



FT-IR, HPLC, GC-MS and wis of *Peucedanum dhana* buch.-Ham. Ex CB Clarke (*Bhojraj*): A rare and endangered medicinal plant of Chotanagpur, Jharkhand

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Abstract

The ethnomedicinal plant, *Peucedanum dhana* Buch-Ham from Jharkhand was extensively investigated for its phytochemical profiling. The preliminary tests revealed the presence of alkaloids, cardiac glycosides, steroids, phenols, tannins, saponins and fixed oils. The FTIR, HPLC and GC-MS analysis revealed several bioactive compounds such as *Lauric acid*; *Elaidic acid*; *1-Phenyl-1-nonyne*; *2-Furanmethanamine* and *1,1,3,3-Tetraallyl-1,3-disilacyclobutane* in the methanolic extract of root, while the leaf exhibited to possess *2,2-Dimethylchromenocoumarin*; *α-Caryophyllene*; *β-Caryophyllene*; *2-Ethylacridine*; *1,1-Diethoxypentane* and *8,8-Dimethyl-2-oxo-9,10-dihydro-2H,8H-pyrano[2,3-f]chromen-9-yl-2-methyl-2-butenolate*. The leaf also possesses *β-Elemente*, an anti-proliferative agent of cancer cells. Moreover, GC-MS analysis detected several compounds, whose bioactivities have not yet been known. *P. dhana* could be a rich source of several drugs used against diseases such as cancer, oral and dental diseases, inflammation, neural damage, depression, alcoholism and epilepsy. Hence, the endangered plant draws urgent attention to conserve and propagate it before it becomes extinct.

Keywords: *Peucedanum dhana*, FT-IR, HPLC, GC-MS, pharmacognostic, Chotanagpur, Jharkhand

1. Introduction

Peucedanum dhana Buch-Ham. ex CB Clarke (Apiaceae) is locally called as *Hukka kanda* or *Mann Tirio* by Oraon tribes, *Huring rengia banam* by Munda tribes and *Bhojraj* by Sadri speakers of Chotanagpur plateau of Jharkhand. The plant has become exotic to Pahar Kapu foot hills of Latehar, Jharkhand as observed during the survey (2015-2017). The root powder of plant is used against male sexual disorders and male impotency along with the roots of Tejraj (*Peucedanum nagpurensis* Prain) and Kamraj (*Byttneria herbacea* Roxb.). The plant is used for the same purpose by the Raj-Gond tribe of Chhattisgarh^[1], whereas the root juice is given orally against arthritis by the tribes of Kalahandi, Odisha^[2]. Nevertheless, some tribes of the same state administer the root paste or pills for enhancing sexual power, vigor and vitality, while the roots are chewed for enhancement of sexual desire^[3].

Moreover, the tribes of Jharkhand use the root powder not only for sexual debilities but also as an ingredient for brewing local drink. However, the ethnomedicinal uses as well as the phytochemical investigation of the plant are being reported for the first time from the land of Jharkhand. Hence, the present study is the scientific investigation of different parts of the plant with various parameters.

Plant description

Glabrous perennial erect herb. Leaves 2-3 pinnate, twice 3-partite, leaflets 3, lanceolate or ovate, terminal longer than the

lateral. Inflorescence of compound umbels with 12-16 rays. Flower small, yellow, pedicellate, ebracteate; bracteoles 4-7. Fruits truncate, apex emarginated, base narrow (Fig.1).



Fig 1: *Peucedanum dhana* a) Habit b) Aromatic Roots c) Herb in bloom

2. Materials and Method

2.1 Collection of plant materials

The herbarium specimen and the roots and leaves of *P. dhana* were collected from the foothills of Paharkapu, Latehar, Jharkhand. The plant was identified and authenticated by Dr. S. John Britto, the Director of Rapinat Herbarium, St. Joseph's College, Trichy, Tamil nadu. The herbarium specimen and the photographs were deposited to the same herbarium with accession number RHT 67786.

2.2 Extraction of phytochemicals

The roots and leaves of *P. dhana* were dried under shade for

two weeks at room temperature, powdered mechanically and kept in air-tight containers. The 10g of the root and leaf powders were extracted with 50 ml of methanol and double distilled water in a rotary shaker for 72 hours.

2.3 Preliminary phytochemical screening

Preliminary analysis of the methanolic and aqueous extracts of roots and leaves of *P. dhana* was carried out by standard methods [4-7]. The bioactive compounds such as alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, steroids, etc. were screened for their presence.

2.4 Anatomy and Powder Microscopy

The microscopic studies of the powders and of the transverse sections of the root and leaf were carried out by following standard procedures [8].

2.5 FT-IR analysis

Fourier Transform-Infrared Spectroscopy (FT-IR) of the powdered materials was carried out by Potassium Bromide (KBr) technique adopted from standard source [9]. The spectra were recorded with Perkin Elmer FT-IR Spectrum RX1 at spectral range of 4000–400cm⁻¹ in room temperature (25±2°C). Interpretations of peaks of the spectra were done by referring to standard FT-IR tables to determine the functional groups of the bioactive compounds [10,11].

2.6 HPLC analysis

High Performance Liquid Chromatography (HPLC) analysis of methanolic extracts of the root and leaf was carried out in Shimadzu HPLC instrument equipped with auto-sampler and

diode array detector adopting the standard procedures and conditions [12, 13]. The Acetonitrile and HPLC grade water were used as solvents for gradient elution. The running time was 30 minutes and the chromatograms were obtained at 254nm.

2.7 GC-MS analysis

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of the extracts of the root and leaf was carried out in GC-MS Shimadzu instrument by adopting standard procedure and conditions [14, 15]. Identification and interpretation of compounds were done by the comparison of mass spectra with the database of NIST library. The molecular formula, molecular weight and molecular structures of the compounds were ascertained from the database of PubChem [16] and Chem Spider [17]. The bioactivities of the compounds were determined from various sources which have been referenced.

3. Results and Discussions

3.1 Phytochemical screening

The results of preliminary phytochemical analysis of root and leaf of *P. dhana* are presented in Table 1. The preliminary tests revealed the presence of following bioactive compounds both in root and leaf– alkaloids, phenolic compounds, tannins, steroids, saponins, fixed oils, proteins, free amino acids and carbohydrates. Moreover, the methanolic extracts of root and leaf showed the high concentration alkaloids. Likewise, the aqueous extract of root contained high amount of starch. The cardiac glycosides were detected in moderate amounts in both the parts of the plant. However, the plant parts showed the absence of flavonoids.

Table 1: Phytochemical screening of *Peucedanum dhana*

S.N.	Plant parts → Phytochemicals ↓	Reagents / Tests	Root		Leaf	
			Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract
1	Alkaloids	Hager's	+++	–	+++	–
		Mayer's	+++	–	+++	–
2	Flavonoids	Pew's	–	–	–	–
		Shinoda	–	–	–	–
3	Phenols & Tannins	FeCl ₃	+	+	+	+
		Lead Ac	+	+	+	+
4	Steroids	Conc. H ₂ SO ₄	–	+	–	+
		Keller Kiliani	–	+	–	+
5	Saponins	Foam test	–	+	–	+
6	Fixed oils	CuSO ₄ Test	+	+	+	+
7	Anthral glycosides	KOH Test	–	–	–	–
8	Cardiac glycosides	Keller Kiliani	++	+	++	+
9	Proteins	Xanthoproteic	++	–	–	–
10	Amino acids	Ninhydrin	+	–	+	+
11	Carbohydrates	Molisch's	+	++	–	+
12	Reducing sugars	Benedict's	+	++	–	+
13	Starch	Iodine test	–	+++	–	–

Very high (++++), high (+++), moderate (++), low (+) and nil (–)

3.2 Powder Microscopy

Powder microscopy of the root exhibited several cell inclusions such as numerous globular starch grains, prismatic crystals of calcium oxalate and oil globules (Fig. 2a+b).

Moreover, it also showed a few rosette crystals. The oil globules may possess bioactive compounds responsible for the bioactivity.

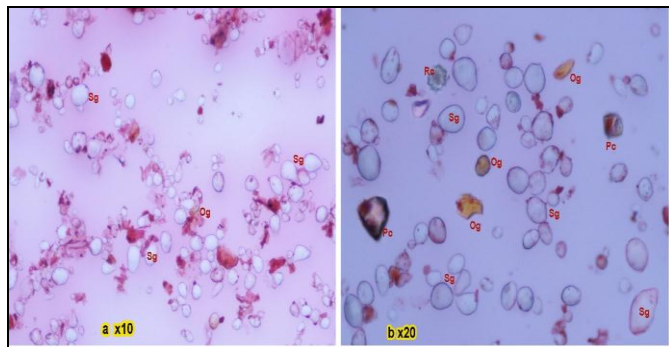


Fig 2(a b): *P. dhana*-powder Microscopy of Root; Pc=Prismatic crystal; og =oil globule; Rc=Rosette crystal; Sg= Starch grain

Likewise, the fine powder of the leaf of *P. dhana* exhibited the presence of prismatic crystals, oil globules, a few rosette crystals, globular starch grains, oleo resins, unicellular trichomes, fibres and spiral xylem vessels (Fig. 3a+b). It also showed some cell debris and undefinable materials.

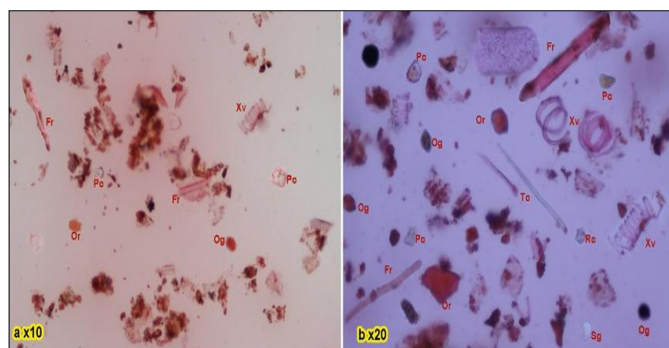


Fig 3(a, b): *P. dhana*-powder Microscopy of Leaf; Pc=Prismatic crystal; og =oil globule; Rc=Rosette crystal; Sg= Starch grain; Or=Oleo resin; Tc=Unicellular trichome; Fr=Fibre; Xylem vessels

3.3 Anatomy

The transverse sections of the root (Fig. 4a-l) exhibited epidermis, followed by cortex, parenchyma, vascular bundles and xylem rays. The vascular bundles are separated by prominent medullary rays. Endodermis is distinct. The secondary metabolites such as oil globules were densely observed in the cortex region. The prismatic and styloid crystals of calcium oxalate were smaller in the medullary rays, while they were larger in other regions.

Similarly, the T.S. of petiole exhibited protective layers of cuticle and epidermis followed by chlorenchyma, ground tissue and pith (Fig. 5a-f). The 6-7 vascular bundles are embedded in the ground tissue and arranged in an irregular ring. The vascular bundles are open and consist of phloem, xylem, cambium and sclereids. A two-layered sclerenchyma acts like a base for each vascular bundle. The distinct oil globules and prismatic crystals are scattered.

The T.S. of the leaflet exhibited upper and lower epidermis covered with cuticle. The mid-rib of leaflet consists of collenchyma and a vascular bundle (Fig. 6a-i). The lamina exhibited palisade mesophyll, spongy mesophyll, vascular bundles and air cavity. The lower epidermis showed several dicytic stomata protected by two guard cells. The leaflet too, exhibited the presence of oil globules and prismatic crystals.

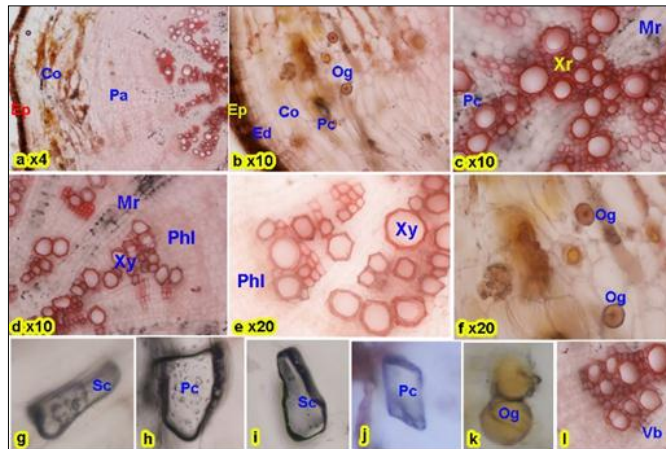


Fig 4(a, b): *P. dhana*-powder T.S of Root; Ep= Epidermis; Ed=Endodermis, Co=Cortex; Parenchyma; Og=Oil globule; Mr= Medullary ray, Xr=Xylem ray, Xy=Xylem; Phl=phloem; Vb=Vascular bundle; Pc =Prismatic crystal; Sc=Styloid crystal

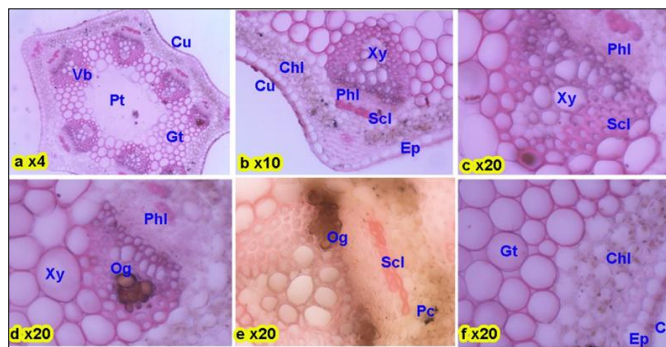


Fig 5(a, f): *P. dhana*-powder - T.S of Petiole; Cu=Cuticle; Ep=Epidermis; Gt=Ground tissue; Pt=Pith; Chl=Chlorenchyma; Scl=Sclerenchyma; Vb=Vascular bundle; Xy=Xylem; Phl=phloem; Pc=Prismatic crystal; Og=Oil globule

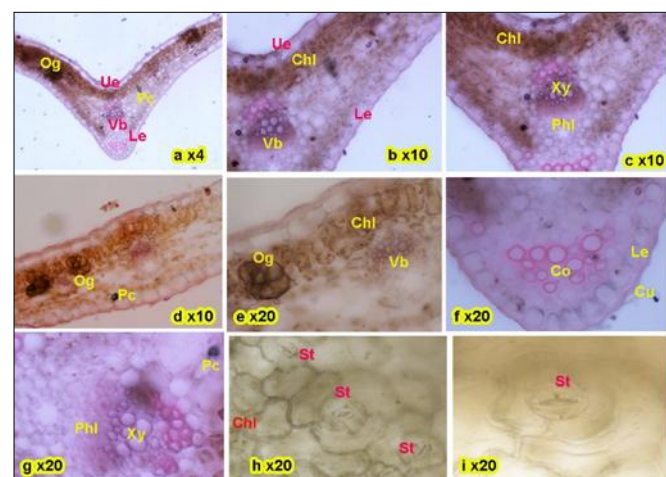


Fig 6(a, i): *P. dhana*- T.S of Leaflet; Cu=Cuticle; Ue=Upper epidermis; Le=Lower Epidermis; Chl=Chlorenchyma; Co=Collenchyma; Vb=Vascular bundle; Xy=Xylem; Phl=phloem; Pc=Prismatic crystal; Og=Oil globule; St=Stomata

3.4 Results of FT-IR Analysis

The FT-IR analysis of the root powder generated 20 peaks, while that of leaf 27 peaks with characteristic absorptions

(Table 2). According to the absorptions, the following functional groups of the unknown compounds were interpreted – alkane, alkene, alkyne, ester, carboxylic acid, benzene ring, amine, ketone, alcohol, ether and alkyl halides. The chromatograms of FT-IR analysis of the root and the leaf are given in Fig. 7 and 8 respectively.

Table 2: FT-IR Analysis of *P. dhana*

Peak #	Root of <i>P. dhana</i> Characteristic Absorptions (cm-1)	Leaf of <i>P. dhana</i> Characteristic Absorptions (cm-1)
1	3390.05	3405.90
2	2928.18	2923.49
3	2094.49	2853.39
4	1639.41	2119.81
5	1419.71	1737.24
6	1384.28	1617.17
7	1339.05	1516.18
8	1242.00	1503.10
9	1202.81	1440.90
10	1157.56	1418.46
11	1080.22	1374.40
12	1019.94	1320.41
13	932.39	1261.97
14	861.24	1208.25
15	765.08	1099.38
16	709.21	1068.93
17	609.33	1036.40
18	575.63	915.49
19	529.75	854.69
20	437.59	834.41
21	-	819.87
22	-	799.44
23	-	768.78
24	-	663.52
25	-	611.30
26	-	536.38
27	-	469.99

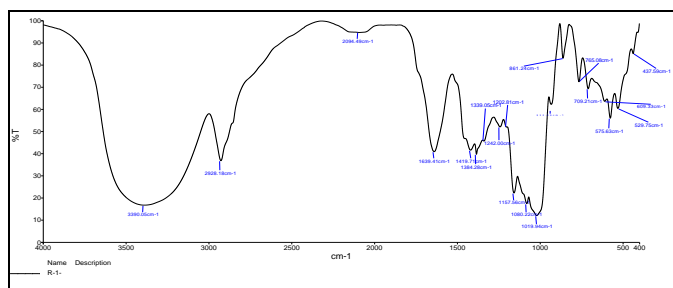


Fig 7: FT-IR Chromatogram of Root of *P. dhana*

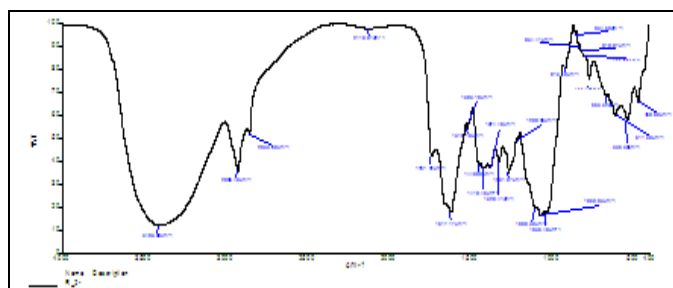


Fig 8: FT-IR Chromatogram of Leaf of *P. dhana*

3.5 Results of HPLC Analysis

The HPLC analysis of extracts of root and leaf of *P. dhana* exhibited 4 peaks each (Table 3 & 4). The chromatograms of the root and leaf are given in Fig. 9 and 10 respectively. The peak #1 of the root showed the highest area percentage (98.665%), indicating the presence of a particular compound in greater amount. The peak #1 of the leaf, too showed the highest area percentage (71.553%). The other higher area peaks of the leaf were #2 (10.945%), and #3 (17.412%). The greatest area and height percentage of peak #1 of the root and the leaf clearly indicate the presence of a specific bioactive compound in large quantity.

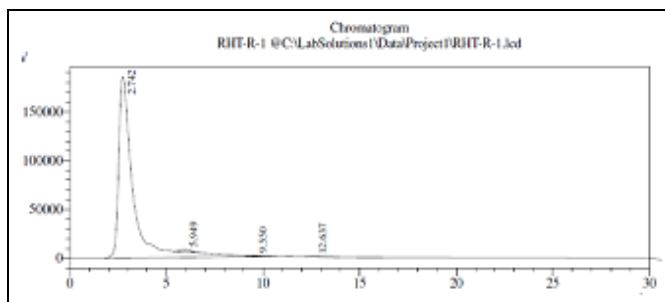


Fig 9: HPLC Chromatogram of **Root** of *P. dhana*

Table 3: HPLC Peak Table of **Root** of *P. dhana*

Peak#	Ret. Time	Area	Height	Area%	Height%
1	2.742	9725794	185874	98.665	98.244
2	5.949	99454	2591	1.009	1.370
3	9.550	15426	390	0.156	0.206
4	12.637	16678	341	0.169	0.180
Tot		9857352	189196	100.00	100.00

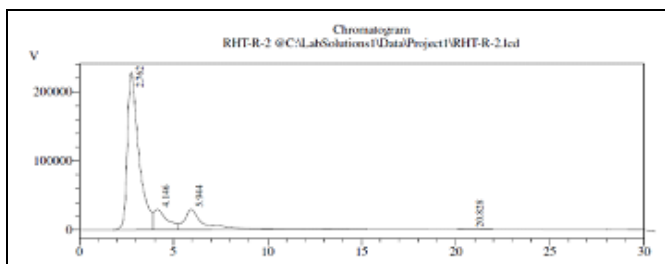


Fig 10: HPLC Chromatogram of **Leaf** of *P. dhana*

Table 4: HPLC Peak Table of **Leaf** of *P. dhana*

Peak#	Ret. Time	Area	Height	Area%	Height%
1	2.762	9763916	228634	71.553	79.379
2	4.146	1493563	29598	10.945	10.276
3	5.944	2375921	29565	17.412	10.265
4	20.828	12297	233	0.090	0.081
Tot		13645697	288030	100.00	100.00

3.6 Results of GC-MS Analysis

The GC-MS analysis of the extracts of root and leaf of *P. dhana* exhibited 8 and 21 bioactive compounds respectively (Table 5 and 6). The bioactive compounds have been tabulated with their peak #, retention time, area percentage, molecular formula, molecular weight and their bioactivities. The chromatograms of the root and leaf extracts have been shown in Fig. 11 and 12 respectively.

The root of *P. dhana* revealed three compounds with greater area percentage, viz. *9-Octadecenoic acid, (E)-* (54.42%); *1-Phenyl-1-nonyne* (27.46%) and *1,1,3,3-Tetraallyl-1,3-disilacyclobutane* (5.10%) (Table 5). The first compound is pharmaceutically important and the bioactivities of the latter two are yet to be determined. The compound *2-Furanmethanamine* showed lesser area percentage (3.56%) which is used for the synthesis of several drugs.

The leaf of *P. dhana* exhibited several compounds with greater area percentages, viz. *2,2-Dimethylchromenocoumarin* (3 peaks - 36.66%, 18.52%, 8.68%); *α-Humulene* (5.41%); *8,8-Dimethyl-2-oxo-9,10-dihydro-2H,8H-pyrano[2,3-f]chromen-9-yl-2-methyl-2-butenolate* (6.81%). The GC-MS analysis has detected several compounds, whose bioactivities have not yet been confirmed or discovered (Table 6).

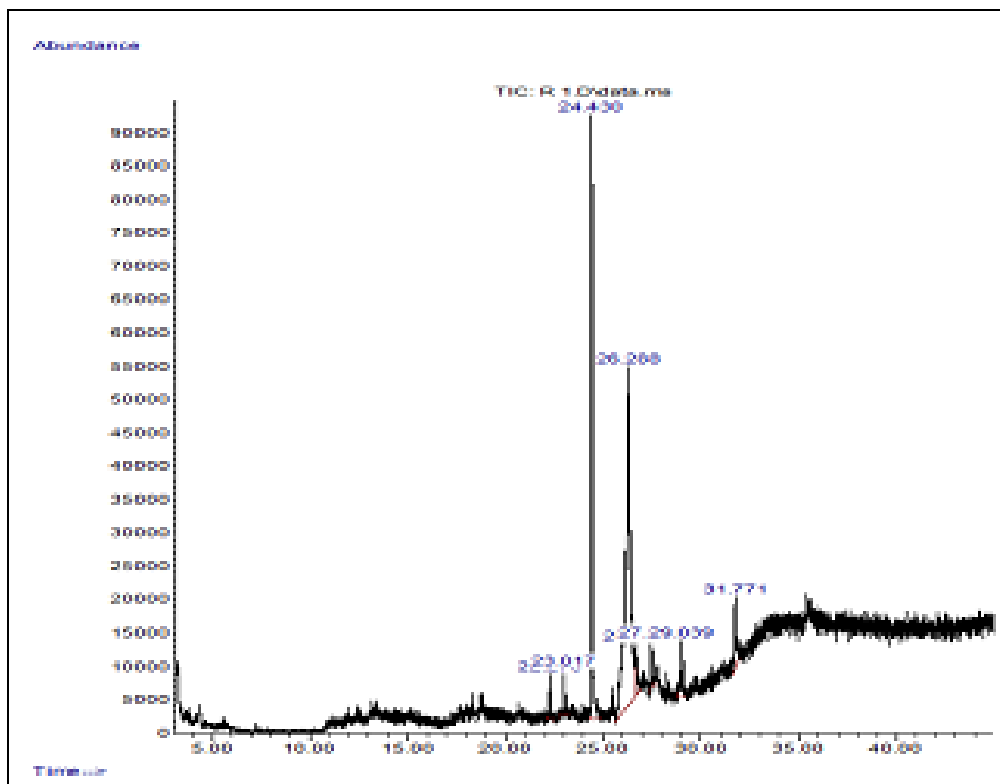


Fig 11: GC-MS Chromatogram of Root of *P. dhana*

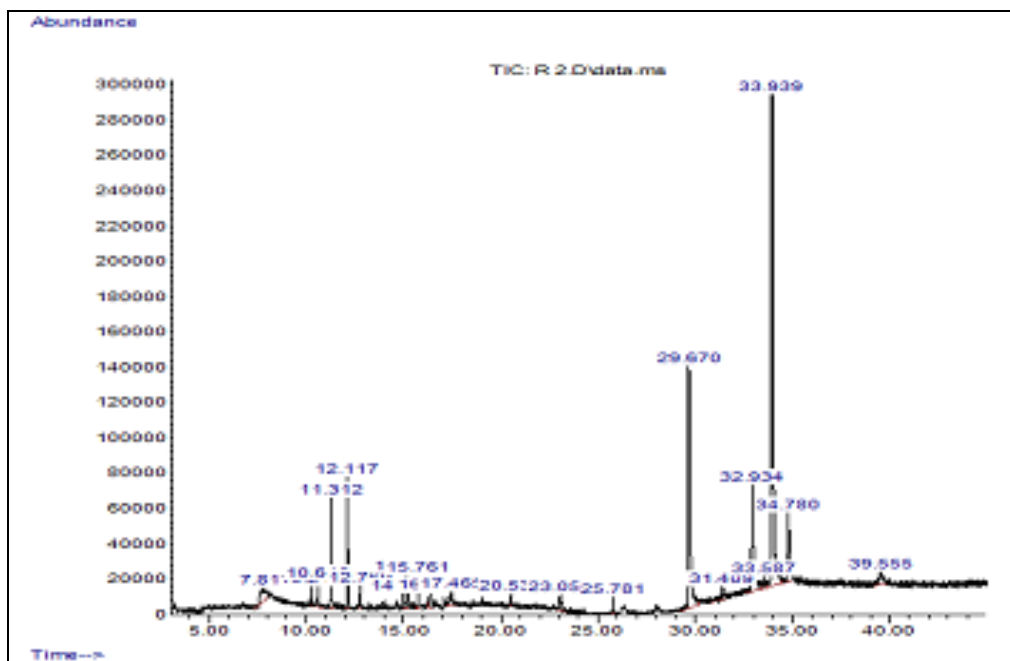


Fig. 12: GC-MS Chromatogram of Leaf of *P. dhana*

Table 5: Compounds detected in Root extract of *P. dhana* by GC-MS analysis.

Peak No.	Ret. Time	Area %	M. F. (M. W.)	Name of Bioactive Compounds (Common name)	Uses/Bioactivity
1	22.273	1.56	C ₁₀ H ₂₀ O ₂ (172.26)	Methyl nonanoate	Flavouring agent in food & alcoholic beverages ^[16]
2	23.017	2.23	C ₁₂ H ₂₄ O ₂ (200.32)	Dodecanoic acid (Lauric acid)	Antimicrobial ^[16] , acne treatment ^[18] , increases cholesterol level due to increase in HDL ^[19] ; in production of soaps, shampoos & cosmetics ^[20]
3	24.430	27.46	C ₁₅ H ₂₀ (200.31)	1-Phenyl-1-nonyne	--
4	26.290	54.42	C ₁₈ H ₃₄ O ₂ (282.46)	9-Octadecenoic acid, (E)- (Elaidic acid)	Increases CETP activity, which lowers HDL cholesterol ^[21] , commercial preparation of oleates & lotions; pharmaceutical solvent ^[16]
5	26.684	3.56	C ₅ H ₇ NO (97.11)	2-Furanmethanamine	Industrially used as intermediates ^[16]
6	27.463	2.19	C ₁₆ H ₂₀ (212.33)	Benzene, 1,4,9-decatrienyl-	--
7	29.036	3.48	C ₁₀ H ₁₁ NO ₂ (177.20)	cis-2-Methyl-β-methyl-β-nitrostyrene	--
8	31.771	5.10	C ₁₄ H ₂₄ Si ₂ (248.51)	1,1,3,3-Tetraallyl-1,3-disilacyclobutane	--

Table 6: Compounds detected in Leaf extract of *P. dhana* by GC-MS analysis.

Peak No.	Ret. Time	Area %	M. F. (M. W.)	Name of Bioactive Compounds (Common name)	Uses/Bioactivity
1	7.807	2.92	C ₉ H ₂₀ O ₂ (160.25)	1,1-Diethoxypentane	Flavoring agent, found in strawberries ^[16]
2	10.239	0.56	C ₁₅ H ₂₄ (204.35)	α-Cubebene	Ayurvedic use in oral and dental diseases, loss of voice, halitosis, fevers, and cough ^[22]
3	10.617	1.41	C ₁₅ H ₂₄ (204.35)	2,4-Diisopropenyl-1-methyl-1-vinylcyclohexane (β-Elementene)	Anti-proliferative effects toward some cancer cell types ^[23,24,25]
4	11.309	3.96	C ₁₅ H ₂₄ (204.35)	β-Caryophyllene	Anti-inflammatory, antinociceptive, neuro-protective, anxiolytic, antidepressant, anti-alcoholism, anti-epilepsy, anticancer, antimicrobial ^[26]
5	12.116	5.41	C ₁₅ H ₂₄ (204.35)	α-Humulene (α-Caryophyllene)	Potential anti-inflammatory ^[27,28]
6	12.768	0.86	C ₁₅ H ₂₄ (204.35)	β-Copaene (β-Ylangene)	--
7	14.988	0.62	C ₁₀ H ₁₆ O ₂ (168.23)	(2R,4R)-p-Mentha-[1(7),8]-diene, 2-hydroperoxide	--
11	17.466	1.38			
8	15.166	1.69	C ₁₀ H ₁₆ (136.23)	5-Ethylidene-1-methyl-cycloheptene	--
9	15.761	1.29	C ₉ H ₁₄ O (138.20)	Spiro[4.4]nonan-2-one	--
10	16.356	0.94	C ₇ H ₁₂ Cl ₂ (167.07)	1,1-Dichloro-2,2,3,3-tetramethylcyclopropane	--
12	20.533	0.54	C ₁₁ H ₁₈ N ₄ O (222.28)	N-(5-Azidopentyl)-4-methyl-4-vinylazetid-2-one	--
13	23.051	1.85	C ₁₃ H ₂₆ O ₂ (214.34)	Tridecanoic acid (Tridecyl acid)	In dairy products, surfactants, industrial intermediates ^[16]
14	25.780	0.64	C ₂₀ H ₄₀ O (296.53)	Phytol	In fragrance industry, in cosmetics, shampoos, toilet soaps, household cleaners, and detergents ^[29] , preparation of vitamins E and K1 ^[16]
15	29.671	18.52	C ₁₄ H ₁₂ O ₃ (228.24)	2,2-Dimethylchromenocoumarin	--
17	32.933	8.68			
19	33.940	36.66			
16	31.410	1.75	C ₁₅ H ₁₃ N (207.27)	2-Ethylacridine	--
18	3.585	1.38			
20	34.781	6.81	C ₁₉ H ₂₀ O ₅ (328.35)	8,8-Dimethyl-2-oxo-9,10-dihydro-2H,8H-pyrano[2,3-f]chromen-9-yl 2-methyl-2-butenate	--
21	39.553	2.14	C ₁₀ H ₂₈ O ₄ Si ₃ (296.58)	Diethyl bis(trimethylsilyl) orthosilicate	--

-- = No reference available

3.7 Discussion

Phytochemical investigations cited above establish clearly that *P. dhana* has pharmaceutically important compounds, i.e. 9-Octadecenoic acid, (*E*)-; 1-Phenyl-1-nonyne; 1,1,3,3-Tetraallyl-1,3-disilacyclobutane; 2-Furanmethanamine; 2,2-Dimethylchromenocoumarin; α -Caryophyllene; β -Caryophyllene; 8,8-Dimethyl-2-oxo-9,10-dihydro-2H,8H-pyrano[2,3-*f*]chromen-9-yl-2-methyl-2-butenolate. The presence of α - and β -Caryophyllene in leaf, is very significant since they are potent agents for anti-inflammatory, anti-nociceptive, neuroprotective, anxiolytic, antidepressant, anti-alcoholism, anti-epilepsy, anticancer and antimicrobial properties. Structurisation of bioactive compounds are given in Fig. 13.

It is to be noted that the root of *P. dhana* seems to produce

different secondary metabolites in different regions and altitude, as reported by Daboria *et al.*, 2013. They have listed entirely different secondary metabolites according to the climatic gradient [30]. Moreover, Babu and Sreenath (2012) have evolved a successful protocol for effective micropropagation of *P. dhana* and as endorsed by them the *in vitro* plants do not exhibit discernible morphological variations from the plant grown *in vivo* [31]. Hence micropropagation has proved to be successful in the conservation of this highly exploited and endangered medicinal plant. Owing to the great demand for the plant there is an urgent need to effectively implement conservation measures by the forest authorities, government and non-governmental agencies.

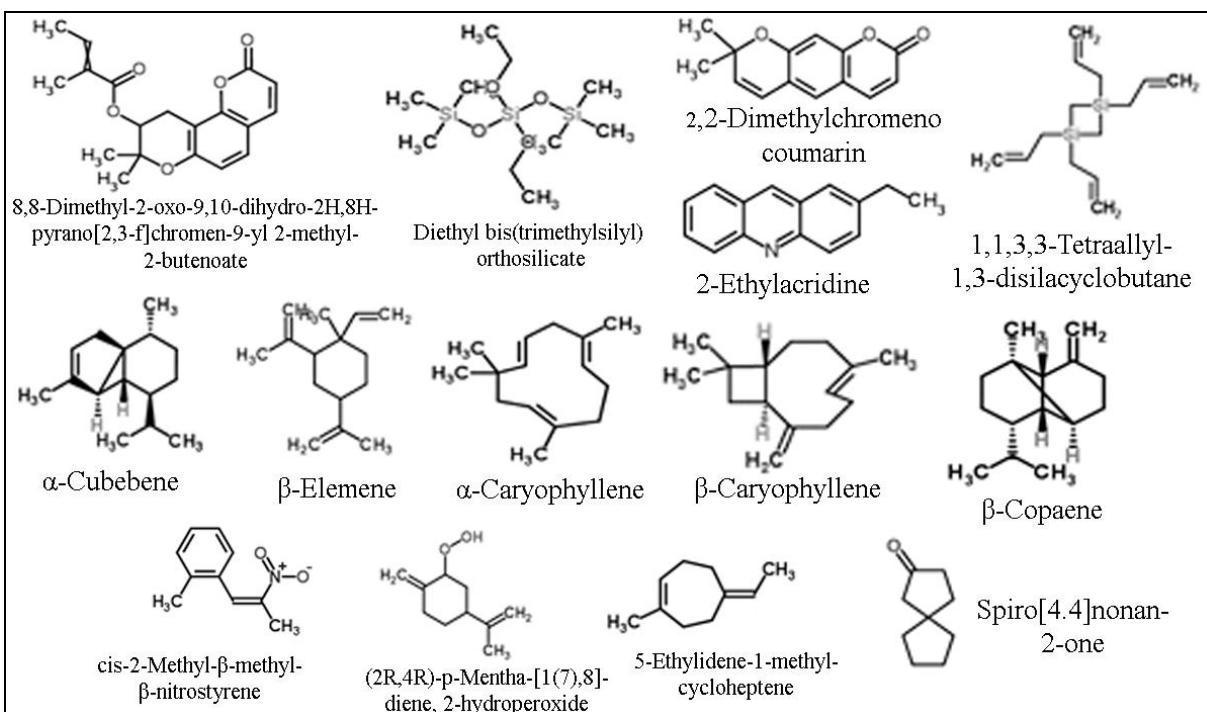


Fig 13: Molecular structure of important compounds detected in root & leaf of *Peucedanum dhana* through GC-MS

4. Conclusion

Our investigations are the first time scientific study of *Peucedanum dhana* Buch.-Ham from Jharkhand, which is being reported with its phytochemical profiles. The preliminary tests of the plant parts exhibited the presence of alkaloids and cardiac glycosides in greater amount, while steroids, phenols, tannins, saponins and fixed oils were present in average amount. The flavonoids and anthral glycosides were absent in the tested plant parts. The advanced phytochemical analysis with FTIR, HPLC and GC-MS revealed several bioactive compounds. The major constituents in the root were Lauric acid; Elaidic acid; 1-Phenyl-1-nonyne; 2-Furanmethanamine and 1,1,3,3-Tetraallyl-1,3-disilacyclobutane; while in the leaf were 2,2-Dimethylchromenocoumarin; α -Caryophyllene; β -Caryophyllene; 2-Ethylacridine; 1,1-Diethoxypentane and 8,8-Dimethyl-2-oxo-9,10-dihydro-2H,8H-pyrano[2,3-*f*]chromen-9-yl-2-methyl-2-butenolate. Moreover, the leaf

exhibited the presence of β -Elemene, a potent agent against proliferation of cancer cells. Additionally, the GC-MS analysis has detected several compounds, whose bioactivities have not been confirmed or discovered. To conclude, the endangered *P. dhana* requires urgent implementation of conservation and propagation so as to make it available for pharmaceutical, pharmacological and therapeutic purposes. The plant is a potential promise leading to drugs against cancer, oral and dental diseases, inflammation, neural damage, depression, alcoholism and epilepsy.

5. Acknowledgments

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