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Analysis of Phytochemical constituents of the chloroform extracts of *Abutilon hirtum* (Lam.) Sweet using GC-MS Method

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Abstract

Abutilon hirtum (Lam.) Sweet (Malvaceae) commonly known as *Vadathuthi*. It is used as one of the most important drugs in traditional system of medicine to treat various ailments. The plant is used for to its various properties as demulcent, diuretics, anti-diabetics, anthelmintic, laxative, wound healing properties, antibacterial and antifungal properties. The present study revealed the presence of phytochemicals like *diethyl phthalate* (19.171%), *benzaldehyde 4-propyl* (5.219%), *methoxyacetic acid 3-tridecyl ester* (5.196%), *sulfurous acid dodecyl 2-propyl ester* (0.455%), *sulfurous acid, butyl dodecyl ester* (0.442%) *etc*., from the chloroform extracts of leaves in *A. hirtum*. In the present study an attempt was made to investigate the phytochemical constituents present in the chloroform extracts of leaves of *A. hirtum* in the preliminary level by using Gas Chromatography coupled with Mass Spectrometry (GC-MS). The study will provide information for the correct identification of the crude drug.
Keywords: *Abutilon hirtum*, GC-MS, *Vadathuthi*, *Diethyl Phthalate*, *Benzaldehyde 4-propyl*

1. Introduction

Medicinal plants have been used by human beings since ages in traditional 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].

Plant based drugs can be used directly, *i.e.*, they may be collected, dried and used as therapeutic agents (crude drug), or their constituents / active principles are separated by various chemical processes, which are employed as medicines. The active principle or compounds with similar structure and activity are manufactured chemically to produce the synthetic drugs used in allopathic or modern system of medicine [2].

The genus Abutilon belonging to the Malvaceae family, comprises about 100-150 species and is distributed in the tropics and subtropics. A small shrub of the *Abutilon hirtum* (Lam.) Sweet. (*vadathuthi*) [Synonym: *Abutilon graveolens* (Roxb. Ex Hornem.) Wight & Arn.] a perennial herb. It is commonly called as Belabenda, Indian mallow, and Florida Keys etc. In Malaysia, A. hirtum is used as a poultice to ease the pain of kidney gravel and often mixed with glutinous rice and applied to ulcers. The leaves or flowers are applied to abscesses [3].The decoction of the leaves is used as mouth wash and to cure bladder inflammations, wounds and ulcers [4-6].

Since alkaloids are reported from the roots of the plant [7,8]. The leaf aqueous extract of A. hirtum possess hepatoprotective activity [9]. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs [10]. Therefore, proper scientific knowledge is required to investigate and explore the exact standardization of such medicinally important plant. Mass spectrophotometry 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 [11]. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [12].

The present investigation was carried out to determine the possible chemical components from the chloroform extracts of leaves in *A. hirtum* by GC-MS.

2. Materials and Methods

2.1 Collection of Plant Materials

The flowering plants of *A. hirtum* (Lam.) Sweet. was collected from location Tiruchirappalli, Tamilnadu situated in the southern region of India during the month of May 2014. It was identified and authenticated by Botanical Survey of India (BSI), Coimbatore. Authentication number: BSI/SC/5/23/07-08/Tech.-1404 (Figure 1).

Figure 1: Abutilon hirtum (Lam.) Sweet.:

2.2 Preparation of the Extracts

The plant material (leaves) was soaked in chloroform solvents for 24 hrs. at room temperature, chopped into small pieces, ground into crude extracts and was placed into the extractor of a centrifuge. The extraction was carried out using chloroform solvent. At the end of the extraction the solvent were concentrated by evaporation. The obtained extracts were stored in a refrigerator at 4^{o}C for further analysis.

Chloroform is an excellent solvent for the separation of many organic compounds. It used mainly on the context of Liquid-Liquid extraction and the separation of homogenous mixture (miscible). Particularly it is used to dissolve Phthalic acid, Benzyl compounds and Lipids. Since the plant material contain Phthalate ester and benzaldehyde compounds, it was used. The work also carried out using ethanolic extract [13].

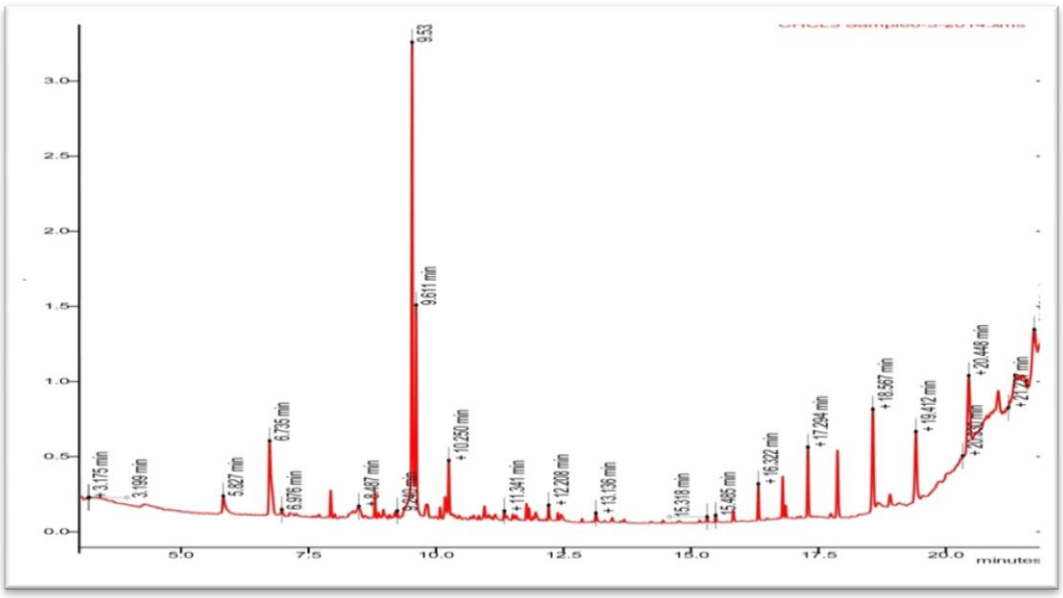
2.3 GC- MS Analysis

GC-MS analysis of chloroform extract of *A. hirtum* was performed in an Agilent-7890 A GC instrument coupled with MS-5975 inert MSD and triple axis mass selective ion detector. The DB-5MS column with dimensions of 30m x 0.2mm capillary column was used for the analysis. The initial temperature was kept at 150 ℃ and the maximum of 300 ° C. One 1µl of sample was injected with split mode (10:1). Helium gas used as a carrier gas at flow rate of 0.8 ml/min and the total run time was 22 minutes. Identification of phytochemical components was conducted using the database of National Institute Standard and Technology MS library (NIST- MS library).

3. Results

The bioactive compounds present in the chloroform extract of *A. hirtum* leaves using Gas chromatography coupled with Mass spectroscopy (GC-MS) reports are given in **Figure 2**.

Figure 2: Chloroform GC-MS Chromatogram:



The leaves sample 12 compounds were identified the highest percentage content of peak area of 19.171 (*Diethyl Phthalate* with R_t 9.546 min), followed by the peak area of 5.219 (*Benzaldehyde, 4-propyl* with R_t 6.737 min), and the lowest percentage content of peak area of 0.442 (*Sulfurous acid, butyl dodecyl ester* with R_t 16.800 min). Phytocompounds with their retention time (RT), molecular formula and molecular weight (MW) in the leaf extract are presented in **Table 1**. The individual fragmentations of the components are illustrated in **Figure 3**.

Figure 3: Phyto-Chemical components in chloroform extract of A. hirtum (Lam.) Sweet

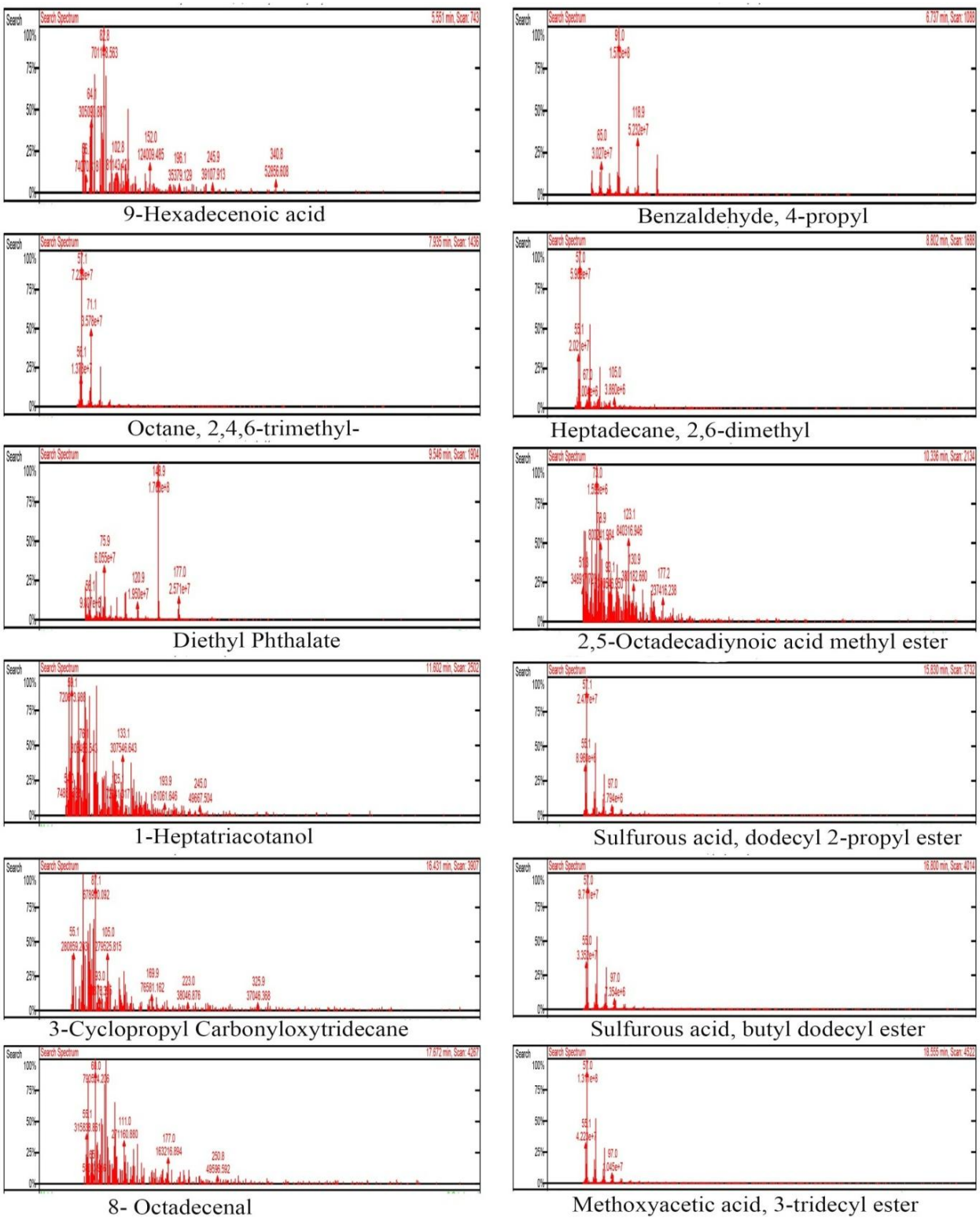


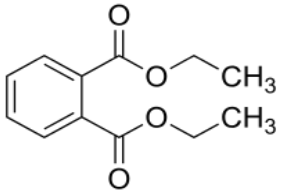
Table 1: Phyto- chemical components in chloroform extract of *A. hirtum* (Lam.) Sweet.

S.No	Rt	Name of the compound	Molecular formula	Mw	Peak area (%)
1	5.551	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	1.523
2	6.737	Benzaldehyde, 4-propyl	$C_{10}H_{12}0$	148	5.219
3	7.935	Octane, 2,4,6-trimethyl-	$C_{11}H_{24}$	156	0.929
4	8.802	Heptadecane, 2,6-dimethyl	$C_{19}H_{40}$	268	0.815
5	9.546	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	19.171
6	10.336	2,5-Octadecadiynoic acid methyl ester	$C_{19}H_{30}O_2$	290	2.782
7	11.602	1-Heptatriacotanol	$C_{37}H_{76}0$	536	0.563
8	15.830	Sulfurous acid, dodecyl 2-propyl ester	$C_{15}H_{32}O_3S$	292	0.455
9	16.431	3-Cyclopropyl Carbonyloxytridecane	$C_{17}H_{32}O_2$	268	1.190
10	16.800	Sulfurous acid, butyl dodecyl ester	$C_{16}H_{34}O_3S$	306	0.442
11	17.672	8- Octadecenal	$C_{18}H_{34}O$	266	2.939
12	18.555	Methoxyacetic acid, 3-tridecyl ester	$C_{16}H_{32}O_3$	272	5.196

4. Discussion

Traditional medicine also known as indigenous or folk medicine comprises medical knowledge systems that developed over generations within various societies before the era of modern medicine. Traditional medicines are prepared from a single plant or combination of more than one plant. Indian contribution to herbal market and emphasis on novel research is continuously increasing. Phytochemical constituents are responsible for medicinal activity of plant species [14].Hence, in the present study phytochemical components in chloroform extract of *A. hirtum* (Lam.) Sweet. leaf extracts the highest percentage content of peak area of 19.171 (*Diethyl Phthalate* with R_t 9.546 min) and then followed by the components like *benzaldehyde 4-propyl* (5.219%), *methoxyacetic acid 3-tridecyl ester* (5.196%), *2,5-Octadecadiynoic acid methyl ester* (2.782%), *sulfurous acid dodecyl 2-propyl ester* (0.455%), *sulfurous acid, butyl dodecyl ester* (0.442%), *etc*., these components mainly used for pharmaceutical and industrial purposes. The result was reported for the first time in *Abutilon hirtum* (Lam.) Sweet.

Molecular structure of *Diethyl phthalate* **($C_{12}H_{14}O_4$):**



Diethyl phthalate is used in nail polish as a solvent for nitrocellulose and cellulose acetate, in perfumes as a fixative and solvent, in toilet preparations as an alcohol denaturant, and in fingernail elongators as a plasticizer [15].

5. Conclusion

Diethyl phthalate components are already reported with Industrial, Pharmaceutical and Antibacterial properties [15].Hence the leaf extracts of *A. hirtum* (Lam.) Sweet. could positively use in the treatment against various ailments for the Herbal Medicine Industry. Further in future, these components can be isolated and pharmacological activity may be studied to determine the traditional use.

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