



Phytochemical Profiling of Dried Palmyrah Haustorium Powder through GC-MS Analysis: Unveiling Novel Bioactive Compounds

Pavithra S. ^a, Irene Vethamoni P. ^{b*}, Pazhanivelan S. ^c,
Venkatesan K. ^a and Anand M. ^d

^a Department of Spices and Plantation Crops, HC&RI, TNAU, India.

^b Department of Horticulture, HC&RI, TNAU, Coimbatore, India.

^c Department of Water Technology Centre, TNAU, Coimbatore, India.

^d Department of Food Process Engineering, TNAU, Coimbatore, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Palmyrah seed haustorium is most beneficial (in human health) with its medicinal properties. It contains various volatile compounds and health benefits, preventing many diseases. The present study was carried out with palmyrah haustorium to evaluate the volatile compounds through GC-MS analysis using methanolic extract. The haustorium extract was used to identify the amount of bioactive substances, secondary metabolites, sugars, amino acids, organic acids, fatty acids, and phenolics found in the Palmyrah haustorium using GC-MS analysis. The results indicated that the palmyrah haustorium contains 50 volatile compounds and secondary metabolites. GC-MS analysis of palmyrah haustorium extract revealed volatile compounds with medicinal properties including

*Corresponding author: E-mail: irenevetha17@gmail.com;

anti-bacterial, anti-microbial, hepatoprotective, antihistaminic, cardiogenic, diuretic, antioxidant, anti-inflammatory, and anti-cancer activity. It may prevent Alzheimer's and nervous system disorders. Haustorium is used in food and perfumery industries as a flavoring agent and food additives. The study highlights its health benefits and disease preventing potential in palmyrah haustorium.

Keywords: *Palmyrah haustorium*; volatile compounds; GC-MS; metabolites.

1. INTRODUCTION

Palmyrah palm (*Borassus flabellifer L.*) belongs to palmae, chromosome number $2n=32$ and native to tropical Africa. It is a dioecious tropical crop of significant commercial importance. Historically, diverse plant parts such the root, leaves, seeds, and fruit have been used for a variety of purposes. Palmyrah is also called as toddy palm and sugar palm. It is cultivated throughout India, Sri Lanka, Bangladesh, Burma, the Philippines, Malaysia, and several east African nations.

In the semi-arid states of Tamil Nadu, Andhra Pradesh, Gujarat, Odisha, West Bengal, Bihar, Karnataka, and Maharashtra, palmyrah brightens the barren environment. At the moment, Tamil Nadu is home to half of India's estimated 102 million palmyrah palms. More than 50% of Tamil Nadu's 51.90 million palm trees are clustered in the Southern district of Thoothukudi. In 1978, the Tamil Nadu government designated palmyrah as its official state tree.

Palmyrah (*Borassus flabellifer L.*), the state tree of Tamil Nadu, is a gift from nature to humankind. It is a plant that provides the community with several ecological, medical, economic, and sociological advantages. It was a plant from heaven that could resist extreme weather and natural disasters. It is one of the most advantageous species and each and every portion of it has economic and medical worth. The plant has a close relationship to India's cottage, agricultural, and rural livelihood sectors. The plant's applications can be broadly divided into non-edible, edible, and value-added uses.

The entire tree can be used for economic purposes as well as to carry out traditional tasks in Tamil culture. It is regarded as nature's eternal gift and is referred to as "Kalpak Tharu" in Tamil, which means the tree that grants all of a person's requests. The ancient palm of palmyrah it is known as the "Wishing Tree" or "a palm that yields anything and everything" because of its extraordinary capacity to produce several

economically significant goods. Every portion of the palm is used almost entirely.

Due to the fruit's therapeutic and nutritional benefits, palmyrah is in high demand worldwide. In Sri Lanka, around 1500 tons of fruits are annually accessible during the seasons when the female palm's inflorescences begin to mature and bear fruits. For one season, a palm may produce 200–300 fruits. Between September and October is the fruiting season. The fruit is big, fibrous, and often has three portions that resemble nuts with a seed encased in each. The juvenile fruit kernel of the palmyrah has a layer of gelatinous endosperm or kernel, as well as some sweet-tasting water, enclosed in a tough shell. The fruit endosperm is referred to as "Nungu," in Tamil which is the juvenile fruit kernel of a palmyrah. Under appropriate conditions, the endosperm of mature fruits hardens.

During August month palmyrah fruit ripens, while September and October the ripe fruits fall from the tree. After using the pulp from the palmyrah fruit, the seeds of the fruits were collected, and sown in beds with three or four levels of seeds and the moisture level of the seeds is properly maintained.

The seed's shoot-root axis descends to the soil during germination, while the distal piece that is still inside the seed grows into the haustorium both during and after germination. The seed generates a stalk that gives rise to the product palmyrah tuber, while the palmyrah seed produces the mouthwatering white, spongy edible portion known as the haustorium. Before being harvested, this shoot reaches a height of 20–30 cm.

Palmyrah haustorium is delicious spongy white edible portion formed at the time of germination. During germination of the tuber, the embryo develops and enlarge to form cotyledon structure, it is called as haustorium. The embryo receives the nutrients through the haustorium. The haustorium will therefore be more nutrient-dense since it will contain carbohydrates, protein content, vital amino acids, and other trace

nutrients and bioactive substances that are very good for our health. The fruit endosperm also has several health promoting activities like anti-inflammatory, antibacterial, anticancer, and anti-diabetic properties [1].

Palmyrah tubers are often collected when they are fully mature. Young fruit kernels, haustoria, and freshly boiled tubers from palmyra are nutrient-rich foods. They are valued for their nutritional value as well as their total phenolic content and vitamin C, both of which exhibit antioxidant characteristics. In order to maintain nutritional needs, prevent chronic diseases including cancer, cardiovascular disease, and age-related pathologies, and boost overall health, people should increase their consumption of these palmyrah products. A proximate analysis revealed that it has a small amount of fat but is a high source of fibre, protein, and carbohydrates.

The various health-promoting and disease preventing effects are due to particular compounds known as phytochemicals, including polyphenols. Different areas of the plant have concentrations of secondary metabolites. The phytochemical like oleic acid and unsaturated fatty acid it possesses cardio-protective effect [2]. Secondary metabolites are commercially exploitable because they are used as active pharmaceutical antioxidant, anti-inflammatory, anti-allergic, anti-microbial, anti-mutagenic, anti-platelet, and vasodilatory functions. Therefore, this study was carried out to identify the volatile compounds and metabolites using GC-MS analysis.

2. MATERIALS AND METHODS

2.1 Plant Material Selection

The local variety of palmyrah seed nuts were selected from the village of Udaiyarkulam, Srivaikuntam of Thuthukudi district. and study was carried out at Department of Spices and Plantation Crops, Horticultural College and Research Institute, TNAU, Coimbatore. from 2021 and 2022. Palmyrah seed nuts with tuber were manually dugout from the seed bed and broken into two parts vertically from the point of plumule growth to collect white spongy delicious haustorium from palmyrah. The haustorium were cut into uniformly sized pieces and dried in a hot air oven at a temperature of 50 to 55 °C for 2-3 days and ground into flour. The dried palmyrah haustorium samples were used to determine GC-MS analysis.

2.2 Metabolite Profiling using GC-MS Analysis

2.2.1 Preparation of palmyrah haustorium methanolic extract

In the hot air oven set to 50 to 55°C, the cut pieces of haustorium were dried. Using an 80-mesh sieve, the dried palmyrah haustorium powder was collected. The powdered samples of 0.5 g were taken, and 1.5 ml of methanol was added and kept in ultrasound sonicator upto 15-20 minutes. Then samples were placed in centrifuge for 10 minutes and the supernatant was collected in a vial for GC-MS analysis.

2.2.1.1 Gas chromatography-mass spectrometry (GC-MS)

This analysis was done using haustorium extract to identify the amount of bioactive substances using GC-MS. Sugars, amino acids, organic acids, fatty acids, and phenolics found in the Palmyrah haustorium were identified using GC-MS analysis. Perkin Elmer Claras SQ8C mass selective detector and TriPlus RSH auto sampler were used for the experiment. The chemical components were separated using a non-polar, 30 m long, 0.25 mm internal diameter, and 0.25 m thick DB-5 MS capillary column. The stationary phase for separation contains around 35% phenyl polysiloxa. The injection volume was one microliter in split-less mode. The injector's temperature was set to 280°C. As a carrier gas, helium was employed at a flow rate of 1 ml/min. After being set to 80°C for 2 minutes, the oven's temperature was ramped up to 220°C at a rate of 10 C/min without holding, then raised to 310°C at a rate of 20 C/min and kept for 10 minutes. Five minutes was chosen as the solvent delay. There was a 1 ml/min flow rate across the column. Based on comparisons between the collected mass spectra and those from the NIST Ver. 11 and WILEY mass spectral libraries, metabolites were identified.

3. RESULTS AND DISCUSSION

The GC-MS analysis of methanolic extract of palmyrah haustorium dried powder revealed the emergence of 50 peaks (Fig. 1). By GC-MS analysis technique it was identified 50 various volatile compounds and secondary metabolites in palmyrah haustorium which has medicinal properties and several health promoting properties. The findings has been shown in

Table 1. Metabolic profiling of palmyrah haustorium powder through GC-MS analysis

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Identified compound name
1	5.374	5.337	5.43	444295	0.28	173921	0.78	2.55	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
2	5.652	5.6	5.747	583214	0.37	166031	0.74	3.51	2H-Pyran-2,6(3H)-dione
3	6.463	6.417	6.51	602569	0.38	274277	1.23	2.2	D-Limonene
4	6.541	6.51	6.59	202588	0.13	91105	0.41	2.22	Eucalyptol
5	6.747	6.713	6.79	145392	0.09	66299	0.3	2.19	3-Thiazolidinecarboxamide, 2-imino-
6	7.535	7.46	7.643	1451544	0.92	343106	1.53	4.23	Thymine
7	8.061	8.01	8.137	964273	0.61	425897	1.9	2.26	Linalool
8	9.213	9.153	9.333	2294697	1.45	649918	2.91	3.53	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
9	10.206	10.16	10.3	618846	0.39	236769	1.06	2.61	Terpinen-4-ol
10	10.57	10.513	10.64	1472242	0.93	593940	2.66	2.48	.alpha.-Terpineol
11	11.447	11.313	12.193	30700212	19.45	4980872	22.27	6.16	5-Hydroxymethylfurfural
12	11.843	11.767	11.887	1011467	0.64	259287	1.16	3.9	3-Acetoxy-3-hydroxypropionic acid, methyl ester
13	11.963	11.887	12.08	1623290	1.03	377461	1.69	4.3	2,6-Octadien-1
14	12.133	12.093	12.173	63110	0.04	23186	0.1	2.72	Methyl cis-11-icosenoate
15	12.418	12.193	12.46	398683	0.25	54312	0.24	7.34	Citral
16	12.717	12.46	12.927	5687805	3.6	325645	1.46	17.47	d-Glycero-d-galacto-heptose
17	13.053	12.927	13.433	3353520	2.12	234146	1.05	14.32	3-(Prop-2-enoyloxy)tridecane
18	13.63	13.487	13.86	1489947	0.94	224730	1	6.63	Acetoacetic acid-meto-TMS
19	14.494	14.433	14.82	13840651	8.77	5241096	23.44	2.64	.alpha.-Terpinyl acetate
20	14.74	14.713	14.78	35554	0.02	21224	0.09	1.68	Methyl linolenate
21	15.236	15.14	15.42	514039	0.33	176301	0.79	2.92	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate
22	15.651	15.433	15.74	1187423	0.75	149533	0.67	7.94	Succinic acid, di(but-2-en-1-yl) ester
23	15.78	15.74	15.953	714975	0.45	123132	0.55	5.81	Cyclohexanediol-2TMS
24	16.767	16.633	16.833	360551	0.23	41314	0.18	8.73	Propionylglycine-TMS
25	16.887	16.833	16.993	521734	0.33	72192	0.32	7.23	2-Furoic acid-TMS
26	17.472	16.993	18.073	14006520	8.88	349526	1.56	40.07	Sucrose
27	18.346	18.073	18.793	5333079	3.38	248635	1.11	21.45	.beta.-D-Glucopyranose, 1,6-anhydro-
28	18.82	18.793	18.953	171955	0.11	37365	0.17	4.6	Isovalerylglycine-TMS
29	19.767	19.673	19.86	549295	0.35	161202	0.72	3.41	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-
30	20.258	19.86	20.42	1458602	0.92	96054	0.43	15.19	Methyl cis-11-icosenoate
31	20.516	20.42	20.633	1065193	0.67	113336	0.51	9.4	Methyl cis-11-icosenoate
32	20.968	20.633	21.327	5763346	3.65	225767	1.01	25.53	1-(.beta.-d-Arabinofuranosyl)-4-O-difluoromethyluracil

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Identified compound name
33	21.407	21.327	21.913	3261562	2.07	188005	0.84	17.35	Methyl caproate
34	22.446	22.26	23.06	1122237	0.71	51592	0.23	21.75	Methyl undecanoate
35	25.695	25.62	26.233	835076	0.53	69254	0.31	12.06	2-Furoic acid-TMS
36	26.814	26.713	27.087	363917	0.23	56845	0.25	6.4	Linoleic acid-TMS
37	27.887	27.753	28.127	301412	0.19	33638	0.15	8.96	Methyl cis-11-icosenoate
38	28.31	28.127	28.953	16361737	10.37	1901608	8.5	8.6	n-Hexadecanoic acid
39	29.14	28.953	29.54	1367852	0.87	67054	0.3	20.4	2-Keto-isovaleric acid-meto-TMS
40	31.476	31.407	32.34	11719053	7.43	1218938	5.45	9.61	9,12-Octadecadienoic acid (Z,Z)-
41	39.453	39.38	39.807	848405	0.54	148943	0.67	5.7	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
42	39.94	39.807	40.1	311719	0.2	60477	0.27	5.15	Methyl oleate
43	42.34	42.207	42.5	313021	0.2	23775	0.11	13.17	Methyl cis-11-icosenoate
44	42.648	42.5	43.06	1435579	0.91	107538	0.48	13.35	Z,Z-3,15-Octadecadien-1-ol acetate
45	44.02	43.94	44.207	1808610	1.15	238812	1.07	7.57	4H-1-Benzopyran-4-one, 2,3-dihydro-5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-, (S)-
46	44.713	44.527	44.873	1017285	0.64	119902	0.54	8.48	Methyl cis-13,16-Docosadienate
47	45.522	44.873	46.5	12254894	7.77	284070	1.27	43.14	1,3:4,6-Dimethylene-d-glycero-d-mannoheptitol
48	50.406	50.34	50.58	795932	0.5	166474	0.74	4.78	Cholest-5-en-3-ol (3.beta.)-, carbonochloridate
49	50.778	50.58	51.007	1649054	1.04	384594	1.72	4.29	Stigmasterol
50	51.675	51.567	52.073	3415486	2.16	682136	3.05	5.01	.beta.-Sitosterol
Total				157813442	100	22361234	100		

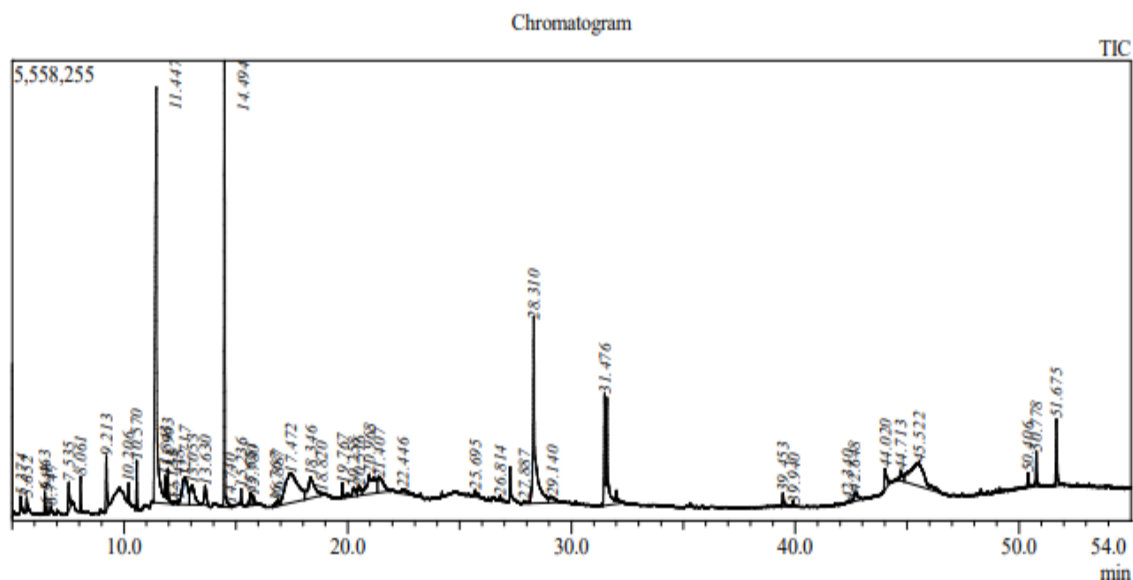


Fig. 1. GC-MS chromatogram of Palmyrah haustorium powder

(Table 1) and it includes data on peak, retention time, area, area percentage of chemical constituents present in haustorium. The identified chemical components having medicinal properties are 5-Hydroxymethylfurfural (19.45 %), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- (1.45 %), 2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)- (1.03 %), d-Glycero-d-galacto-heptose (3.60 %), 3-(Prop-2-enoyloxy)tridecane (2.12 %), .alpha.-Terpinyl acetate (8.77 %), Sucrose (8.88 %), .beta.-D-Glucopyranose, 1,6-anhydro- (3.38 %), 1-(.beta.-d-Arabinofuranosyl)-4-O-difluoromethyluracil (3.65 %), Methyl caproate (2.07 %), n-Hexadecanoic acid (10.37 %), 9,12-Octadecadienoic acid (Z,Z)- (7.43 %), 4H-1-Benzopyran-4-one, 2,3-dihydro-5-hydrox (1.15 %), 1,3:4,6-Dimethylene-d-glycero-d-mannoheptito (7.77%), Stigmasterol (1.04%) and .beta.-Sitosterol (2.16%) and these were identified using GC-MS analysis.

The various volatile compounds and metabolites present in palmyrah haustorium dried powder were identified by GC-MS analysis. The structural chemical compounds and metabolites 5-Hydroxymethylfurfural identified in trial have been used in the treatment of preventing Hypoxia, Anemia, Sick Cell Disease, and antioxidant properties [3]. It is also utilized as a food additive and flavoring agent in the food industry [4] 5-hydroxymethylfurfural (HMF) is considered as an important primary renewable building blocks and intermediate due to its rich chemistry and potential availability from carbohydrates such as fructose, glucose,

sucrose, cellulose and inulin [5], 5-hydroxymethylfural (HMF) can be transformed to various value-added chemicals [6], The compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- used as Anti- microbial, Anti-inflammatory properties [7], It possesses Anti- bacterial activity [8]. 2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)- compound which is a monoterpene used in perfumery industries [9] and it possesses anticancer activity [10].

Another one compound d-Glycero-d-galacto-heptose is used as a dietary supplement [11] and having a higher docking score than many commercially available COX2 inhibitors [12]. Yet another 3-(Prop-2-enoyloxy)tridecane identified by GC-MS analysis has many industrial applications [13].The compound α -terpinyl acetate have anti-bacterial and anti-biofilm activity [14] and many pharmacological activities like antiseptic (pulmonary), antispasmodic (neuromuscular), aphrodisiac, expectorant, anthelmintic, antibacterial (variable), cephalic, cardiogenic, diuretic, emmenagogue, sialagogue and stomachic. It also act as a stimulant of nervous system [15] and it has been used as a suitable lead to develop a molecule that might have multi-targeted directed ligand (MTDL) potential and disease amelioration effects in AD (Alzheimer Diseases) [16].

Sucrose hydrolytic enzymes, present in palmyrah haustorium is widely used in a variety of food industries, employ sucrose as a substrate. In

addition to their hydrolysis activities [17] it has production potential of anti-oxidant secondary metabolites [18]. The compound Beta.-D-Glucopyranose, 1,6-anhydro- known as Levoglucosan acts as a preliminary material for the synthesis of stereoregular polysaccharides and have anti-human immunodeficiency virus and blood coagulant activities [19]. Presence of n-Hexadecanoic acid is used in the treatment of rheumatic symptoms in the traditional medical system of India, and it has anti-inflammatory, anti-oxidant, anti-bacterial properties [20] and It also have anti-microbial properties [21].

9,12-Octadecadienoic acid (Z,Z)- compound have Hepatoprotective, antihistaminic, hypocholesterolaemic, antieczemic, Catechol-O-Methyl-Transferase-Inhibitor, Methyl Guanidine-Inhibitor activity [22]. 1,3:4,6-Dimethylene-d-glycero-d-mannoheptitol has antioxidant [23] and antimicrobial properties [24]. The presence of Stigmasterol compound act as antibacterial and antifungal agent and as such may serve as a lead compound in the development of novel antimicrobial drugs [25] and it has chemo preventive anti-cancer activity [26]. Beta.-Sitosterol(BS), is an important plant derived nutrient with anti-cancer agent for chemoprevention and chemotherapy [27] and anti-microbial properties [28].

4. CONCLUSION

The GC- MS analysis of methanolic extract of palmyrah haustorium dried flour contains various volatile compounds, chemical constituents, medicinal properties and therapeutic compounds with anti- bacterial, anti- microbial, Hepatoprotective, antihistaminic, cardiotoxic, diuretic, anti-oxidant, anti-inflammatory, and anti-cancer activity. It prevents Alzheimer disease and nervous system disorders. It is also used in food additives and flavoring agent in food industry and perfumery industries. Due to the presence of various health promoting benefits and secondary metabolites with chemical constituents and volatile compounds. Palmyrah haustorium production may be enhanced and its multivarious potential may be tapped through making value added product using palmyrah haustorium flour.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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