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CHEMICAL CHARATERISATION OF GCMS ANALYSIS OF *TABERNAEMONTANA DIVARICATA*

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ABSTRACT
Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value. The present study was carried out to identify the phytochemical components of the *Tabernaemontana divaricata* using ethanol, chloroform and water extract. The phytochemical screening showed the presence of alkaloids, tannins, steroids, flavonoids, saponins, tannins and phenolics. In GC MS, the mass spectrum of the unknown component was compared and interpreted with the spectrum of the known components stored in the National Institute Standard and Technology (NIST) library. The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners.

KEYWORDS: *Tabernaemontana divaricata*, phytochemical, GC MS, NIST library.

INTRODUCTION

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects**.**

Collection of Plant Material

The healthy plant samples of *Tabernaemontana divaricata* was collected from Trichy. The collected plant materials were transported to the laboratory. The plant materials were identified and authenticated at Department of Botony, St. Joseph's college, Trichy.

Preparation of Leaf Powder

The leaves of *Tabernaemontana divaricata* was collected, washed and cut into small pieces and dried at room temperature for two weeks and made in to powder for further analysis.

Extraction of Plant Material

Anand, *et al*., (2012) Aqueous, chloroform and alcoholic extracts were prepared according to the methodology of Indian Pharmacopoeia. The shady dried plants materials were subjected to pulverization to get coarse powder. The coarse powder material was subjected to soxhlet extraction separately and successively with alcohol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{0}C). The aqueous and alcohol extracts put in air tight containers stored in a refrigerator.

Phytochemical screening

Evan *et al*., (2002), The *Tabernaemontana divaricata* was tested for steroids, alkaloids, sugar, phenolic compounds, flavonoids, saponins, tannins, anthraquinone and amino acids. Phytochemical screening of the extract was carried out according to the standard method.

GCMS analysis

Merlin *et al*., (2009), The GCMS analysis of ethanolic crude extract of *Tabernaemontana divaricata* was performed using a GCMS equipment Thermo GC-TRACE ultra ver: 5.0, Thermo MS DSQ II. Experimental conditions of GCMS system were as

follows: TR 5-MS capillary standard non-polar column, dimension: 30 m, ID: 0.25 mm, Film: 0.25 mm was used and flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature program (oven temperature) was 40^0C raised to 250^0C at 5^0C/min and injection volume was 1mL. Samples which dissolved in chloroform were run fully at a range of 50e 650m/z and the results were compared by using Wiley Spectral library search program. The mass spectra detected in 36 min.

RESULTS AND DISCUSSION

Table-1: Preliminary Phytochemical Screening Of *Tabernaemontana Divaricata.*

S.NO	TEST	WATER	CHLOROFORM	ETHANOL
1	Alkaloids	+	-	_
2	Anthaquinone	-	+	-
3	Coumarin	+	+	+
4	Flavonoids	-	+	+
5	Glycosides	-	-	+
6	Phenols	+	+	+
7	Saponin	+	+	-
8	Steroids	-	+	-
9	Tannins	+	+	+
10	Terpenoids	-	+	-

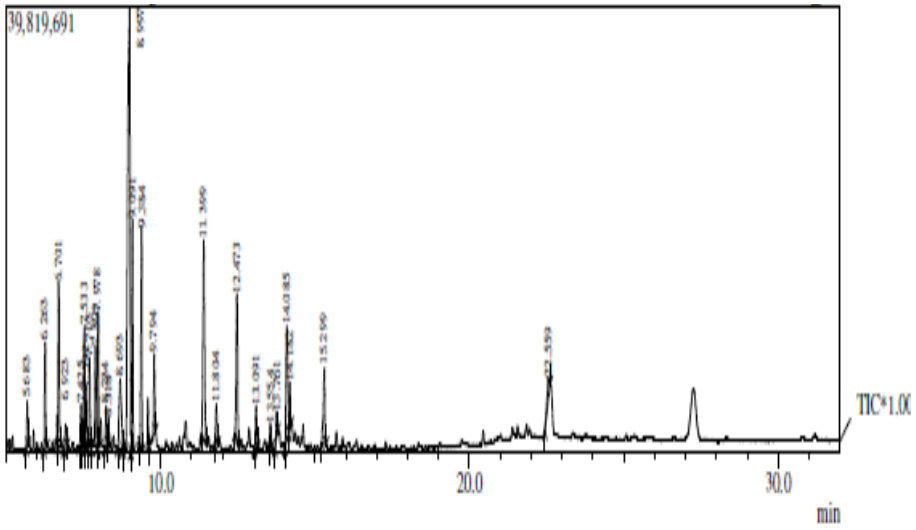
Phytochemical Analysis

The preliminary phytochemical analysis was carried out in the *Tabernaemontana divaricata*.The phytochemical analysis was carried out in the three different extract (Table I). The qualitative analysis of the ethanolic, chloroform and water extracts *Tabernaemontana divaricata* revealed the presence of alkaloid, flavanoid, terpenoid, steroid, tannin, and phenolic compounds, whereas cardiac glycosides were absent. The Chloroform extract of *Tabernaemontana divaricata* contain more compound than compared to other solvent. Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value. The development of microbial resistance to antibiotics has led the researches to investigate the alternative sources for the treatment of resistant strains.

The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds **(Hill, 1952**). Many of these indigenous medicinal plants are used as spices. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes **(Okwu, 1999, 2001).**

According to **Middleton and McLaughlin, (1992),** the flavonoids have long been recognized to possess antiallergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic activities as well as to affect some aspects of mammalian metabolism. Farwuar (1996) included protection against free radicals, platelet aggregation, microbes, ulcers and hepatoxins. The phytochemical analysis revealed the presence of flavonoids in the herbal plants.

GCMS ANALYSIS OF Tabernaemontana divaricata



GCMS Analysis of *Tabernaemontana divaricata*

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area%	Height%	A/H	Mark	Name
1	5.683	5.650	5.708	1.11	1.65	2.15		D-Limonene $$ Cyclohexene, 1-methyl-4-(1-r
2	6.263	6.217	6.308	2.53	3.85	2.10		4-Nonanone $$ Propyl amyl ketone $$ Nonan
3	6.701	6.667	6.783	4.54	5.97	2.43	V	1,6-Octadien-3-ol, 3,7-dimethyl- $$ .beta.-Lin
4	6.923	6.867	6.975	0.81	0.87	2.98	MI	Carane, 4,5-epoxy-, trans
5	7.425	7.375	7.467	1.28	1.54	2.65		Neogermylidenecyclohexane $$ (2,2-Dimethyl
6	7.533	7.467	7.567	3.26	4.41	2.35	V	Citronellal $$ 6-Octenal, 3,7-dimethyl- $$ .bet
7	7.592	7.567	7.633	1.34	2.16	1.98	V	trans-Chrysanthemal
8	7.703	7.642	7.750	2.40	3.29	2.32		Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-,
9	7.893	7.750	7.942	3.48	3.75	2.96	V	TRICYCLO[4.3.1.1(3,8)]UNDECAN-1-OL
10	7.978	7.942	8.025	3.63	4.92	2.35	V	5,8,8-TRIMETHYL-3-OXATRICYCLO[5.1.(
11	8.234	8.183	8.283	1.12	1.44	2.46	MI	3-CYCLOHEXENE-1-METHANOL, .ALPH
12	8.318	8.292	8.358	0.56	1.01	1.79	MI	DECANAL $$ CAPRALDEHYDE $$ 1-DEC
13	8.693	8.617	8.783	4.12	2.58	5.11		Citronellol $$ 6-Octen-1-ol, 3,7-dimethyl- $$
14	8.997	8.783	9.042	27.68	16.27	5.43	V	2,6-Octadienal, 3,7-dimethyl-, (Z)- $$ .beta.-C
15	9.091	9.042	9.125	6.16	8.50	2.31	V	Geraniol $$ 2,6-Octadien-1-ol, 3,7-dimethyl-,
16	9.384	9.325	9.417	5.64	8.13	2.21		2,6-OCTADIENAL, 3,7-DIMETHYL-
17	9.794	9.625	9.833	2.18	2.93	2.38	V	BICYCLO[2.2.1]HEPTAN-2-OL, 1,7,7-TRIM
18	11.399	11.325	11.500	8.37	7.29	3.66		Geranyl acetate $$ 2,6-Octadien-1-ol, 3,7-dim
19	11.804	11.750	11.858	1.39	1.44	3.09	MI	CYCLOHEXANE, 1-ETHENYL-1-METHYL
20	12.473	12.408	12.533	5.35	5.40	3.16		Caryophyllene
21	13.091	13.033	13.142	1.32	1.58	2.67	MI	1,4,8-CYCLOUNDECATRIENE, 2,6,6,9-TE
22	13.554	13.517	13.600	0.60	0.80	2.42	MI	1,6-CYCLODECADIENE, 1-METHYL-5-MI
23	13.761	13.692	13.792	0.63	0.92	2.11	MI	cubebol
24	14.085	14.025	14.150	5.04	4.16	3.87		Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-m
25	14.182	14.150	14.225	1.25	1.69	2.36	V	NAPHTHALENE, 1,2,3,5,6,8A-HEXAHYDR
26	15.299	15.217	15.358	2.90	2.76	3.35		Caryophyllene oxide
27	22.359	22.433	22.608	1.28	0.70	5.07	MI	HEXATRIACONTANE $$ AI3-52389 $$ EI
				100.00	100.00			

The analysis and extraction of plant material play an important role in the development, modernization and quality control of herbal formulations. Hence the present study was aimed to find out the bioactive compounds present in the chloroform extract of *Tabernaemontana divaricata* by using Gas chromatography and Mass spectroscopy. GC-MS chromatogram of the chloroform extract of *Tabernaemontana divaricata* showed 27 peaks indicating the presence of 27 phytochemical constituents. All the constituents were characterized and identified by comparison of the mass spectra of the constituents with the NIST library. The active compounds with their peak number, concentration (peak area %), and retention time (RT) are presented in Table 1. The more precise information in qualitative analysis can be obtained by gas-chromatography coupled with mass spectrometry (GC-MS) **(Cong *et al.*, 2007).** For quantitative determination, gas-chromatography with flame ionization detector (GC-FID) and GC-MS are preferred **(Lee *et al.*, 2005).**

GCMS analysis revealed that - limonene, Nonanone, 1,6-Octadien-3-ol, 3,7-dimethyl- Carane, Citronellal trans-Chrysanthemal, Bicyclol and geranial etc.

The identified compounds possess many biological properties. For instance, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- Linolenic acid (R/T 20.06) possesses anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties. N-Hexadecanoic acid - palmitic acid (R/T 17.25) can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors. Phytol- Diterpene (R/T 19.67) is an antimicrobial, anticancer, anti-inflammatory and diuretic agent **(Praveen kumar *et al.*, 2010).** 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-, n-Hexadecanoic acid, 1, 2-Benzenedicarboxylic acid and di-isooctyl ester were present in *Caesalpinia sappan* ethanol extract **(Sarumathy *et al.*, 2011).** Similar types of compounds were identified among the seventeen compounds of this present study.

Hexadenoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata* **(Grace *et al.*, 2002)** and *Melissa officinalis* **(Sharafzadeh *et al.*, 2011).** (**Parasuraman *et al.*, (2009))** identified 17 compounds with n-Hexadecanoic acid and Octadecanoic acid as the major compounds in the leaves of *Cleistanthus collinus.* GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-Hexadecanoic acid N-hexadecanoic acid, Hexadecanoic acid, Phytol, 9, 12 - Octadecadienoic acid, 9, 12, 15-Octadecatrienoic acid and Squalene were identified in the ethanol leaf extract of *Aloe vera* **(Arunkumar and Muthuselvam, 2009)** and *Vitex negundo* **(Praveen kumar *et al.*, 2010).** Squalene is used in cosmetics as a natural moisturizer**. Devi *et al.* (2009)** reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and 9, 12-Octadecadienoic acid. These reports are in accordance with the result of this study.

SUMMARY AND CONCLUSION

The preliminary phytochemical analysis was carried out in the extracts of *Tabernaemontana divaricata*. The extracts showed an indication of the presence of alkaloids, reducing sugar, coumarin, tannin and, phenolic compound.The bioactive compounds were identified in the GC-MS analysis.

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