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GC-MS study and isolation of a sesquiterpene lactone from *Artemisia pallens*

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ABSTRACT

Artemisia pallens wall is a potant medicinal plant used in Ayurvedic system of medicines since ancient times. Taking into consideration the medicinal importance of the plant, a fraction of acetone extract was analyzed using GC-MS and the structures were confirmed by genesis. The major constitutents were alpha santonin, diisobutyl phthalate, tetradecane etc. The presence of alpha santonin and its isolation has been shown for the first time. The structure of the isolated compound has been elucidated by spectral analysis (MS, ^{1}H-NMR, ^{13}C-NMR, DEPT etc.). It is further confirmed by single crystal X-Ray crystallography.

Key words: GC-MS, sesquiterpene lactone, *Artemisia pallens.*

INTRODUCTION

Ayurveda is a 5000 year-old system of natural healing that has its origins in the Vedic culture of India. Medicinal plants and herbs contain substances known to modern and ancient civilizations for their healing properties.

Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times[1]. Over 50% of all modern clinical drugs are of natural product origin[2] and natural products play an important role in drug development programs in the pharmaceutical industries[3].

A. Pallens is a small and aromatic herbaceous plant which is native to the southern part of India, especially to the states of Karnataka, Tamil Nadu, Andhra Pradesh and in Maharashtra. In the regional languages of the south, it is known by several names as “davanam” in Tamil, “davanamu” in Telugu and “davana” in Kannada. Its leaves and flowers are highly valued in the making of floral decorations and oils. Leaves are very small, bluish green with yellow flowers and inconspicuous. It is utilized in traditional Ayurvedic medicinal formulations. Oral administration of the methanol extract of the aerial parts of Artemisia pallens Wall (Used in Indian folk medicine for the treatment of diabetes mellitus) led to significant blood glucose lowering effect in glucose-fed hyperglycemic and alloxan-induced diabetic rats[4]. Essential oil of davana is useful as antiseptic and disinfectant[5]. The present work is carried out in order to evaluate phytochemicals of therapeutic potential.

MATERIAL AND METHODS

Plant Material

The plant material was collected from Jejuri, Maharashtra state, India. It was authenticated at Botanical Survey of India, Pune. Its authentication number is BSI/WC/Tech/2008/1059.

Extraction

Air shade dried and powdered plant material (100 g) was extracted with acetone by stirring for 18 hours at room temperature. Solvent was recovered under reduced pressure to obtain crude extract. The extract produced was 7.27 % The crude extract (7g) was broad fractioned on silica gel (60-120, 10 g) by n-hexane , non-polar solvent, with increasing percentage of acetone.Thus seven broad fractions(A – G) were collected. The details of it are summarized in Table 1.

GC-MS of fraction (B) was carried out. Fraction (B) (650 mg) was adsorbed on 2 g silica and column was eluted through hexane: ethyl acetate with increasing polarity of ethyl acetate. Details of it are given in Table 2. The compound was further purified by repeated crystallization using mixed solvent system.

GC-MS analysis

Gas chromatography analysis was performed by Agilent 6890N with FID using HP-5 capillary column. GC-MS analysis was performed using a Shimadzu QP 5050A mass spectrometer coupled with a Shimadzu 17A gas chromatograph fitted with a split-splitless injector and a DB-5 fused silica capillary column (30m × 0.25 mm i. d., 0.25 µm film thickness). Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The injection port was maintained at 250 ^{o}C, and the split ratio was 40:1 . Oven temperature programming was done from 50 to 280 ^{o}C, at 10 ^{o}C/min, and it was kept at 280 ^{o}C for 5 min. Interface temperature was was kept at 250 ^{o}C. Ionization mode was electron Impact ionization and the scanning range was from 40 amu to 400 amu. Mass spectra were obtained at 0.5 sec. Interval. The spectra of the compounds were matched with NIST and Wiley library. Their structures were defined by the % similarity values.

RESULTS AND DISCUSSION

GC-MS indicates presence of different chemical constituents in it. The compounds identified are listed in Table 3. The major constituents were alpha santonin, tetradecane, hexadecane, diisobutyl

Table 1: Broad Fractionation of Acetone Extract

Fr. NO.	Eluent	Total volume collected (ml)	Weight (g)	Approximate composition
A	Hexane (100%)	5 × 100	2.086	Mixture of unidentified compounds
B	Hexane:Acetone (95:5)	6 × 100	0.678	Mixture of unidentified compounds + **compound 1**
C	Hexane : Acetone (90:10)	5 × 100	0.415	Mixture of unidentified compounds
D	Hexane : Acetone (80:20)	6 × 100	0.725	Mixture of unidentified compounds
E	Hexane : Acetone (70:30)	6 × 100	0.988	Mixture of unidentified compounds
F	Hexane : Acetone (60:40)	5 × 100	0.519	Mixture of unidentified compounds
G	Hexane : Acetone (50:50)	5 × 100	0.227	Mixture of unidentified compounds

phthalate, pentatriacontane etc. Out of these alpha santonin was isolated as a white crystalline solid. It showed sharp melting nature at 172 ^{0}C. LC-MS of the compound exhibited a molecular ion peak at *m/z* 247 which suggested the molecular formula $C_{15}H_{18}O_3$. The IR spectrum showed the absorption bands at 1785 cm^{-1} (lactone carbonyl), 1658 cm^{-1} (disubstituted alkene) and 1629 cm^{-1} (carbonyl group in conjugation).

Methyl protons at C-3 showed a doublet at 1.19 δ. Methyl protons of 5a at tetra substituted carbon showed a singlet at 1.27 δ. Methyl protons at C-9 (sp^2 hybridized carbon) showed a singlet at 2.05 d. A doublet for lactone proton appeared at 4.77 δ. Doublets of olefinic protons on C-6 and C-7 are noticed at 6.67 and 6.18 δ respectively. A multiplet at 2.46 δ is observed for C-3 proton.

The ^{13}C NMR reveled presence of total 15 carbon atoms. After recording DEPT spectrum it was clear that the molecule showed three quartets, one triplet, six doublets and five singlets. The structure of the compound was established on the basis of ^{1}H and ^{13}C NMR as [3S – (3, 3a , 5a, 9b)] – 3a, 5, 5a, 9b – tetrahydro- 3, 5a, 9 – trimethyl naphtha [1, 2 – b] furan – 2, 8 (3H, 4H) – dione.

Crystal Data for the Compound 1

Empirical formula	C15 H18 O3
Formula weight	246.29
Temperature	273(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic

Table 2: Rechromatography of Fraction (B)

Fr. NO.	Eluent	Total volume collected (ml)	Weight (g)	Approximate composition
B-1	Hexane (100%)	3 × 100	12	Mixture of unidentified compounds
B-2	Hexane : Ethyl Acetate (90:10)	7 × 100	96	Mixture of unidentified compounds
B-3	Hexane : Ethyl Acetate (80:20)	8 × 100	95	Mixture of unidentified compounds
B-4	Hexane : Ethyl Acetate (80:20)	6 × 100	128	Mixture of unidentified compounds +compound 1
B-5	Hexane : Ethyl Acetate (70:30)	6 × 100	150	Mixture of unidentified compounds
B-6	Hexane : Ethyl Acetate (60:40)	6 × 100	58	Mixture of unidentified compounds

Table 3: GC-MS Analysis Data

S. No	Retention Time (minutes)	Name of Compound	% Similarity	Molecular ion peak (amu)	Base peak (amu)
1.	8.9’	Tetradecane	96 %	198	57
2.	10.4’	Hexadecane	98 %	226	57
3.	11.8’	Octadecane	97 %	254	57
4.	12.4’	Di-isobutyl phthalate	94 %	278	149
5.	13.0’	Pentatriacontane	89 %	492	57
6.	15.0’	Alpha santonin	94 %	246	173

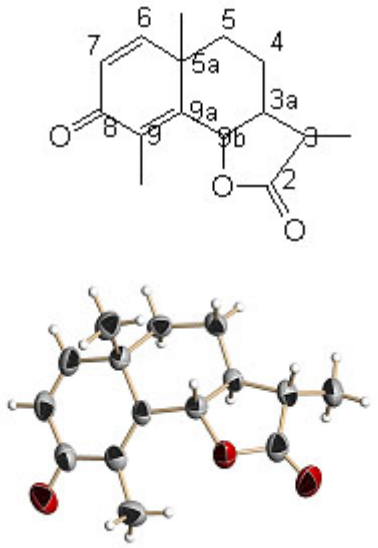
Space group	P212121
Unit cell dimensions	a = 6.9962(5) Å α= 90°.
	b = 10.7176(8) Å β= 90°.
	c = 34.572(3) Å γ = 90°.
Volume	2592.3(3) Å3
Density (calculated)	1.262 Mg/m3
Crystal size	0.33 x 0.10 x 0.06 mm3
Completeness to θ 25.00° 99.9 %	
Final R indices [I>2sigma(I)]	R1 = 0.0438, wR2 = 0.0915

^{13}C NMR Spectral data of the compound ($CDCl_3$ at 100 MHz)

Carbons	Chemical shift in ppm-d
C – 2	177.65 (*s*)
C – 3	40.87 (*d*)
C – 3a	53.53 (*d*)
C – 4	23.06 (*t*)
C – 5	37.83 (*d*)
C – 5a	41.38 (*s*)
C – 6	155.13 (*d*)
C – 7	125.86 (*d*)
C – 8	186.34 (*s*)
C – 9	128.68 (*s*)
C – 9a	151.30 (*s*)
C – 9b	81.40 (*d*)
C3 - $\underline{C}H_3$	12.51 (*q*)
C9 - $\underline{C}H_3$	10.93 (*q*)
C5a - $\underline{C}H_3$	25.14 (*q*)

^{1}H NMR Spectral data of the compound ($CDCl_3$ at 400 MHz)

Protons	Chemical shift in ppm- δ
C3-CH_3	1.19 (*d*, 6.8 Hz, 3 H)
C9-CH_3	2.05 (*s*, 3 H)
C5a-CH_3	1.27 (*s*, 3 H)
H-9b	4.77 (*d*, 10.8 Hz, 1 H)
H-6	6.67 (*d*, 10 Hz, 1 H)
H-7	6.18 (*d*, 10 Hz, 1 H)
H-3	2.46 (*m*, 1 H)



Compound 1

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