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Research Article

IDENTIFICATION OF BIOACTIVE COMPOUNDS IN FLOWER OF *TABERNAEMONTANA DIVARICATA* (L.) USING GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS

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ABSTRACT

Objective: Herbs are a key resource with therapeutic properties. Nowadays, there is a focus on the identification of bioactive compounds with the ability to act against various disorders.

Methods: In the present study, gas chromatography–mass spectrometry analysis was conducted to determine the occurrence of different phytochemical compounds in ethanolic flower extract of *Tabernaemontana divaricata* (L.).

Results: The ethanol extract of flower revealed the presence of several bioactive compounds such as n-hexadecanoic acid, squalene, Vitamin D3, Vitamin A aldehyde, desulfosinigrin, and Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-.

Conclusion: The perceived compounds from ethanolic extract of flower have diverse beneficial properties such as antimicrobial, antioxidant, cancer anticipatory effect, pesticide, and antiarthritic.

Keywords: *Tabernaemontana divaricata* (L.), Gas chromatography–mass spectrometry analysis, Bioactive compounds.

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INTRODUCTION

Plants are posh bequest to every individual because of the presence of curative possessions. Medicinal plants can act as wealthy mine of phytochemicals (secondary metabolites) with diverging natal actions [1]. These compounds defend human being through the action against unremitting and contagious disorders [2-4].

The World Health Organization stated that above 80% of populace depend on conventional basis plant drugs for their principal well-being penury [5-7]. Herbal medications are inoffensive than synthetic medicines since in attendance with the phytochemical constituents in herbal extract that intent biochemical path [8].

At present, these plant-based drugs come to attention notably in developing countries for the treatment of copious diseases owing to its certainty that green linctus is secure, economic, simply accessible as well as few side effects. As a consequence of these reasons, awareness about its exploitation can be spreaded throughout the world [9].

Disposition of various dynamic phytochemical compounds in these green medicinal plants has bestowed origin for maximum activity containing drugs. Most significant chemical active components of shrubs are alkaloids, flavonoids, and tannin likewise some phenolic compounds. Right now, the exigency for herbal products has augmented drastically. Hence, nowadays, pharmaceutical companies are also produce health-care products from therapeutic herbs [2] which have the ability to provide naturally active compounds and it can act against numerous syndromes [10].

In India, one of the ornamental as well as shrine plants is *Tabernaemontana divaricata* (L.), it belongs to *Apocynaceae* family. It has dichotomous branch containing herb, or small tree is extensively dispersed all over in India, Bangladesh, and some elements of South East Asia. It engenders gorgeous white-colored aroma flowers and may become visible intermittently during the year. The leaves are sleek, hefty, and deep green in color. It holds variety of beneficial actions such as antimicrobial, antioxidant along with antidiabetic properties [11-13].

Gas chromatography–mass spectrometry (GC-MS) is the better process to find the biochemical components of long-chain hydrocarbons, volatile essential oil, fatty acids, lipids, alkaloids, steroids, amino, nitro compounds, alcohols, esters, etc. [14,15]. Normally, GC-MS is used for detection and enumeration of compounds in various extracted samples. The known compound spectrum can be matched with unknown components in complex mix. This analysis used to detach diverse compounds in the sample based on their wavering [16]. From the literatures, it is evident to analyze herbal value of the plant *T. divaricata* (L.). In this study, GC-MS technique was applied to find the presence of several bioactive compounds in the ethanolic extract of *T. divaricata* (L.) flowers to give better pharmacological activities.

MATERIALS AND METHODS

Plant material

T. divaricata (L.) plant was identified with the help of book and literature references [17-19], and the herbarium was deposited in the Department of Biotechnology, Marudupandiyar College of Arts and Science, Thanjavur, Tamil Nadu, India. *T. divaricata* (L.) flowers (double layer flowering plant variety) were gathered in and around area of Thanjavur, Tamil Nadu, India. Collected flowers were cleansed and dried in shadow for 2 weeks. Dried plant material was pulverized with electrical beater into fine powder and then utilized to prepare extracts.

Preparation of extract for GC-MS

25 g flower powder was treated with 50 ml of ethanol for 12 h and sieved by means of Whatman Filter Paper No. 41 among sodium sulfate (2 g). Beforehand this separation, filter paper along with Na_2SO_4 was damped with 95% ethanol. This filtration used for the elimination of sediments and water in the filtrate. The filtrate was abridged by bubbling nitrogen gas. 2 µl of extract was injected into GC-MS apparatus [20].

GC-MS program

GC-MS technique was performed in a GC Clarus 500 Perkin Elmer system. GC-MS operated using the following conditions: Column: Elite-5MS (30×0.25 mm×0.25 m df, made up of 5% diphenyl/95% dimethyl polysiloxane), electron energy - 70 eV; helium was utilized as hauler gas and at a incessant surge of 1 ml/min. 2 µl of injection volume was employed (split ratio of 10:1). The temperature of injector was 250°C; temperature of inlet and source was 200°C. The oven temperature was encoded up to 200°C at the rate of 10°C/min (no hold), to 5°C/min - 9 min hold up to 280°C. Mass spectra were taken at 70 eV; mass scan (m/z) fragments were obtained from 45 to 450 Da. Total working time of GC and MS was 36 min [21].

Identification of components

GC-MS spectrum was interpreted with the database of National Institute of Standard and Technology (NIST) containing above 62,000 paradigms. Spectrum of familiar compounds accumulated in the NIST library and unknown components spectrum identified from GC-MS method was correlated. After comparison, name, molecular weight (MW), and structure of the tested components were ascertained [16].

RESULTS AND DISCUSSION

GC-MS analysis

Through GC-MS study, 16 active compounds were determined in the flower extract of *T. divaricata* (L.). Identified components with their molecular formula, MW, retention time (RT), and concentration (% peak area) are specified in Table 1 as the same GC-MS chromatogram of above compounds perceived is shown in Fig. 1.

Most phytochemicals have antioxidant capacity and safeguard our cells against oxidative damage and decrease the threat of emerging definite types of cancer. Antioxidants retard the feat of free radicals which have been responsible for the pathogenesis of copious syndromes [22,23].

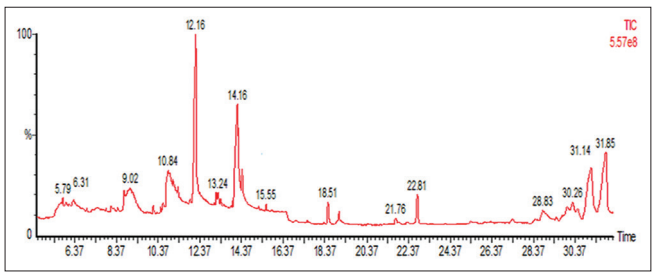

Fig. 1: Gas chromatography–mass spectrometry chromatogram for ethanolic flower extract of *Tabernaemontana divaricata* (L.)

Vitamin D3 is a liposoluble vitamin. Very few dietary supplements possess this vitamin naturally also fabricated by the skin when rendered to the sunlight. Right now, Vitamin D3 deficiency is a big problem because of the vast proportion of world civilization. In both smokers and non-smokers, low level of this vitamin raises propensity to respiratory infections and destitute mineralization of the skeleton owing to calcium besides phosphate diminution [24,25]. This kind of calcium depletion is the starting point for bone-related complaints. It has the capacity to act against cancer as well as decrease blood pressure and increase insulin secretion [26-28]. For these reasons, Vitamin D3 demand is still increasing.

Vitamin A aldehyde is otherwise known as retinal. Huge amount of compounds can come under the category of Vitamin A. Retinol and retinal are called as preformed Vitamin A. Deficiency of Vitamin A is linked with severe physical tribulations such as night blindness [29], complexity in epithelium formation [30], elevated threat in respiratory passages [31], and play a role as antioxidant [32,33].

1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)- is a sesquiterpene and it has anti-tumor, antibacterial, anti-inflammatory properties [34]. Alpha-D-Glucopyranoside, O-à-D-glucopyranosyl-(1. fwdarw.3)-á-D-, fructofuranosyl is a sugar molecule; furthermore, it possesses anti-inflammatory and diuretic effect [35]. Palmitic acid, linoleic acid, cholestan-3-ol, 2-methylene-, (3á,5à)-, and 1-Heptatriacotanol acquires antioxidant, anti-inflammatory hypocholesterolemic, antimicrobial, and anticancer properties [36,37].

Cyclopropane tetradecanoic acid, 2-octyl-, methyl ester, has antimicrobial activity [38]. 9,12,15-Octadecatrienoic acid, 2,3- bis(acetyloxy)propyl ester, (Z,Z,Z)- is an unsaturated fatty acid ester compound which possesses anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, antihistaminic, antieczemic, antiacne, antiarthritic, and antiandrogenic activities [39]. Terpenoids can act as controller of metabolism and play a defensive role as antioxidants along with it acquires antimicrobial, antiallergic, and anti-inflammatory activity [40]. Likewise, triterpene squalene retains antimicrobial, antioxidant as well as anticancer activities [41,42]. Desulphosinigrin holds antioxidant capacity and also acts against urinary tract infections [35,43].

Swamy *et al.* [44] reported the GC-MS analysis of methanolic leaf extract of *Alstonia scholaris* (*Apocynaceae* family). Through this method, only nine compounds were identified. Similarly, Papitha *et al.* [45] investigated that chemical compounds present in chloroform extract of leaves and flowers of *Spermadictyon suaveolens* Roxb., and they got ten and eight compounds, respectively. RT and peak area percentage was slightly matched with squalene compound recognized from methanolic extract of *Adiantum capillus-veneris* L. [6]. Kalaivani

Table 1: RT, MW, and peaks of various components identified in flower by GC–MS

S. No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1.	5.79	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-	$C_{15}H_{24}$	204	4.55
2.	6.31	Cyclohexanepropanoic acid, 3-oxo-, methyl ester	$C_{10}H_{16}O_3$	184	5.94
3.	9.02	a-D-Glucopyranoside, O-à-D-glucopyranosyl-(1.fwdarw. 3)-á-D-fructofuranosyl	$C_{18}H_{32}O_{16}$	504	10.80
4.	10.11	Vitamin D3	$C_{27}H_{44}O$	384	0.43
5.	10.84	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	8.08
6.	11.06	Lactose	$C_{12}H_{22}O_{11}$	342	4.11
7.	12.16	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	16.16
8.	13.24	Cyclopropane tetradecanoic acid, 2-octyl-, methyl ester	$C_{26}H_{50}O_2$	394	0.85
9.	14.16	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280	15.49
10.	18.51	4-(4-Methyl-2-biphenylyloxy) phthalonitrile	$C_{21}H_{14}N_2O$	310	1.40
11.	21.76	9,12,15-Octadecatrienoic acid, 2,3-bis (acetyloxy) propyl ester, (Z, Z, Z)-	$C_{25}H_{40}O_6$	436	0.47
12.	22.81	Squalene	$C_{30}H_{50}$	410	2.29
13.	28.83	Cholestan-3-ol, 2-methylene-, (3á,5à)-	$C_{28}H_{48}O$	400	3.55
14.	30.26	Vitamin A aldehyde	$C_{20}H_{28}O$	284	2.80
15.	31.14	1-Heptatriacotanol	$C_{37}H_{76}O$	536	11.36
16.	31.85	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	$C_{31}H_{48}O_3$	468	11.72

RT: Retention time, MW: Molecular weight, GC–MS: Gas chromatography–mass spectrometry

et al. [46] examined and detected thirteen compounds from ethanolic leaf extract of *Andrographis paniculata* using GC-MS technique, and percentage of peak area was very low in some compounds such as n-Hexadecanoic acid (4.44), 9,12-Octadecadienoic acid (Z,Z)- (0.86), and squalene (0.87).

By comparing with the literatures stated above, the present work that means the ethanolic flower extract of *T. divaricata* (L.) exhibits sixteen different phytochemical compounds. Hence, it can be used for pharmacological activities.

CONCLUSION

The current investigation concludes that the flower has engendered numerous phytochemical compounds with natal action. Because of this reason, it can be used for the improvement of novel drugs to treat several disorders. The elucidation of specific compound is responsible for treating particular ailment which will be helpful for pharmaceutical industries to synthesize those medications in the future.

AUTHOR CONTRIBUTION

Author contributed to the paper.

CONFLICTS OF INTEREST

No conflicts of interest.

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