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ISSN: 0976-3031

International Journal of Recent Scientific Research
Vol. 6, Issue, 4, pp.3520-3522, April, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

BIOACTIVE COMPOUNDS FROM THE LEAVES OF TABERNAEMONTANA DIVARICATA (L.)

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ARTICLE INFO

Article History:

Received 5th, March, 2015
Received in revised form 12th, March, 2015
Accepted 6th, April, 2015
Published online 28th, April, 2015

Key words:

Tabernaemontana divaricata (L.), GC-MS analysis, Phytocomponents

ABSTRACT

Plants are the main source with therapeutic significance from thousands of years. Ethanol extract of *T. divaricata* leaf (double flower variety) was evaluated by Gas Chromatography–Mass Spectrometry. The mass spectra of the unknown compounds found in the extract was matched with the known components of National Institute of Standards and Technology (NIST) library. GC-MS analysis exposed the presence of 9 compounds. From the GC-MS analysis, the phytocomponents such as 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Lactose, n-Hexadecanoic acid, Phytol, 9,12-Octadecadienoic acid (Z,Z), Squalene, Cedrol, Vitamin E and Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)- were screened. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. Many of them used for several purposes like antioxidant, anti-inflammatory, antimicrobial, pesticide and cancer preventive.

INTRODUCTION

Medicinal plants used for conventional medicine have extensive series of substances that can be utilized to treat chronic and infectious disorders (Periyasamy *et al*., 2010). Even the World Health Organization (WHO) encourages the use of medicinal plants, because it is proven to be efficacious, safe, less toxic, accessible and reliable natural source (Easwari *et al*., 2013).

Tabernaemontana divaricata (L.), a glabrous, evergreen, dichotomously branched shrub or small tree. It bears attractive, white coloured fragrant flowers, double layered and may appear sporadically throughout the year. The leaves are large, shiny and deep green in colour and the size is about 6- inches in length and 2-inches in width. *Tabernaemontana divaricata* (L.) is widely distributed throughout in India as ornamental plant. It is also found in Bangladesh and other parts of South East Asia. It possesses wide range of valuable activities like anti-infection, antioxidant, anti-inflammation, anticancer, anticonvulsant and antidiabetic properties (Ashikur *et al*., 2011, Rumzhum *et al*., 2012, Qamruzzama *et al*., 2012, Hari *et al*., 2012, Basavaraj *et al*., 2011, Rahman *et al*., 2012).

In recent years, Gas Chromatography–Mass Spectroscopy (GC-MS) is utilized for exact analysis of components present in the medicinal plants (Dubal *et al*., 2013). For the reason that it is an efficient method to analyse the primary and secondary metabolites (Cowan, 1999).

MATERIALS AND METHODS

Preparation of extract

Tabernaemontana divaricata (L.) leaves were collected from the plant then washed, shade dried and powdered. 25g leaf powder was treated with 50ml of ethanol for 12 hours and filtered through a Whatmann No.41 filter paper along with 2g sodium sulphate to remove the deposits and water in the filtrate. The filtrate is concentrated to 1ml by bubbling nitrogen gas into the solution. The extract contains both polar and non-polar components of the material. 2µl of sample was used for injection into GC-MS instrument.

GC-MS Programme

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system. GC-MS operated using the following conditions: Column: Elite-5MS (30 x 0.25mm x 0.25 m df, composed of 5% Diphenyl / 95% Dimethyl poly siloxane), working in electron energy at 70 eV; helium was used as carrier gas at a constant flow of 1ml/min and an injection volume of 2µl was employed (split ratio of 10:1). The injector temperature - 250°C; inlet and source temperature - 200°C. The oven

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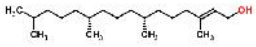
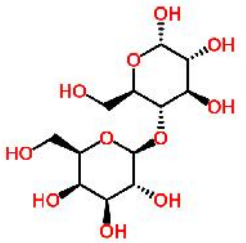
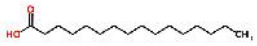
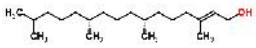
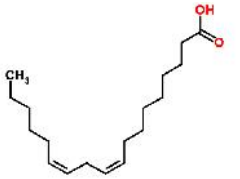
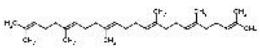
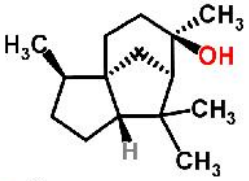
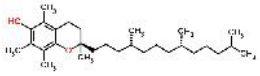
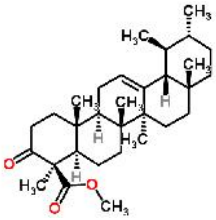
temperature was programmed from upto 200 °C at the rate of 10 °C/min (no hold), to 5 °C/min - 9 min hold upto 280°C. Mass spectra were taken at 70 eV; a mass scan (m/z) fragments from 45 to 450 Da. Total GC and MS running time was 36min.

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 determined.

Table 1 Retention time, molecular weight and peaks of various Components identified in Leaves by GC – MS

Sl.No	RT	Name of the Compound	Molecular formula	MW	Peak area %
1.	10.53	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	5.46
2.	11.42	Lactose	$C_{12}H_{22}O_{11}$	342	20.31
3.	12.23	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	27.37
4.	13.43	Phytol	$C_{20}H_{40}O$	296	8.24
5.	14.15	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	8.53
6.	22.33	Squalene	$C_{30}H_{50}$	410	17.92
7.	25.61	Cedrol	$C_{15}H_{26}O$	222	1.12
8.	26.49	Vitamin E	$C_{29}H_{50}O_2$	430	6.62
9.	30.75	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	$C_{31}H_{48}O_3$	468	3.50

Table 2 Bioactivity of phytocomponents identified in the ethanolic leaf extract of *Tabernaemontana divaricata* (L.)

Sl.No	Name of the Compound	Structure	Activity
1.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		Antimicrobial, Antiinflammatory
2.	Lactose		Preservative, Nutritive
3.	n-Hexadecanoic acid		Antioxidant, Pesticide, Antiandrogenic
4.	Phytol		Antimicrobial, Anticancer Antiinflammatory,
5.	9,12-Octadecadienoic acid (Z,Z)-		Hypocholesterolemic, Cancer preventive, Hepatoprotective Nematicide, Antiacne, Antiarthritic
6.	Squalene		Anticancer, Antimicrobial, Antioxidant, Pesticide
7.	Cedrol		Anti-tumour, Antibacterial, Anti-inflammatory, Fungicide, Nematicide
8.	Vitamin E		Antiaging, Antidiabatic, Antioxidant, antileukemic, Hepatoprotective, Antiulcerogenic, Antibronchitic,
9.	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-		No activity reported

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

RESULTS AND DISCUSSION

GC-MS analysis was done in the leaf extract of double flower variety of *T. divaricata* (L.). The active components with their molecular formula, molecular weight (MW), retention time (RT), and concentration (% peak area) are given in Table 1. The GC-MS chromatogram of 9 compounds were identified are

shown in fig.1. The most dominant compounds are n-Hexadecanoic acid (27.37%), Lactose (20.31%) and followed by Squalene (17.92%).

GC MS Chromatogram

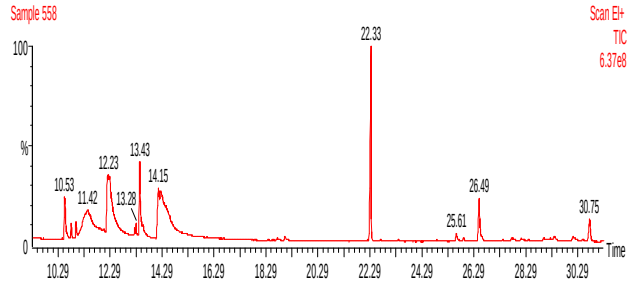


Figure 1 GC-MS Chromatogram for ethanolic leaf extract of *T. divaricata* (L.)

The structure and biological activities of the detected phytocomponents are specified in Table 2. n-hexadecanoic acid (palmitic acid) acquires antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic and hemolytic properties. Phytol holds antimicrobial and anticancer property (Sermakkani *et al*., 2012). 9-12, Octadecadienoic acid (Z, Z) have antiinflammatory and antiarthritic property (Rani *et al*., 2009). Squalene retains preventive action against colon carcinogenesis (Rao *et al*., 1998). Cedrol contains antitumour, antibacterial and fungicidal activities (Vaitheeswaran *et al*., 2014). Likewise Vitamin E comprises antioxidant, antidiabetic and antiulcerogenic and antibronchitic activities (Rajeswari *et al*., 2012).

CONCLUSION

The GC-MS analysis revealed that nine phytochemical constituents had been identified from the leaf extract of *T. divaricata* (L.) and for further investigation, specific phytochemical compounds has to be isolated and their characteristic nature will be analysed.

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How to cite this article:

Kalaimagal C and Umamaheswari G., Bioactive Compounds from the leaves of Tabernaemontana Divaricata (l.). *International Journal of Recent Scientific Research Vol. 6, Issue, 4, pp.3520-3522, April, 2015*

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