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RESEARCH ARTICLE

Phytochemical screening and GC-MS analysis of methanolic root extract of *Decalepis hamiltonii* Wight & Arn.

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Abstract:
Decalepis hamiltonii (swallow roots) belongs to Asclepiadaceae and is an endangered shrub. It is generally considered as a tubers root food mostly in the southern part of India. The medicinal value of rhizomatous tubers is yet unexplored, hence this study forms a basis for the active components present in it and further isolation of the compound. The aim of this study is to screen the phytochemicals present in the root of *Decalepis hamiltonii* and further analysis of the components present in it by GC-MS analysis. The roots were sequentially extracted based on the polarity viz., petroleum ether, chloroform, ethyl acetate and methanol. The methanolic extract showed the presence of all phyto constituents studied. The GC-MS analysis of the methanolic extract revealed the presence of ten major compounds. This study forms a basis for the biological characterization and importance of the compounds identified

Keywords: *Decalepis hamiltonii*, phytochemical screening, GC-MS analysis

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available and with less side effects. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products (1).Extraction and characterization of several active phytocompounds from these green factories have given birth to some high activity profile drugs (2). Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, flavonoids, tannins, steroids, glycosides and Saponins. Secondary metabolites from plant serve as defense mechanisms against predation by many microorganisms, insects and herbivores (3).Hence it is obligatory to screen the secondary metabolites, the key factor in therapeutics.

GC-MS is one of the best techniques to identity the bio active constituents, long chain, branched chain hydrocarbons, alcohols, acids, ester etc. Identification of pure components even at low concentration, less than 1mg in made possible by gas chromatography.

Decalepis hamiltonii Wright & Arn an endemic endangered, climbing shrub and native of southern peninsula. This plant has been used in Ayurveda, the ancient Indian traditional system of medicine to stimulate appetite, relieve flatulence and as a general tonic. It is also useful as a blood purifier, preservative and as a source of bioinsectcide for stored food grains(4, 5).Earlier studies have shown that roots contain aldehyde, inostiols, Saponins, amyrins and lupols (6, 7-8)as well as volatile compounds such as 2-hydroxy-4-methoxybenzaldehyde, vanillin, 2-phenyl ethyl alcohol benzaldehyde and others.

These chemical preservatives act as antimicrobial compounds which inhibit the growth of undesirable microorganisms. To explore the medicinal importance, the root of *Decalepis hamiltonii* was screened primary to the phytochemicals present in it and it was analysis using GC-MS.

MATERIALMETHODS

Fresh and healthy *Decalepis hamiltonii* roots were collected from Kolli hills of Namakkal district, Tamilnadu. The plant was taxonomically identified by using flora of Madras presidency. In the laboratory, the roots were washed 2-3 times with running fresh water, then air dried under shade drying was grinded with mechanical grinder, the powder was kept in small labelled plastic bags. 100g of roots of *Decalepis hamiltonii* were subjected to successive extraction with different solvents in increasing polarity *viz*. petroleum ether, chloroform, ethyl acetate and methanol using soxhlet apparatus for phytochemical screening as per the method given by (9). The solvents were evaporated under reduced pressure and stored in desiccator at 4ºC.The methanolic extracts was used for GC-MS analysis

GC-MS Analysis

Methanolic extract of *Decalepis hamiltonii* roots was analyzed with the help of GC-MS analyzer (GC Clarius 500 Perkin Elmer). On Elite-1 column the data was generated. The carrier gas helium (99.999%) was used at flow rate of 1ml per min in split mode (10: 1). 8 µl of methanolic sample was injected to column at 250ºC injector temperature. Temperature of oven starts at 80ºC and hold for 2 min and then it was raised at rate of 10ºC per min to 200ºC without holding. Holding was allowed for 9 min at 280ºC at program rate of 5ºC per min. Temperature of ion source was maintained at 200ºC. The injector temperature was set at 230ºC and detector temperature was set at 260ºC. The mass spectrum of compounds present in samples was obtained by electron ionization at 70 eV and detector operates in scan mode from 45 to 450 Da atomic mass units. A 0.5 seconds of scan interval and fragments from 45 to 450 Da was maintained. Total running time was 40 minutes.

Identification of components

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version (2005), software, Turbomas 5.2.This is done in order to determine whether this plant species contains any individual compound or group of compounds, which may substantiate its current commercial and traditional use as an herbal medicine. Further it helps to determine the most appropriate methods of extracting these compounds. These results will consequently be discussed in the light of their putative biological or therapeutic relevance.

RESULT AND DISCUSSION

Phytochemical screening

In the present study, the phytochemical screening was studied with petroleum ether, chloroform, ethyl acetate and methanol extract of the roots of *Decalepis hamiltonii*. The results revealed, Methanolic root extracts of *Decalepis hamiltonii* were rich in saponins, steroids flavonoids, phenols, terpenoids, and tannins followed by other extracts Table 1.

Photochemical constituents such as tannins, flavonoids and several other aromatic compounds or secondary metabolites of plants serve as defense mechanism against predation by many microorganisms. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins and steroids (10). The presence of Saponins, flavonoids, phenols and terpenoids in the root extract are very important and are used in analgesic, anti Plasmodic and bactericidal activities (11). Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

GC – MS analysis

The compounds present in the methanolic root extract of *Decalepis hamiltonii* were identified by GC-MS analysis presented in figure 1. The active principle, Molecular Weight (MW), Concentration (%), Molecular Formula (MF), and Retention Time (RT) is presented in Table 2. More than ten major compounds were identified in the extract being Oleic Acid (32.64%), Benzaldehyde, 2-hydroxy-4- methoxy (19.70 %),n- Hexadecanoic acid(13.37 %), Mome inositol (4.71%), Thunbergol (2.58%)glycerin (2.59 %), Pentadecanoic acid, 14-methyl-, methyl ester(1.61 %), 11-Octadecenoic acid, methyl ester (1.85),9, 12-Octadecadienoic acid, methyl ester (1.97%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (1.23%) respectively along with other minor constituents.

The identified compounds in the roots of methanolic extract of *Decalepis* hamiltonii possess many biological properties. Among the identified phytochemicals, 2 hydroxy-4-methoxybenzaldehyde a phenolic compound have the property of antioxidant and

Table 1. Preliminary Qualitative Phytochemical analysis of root extracts of *Decalepis hamiltonii* Wright & Arn

No.	Name of the Test	Root			
	Solvents	Petroleum	Chloroform	Ethyl acetate	Methanol
1	Carbohydrate	+	-	-	-
2	Saponins	+	-	-	++
3	Tannins	+	-	-	++
4	Steroids	+	+	+	++
5	Flavonoids	+	-	+	++
6	Alkaloids	+	-	-	+
7	Phenol	+	+	+	++
8	Glycosides	+	-	+	+
9	Gums	+	-	+	+
10	Terpenoids	+	+	+	++

++=Strong positive + =Positive - = Negative

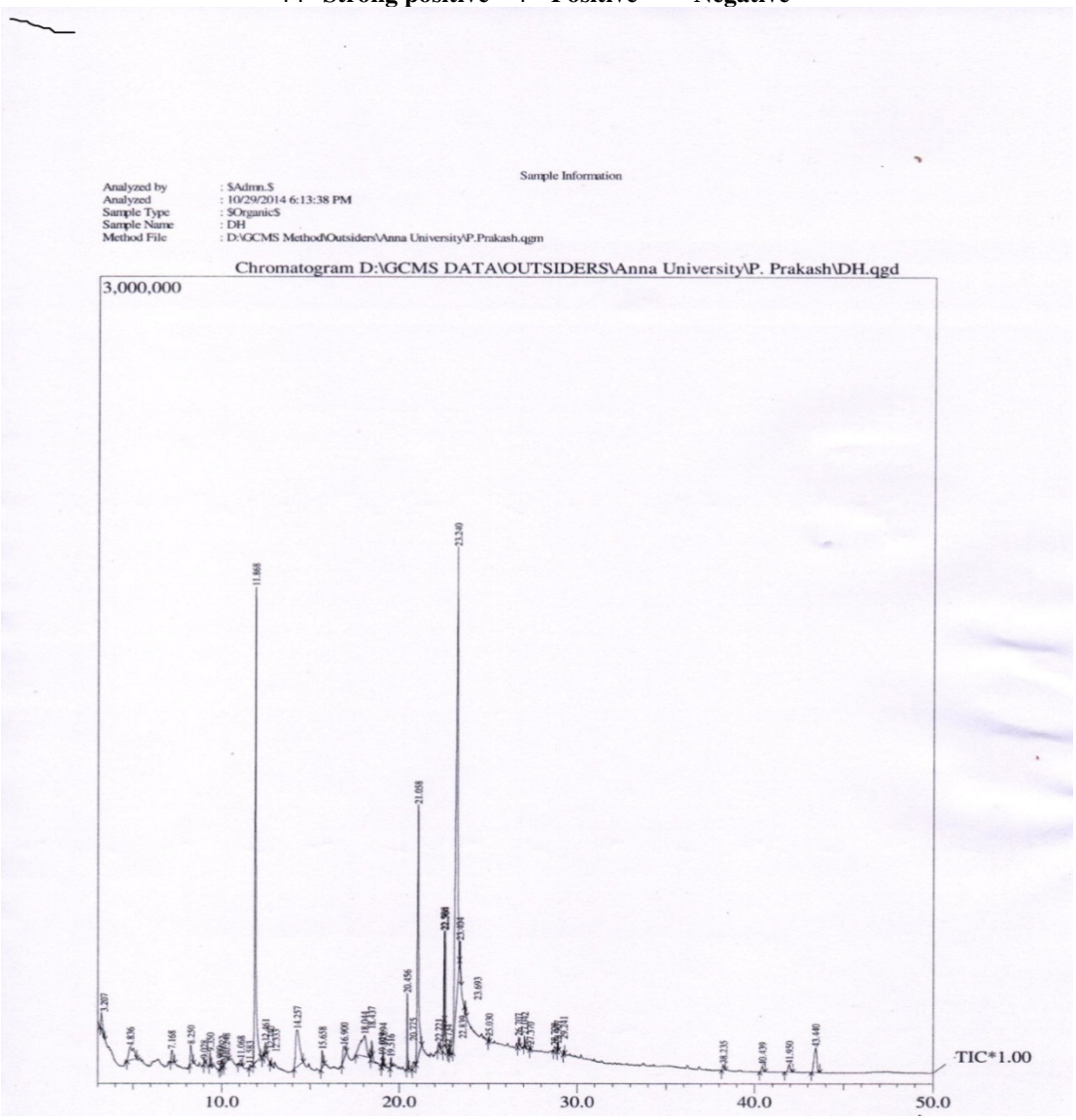

Table.2 Compounds identified in methanolic root extract of *Decalepis hamiltonii*

S.No	RT	Name of compound	Molecular formula	Molecular weight	Peak of area (%)
1	4.836	Glycerin	$C_3H_8O_3$	92.09	2.59
2	8.250	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144.12	1.23
3	11.868	**Benzaldehyde, 2-hydroxy-4- methoxy**	$C_8H_8O_3$	152.1473	19.70
4	18.044	**Mome inositol**	$C_6H_{12}O_6$	180.2	4.91
5	20.456	**Pentadecanoic acid, 14-methyl-, methyl ester**	$C_{16}H_{32}O_2$	256.42	1.67
6	21.058	**n- Hexadecanoic acid**	$C_{16}H_{32}O_2$	256.42	13.37
7	22.506	**9, 12-Octadecadienoic acid, methyl ester**	$C_{19}H_{34}O_2$	294.47	1.95
8	22.564	**11-Octadecenoic acid, methyl ester**	$C_{19}H_{36}O_2$	296.48	1.89
9	23.240	**Oleic Acid**	$C_{18}H_{34}O_2$	282.46	32.64
10	43.440	**Thunbergol**	$C_{20}H_{34}O$	290.48	2.58

antifungal activity (12) Oleic acid a common monounsaturated fat has been associated with decreased low density lipoprotein (13) and hypotensive (14) as reported by earlier worker. The structure and kinetics studies of n-Hexadecanoic acid(Palmitic acid) revealed that it is an inhibitor of phospholipase, hence an anti-inflammatory compound(15) Mome Inositol a inositol compound has antialopecic, anticirrhotic, antineuropathic, cholesterolytic, lipotropic and sweetener activity as reported by(16).Thus, this type of GS-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

Conclusions

From the present study it is concluded that the maximum extraction of phytochemicals was observed in methanol extract than petroleum ether, chloroform, and ethyl acetate. More over Gas Chromatography and Mass spectrometry analysis showed the existence of various compounds with variable chemical structures which suggests the contribution of these compounds on pharmacological activity. Hence, the roots, of *Decalepis hamiltonii* might be utilized for the development of traditional medicines and further investigation is in need to elute novel active compounds which may create the new way to treat many incurable diseases.

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