GCMS analysis of phytochemicals from methanol leaf extract of *Artemisia pallens*

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Abstract

The plant *Artemisia pallens* is cultivated throughout Tamilnadu for ritual purpose.The peoples in Tamilnadu, particularly the women are wearing these leaves on their scalp hair for their fragrance. The perfume produced from these plant to create fragrances with truly individual notes.At the same time, they were used as medicines to cure some disorders mental illness,skin disesaes and actas antihelmintic, antipyretic, antibacterial, antifungal, tonic properties, wound healing medicine. Hence in the present investigation, ethanolic extract of *Artemisia pallens* were prepared and analysed by GCMS to evaluate their phytoconsitituents. The GCMS results showed ten major peaks with nine known compounds such as(1)7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin,(2) 2,6,6-Trimethyl-bicylo [3.1.1] hept-3-ylamine (3) 7-Dehydrocholesteryl isocaproate (4) N-Hexadecanoic acid (5) Beta carotene (6) Cholst-8-en-3-ol, 14-methyl_ (3-beta,5-alpa (7) 3,6-Methano-8h-1.5.7-trioxacyclopenta [ij] cycloprop (a) azulene-4.8 (3h)(8) Cholesta-8, 24-dien-3-ol, 4-methyl (3-beta., 4-alpa)(9) Retinol, acetate.

Key Words: GCMS, Methanolic Extract, *Artemisia pallens, Davanam.*

Introduction

Even though, allopathic medicinal system has all facilities to cure various diseases, still peoples are more interested and preferred plant-based natural and traditional medicines to cure their diseases over synthetic medicines because of these traditional herbals are more natural, environment-friendly and devoid of side effects (Philomena, 2011., Sahoo and Manchikant, 2013). In Indian medical history, from the period of time immemorial, they have been used 80,000 species of plants for the treatment of various diseases and disorders (Pandey et al, 2013). In this way, the Indian traditional medical practitioners designed nearly 25,000 formulations which are prescribed by about 1.5 million practitioners in preventive, persuasive and

healing applications. However, this much of medicinal formulations are under in usage, there is no any concrete and scientific evidence to explain the mechanism and factors behind this cure. Hence, the bio-researchers now exert their interests and used modern technology to extract and characterize the bioactive compounds present in various medicinal plants to the delivery of herbal based medicines with high-activity profle. Hence in the present investigation, GC-MS were used to identify the important functional groups and phytochemical constituents present in the ethanolic leaf extract of *Artemisia pallens*. The gas chromatography-mass spectrometry (GC–MS) is one of the best, fast and accurate method to detect various compounds present in the samples.

Materials and method

The study plant *Artemisia pallens* leaves were collects from the herbal garden of Thiruvalluvar University. The information about local name and their medicinal uses were collected from local siddha practitioners and traditional healers. Immediately they were brought to the laboratory. This fresh leaves washed thoroughly with distilled water two to three times to remove the dirty particles, insect eggs and larva adhere on the leaves.Then they were chopped into small pieces for easy drying. The chopped leaves were spread on the newspaper and kept at room condition in a shade place. The entire setup left for two week for shade drying. The leaves were turned over of at least twice a day. This processes permits rapid and uniform drying of leaves. After the conformation of complete drying the leaves were made into fine powder with a help of electrical blender.

Plant extract preparation

The 5grams of fine powder was taken to the maceration process to get a plant extract. Maceration is a simple technique widely used in plant extract preparation worldwide. In this maceration process the 5grams of fine powder of *Artemisia pallens* was taken in stopped container in a ethanol solvent and allowed to stand at room temperature for in a magnetic stirrer. This process enhances the release of soluble phytochemicals from the plant cells by break the plant cell wall. After three days the ethanol extract was filtered with Whattman No-1 filter paper. The filtered plant extracts was transferred into petri plate and kept in room temperature in open condition for complete drying. After the conformation for complete drying the dried powder collected from the petri plate was transfer to small container and sent it for GC-MS analysis.

GC-MS analysis

The quantitative and qualitative analysis of phytocompounds of ethanol leaf extract of the plant *Artemisia pallens* were analysed by using GC-MS method to detect the active phytocompounds for this plant.

Plant description

Davana (*Artemisia pallens*) is an important annual aromatic herb belonging to asteraceae family, which is much priced in India for its delicate fragrance. The davana springs are commonly used in garlands, bouquets and religious offerings in most parts of the year. It is a highly fragrant herb among the various species of Artemisia, yielding essential oil of commercial importance. *A. pallens* is used in Ayurvedic systems of medicines from ancient times.

Classification

Kingdom: Plantae

Class: Eudicots

Order: Asterales

Family: Asteraceae

Genus:*Artemisia*

Species:*Pallens*

Names in various languages

In tamil the plant called as 53 names such as anantakarantam, anantapitam, cilesmanarapani, cukantacutcam, cukatmarankara, cutcampiccam, davanam, irucukantappuntu, kantotkatam, manali, manalicacceti, manalicam, manamali, manamalikkoluntu, mancarikam, manci, mancitavikam, manmali, manmalikkoluntucceti, marikkoluntu, marukkam, marukkarakkoluntu, marukkoluntu, maruvakam, maruvu, maruvucceti, mayirkkoluntu, mentiyakkoluntu, mentiyam, municatilam, napacariyacceti, napacariyam, panitanki, pintitakam, pintitakkoluntu, pintitam, pintitam, pompani, pompanicceti, puccaratturakam, talamalam, tamanakantam, tamanam, tavanakam, tavanam1, tipanicam, tipanicapputu, vacikaram, vacikaramaruvu, vauciki, vaucikikkoluntu, venkapattirakam, virai; Sanskrit- Davanam; Hindi –Davana; Kannada-Davana and manji pathre; Marathi- devna; English- Wormwood; Malayalam- Davanam.

Distribution and Habitat

The plant shares its habitat with that of the Sandalwood trees of Mysore. It is an aromatic herb found abundantly in humid habitats in the plains all over India.

Plant Morphology

A. pallens is an aromatic perennial shrub, hairy, pubescent, erect, stem, angled, ribbed and leaves are very small bluish green with yellow flower and inconspicuous belongs to the family Compositeae. The leaf is dorsiventral with isolateral mesophyll tissue. The surface of the leaf is even and uniform. The mid rib is fairly prominent and spindle shaped in cross sectional view, projecting equally on the upper and lower sides. There is a small furrow on the lower side of the mid rib. The epidermal layer of the mid rib is thin and distinct with squarish cells and smooth cuticle. There is a single large vascular bundle which is surrounded by compact parenchymatous tissue; the vascular bundle is collateral with adaxial parallel rows of xylem and abaxial are of phloem. Thick mass of sclerenchyma cells occurs both on the upper and lower sides of the vascular bundle. The mid rib is 550 µm thick. The lamina has even upper and lower surfaces. It has wide; semicircular margin .The lamina is 350 µm thick. The epidermal layers are thin with spindle shaped fairly thick walled cells. The epidermis is stomatiferous, both on the upper and lower sides. The mesophyll consists of a central horizontal layer of two or three rows of cells. On the upper and lower sides of the central row are wide, thin walled palisade cells .The vascular bundles of the lateral veins are located in the median part of the lamina. The bundles become smaller towards the margin. They are collateral with adaxial xylem cluster and abaxial phloem. The lateral veins are thin and less and form less distinct vein-islets. The islet is distinct; it is wide and inconsistent in shape. Vein terminations are present sporadically; they are simple, short and thin. Epidermal trichomes are prevalent on the surface of the leaf. There two types of trichomes, both of which are glandular in nature. This type of trichome has short unicellular stalk with two terminal cells placed end to end forming a spindle shape .The spindle shaped trichomes are diffusely distributed all over the lamina; they are 50 × 30 µm in size .. These types of trichomes are less common. The trichome has a short, unicellular stalk with a circular thin plate of eight or more triangular cells. The cells have prominent nuclei.The orbicular trichomes are random in distribution. They are 35 µm in diameter In cross sectional view, the petiole of the leaf is boat shaped with wide shallow concavity on the adaxial side and wavy and convex on the abaxial side. The epidermis is prominent and stomatiferous; the epidermal cells are squarish with thick cuticle. The ground tissue consists of a central horizontal band of compact parenchyma tissue and

narrow bands of palisade tissue, both on the adaxial and abaxial. These are larger median vascular bundle and three or more small, less prominent vascular strands on either side of the central strand. The vascular strands are collateral surrounded by a single whole of parenchymatous sheath cells. The central bundle has wide mass of extension both on the adaxial and abaxial sides. Dense epidermal trichomes are spread all along the surface of the petiole. The stem is young with primary vascular tissue. The stem surface bears dense trichomes. Epidermis is thin and distinct, comprising of small squarish cells with thick cuticle. Cortex is homogeneous and parenchymatous with small air-chambers. The stele consists of several discrete vascular bundles arranged in elliptical circle. The vascular bundle is collateral with a row of parallel rows of xylem elements. Phloem is in wide mass. Pith consists of a central elliptic cavity, with wide parenchymatous borders.

Medicinal Properties:

Artemisia pallens commonly known as "Davana" has been traditionally used in Indian folk medicine for the treatment of diabetes mellitus, wound healing and immunomodulating, antihelmintic, antipyretic, antibacterial, antifungal, tonic properties, wound healing and also as stimulant (Drurey and Wallington, 1980). It is also considered a good fodder. The oil possesses antispasmodic, antibacterial, antifungal and stimulant properties.

Phytochemical Properties

Davanone, Davana-Ether, Davana Furan and linalol are the major constituents of davana oil. Methyl cinnamate, ethyl cinnamate, bicyclogermacrene, davana ether, 2-hydroxyisodavanone, farnesol, geranyl acetate, sequiterpene lactones, germacranolides etc. are also found. The contents of davanone, the major constituent of davana oil, and linalool decreased while those of (Z) − and (E) − methyl cinnamate, (E) − ethyl cinnamate, bicyclogermacrene, davana ether, 2-hydroxyisodavanone, and farnesol increased from flower heads emergence stage to the initiation of seed set stage. Five compounds, viz., (Z) − and (E) − methyl cinnamates, (Z) − and (E) − ethyl cinnamates, and geranyl acetate, were identified for the first time in davana oil.

Photo-1. The plant *Artemisia pallens* with leaves

Results and discussion

In Indian tribal medicine and Ayurvedic literature *Artemisia pallens* is placed in a unique place because it is used for various treatment such as sour curevata, cough, skin diseases and stomachic, and also act as stimulant, flavoring, antioxidant, antihelmintic, antibacterial, anti-inflammatory, antispasmodic and carminative. Addition to this plant is used to treat diabetes mellitus, healing of wounds, modulating immune system, antihelmintic, antimicrobial, stimulant and as a tonic (Drury and Wallington, 1980). The volatile oil of this plant has been reported as antispasmodic, antifungal, antimicrobial, antibacterial, stimulant, antiseptic, disinfectant and commercially important due to its fragrance (Suresh et al,2007., Misra et al, 1991 and Ruikar et a, 2009). Naturally, this plant has rich antioxidants and good sources of antibiotics against various bacterial and fungal pathogens (Falodun, *et al.*, 2006). Hence in the present investigation, the ethanolic leaf extract of *Artemisia pallens* was analyzed by FTIR to evaluate the bioactive phytocompounds. The obtained results showed 10 major peaks. On comparison of the mass spectra of the constituents with the NIST library the nine phytocompounds were characterized and identified. Among these, nine compounds are known (phytochemical constituents) and one is unknown. The identification of the phytochemical

compounds was further confirmed based on the peak area, retention time and molecular formula of the GCMS results. They are namely (1)7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin,(2) 2,6,6-Trimethyl-bicylo [3.1.1] hept-3-ylamine (3) 7-Dehydrocholesteryl isocaproate (4) N-Hexadecanoic acid (5) Beta carotene (6) Cholst-8-en-3-ol, 14-methyl_ (3-beta,5-alpa (7) 3,6-Methano-8h-1.5.7-trioxacyclopenta [ij] cycloprop (a) azulene-4.8 (3h)(8) Cholesta-8, 24-dien-3-ol, 4-methyl (3-beta., 4-alpa)(9) Retinol, acetate. Each and every phytocompounds identified in the methanolic extracts of leaves of *Artemisia pallens* have some valuable medicinal use. The healing activity of the medicinal plants depends upon the nature of phytoconstituents present in the plant (Sen, *et al*., 2016). A large number of naturally occurring phytoconstituents have been identified from various plants. These phytoconstituents show lot of beneficial effects with advantages in treating diseases and disorders synthetic chemical drugs. In many researches, several bioactive phytocompounds have been detected by GC-MS (Hema, *et al*., 2011). In the recent years, the instrument gas chromatography- mass spectrometry (GC-MS) has established as a key technique in identifying and profiling the secondary metabolite in both plant and non-plant species. The phytocompound 7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin obtained in the present results is otherwise known as coumarin and 7-hydroxycoumarin. This compound act as anti-cancer agents and used to cure advanced malignancies in some patients with (Myers et al, 1994) . The phytocompound dehydrocholesteryl function as cholesterol precursor in serum. In animal skin this compound is converted into vitamin D3. The presence of this compound in human skin enables humans to manufacture vitamin D3 (cholecalciferol) from ultraviolet rays in the sun light, via an intermediate isomer pre-vitamin D3. It is also found in the milk of several mammalian species. In insects it is a precursor for the hormone ecdysone, required for reaching adulthood (Young, 2012).

The n-Hexadecoic acid present in this plant has chemical formula C16H32O2, molecular weight-256.4241 and CAS Registry Number: 57-10-3. The other names for this compound is Hexadecanoic acid;; Palmitic acid; Pentadecanecarboxylic acid; 1-Pentadecanecarboxylic acid; Cetylic acid; Emersol 140; Emersol 143; Hexadecylic acid; Hydrofol; Hystrene 8016; Hystrene 9016; Industrene 4516; Glycon P-45; Prifac 2960; NSC 5030; Palmitinic acid; Kortacid 1695; Hexadecanoic acid (palmitic acid); Hexadecanoic (palmitic) acid; Palmitic acid (hexadecanoic acid). It has lot of medicinal properties, it act as anticancer (Harada, *et al.* ,2002), antioxidant, hyperchloesterolemic, nematicide, antiandrogenic flavour,haemolytic and

5- alpha reductase inhibitor. Hexadenoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata* (Grace,*et al*., 2002) and *Melissa officinalis* (Sharafzadeh ,*et al*., 2011). (Parasuraman, *et al*., 2009) identified 17 compounds with n-Hexadecanoic acid and Octadecanoic acid as the major compounds in the leaves of *Cleistanthu scollinus.* GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-Hexadecanoic acid (Siddig Ibrahim *et al.,* 2009). N-hexadecanoic acid, Hexadecanoic acid, Phytol, 9, 12 - Octadecadienoic acid, 9, 12, 15-Octadecatrienoic acid and Squalene were identified in the ethanol leaf extract of *Aloe vera* (Arunkumar and Muthuselvam, 2009) and *Vitex negundo* (Praveen kumar, *et al*., 2010). Squalene is used in cosmetics as a natural moisturizer**.** (Devi, *et al*. 2009) reported that *Euphorbia long an* leaves mainly contained n-hexadecanoic acid and 9, 12-Octadecadienoic acid. The compound beta-carotene obtained is mainly used to decrease asthma symptoms caused by exercise; to prevent certain cancers, heart disease, cataracts, and age related macular degeneration (AMD); and to treat AIDS, alcoholism, Alzheimer's disease, depression, epilepsy, headache, heartburn, high blood pressure, infertility, Parkinson's disease, rheumatoid arthritis, schizophrenia, and skin disorders including psoriasis and vitiligo. The compound retinol identified in plant *A.pallens* is a synthetic derivative of vitamin A, the group of fat-soluble vitamins commonly present in carrots, eggs and sweet potatoes and used as a dietary supplement. The compound retinol is incorporated into age-preventive, skin care routines, accelerate skin renewal, enhance collagen production and reduce the aging appearance, uneven texture and age spots on skin. The compound retenoid is present in many form such as acitretin, adapalene, alitretinoin, isotretinoin, tazarotene and tretinoin. Among these, alitretinoin used for the treatment of skin lesions in AIDS patients with Kaposi's sarcoma (a type of skin cancer). Bexarotene used for the treatment of cutaneous T-cell lymphoma (CTCL, a rare cancer of the lymph Updated measures for pregnancy prevention during retinoid use). The compound 4alpha-hydroxymethyl-4beta-methylzymosterol identified in this plant extract is a 3beta-sterol that consists of 4beta-methylzymosterol in which the 4alpha-hydrogen is replaced by a hydroxymethyl group. This compound is act as a metabolite in human.

Table.1. Retention time and related bioactive phytocompounds in the ethanolic leaf extract of *Artemisia pallens*

S.NO	R/T	Name of the compounds	Molecular formula	MW	Peak area%
1	19.840	7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin_	$C_{14}H_{14}O_3$	230	2.997
2	20.321	2,6,6-Trimethyl-bicylo [3.1.1] hept-3-ylamine	$C_{10}H_{19}N$	153	2.709
3	20.516	7-Dehydrocholesteryl isocaproate_	$C_{33}H_{54}O_2$	482	1.668
4	21.746	N-Hexadecanoic acid_	$C_5H_{11}O_3N$	133	11.299
5	22.992	Unknown-	-	-	4.595
6	25.958	Beta carotene_	$C_{40}H_{56}$	536	1.913
7	26.208	Cholst-8-en-3-ol, 14-methyl_ (3-beta,5-alpa)_	$C_{28}H_{48}O$	400	4.062
8	26.808	3,6-Methano-8h-1.5.7-trioxacyclopenta [ij] cycloprop (a) azulene-4.8 (3h)_	$C_{15}H_{18}O_6$	294	9.815
9	27.729	Cholesta-8, 24-dien-3-ol, 4-methyl_ (3-beta., 4-alpa)_	$C_{28}H_{46}0$	398	8.356
10	28.679	Retinol, acetate_	$C_{22}H_{32}O_2$	328	52.585

Qualitative Report

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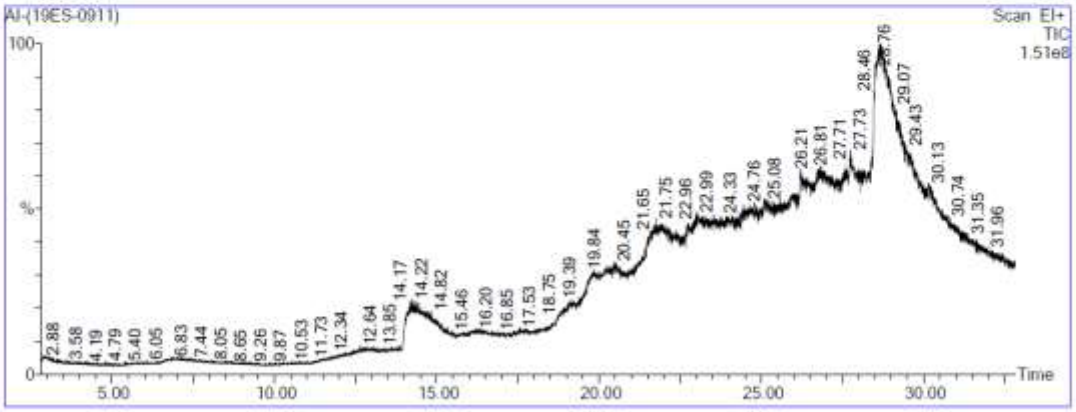

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1	19.840	3407	11,406,531	3,421,281.0	2.997	5.70
2	20.321	3503	9,528,002	3,092,729.8	2.709	5.15
3	20.516	3542	9,541,543	1,904,163.8	1.668	3.17
4	21.746	3788	19,100,292	12,897,470.0	11.299	21.49
5	22.992	4037	12,071,302	5,244,779.5	4.595	8.74
6	25.958	4630	8,839,346	2,183,478.0	1.913	3.64
7	26.208	4680	19,454,242	4,636,891.5	4.062	7.73
8	26.808	4800	20,109,688	11,203,440.0	9.815	18.66
9	27.729	4964	24,832,960	9,538,259.0	8.356	15.89
10	28.679	5174	72,261,008	60,024,016.0	52.585	100.00

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 60°C for 2.80 min, ramp 10°C/min to 300°C, hold 6 min, InjAauto=260°C, Volume=0 µL, Split=10:1, Carrier Gas=He, Solvent Delay=2.80 min, Transfer Temp=230°C, Source Temp=230°C, Scan: 40 to 600Da, Column 30.0m x 250µm

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Page 1 of 1
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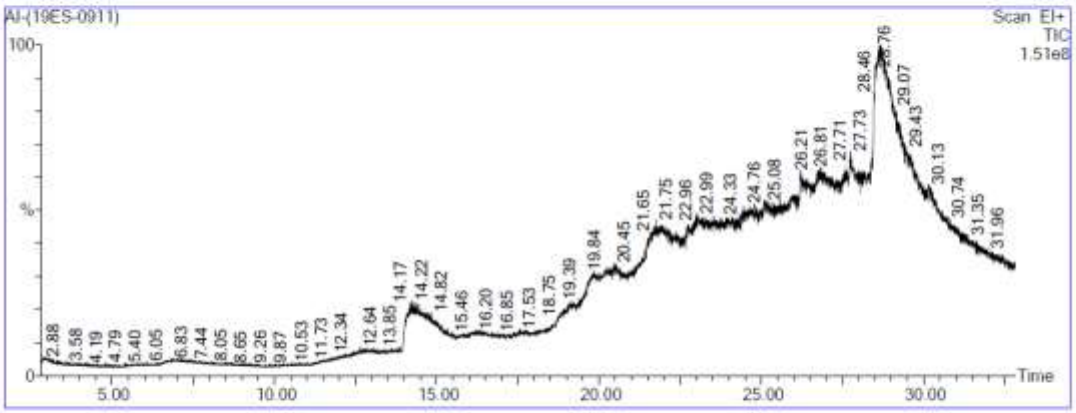

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Spectrum peaks for possible Phyto-compounds in the ethanol extract of *Artemisia pallens*