ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 2, 2015

ISSN - 0974-2441

Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRY DETERMINATION OF BIOACTIVE COMPONENTS OF THREE TRAITS OF VILVAM (BAEL)

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Received: 19 January 2015, Revised and Accepted: 03 February 2015

ABSTRACT

Vilvam is an important medicinal plant, which is used to treat many ailments of human beings in the traditional system of medicine. As one of the important conservation aspect of traditional medicinal plant, it is mostly grown in the Hindu Siva temples, where the leaves are mostly used for pooja purpose and medicinal aspects. Based on the morphological features of the leaves, three different traits of plants were identified and selected for the study. This study aimed to screen the phytochemical compound present in the three traits of Vilvam by gas chromatography-mass spectrometry (GC-MS) method. The GC-MS analysis revealed that the phytochemical components present in the trait 1 is different from the other two traits. The trait 2 and 3 are having almost similar components. Among them, trait 3 has more number of bioactive compounds, which can be exploited for the medicinal use in the future.

Keywords: Gas chromatography-mass spectrometry, Medicinal plant, Ethnobotany, Vilvam.

INTRODUCTION

Medicinal plants have been used by the human being since ages in the traditional system of medicine due to their therapeutic potential and the search on medicinal plants have led to the discovery of novel drug candidates used against diverse diseases. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [1]. Natural products from microbial sources have been the primary source of antibiotics. However with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant. Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemotherapeutants, these are non-phytotoxic, more systemic and easily biodegradable [2-4]. Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties [5]. A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies [6]. Hence, a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action. Mass spectrometry, coupled with chromatographic separations such as gas chromatography (GC-MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oil, fatty acids, lipids [7] and alkaloids [8].

GC is one of the most widely used techniques. It was first described by James and Martin in 1952 and has become one of the most important tools for the separation of volatile compounds. GC has gained widespread acceptance in numerous application areas, such as process control in chemical plants, quality control in the food industry, monitoring sample composition in the oil-industry, environmental and bio medical sciences. These are just a few examples in which GC has been applied. The combination of speed, sensitivity and high resolving power in GC provides an adequate technique for the separation of complex samples. Moreover, the coupling to spectrometric methods such as MS for direct identification of unknown compounds is easy to establish. Therefore, the purpose of this study was to determine the bioactive compounds and volatile compounds in Vilvam (Bael), a traditional medicinal plant. *Aegle marmelos* is one of the most important medicinal plants used to cure most of the common ailments of human beings. *A. marmelos* Linn. is commonly called as Bael in Hindi, Vilvam in Tamil and Bilva in Sanskrit. It belongs to the family Rutaceae. It is indigenous to India and is used in folk medicines. The Ayurvedic practitioners use almost all of their parts, but the greatest medicinal value ascribed to its fruits and leaves [9,10]. Vilvam is a perennial tree, wild in the sub Himalaya tract, Central, and South India. *A. marmelos* is a medium sized armed deciduous tree grows up to a height of 9-10 m with straight, sharp, axillary thorns and yellowish brown shallowly furrowed corky bark. The leaves are trifoliate alternate, leaflets are ovate to lanceolate with pellucid - punctuate aromatic oil glands [11]. The lateral leaves are sub sessile, and the terminal one is long petioled. The flowers are greenish white sweet scented present in the axillary panicles. The fruits are globus woody berry with yellowish ring, seeds are numerous embedded in orange brown sweet gummy pulp. The leaves are used as astringent, laxative, febrifuge and expectorant. The leaves are useful in opthalamia, inflammations, catarrh, diabetic and asthmatic complaints [12]. The leaves are used for the heart and brain disorders. The confection called "ilakam" is made of fruit is used to treat tuberculosis and loss of appetite [5]. Stress is produced during normal metabolic process in the body as well as induced by a variety of environmental and chemical factors, which cause generation of various reactive free radicals and subsequent damage to macromolecules like DNA, proteins and lipids could be cured by Vilvam. No specific scientific evaluation of antioxidant activity of *A. marmelos* fruit pulp has been reported so

far. Therefore, it was thought worthwhile to evaluate antioxidant activity of *A. marmelos* fruit pulp to confirm its folk medicinal claim [13]. Many naturally occurring products have been reported to contain a large amount of antioxidant compounds other than vitamin C, E and carotenoid [14]. These antioxidants play a vital role in delaying, intercepting or preventing oxidative reactions catalyzed by free radical. Antioxidant activity of medicinal plants might be due to the presence of phenolic compounds such as flavonoids, phenolic acids and phenolic diterpine [15]. Synthetic antioxidants such as butylatedhydroxy anisole butylatedhydroxy toluene, tertiary butylated hydroxyl quinone, and gallic acid esters have been suspected to be carcinogenic. Hence, strong limitations have been placed on their use and there is a trend to replace them with naturally occurring antioxidants [16]. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity [17]. Hence, search for natural antioxidant has greatly been increased in the recent scenario. The *A. marmelos* contains more of natural antioxidant in the leaves [13] and the fruit pulp. Because of this specific property the Vilva is used in the treatment and preventive of all common ailment of mankind [18,19].

METHODS

Collection of samples

The leaves of three traits of Vilva were collected from, Agasthiyamalai Biosphere Reserve forest region of South Western Ghats, Agasthiyamalai biosphere reserve. The collected leaves were cleaned and dried under shade. The powdered leaves were stored in the refrigerator for further use.

GC-MS

The experiment was carried out in the sophisticated analytical instrumentation facility, IIT, Chennai, India. JEOL GCMATE II GC-MS (Agilent Technologies 6890N Network GC system for GC). The column (HP5) was fused silica 50 m × 0.25 mm I.D. Analysis conditions were 20 minutes at 100°C, 3 minutes at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 µl) was evaporated in a split less injector at 300°C. Run time was 22 minutes. The components were identified by GC coupled with MS.

Identification of components

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

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters, etc. The identification of phytochemical compounds was based on the peak area, retention time and molecular formula. The GC-MS analysis of three traits of Vilvam showed the presences of 5, 9 and 11 bioactive compounds in the trait 1, trait 2, and trait 3, respectively. 11 compounds in the trait 3 reveal that these are the bioactive compounds (phytochemical constituents) that could contribute the medicinal properties of the plant.

The GC-MS studies confirm the presence of five major components in the trait 1, the retention time, the name of the compound, frequency molecular weight and molecular formula are given in the Table 1. The GC-MS spectrum was given in Fig. 1a, and the possible structure was given in Fig. 1b. Out of the five major components, two components are present in large quantity, and other three was present in small quantity. Among the structure prediction, three components are aromatic ring compounds, and two are aliphatic chain compounds.

There are nine major components in the trait 2, the retention time, the name of the compound, frequency; molecular weight and molecular formula were given in the Table 2. The GC-MS spectrum was given in Fig. 2a and the possible structure was given in Fig. 2b. Out of the nine components two major components are present in large quantity, and other seven were present in less quantity. Among the structure

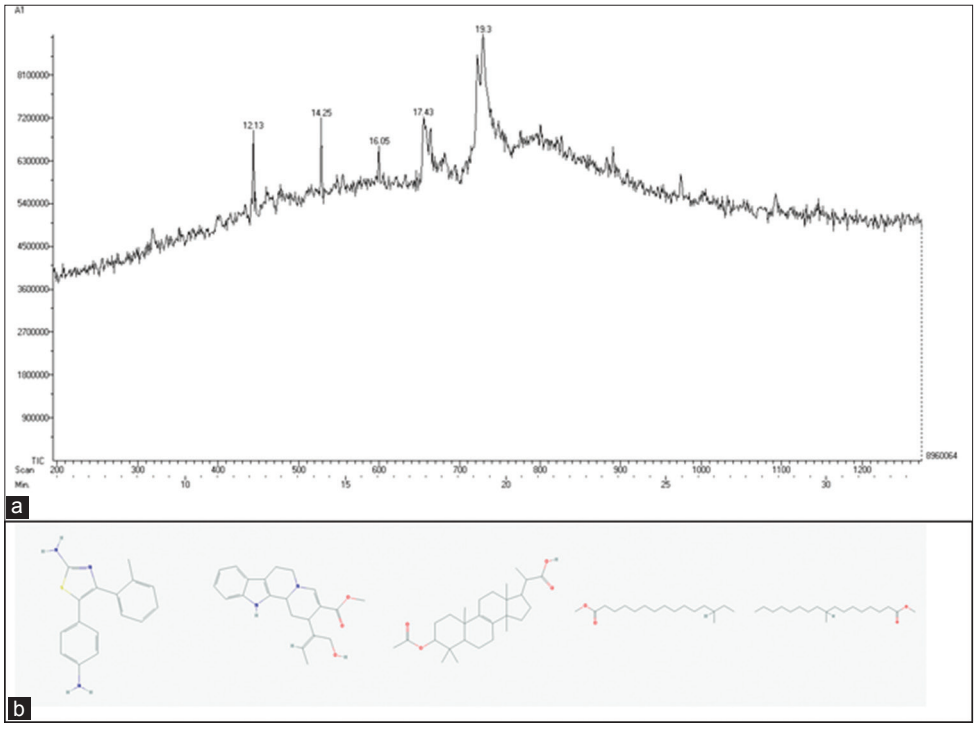

Fig. 1: (a) Gas chromatography-mass spectrometry spectrum of trait 1 Vilvam, (b) the structure of bioactive compounds present in trait 1 Vilvam

Table 1: Phytocomponents detected by GC-MS of trait 1 Vilvam

S.No	RT	Name of the compound	Frequency	Molecular weight g/mol	Molecular formula
1	12.13	5-(p-aminophenyl)-4-(o-tolyl)-2-thiazolamine	444	281.3754	C16H15N3S
2	14.25	18,19-Seco-15a-yohimban-19-oic acid, 20,21-didehydro-16a-(hydroxymethyl)-, methyl ester	528	352.42686	C21H24N2O3
3	16.06	Propanoic acid, 2-(3-acetoxy-4,4, f 4-tri methyl androst-8-en-17-yl)-	600	430.61998	C27H42O4
4	17.43	Pentadecanoic acid, 13-methyl-, methyl ester	655	270.45066	C17H34O2
5	19.3	Heptadecanoic acid, 9-methyl-, methyl ester	729	298.50382	C19H38O2

GC-MS: Gas chromatography-mass spectrometry

Table 2: Phytocomponents detected by GC-MS of trait 2 Vilvam

S.No	RT	Name of the compound	Frequency	Molecular weight	Molecular formula
1	8.12	2-(2-Methyl-propenyl)-cyclohexylamine	284	153.265 Da	C10H19N
2	12.12	Benzo[b] thiophene, 5-methyl-	443	148.22482 g/mol	C9H8S
3	14.22	Pyrimidine-5-carboxylic acid, 1,4-dihyo-4-(2furyl)-1,6-dimethyl-2-methylthio-, ethyl ester	527	294.36932 g/mol	C14H18N2O3S
4	16.03	9-lsopropyl-1-methyl-2-methylene-5-oxatricyclo[5.4.0.0[3,8]) undecane	599	220.35046 g/mol	C15H24O
5	17.38	Pentadecanoic acid, 13·methyl-, methyl ester	653	270.45066 g/mol	C17H34O2
6	17.63	Acetamide, N-(acetyloxy)-N-9H-fluoren-2-yl-	663	281.3059 g/mol	C17H15NO3
7	19.12	16-octadecenoic acid, methyl ester	722	296.48794 g/mol	C19H36O2
8	19.27	Heptadecanoic acid, 9-methyl-, methyl ester	728	298.50382 g/mol	C19H38O2
9	20.45	2,7- Dihenyl-1,6-dioxopyridazino[4,5:2'3']pyrrolo[4',5'-d] pyridazine	775	355.34952 g/mol	C20H13N5O2

GC-MS: Gas chromatography-mass spectrometry

Table 3: Phytocomponents detected by GC-MS of trait 3 Vilvam

S.No	RT	Name of the compound	Frequency	Molecular weight	Molecular formula
1	1.97	o-Xylene	39	106.165	C8H10
2	10.7	1-Acetyl-6,8-dimethoxy·5-nitro-1,2,3,4-tetrahydroquinoline	387	280.27654	C13H16N2O5
3	11.93	1H-Pyrrolo[2,3-c] pyridine-3-propanoic acid, 5[4H]-oxo-6,7-dihydro-, methyl ester	436	222.24046	C11H14N2O3
4	12.82	Pentadecanoic acid. 14-methyl-, methyl ester	471	256.42	C16H32O2
5	14.02	16-octadecenoic acid, methyl ester	519	296.48794	C19H36O2
6	14.12	Heptadecanoic acid, 16-methyl-, methyl ester	523	298.50382	C19H38O2
7	15	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	558	430.61998	C27H42O4
8	16.13	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	603	430.61998	C27H42O4
9	17.62	1h-Pyrrole-3,4-diacetic acid, 2-acetoxymethyl-5-methoxycarbonyl-, dimethyl ester	662	341.31326	C15H19NO8
10	19.65	Acetic acid, 17-(1,5-dimethylhexyl)-4,4,10,13,17-pentamethyl-2,3,4.5,6,7,10,11,12,13,16,1 7-dodecahydro-1H-cyclopenta[a] phenanthrene-3-0l (ester)	743	430.61998	C27H42O4
11	22.13	Lanost-9 (11)-en-18-oic acid, 3,20,23-trihydroxy-,(3a',20 .xi.)	858	472.69972	C30H48O4

GC-MS: Gas chromatography-mass spectrometry

prediction six components are aromatic ring compounds, and three are aliphatic chain compounds.

There are 11 major components were present in the trait 3, the retention time, the name of the compound, frequency molecular weight, and molecular formula were given in Table 3. The GC-MS spectrum was given in Fig. 3a, and the possible structure was given in Fig. 3b. Out of the 11 major components two components are present in large quantity, and other nine was present in small quantity. Among the structure prediction eight components are aromatic ring compounds and three are aliphatic compounds.

DISCUSSION

The more precise information in qualitative analysis can be obtained by GC couples with MS. For the quantitative determination, GC and GC-MS are preferred. Based on these results of the 3 traits of Vilvam, the traits 3 have more the medicinal properties. Due to the presences of 11 phytochemical components. The GC-MS analysis of Vilvam the presences of 11 phytochemical components. The identified compounds may possess many biological properties. The trait 2 and 3 has close similarity and trait 1 differs from the trait 2 to 3. The previous antioxidant studies on the three trait of Vilvam [13], the antimicrobial studies on the three traits of Vilvam [20] and the FTIR studies on the three traits of Vilvam [21] showed the trait 3 possess the maximum activity in all the three experimental studies. The trait 3 is the traditional type of plant which is more commonly found in the temple are and the forest area. A survey was made in 24 locations in south Tamil Nadu and Kerala where the trait 3 type of plant was located in all 24 places. Among the 24 locations only 5 localities the trait 1 and trait 2 was located in two places. The GC-MS studies also confirms that the trait 3 is having more number of bioactive compounds and it is the traditional type of Vilvam commonly found in natural forest and Sthala Virusha, which could be exploited for the future use. The morphological variation in the bale may be genetical than geographical condition. Further studies on genomic aspects and germplasam evaluation are necessary to conform the genetical variation.

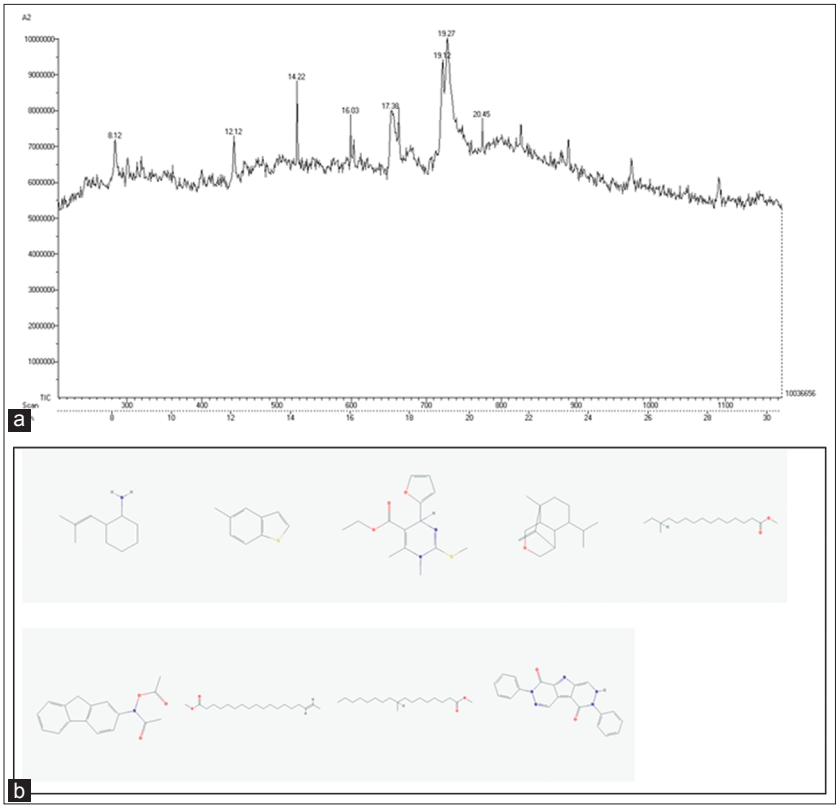

Fig. 2: (a) Gas chromatography-mass spectrometry spectrum of trait 2 Vilvam, (b) the structure of bioactive compounds present in trait 1 Vilvam

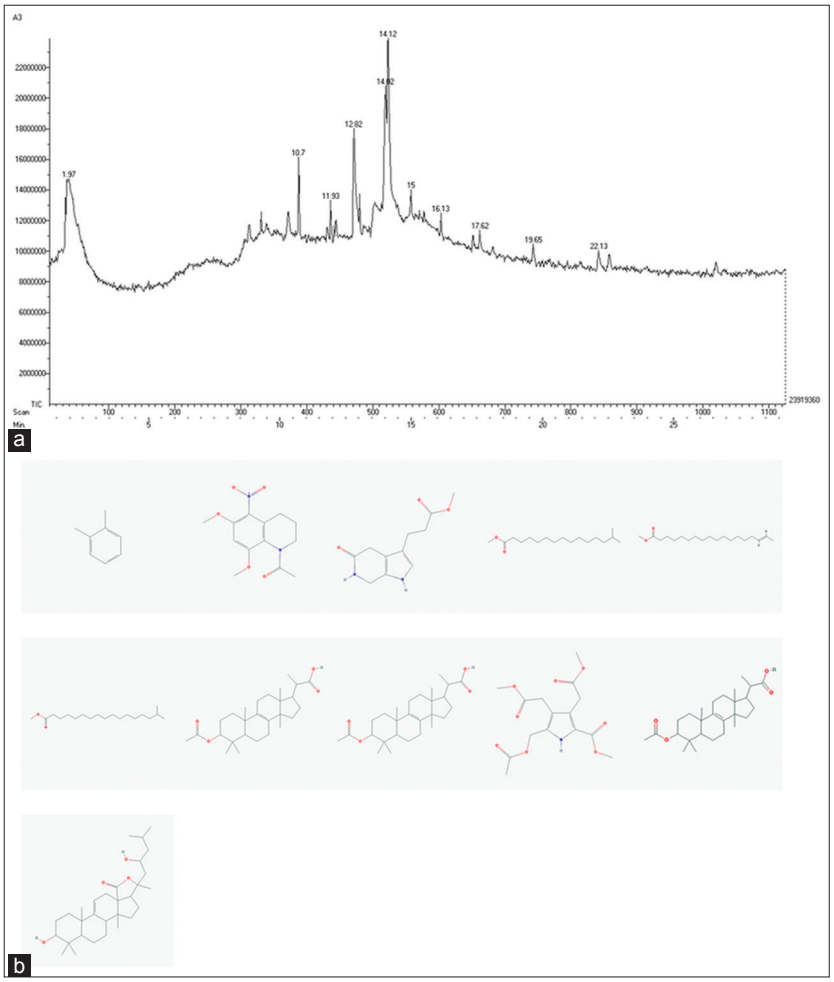

Fig. 3: (a) Gas chromatography-mass spectrometry spectrum of trait 2 Vilvam, (b) the structure of bioactive compounds present in trait 3 Vilvam

CONCLUSION

Therefore, GC-MS method is a direct and fast analytical approach for identification of terpinoids and steroids, flavonoids, tannin, etc. For the GC-MS analysis only a few grams of plant material is required. The importance of the study is to detect the bioactive components and to screen the biological activity of some of these compounds. Further the pharmacological activity of the bioactive components should be evaluated. The present study concluded that the maximum presences of phytochemicals were observed in trait 3 of Vilvam, when compared with trait 2 and 1. The trait 1 possesses very less amount of phytochemical component when compared with trait 2. Only one phytochemical component present in trait 1 is also found in trait 2 and 3. The other four components are unique. But both in trait 2 and trait 3, nine components are common. The presence of more number of phytochemicals components in the trait 3 of Vilvam is valuable and highly useful for the treatment of various human ailments. The identified phytocomponents needs further research on toxicological aspects to develop safe drug.

ACKNOWLEDGMENT

The authors are thankful to the Department of Science and Technology Govt. of India (DST/TSG/AF/2012/01 dated 31 December 2012) for financial assistance to carry out this investigation. The authors are indebted to the chancellor, vice chancellor and the management, Noorul Islam University, Kumaracoil for their valuable guidance, constant encouragement and providing basic facilities throughout the study.

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