii* extract was recorded at 70 eV, with a scan interval of 0.5 seconds and a fragment from 45 to 450 Da. Total GC running time was 36 min. The relative percentage amount of each compound present in the GC-MS spectrum was calculated by comparing the individual compounds average peak area to their total areas. Turbo mass 5.2 software was used to handle mass spectra and chromatograms.

Identification of compounds

The identification of the compounds in the GC-MS of *A. bourdillonii* was based on the database of National Institute Standard and Technology (NIST), which had standards for more than 62,000 compounds. The known components in the mass spectrum were compared with the spectra of known components stored in the NIST library, through which the name, molecular weight and structure of the compounds were disclosed.

RESULTS AND DISCUSSION

The compounds present in the methanolic extract of leaves of *A. bourdillonii* were identified by GC-MS analysis. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention Time (RT), Molecular formula, Molecular Weight (MW) and peak area in percentage are presented in Table 1 and Figure 1. This analysis of methanolic extract of leaves of A. *bourdillonii* revealed the presence of four major phytochemical constituents. They were a Tobacco compounds- 4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)- (33.47%), Terpene alcohol compounds - 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (10.78%), Myirstic acid - Tetradecanoic acid (9.89%) and Sugar moiety compounds -1,6-Anhydro-2,4-dideoxy-á-D-ribo-hexopyranose (7.93%). All identified compounds were generally reported to have antimicrobial activities. In addition, the Tobacco compounds are having Estrogen like activity, while Terpene alocohol compounds reported to have antimicrobial and antiinflammatory activities. Antioxidant, Cancer preventive, Nematicide, Lubricant Hypocholesterolemic properties were reported for Myristic acid compounds and Sugar moiety compounds possess preservative properties. No such activities have been reported in two alcoholic compound 9-Tetradecen-1-ol, acetate, (E)- and E-2-Tetradecen-1-ol and sugar moiety compound 1,6-Anhydro-2,4-dideoxy-á-D-ribo-hexopyranose.

Table 1: Components detected in the methanolic extract of leaves of *Actinodaphne bourdillonii* Gamble

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	8.93	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	$C_6H_7N_3O_2$	153	4.20
2.	10.18	1,6-Anhydro-2,4-dideoxy-á-D-ribo-hexopyranose	$C_6H_{10}O_3$	130	7.93
3.	10.80	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	10.78
4.	11.06	9-Tetradecen-1-ol, acetate, (E)-	$C_{16}H_{30}O_2$	254	3.72
5.	11.24	E-2-Tetradecen-1-ol	$C_{14}H_{28}O$	212	5.92
6.	12.43	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	9.89
7.	14.59	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	6.57
8.	17.55	2,6,6-Trimethyl-bicyclo[3.1.1]hept-3-ylamine	$C_{10}H_{19}N$	153	0.46
9.	18.91	Methoxyacetic acid, 3-tridecyl ester	$C_{16}H_{32}O_3$	272	0.71
10.	20.29	Methoxyacetic acid, 4-tridecyl ester	$C_{16}H_{32}O_3$	272	0.69
11.	23.08	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222	1.25
12.	23.16	Squalene	$C_{30}H_{50}$	410	3.03
13.	24.97	Z,Z,Z-4,6,9-Nonadecatriene	$C_{19}H_{34}$	262	1.13
14.	26.24	5à-Androstan-16-one, cyclic ethylene mercaptole	$C_{21}H_{34}S_2$	350	0.89
15.	26.69	Cholest-5-ene, 3-bromo-, (3á)-	$C_{27}H_{45}Br$	448	5.33
16.	27.36	Vitamin E	$C_{29}H_{50}O_2$	430	2.94
17.	30.04	Urs-12-en-28-ol	$C_{30}H_{50}O$	426	1.09
18.	30.97	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	$C_{20}H_{34}O_2$	306	33.47

*Parameters tested are not covered under the scope of NABL accreditation

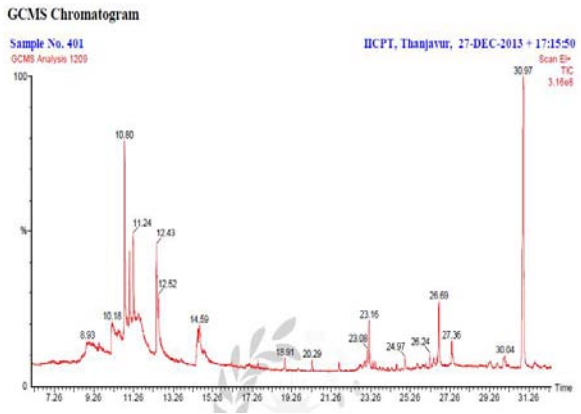

Fig. 1: GC-MS Chromotogram of the methanolic extract of leaves of *Actinodaphne bourdillonii*

CONCLUTION

The present study revealed that 18 compounds were identified by Gas Chromatography –Mass Spectrometry (GC-MS) analysis. *A. bourdillonii* is a valuable tree with numerous medicinal properties. *A. bourdillonii* contains various bioactive principles. However, isolation of individual Phytochemical constituents and subjecting them to biological activity will definitely give fruitful results. Such studies will be very much help full in designing new drug for the therapeutic values.

CONFLICT OF INTERESTS

Declared None

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