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GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF *HUGONIA MYSTAX* LEAVES

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Keywords:

Hugonia mystax, Phytochemical analysis, Soxhlet extraction, GC-MS

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ABSTRACT: *Hugonia mystax* is a woody evergreen plant that grows in the dry forest areas of India and Sri Lanka. Often found in the forests of Kerala and Tamil Nadu in southern India. It is locally known as modirakanni (tamil) and kamsamaraha and tribals from Tamil Nadu, India, have used it in primary health care. The plant parts are used ethnobotanically to treat rheumatism, skin diseases and inflammations, snake bites, fever, and worm infestation. *Hugonia mystax* is an antibiotic, anthelmintic, febrifuge, astringent, peptic ulcer and verminosis remedy. *Hugonia mystax* has been practising herbal medicine for a long time. This plant is a member of the linaceae family and is an essential medicinal plant in Indian medicine. To evaluate the extractive values, powdered materials were subjected to successive extractions with ethanol using the Soxhlet process. The existence of several compounds such as flavonoids, phenolic elements, steroids, tannins, saponins, terpenoids, and carbohydrates was revealed by qualitative phytochemical screening of the ethanolic extracts of the leaves, as well as GC-MS analysis to examine the chemical components present in it. A total of twenty-seven compounds were discovered in the ethanolic extract. The findings of this study would form the basis for the manufacture of herbal medicines for a variety of ailments using *Hugonia mystax* leaves.

INTRODUCTION: In India there are about 7500 wild plants used for medicinal purposes by different tribal inhabitants. Treatment with medicinal plants were considered very safe as there is no or minimal side effects. The golden fact is that, use of herbal treatment is independent of any age group [1]. Most of the drugs thus formulated are free of side effects or reactions. One of the phenomena of the last three decades has been the huge increase in use of herbal products [2].

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These can be defined as plants, parts of plants or extracts from plants that are used in health care or in combating diseases [3]. Traditional plant-based medicines for primary healthcare used is followed in underdeveloped countries of about 80% of world's population.

Hugonia mystax leaves were associated with different biological activities such as anti-microbial, anthelmintic, anti-oxidant, hepato-protective and cytotoxic actions. This species has a restricted global distribution, occurring only in India and Sri lanka. Within India, it has been recorded in Maharashtra, Karnataka, Tamil nadu and Andhra Pradesh. It is an unexplored medicinal plant in the Indian medicinal system. Medicinal plants are the richest bio resources of drugs of traditional systems of medicine, modern medicines,

neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [4]. The plant kingdom holds a great promise for medicinal substances in many plant species and still unexplored [5].

According to ethnobotanical information, the leaves are used in the treatment of peptic ulcers and as anthelmintic, febrifuge, antidote and its fruits are used in diarrhea and dysentery [6]. Stem bark is used in the treatment of jaundice and skin diseases [7].

All these medicinal properties of *Hugonia mystax* is due to the presence of its phytochemical constituents which is not yet explored thoroughly [8]. In this study the gas chromatogram mass spectrometric method was carried out in the ethanolic extract of dried leaves for phytochemical analysis [9].

There are thousands of phytochemicals each have their pharmacological properties of their own. The plant possesses various medicinal properties. The aim of this study was to identify the phyto-compounds in the ethanolic leaf extract of the *Hugonia mystax* by qualitative screening of phytochemicals and to identify each specific compound by GC-MS analysis. A major part of the world population mainly in the developing countries still uses traditional, folk medicine to avoid synthetic drugs treatment.

FIG. 1: *HUGONIA MYSTAX*

Recently many of the research were being carried out in medicinal plants. The main reason was that the synthetic drugs which was now taking up by the human have many side effects that often lead to serious complications. The development of herbal medicine was done by the primary screening of the compounds in the plant extracts.

Comparing to modern medicine the herbal medicine was the lifesaving drug [10]. The non-nutritive plant chemicals are called as phyto-chemicals which have the properties to protect or prevent diseases. Plant produces these chemicals to protect themselves but the research shown that they have the capacity to treat human diseases in an effective way [11]. Review of literature revealed less work on this plant. Hence in the present study, the successive extractive value and Gas chromato-graphy-Mass spectrometry analysis of ethanol extract of leaves of *Hugonia mystax* were done.

MATERIALS AND METHODS:

Collection of Plant Material: The leaves of *Hugonia mystax* were collected from Dindigul District, Tamil Nadu, India. The collected plant material was authenticated by Botanical Survey of India (BSI/SRC/5/23/2018/Tech/149). The herbarium specimen was prepared and deposited at the Natural Product Research Laboratory, J.K.K. Nattraja College of Pharmacy, Namakkal, Tamil Nadu for future reference.

Preparation of the Extract: The plant material was collected and chopped into small pieces, dried under shade condition and coarsely powdered [12, 13]. The coarse powder was subjected to successive extraction with ethanol solvent by Soxhlet extraction method [14]. The extract was collected and distilled at atmospheric pressure and the trace of solvent was removed in vacuo and stored at 4 ºC. The resulted extract was subjected to GC-MS analysis [15]. **Table 1** shown the preliminary phyto-chemical analysis of dry leaf extract of *Hugonia mystax* Linn [16].

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF DRY LEAF EXTRACT OF *HUGONIA MYSTAX*

Phytochemical Constituents	Ethanol extract
Alkaloids	-
Flavonoids	+
Phenolic Compounds	+
Fats and oils	-
Steroids	+
Tannins	+
Amino acids	-
Saponins	+
Terpenoids	+
Carbohydrates	+
Glycosides	-

+ ---- Present, - ---- Absent

TABLE 2: GAS CHROMATOGRAPHY-MASS SPECTROMETRY [17]

GCMS Details:	
System	GCMS
Make	Agilent 7890 B with Mass Detector
Column	HP-5MS, 30m × 0.250 mm, 0.250µm
Collision gas (N2)	1.50 mL/minute
Quench gas (He)	2.25 mL/minute
Injection Volume	1.0 µL with split
Inlet Temperature	200 °C
Detection	Mass Spectrometry
GC Parameters:	
Initial Temperature	90 °C
Initial Time	6 minutes
Program rate 1	6°C/minute
Temperature 1	280 °C
Hold Time 1	23 minutes
Run time	60.667 Minutes
Injection mode	Split 20:1
Auxiliary temperature	230 °C
MS parameters:	
Ion source Temperature	230 °C
Solvent cut time	3 minutes
Detector Gain Mode	Relative to Tuning result
Mode	MS1 SCAN
M/Z Range	50-600

The details of gas chromatography mass spectrometry are shown in **Table 2** [18].

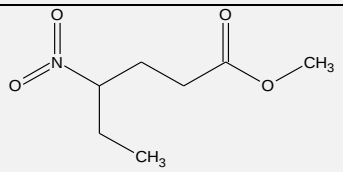
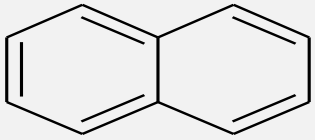
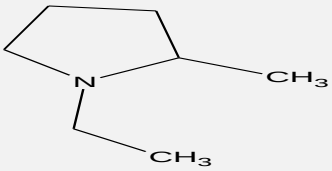
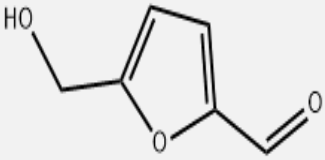
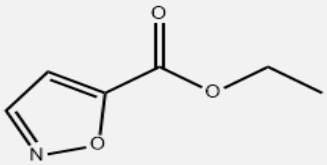
Identification of Compounds: The individual compounds were identified from ethanol extract based on direct comparison of the retention times and their mass spectra with the spectra of known compounds stored in the spectral database [19, 20], NIST version 2.2 mass spectral library and comparing the spectrum obtained through GC-MS compounds present in the plant samples were identified [21].

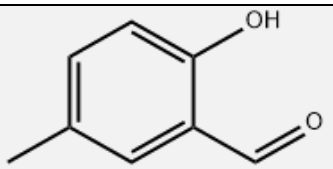
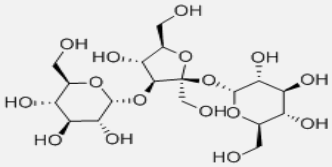
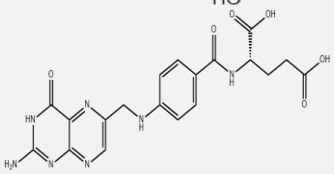
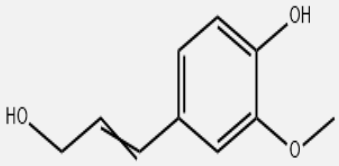
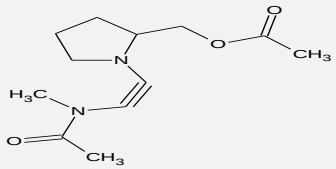
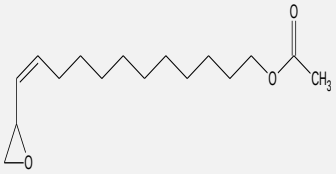
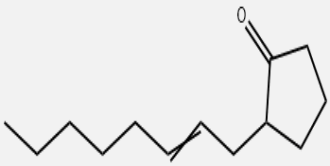
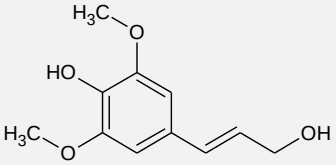
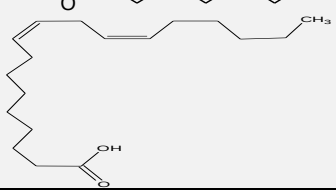
Compounds Identification by GC-MS: The samples eluted using column chromatography has been analysed using gas chromatography equipped with mass spectrometry.

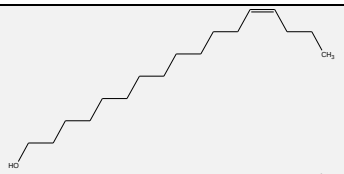
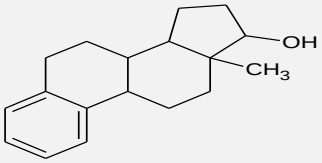
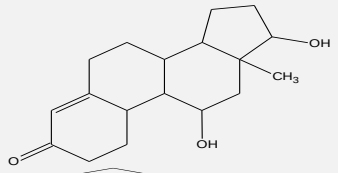
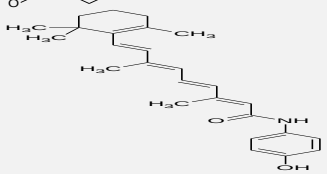
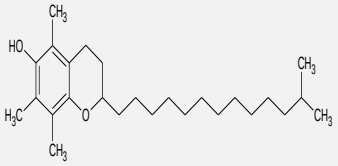
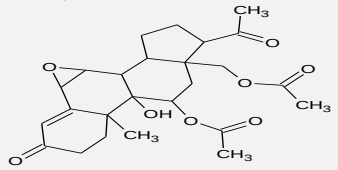
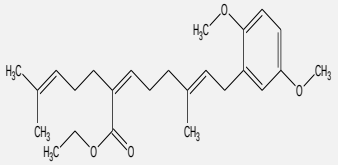
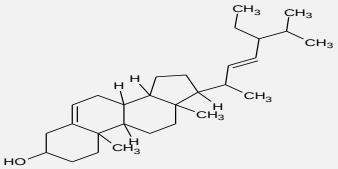
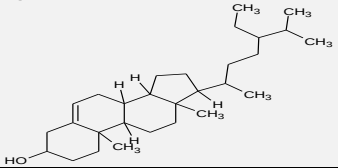
Elution solvent of ethanol was used [22, 23]. Hence the blank solvents and separated samples were injected in the GCMS and chromatograms were obtained [24]. The result of the sample was tabulated.

RESULTS AND DISCUSSION:

TABLE 3: COMPONENTS DETECTED IN THE LEAF OF ETHANOL EXTRACT OF HUGONIA MYSTAX

Peak Retention Time	Peak Area %	Molecular Weight	Molecular Formula	Compound Name	Chemical structure
7.57	13.23	175.08	$C_7H_{13}NO_4$	Methyl 4-nitrohexanoate	
8.84	15.22	128.06	$C_{10}H_8$	Naphthalene	
9.80	59.91	113.12	$C_7H_{15}N$	2-Methyl-1-ethylpyrrolidine	
10.45	23.19	126.03	$C_6H_6O_3$	5-Hydroxy methyl furfural	
11.22	41.15	141.04	$C_6H_7NO_3$	Ethyl isoxazole-5-carboxylate	

15.54	19.53	136.05	$C_8 H_8 O_2$	2-Hydroxy-5-methylbenzaldehyde	
20.01	23.96	504.17	$C_{18} H_{32} O_{16}$	Melezitose	
21.36	4.21	441.4	$C_{19} H_{19} N_7 O_6$	Folic Acid	
21.58	37.38	180.08	$C_{10} H_{12} O_3$	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	
22.53	3.51	266.16	$C_{14} H_{22} N_2 O_3$	Acetamide, N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-	
23.33	13.86	268.20	$C_{16} H_{28} O_3$	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate	
23.754	1.67	194.17	$C_{13} H_{22} O$	Cyclopentanone, 2-(2-octenyl)-	
25.37	29.45	298	$C_{19} H_{38} O_2$	Isopropylpalmitate	
25.53	6.46	244	$C_{13} H_{24} O_4$	Tridecanedioic acid	
25.90	13.99	210	$C_{11} H_{14} O_4$	Trans Sinapyl alcohol	
27.73	5.62	280	$C_{18} H_{32} O_2$	9, 12-octa decadienoic acid	

28.10	11.06	252	C_{17} H_{32} O	13-heptadecyn-1-ol	
28.43	5.92	284	C_{18} H_{36} O_2	Hexadecanoic acid, ethyl ester	
31.20	1.12	282	C_{18} H_{34} O_2	Oleic acid	
33.24	4.01	256	C_{18} H_{24} O	Estra-1,3,5 (10)-trien-17β-ol	
35.08	2.11	290	C_{18} H_{26} O_3	11 β, 17 β-dihydroxy-19-nortestosterone	
40.40	3.31	391	C_{26} H_{33} N O_2	Fenretinide	
41.36	2.41	430	C_{29} H_{50} O_2	Vitamin E	
42.60	14.13	460	C_{25} H_{32} O_8	6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione,11, 18-diacetate	
42.96	23.73	400	C_{25} H_{36} O_4	8-(2,5-Dimethoxyphenyl)-6-methyl-2-(4-methylpent-3-enyl) octa-2,6-dienoic acid, ethylester	
43.78	8.14	412	C_{29} H_{48} O	Stigmasterol	
45.08	18.41	414	C_{29} H_{50} O	Beta sitosterol	

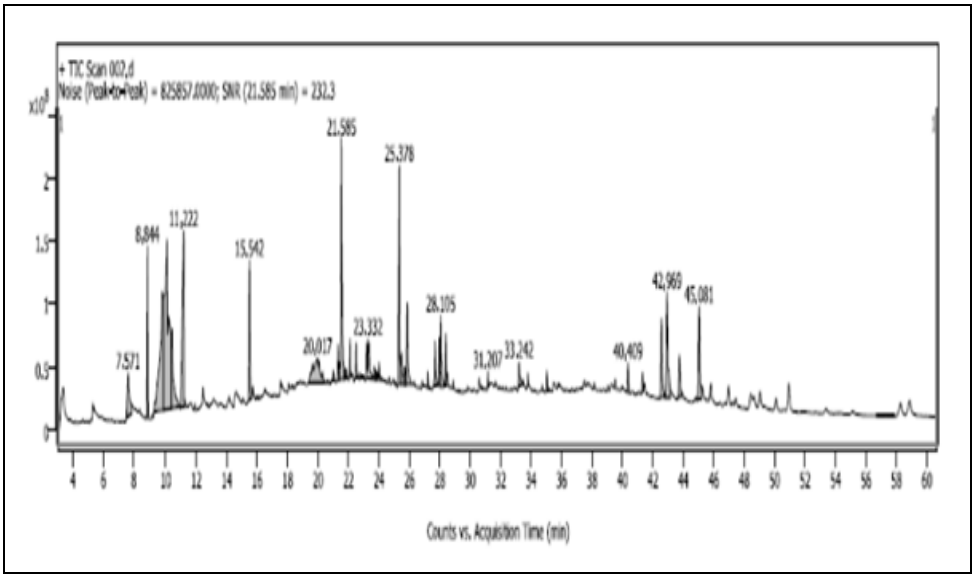


FIG. 2: GAS CHROMATOGRAPHY MASS SPECTROMETRY ANALYSIS25

TABLE: 4 BIOLOGICAL PROPERTIES OF THE PHYTOCOMPONENTS

Name of the compound	Compound nature	Activity
Methyl 4-nitrohexanoate	Aliphatic ester	Lubricant
Naphthalene	Polycyclic Aromatic hydrocarbon	Anti-microbial
2-Methyl-1-ethylpyrrolidine	Cyclic amine	Anti-bacterial activity
5-Hydroxy methyl furfural	Aldehyde	Anticancer
Ethyl isoxazole-5-carboxylate	Heterocyclic ester	Anti-oxidant, anti-cancer, anti-tubercular
2-Hydroxy-5-methylbenzaldehyde	Aromatic aldehyde	Anti-fungal
Melezitose	Non reducing sugar	Stabilizing human blood plasma proteins
Folic Acid	Vitamin compound	Treatment of anemia
(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	Aromatic alcohol	Anti-microbial
Acetamide, N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-	Cyclic ester	Anti-biotic, anti-cancer
Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate	Cyclic ester	Anti tumor
Cyclopentanone, 2-(2-octenyl)-	Cyclic ketone	Pesticide
Isopropylpalmitate	Aliphatic Ester	Emollient, moisturiser
Tridecanedioic acid	Aliphatic dicarboxylic Acid	Anti-biotic
Trans Sinapyl alcohol	Aromatic Alcohol	Hepatoprotective agent
9, 12-octa decadienoic acid	Linoleic acid	Anti-inflammatory, ant histaminic, antiandrogenic, antiarthritic
13-heptadecyn-1-ol	Terpenes	Anti-inflammatory, anticancer, antimalaria
Hexadecanoic acid, ethyl ester	Palmitic acid ester	Anti-oxidant, lubricant, pesticide, anti-androgenic, antibacterial
Oleic acid	Unsaturated aliphatic Acid	Anti-inflammatory, Pulmonaryedema, cancer preventive
Estra-1,3,5 (10)-trien-17beta-ol	Alcohol	Act as a human metabolite
11beta, 17beta-dihydroxy-19-nortestosterone	Steroid	Anti-cancer and anemia
Fenretinide	Retinoids	Anti-cancer activity
Vitamin E	Vitamin compound	Anti-ageing, anti-diabetic, anti-cancer, hepatoprotective
6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione,11, 18-diacetate	Steroid	Anti-microbial, anti-cancer, anti-inflammatory
8-(2,5-Dimethoxyphenyl)-6-methyl-2-(4-methylpent-3-enyl) octa-2,6-dienoic acid, ethylester	Aromatic acid and ester	Treatment of respiratory diseases
Stigmasterol	Steroids	Lowering the LDL, anti hypercholesterolemic, antitumor
Beta sitosterol	Steroids	Cardiac disorder, anti-cancer

The results of GC-MS analysis on an ethanol extract of leaves are shown in **Table 3** [26]. Preliminary phytochemical analysis was carried out to qualitatively analyze the phytoconstituents in the extracts.Twenty-seven compounds were identified with ethanol extract of leaves of Hugoniamystax. All 27 compounds are medicinally important.The presence of various bioactive compounds confirms the application of Hugoniamystax for various ailments by traditional practitioners [27].

Isolation of individual phytochemical constituents may proceed to find a novel drug. In addition to this, the results of the GC-MS profile can be used as phytochemical tool for the identification of the bioactive components. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanolic extract of Hugoniamystax are presented in **Table 3**.

Methyl 4-nitrohexanoate (13.23%), Naphthalene (15.22%), 2-Methyl-1-ethylpyrrolidine (59.91%), 5-Hydroxy methyl furfural (23.19%), Ethyl isoxazole-5-carboxylate (41.15%), 2-Hydroxy-5-methylbenzaldehyde (19.53%),Melezitose (23.96%), (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol (37.38%), Z-(13, 14-Epoxy) tetradec-11-en-1-ol acetate (13.86%), Isopropyl-palmitate (29.45%), Trans Sinapyl alcohol (13.99%), 13-heptadecyn-1-ol (11.06%), 6,7-Epoxypregn-4-ene-9, 11, 18-triol-3, 20-dione, 11, 18-diacetate(14.13%), 8-(2, 5-Dimethoxy-phenyl)-6-methyl-2-(4-methylpent-3-enyl)octa-2,6-dienoic acid, ethylester (23.73%), Beta sitosterol (18.41%). All these compounds present in Hugoniamystax leaf extract support the medicinal application of the plant.The study revealed major bioactive compounds present in the ethanol extract. Identification of these compounds in the plant serves as the basis in determining the possible health benefits of the plant leading to further biologic and pharmacologic studies.

CONCLUSION: According to the findings of this report, the plant Hugoniamystax leaves, as well as the chemical constituents contained in them, are highly valuable in medicinal use for the treatment of various human ailments.The existence of medicinally valuable bioactive components is revealed by GC-MS study of the ethanolic extract of Hugoniamystax leaves. To develop safe drugs, further research on toxicological aspects is needed, as well as the isolation, purification and identification of the bioactive molecules responsible for the activities in order to develop novel pharmaceutical leads.

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CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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