

GC MS determination of bioactive components of *Peperomia pellucida* (L.) Kunth

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Article Info

Received: 25-05-2015,

Revised: 21-06-2015,

Accepted: 23-06-2015

Keywords:

Bio-active components,
GC-MS Analysis,
Ethanol extract,
Peperomia pellucida

Abstract

In this investigation, whole plant of *Peperomia pellucida* (L.) Kunth (Piperaceae) was subjected to the identification of bioactive compounds by using Gas Chromatography - Mass Spectrum technique. The plant sample was extracted with 99% of ethanol. Extracted sample was injected; based on the retention time and peak formation, the bioactive compounds were screened. Interpretation was done using the database of National Institute Standard and Technology (NIST). Thirty two compounds were identified. Among them, Apiol (22.64%) was found to be the major component followed by (3-Methoxy-nitrophenyl) acetic acid, methyl ester (8.14%), Phytol (7.47%), n-Hexadecanoic acid (7.29%), E-2-Tetradecen-1-ol (6.92%), 5H-Cyclopropa (3,4) benz (1,2-e) azulen-5-one, 4,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]- (5.89%), Stigmasterol (4.60%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (4.00%), Campesterol (3.19%), a-Sitosterol (2.92%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (2.79%), Z,Z-2,5-Pentadecadien-1-ol (2.53%) and 3-Hydroxy-4-methoxycinnamic acid (2.01%).

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Leaves, flowers, stems, roots, seeds, fruit and bark can all be constituents of herbal medicines. The medicinal values of these plants lie in their component phytochemicals, which produce definite physiological actions on the human body (Hill, 1952). India is bestowed with a very rich wealth of medicinal plants which in turn are a source of

genetic diversity. Many of these indigenous medicinal plants are also used for medicinal purposes (Edeoga *et al.*, 2005). *Peperomia pellucida* (L.) Kunth, an important medicinal plant belonging to the family Piperaceae has been selected for the present study because of its therapeutic value in treating abscesses, boils, abdominal pain, acne, colic, fatigue, gout, headache, renal disorders impotence, measles, mental disorders, wounds and bleeding (Aziba *et al.*, 2001;

Arrigoni-Blank *et al.*, 2002, 2004), cancer (Xu *et al.*, 2006) eczema and fever (Ghani, 1998), rheumatic pain (Khan and Omoloso, 2002) and skin infections (Ragasa *et al.*, 1998). In Ayurveda, the plant is used to pacify vitiated cough, pitta, constipation, kidney diseases, urinary retention (Puluk Majumder *et al.*, 2011) dysuria, urinary tract infection, emaciation, edema and general weakness. Pounded whole plant used as warm poultice for boils, pustules and pimples. In Jamaica and the Caribbean used for colds and as a diuretic for kidney problems. In South America, fresh juice of stem and leaves are used for eye inflammation. Infusion and decoction of leaves and stems are used for gout and arthritis (dos Santos *et al.*, 2001). This plant is widely distributed in South America and Asia and is known as silver bush. Fresh leaves and stems are eaten as vegetable (Hua *et al.*, 1999). In this study, whole plant ethanol extract of *P. pellucida* was used for determining the various bioactive components using GC-MS analysis.

MATERIALS AND METHODS

Peperomia pellucida (L.) Kunth plants were collected from a garden in Senthil Nagar, Coimbatore, Tamilnadu and authenticated by Botanical Survey of India (No. BSI/SRC/5/23/2015/tech-1169 dt. 22.5.2015).

Preparation of plant extract

The whole plant materials collected were washed thoroughly in tap water followed by distilled water to remove the dirt and soil particles then shade dried and pulverized to fine powder using a mechanical grinder. 25 grams of powdered whole plant sample was transferred to stoppered flask with thirty ml of ethanol and kept it for overnight soaking. The flask was shaken frequently. Then the sample was filtered using Whatmann filter paper with sodium sulphate to remove the sediments and traces of water in the filtrate. The filtrate was concentrated with the help of nitrogen flushing. 2 µl of purely prepared sample was injected into the programme GC-MS instrument.

GC Programme

Column: Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30 x 0.25mm x 0.25µm df
Equipment: GC Clarus 500 Perkin Elmer; Carrier gas: 1ml per min, Split: 10:1; Detector: Mass detector Turbo mass gold-Perkin Elmer; Software: Turbomass 5.2; Sample injected: 2µl

Oven temperature Programme

110° C -2 min hold; Up to 200° C at the rate of 10° C /min-No hold; Up to 280° C at the rate of 5° C / min-9 min hold; Injector temperature 250° C; Total GC running time 36 min.

MS Programme

Library used NIST Version-Year 2005; Inlet line temperature 200° C; Source temperature 200°C Electron energy: 70 eV; Mass scan (m/z): 45-450; Solvent Delay: 0-2 min; Total MS running time: 36 min. Interpretation on GC mass spectrum was conducted using the database of National Institute Standard and Technology (NIST) having more than 62, 000 patterns. The spectrum of the unknown component 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 AND DISCUSSION

The results obtained from GC-MS analysis of whole plant ethanol extract of *Peperomia pellucida* led to the identification of number of phytochemicals. The GC-MS chromatogram shows the presence of 32 major peaks with the retention time range between 2.39 and 29.90 (Figure 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW), the concentration (peak area percentage) and activity are presented in Table 1. Among the identified components, Apiol (C₁₂H₁₄O₄) attained the largest peak (22.64%) with the retention time 8.71 followed by (3-Methoxy-nitrophenyl) acetic acid methyl ester (C₁₀H₁₁NO₅) with the retention time 7.96 having the peak area percentage of 8.14. The third significant peak (7.47%) was attained by Phytol (C₂₀H₄₀O) in the retention time 13.84. n-Hexadecanoic acid (C₁₆H₃₂O₂) with the peak area percentage of 7.29 had the retention time 12.40.

The nature of the compounds obtained are of organic compound, sesquiterpene, ether compound, alcoholic compound, palmitic acid, diterpene, linoleic acid, linolenic acid, ketone compound, phenolic compound, unsaturated fatty acid ester, sulfur compound and vitamin compound and steroids. Sesquiterpene is found to have the activities of Anti-tumor, Analgesic Antibacterial, Anti-inflammatory, Sedative and Fungicide. Alcoholic and diterpene compounds are having antimicrobial activities.

Fig.1: Chromatogram of ethanol extract of *Peperomia pellucida* whole plant

Sample No. 562

HCPT, Thanjavur, 25-MAR-2014 + 18:51:47

GCMS Analysis 1287

Scan E+
TIC
1.08e10

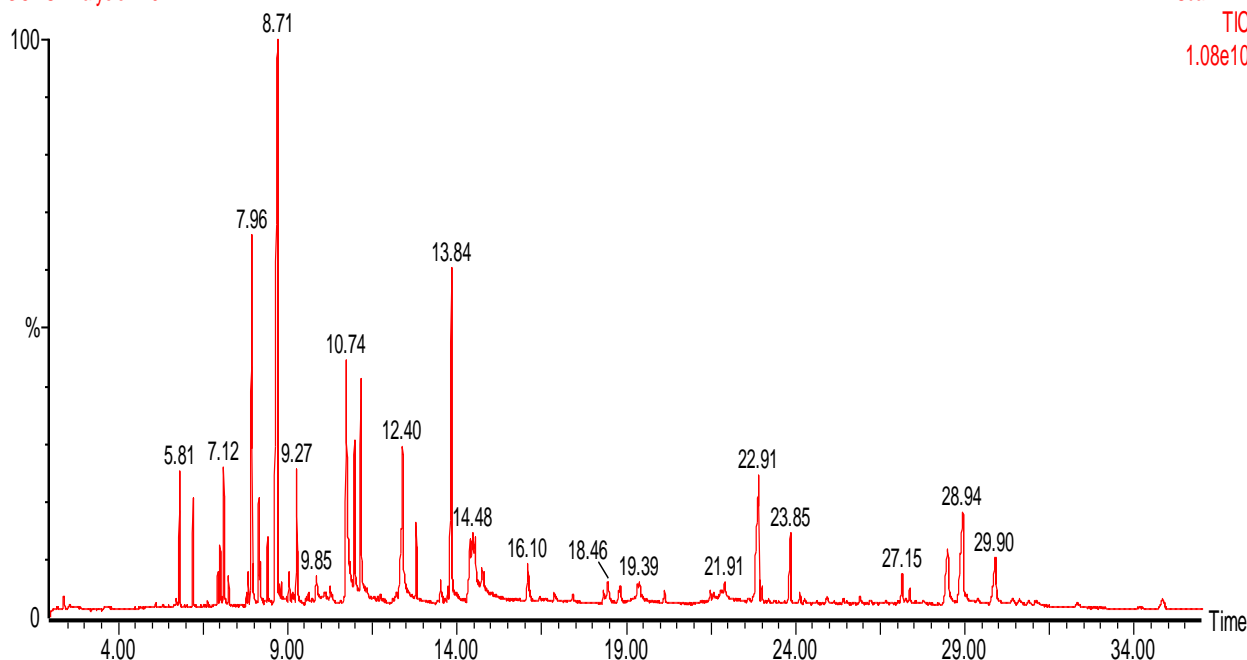


Table 1: Activity of the components identified in the ethanol extract of *P. pellucida* whole plant

No	RT	Name of the compound	Molecular Formula	M W	Peak Area %	Compound Nature	**Activity
1	2.39	Propane, 1,1,3-triethoxy-	C ₉ H ₂₀ O ₃	176	0.30	Ether compound	No activity reported
2	5.81	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	C ₁₅ H ₂₄	204	1.81	Sesquiterpene	Anti-tumor, Analgesic, Anti-bacterial, Anti-inflammatory, Sedative, Fungicide
3	6.21	Caryophyllene	C ₁₅ H ₂₄	204	1.46	Sesquiterpene	Anti-tumor, Analgesic, Anti-bacterial, Anti-inflammatory Sedative, Fungicide
4	6.94	1H-Cyclopenta[1,3]cyclopropano[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3aà,3bá,4á,7à,7aS*)]-	C ₁₅ H ₂₄	204	0.40	Sesquiterpene	Anti-tumor, Analgesic, Anti-bacterial, Anti-inflammatory Sedative, Fungicide
5	7.12	ç-Elemene	C ₁₅ H ₂₄	204	1.84	Sesquiterpene	Anti-tumor, Analgesic, Antibacterial, Anti-inflammatory Sedative, Fungicide
6	7.26	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-	C ₁₅ H ₂₄	204	0.34	Sesquiterpene	Anti-tumor, Analgesic, Anti-bacterial, Anti-inflammatory Sedative, Fungicide

		methylethenyl)- ,[2R-(2à,4aà,8aá)]-					
7	7.96	(3-Methoxy-2-nitrophenyl)acetic acid, methyl ester	C ₁₀ H ₁₁ NO ₅	225	8.14	Ester compound	No activity reported
8	8.15	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aà,4aà,7á,7aá,7bà)]-	C ₁₅ H ₂₄ O	220	1.61	Sesquiterpene oxide	Anti-tumor, Analgesic, Antibacterial, Anti-inflammatory Sedative, Fungicide
9	8.41	Carotol	C ₁₅ H ₂₆ O	222	0.90	Sesquiterpene alcohol	Anti-tumor, Analgesic, Antibacterial, Anti-inflammatory Sedative, Fungicide
10	8.71	Apiol	C ₁₂ H ₁₄ O ₄	222	22.64	Organic compound	Used in abortion
11	9.27	1,4-Benzenediol, 2,6-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O ₂	222	2.31	Alcoholic compound	Antimicrobial
12	10.74	E-2-Tetradecen-1-ol	C ₁₄ H ₂₈ O	212	6.92	Unsaturated alcoholic compound	No activity reported
13	10.97	Z,Z-2,5-Pentadecadien-1-ol	C ₁₅ H ₂₈ O	224	2.53	Unsaturated alcoholic compound	No activity reported
14	11.16	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	4.00	Terpene alcohol	Antimicrobial, Anti-inflammatory
15	12.40	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	7.29	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
16	12.80	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-	C ₁₅ H ₂₆ O	222	1.20	Sesquiterpene alcohol	Anti-tumor, Analgesic, Antibacterial, Anti-inflammatory Sedative, Fungicide
17	13.84	Phytol	C ₂₀ H ₄₀ O	296	7.47	Diterpene	Antimicrobial, Anti-inflammatory Anticancer, Diuretic
18	14.39	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	1.84	Linoleic acid	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Insectifuge
19	14.48	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	278	2.79	Linolenic acid	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge

20	16.10	1-Hexadecanol, 2-methyl-	C ₁₇ H ₃₆ O	256	1.31	Alcoholic compound	Antimicrobial
21	19.39	10-Undecenoic acid, octyl ester	C ₁₉ H ₃₆ O ₂	296	1.01	Unsaturated fatty acid ester	No activity reported
22	20.13	1,4-Dioxaspiro[4.5]decane, 8-(methylthio)-	C ₉ H ₁₆ O ₂ S	188	0.34	Sulfur compound	Antimicrobial
23	21.49	7-Methyl-Z-tetradecen-1-ol acetate	C ₁₇ H ₃₂ O ₂	268	0.43	Acetate compound	No activity reported
24	21.91	6,9,12,15-Docosatetra-enoic acid, methyl ester	C ₂₃ H ₃₈ O ₂	346	1.08	Unsaturated fatty acid ester	Cardio protective Hypocholesterolemic
25	22.91	5HCyclopropa[3,4]benz[1,2-e]azulen-5-one, 4,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-,1a,1b,4,4a,7a,7b,8,9,9a-decahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-	C ₂₈ H ₃₆ O ₁₁	548	5.89	Ketone compound	No activity reported
26	23.02	á-D Mannofuranoside, farnesyl-	C ₂₁ H ₃₆ O ₆	384	0.32	sugar moiety	preservative
27	23.85	3-Hydroxy-4-methoxycinnamic acid	C ₁₀ H ₁₀ O ₄	194	2.01	Phenolic compound	Antimicrobial, Antioxidant Anti-inflammatory
28	27.15	Vitamin E	C ₂₉ H ₅₀ O ₂	430	0.76	Vitamin compound	Antiageing, Analgesic, Anti-diabetic, Anti-inflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, Anticoronary
29	27.37	12-Methyl-E,E-2,13-octadecadien-1-ol	C ₁₉ H ₃₆ O	280	0.35	Unsaturated fatty alcohol compound	No activity reported
30	28.49	Campesterol	C ₂₈ H ₄₈ O	400	3.19	Steroid	Antiarthritic, Hepatoprotective Antiasthma, Anti-inflammatory Diuretic, Cancer preventive Antioxidant, Hypocholesterolemic
31	28.94	Stigmasterol	C ₂₉ H ₄₈ O	412	4.60	Steroid	Hypocholesterolemic, sedative Antiviral, Antioxidant, Antihepatotoxic, Anti-inflammatory Diuretic, Cancer preventive
32	29.90	á-Sitosterol	C ₂₉ H ₅₀ O	414	2.92	Steroid	Antiarthritic, Hepatoprotective Antiasthma, Anti-inflammatory Diuretic, Cancer preventive Antioxidant, Hypocholesterolemic

**Activity Source: Dr Duke's Phytochemical and Ethnobotanical databases

Linoleic acid and linolenic acid are having many activities such as Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary and Insectifuge.

Sheikh *et al.* (2013) explored the hypoglycemic, analgesic and anti inflammatory activity of ethyl acetate extract of *Peperomia pellucida*. Various solvent extracts of *Peperomia pellucida* were found to have broad spectrum antimicrobial activity (Oloyede *et al.*, 2011). Isolation of linoleic acid and linolenic acid from *P. pellucida* were reported by Heinrich *et al.* (1998) using ¹³C NMR spectrum. Presence of steroids and triterpenoids in the present study is in accordance with the reports of Puluk Majumder (2011). Vitamin E acts in cell membrane and prevents the propagation of free radical reaction (Sheela and Uthayakumari, 2013). The presence of various bioactive components justifies the use of whole plant for various ailments by traditional medical practitioners.

Based on the results obtained, *Peperomia pellucida* could be selected for further investigation for the isolation these bioactive components for the development of effective new drugs.

ACKNOWLEDGEMENT

Authors express sincere thanks to Dr. S. Kumaravel, Senior Scientist, Indian Institute of Crop Processing Industries, Government of India, Thanjavur, Tamilnadu for providing the facilities and support to carry out the work

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