Research Article

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GC-MS ANALYSIS OF CHEMICAL COMPONENTS OF TWO DIFFERENT STAGES OF ROOT PART OF MOOLAK (*RAPHANUS SATIVUS* LINN.)

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ABSTRACT

Moolak or Radish (*Raphanus sativus* Linn.) is grown all over the world. Radish is eaten raw as salad due to its pungent flavor. Tender radish root is consumed with leafy vegetable and radish in its starting stage of bolting is consumed as stew. After late bolting stage, only new leaves produced in inflorescence axis and seeds are consumed as edible substance and mature seeds are used for medicinal purpose like Kushtha (Skin disease). Formulations of Moolak seed is already mentioned in Ayurvedic literatures. Here we are taking tender and early bolt stage of radish where tender is balmoolak and Bolt is vriddha moolak. This study shows the differences in chemical composition of two stages of moolak to know about their medicinal properties, because medicinal properties of herbs are due to phytoconstituents. GC-MS (Gas chromatography - mass spectrometry) technique is used to identify bioactive compounds in methanolic extract of *Raphanus sativus*. Tender radish (balmoolak) has shown total 38 peaks and Bolt radish (vriddha-moolak) showed total 41 peaks of phytoconstituents. 15 compounds are common among both samples.

Keywords: Radish, balmoolak, vriddha moolak**,** moolak

INTRODUCTION

Raphanus sativus Linn. is known as moolak in Ayurvedic literatures and commonly known as radish. Moolak is mentioned in Ahara dravya as well as ausadhdravya[1]. Due to its medicinal properties, it is used in different formulations in ayurvedic literatures. Literature's mention balmoolak, moolak and vriddha moolak term in different places for various purposes[2]. These different terms denote different stages of moolak. Bolting is the stage when a plant has used its entire energy to produce its next generation. Flowering stalk is visible sign for it[3]. After some time the root becomes woody, tough and cannot be used for consumption. There are numerous varieties of *Raphanus sativus* according to their size, shape, color and harvesting season[4]. This is used in vata vyadhi (Joint pain), Adhmana (Flatulence) and Ashmari (Stone). Glucosinolate, Myrosinase and isothiocyanates gives sharp flavor and raphanin is a major content of radish which has antibacterial and antifungal activity to moolak[5] (radish). This GC-MS study shows clear difference in chemical composition of both stages of radish.

MATERIAL AND METHODS

Collection of plant material

Here two stages of *Raphanus sativus* Linn. are taken for the experimental study from Raipur, Chhattisgarh which latitude is 21.25 N and longitude is 81.63 E[6].

Balmoolak (1st sample) is taken just before harvesting time (approximately 6 weeks or one and half month) in December after sowing and vriddha (2nd sample) one is taken after flowering stalk appeared till fruiting stage (season: January - February). Fruit maturing stage and ripen seed stage was not taken because after that degeneration process started in roots.

Authentication of plant

Authentication of collected plant material was done in the Raw Material Herbarium & Museum, Delhi (RHMD), National Institute of Science Communication and Information Resources (CSIR-NISCAIR). Ref. No.- NISCAIR/RHMD/ Consult/2019/3545-46 Date - 17/12/2019.

Processing of the plant material

The collected plant materials (Roots) were separated with extraneous matter then cleaned with distilled water. They were dried in shade at room temperature until they were free from moisture. Completely dried plant materials were pulverized in a mechanical grinder. The coarse powdered sample was stored in airtight container for further use.

Preparation of Extract

Macerate 5 gram of air-dried drug, coarsely powdered, with 100 ml of methanol the specified strength in a closed flask for 24 hours. Shaking frequently for 6 hours and allowing to stand for 18 hours. Filter rapidly, taking precaution against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105º, to constant weight and weigh.

GC- MS (Gas Chromatography - Mass Spectrometry)

- **Preparation of stock solution**

Methanol extract (10 mg) was reconstituted in 1000 µL Methanol. Methanol extracts (1 µl) was injected for GC-MS analysis.

- **GC-MS Analysis**

Instrumental Used: Thermo Scientific GC Trace 1310 Equipped with Thermo
Scientific MS TSQ 8000.
Type: Acquisition - General
Method MS transfer line temperature: 300°C
Ion source temperature: 230°C
Ionization mode: EI
Temperature program: Initial 60 degree C hold for 5 min Ramp at 10 degree C to 240 degree C Ramp at 10 degree C to 300 degree C hold for 5min.
Flow rate: 1 ml/min.
Carrier Gas: Helium
Column Used: Agilent DB 5MS (30-meter X 0.25 mm)

- **Data Interpretation**

The sample subjected to GC-MS and the total separated peak are shown in Figure 1. Extracted ion. O-chromatograms were obtained from all the 26 peaks (provided as attachment). The mass of the compounds and fragments recorded were matched with NIST database for identification of probable compounds present in the samples.

- **Identification of components**

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns.[7]

RESULT AND DISCUSSION

GC-MS Reports

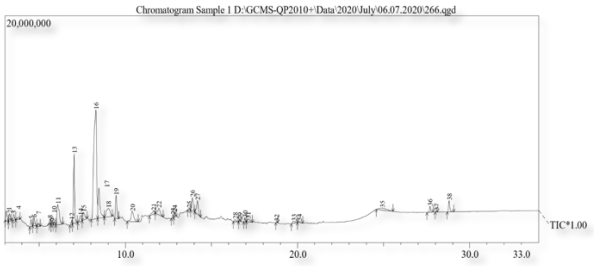

Figure 1: GC-MS Report of Sample 1

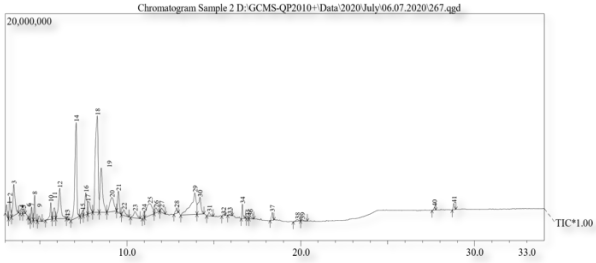

Figure 2: GC-MS Report of Sample 2

Table 1: Compounds of Sample 1 (Balmoolak or tender Root)

Peak N.	R. Time (in min. sec.)	Peak Area%	Peak name (Compound Name)
1.	3.087	1.45	Furfural
2.	3.273	1.20	2-Furanmethanol
3.	3.526	0.86	Butanoic acid, 2-ethyl-3-oxo-, methyl ester
4.	3.785	0.41	dl-Glyceraldehyde dime
5.	4.525	0.90	2-Furancarboxaldehyde, 5-methyl-
6.	4.703	1.37	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
7.	4.963	0.45	-
8.	5.618	0.52	Pentanoic acid, 4-oxo-
9.	5.760	0.27	2,5-Dimethyl-4-hydroxy-3(2H)-furanone
10.	5.866	0.35	-
11.	6.075	5.76	1,3,5-Triazine-2,4,6-triamine
12.	6.883	0.69	2-Propanamine, N-methyl-N-nitroso-
13.	7.026	10.22	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-
14.	7.412	1.54	-
15.	7.583	1.92	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-
16.	8.288	36.61	-
17.	8.468	6.74	1,2,3-Propanetriol, monoacetate
18.	9.015	3.86	d-Glycero-d-ido-heptose
19.	9.469	3.68	N-Nitroso-2-ethyl-1,3-tetrahydrooxazine
20	10.410	3.42	Tetrahydro [2,2'] bifuranyl-5-one
21	11.643	1.03	2,2,3,3-Tetramethylcyclopropane-carboxylic acid 4-methylphenyl ester
22	11.392	2.03	1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan)
23.	12.779	0.38	2,3,3-Trimethyl-2-(4-methylpentanoyl)-cyclopentanone
24.	12.857	0.78	Diethyl Phthalate
25.	13.700	0.26	3-Deoxy-d-mannoic lactone
26.	13.893	2.51	Pyrimidine-2,4(1H,3H)-dione,6-hydroxy-5-methyliminomethylIADFAC
27.	14.187	3.17	1,4-Dioxaspiro[4,5]decane, (E)-2,3-dimethyl-7-t-butyl-
28.	16.369	0.46	2,2,3,3-Tetramethylcyclopropane-carboxylic acid, pentyl ester
29.	16.638	0.19	l-(+)-Ascorbic acid 2,6-dihexadecanoate
30.	16.943	0.49	Furfuryl heptanoate
31.	17.126	0.82	3,4-Furandimethanol
32.	18.793	0.16	3,7-Dimethyloctyl isopropylphosphono-fluoridate
33.	19.794	0.62	-

34.	20.086	0.36	Hexadecanoic acid, 4-nitrophenyl ester
35.	20.292	1.85	-
36.	27.686	0.88	Ergost-5-en-3-ol, (3.beta.)-
37.	28.054	0.14	Stigmasterol
38.	28.801	1.66	gamma.-Sitosterol[8]

Table 2: Compounds of Sample 2 (Vriddha moolak or Bolt root)

Peak N.	R. Time (in min. sec.)	Peak Area%	Peak name (Compound Name)
1.	3.084	0.83	Furfural
2	3.192	1.86	3-Furanmethanol
3.	3.506	4.98	Propane, 1-(1-methylethoxy)-
4.	3.931	0.54	2-Hexene, (E)-
5.	4.063	0.39	Acetic acid, 1-(2-methyltetrazol-5-yl)ethenyl ester
6.	4.358	0.14	2-Furanmethanol, 5-methyl-
7.	4.523	1.02	2-Furancarboxaldehyde, 5-methyl-
8.	4.693	2.05	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
9.	4.955	0.64	Glutaconic anhydride
10.	5.621	1.59	Pentanoic acid, 4-oxo-
11.	5.834	1.26	Furanone, 3-hydroxy-4,5-dimethyl
12.	6.137	5.49	4-Methylpiperidine-1-carboxylic acid, phenyl ester
13.	6.581	0.17	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
14.	7.089	13.62	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
15.	7.462	0.61	Erythritol
16	7.641	3.35	4H-Pyran-4-one, 5-hydroxy-2-(hydroxymethyl)-
17.	7.757	2.17	2-Acetonyl-9-[3-deoxy-.beta.-d-ribouranosyl]hypoxanthine
18.	8.300	18.47	Furancarboxaldehyde
19.	8.531	8.05	1,2,3-Propanetriol, monoacetate
20.	9.141	4.30	d-Glycero-d-tallo-heptose
21.	9.408	2.33	1-Amino-4-methylpiperazine
22.	9.834	0.95	Nonanoic acid, 6-oxo-, ethyl ester
23.	10.468	1.47	3-Cyclopentylpropionic acid, 2-tetrahydrofurylmethyl ester
24.	10.986	0.56	Isopropylphosphonic acid, dicylcopentyl ester
25.	11.334	5.01	Butanamide, 2-hydroxy-N,2,3,3-tetramethyl-
26.	11.681	0.84	2,2,3,3-Tetramethylcyclopropane-carboxylic acid, 4-methylphenyl ester
27.	11.999	0.70	Anhydro-.beta.-D-glucopyranose
28.	12.857	0.64	Diethyl Phthalate
29.	13.909	8.16	Pyrimidine-2,4(1H,3H)-dione, 6-hydroxy-5-methyliminomethyl-
30.	14.202	3.41	1,4-Dioxaspiro[4,5]decane, (E)-2,3-dimethyl-7-t-butyl-
31.	14.735	0.51	Acetate, [4-hydroxy-4-(1-methylethyl)-5-methyl-2-hexynyl] ester
32.	15.551	0.17	Borane, silane
33.	15.917	0.18	Epi-Inosito
34.	16.647	0.96	l-(+)-Ascorbic acid 2,6-dihexadecanoate
35.	16.946	0.15	Furfuryl heptanoate
36.	17.099	0.31	Cyclobutanecarboxylic acid, 2-ethylcyclohexyl ester
37.	18.366	0.73	cis,cis,cis-7,10,13-Hexadecatrienal
38.	19.807	0.34	Octahydroindene
39.	20.086	0.22	Hexadecanoic acid, 4-nitrophenyl ester
40.	27.687	0.27	Ergost-5-en-3-ol, (3.beta.)-
41.	28.801	0.52	gamma. -Sitosterol

Table 3: Comparison between Compounds of Balmoolak and Vriddha moolak

S. N.	Peak name (Compound)	Sample 1	Sample 2
1.	Furfural	1.45	0.83
2.	2- furancarboxaldehyde-5-methyle-	0.90	0.14
3.	2,4-dehydroxy-2,5-dimethyle-3(2H)-furan	1.37	2.05
4.	Pantanoic acid,4-oxo-	0.52	1.59
5.	4H-Pyra-4-ene,2,3-dihydro-3,5-dihydroxy-6-methyle	10.22	13.62
6.	1,2,3-Propanetriol, monoacetate-	6.74	8.05
7.	2,2,3,3 Tetramethylcyclopropanecarboxylic acid-4-methylephenyle ester	1.03	0.84
8.	1,6-Anhydro beta D glucopyranose (levoglucosan)	2.03	0.70
9.	Pyridine-2,4 (1H,3H) - dione 6-hydroxy-5-methyliminomethyle	2.51	8.16
10.	1,4 Dioxaspiro [4,5] decane	3.14	3.41
11.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	0.19	0.96
12.	furfuryl heptanoate	0.82	0.15
13.	Hexadecanoic acid, 4-nitrophenyl ester	0.36	0.22
14.	Ergost-5-en-3-ol, (3.beta.)-	0.88	0.27
15.	gamma. -Sitosterol	1.66	0.52

By the analysis of GC-MS report we got 38 peaks in Sample 1 and 41 peaks in sample 2. 15 common compounds are present in both samples but in different amount. This study shows the difference in chemical composition of two different stages of *Raphanus sativus* Linn. Compounds which are present only in Sample-1: 2-Furanmethanol, Butanoic acid, 2-ethyl-3-oxo-, methyl ester, dl-Glyceraldehyde dimer, 4-oxo-, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 1,3,5-Triazine-2,4,6-triamine, 2-Propanamine, N-methyl-N-nitroso-, 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-, d-Glycero-d-ido-heptose, N-Nitroso-2-ethyl-1,3-tetrahydrooxazine, Tetrahydro[2,2']bifuranyl-5-one, 2,3,3-Trimethyl-2-(4-methyl-pentanoyl)-cyclopentanone, Diethyl Phthalate, 3-Deoxy-d-mannoic lactone, 2,2,3,3-Tetramethylcyclopropanecarboxylic acid, pentyl ester,3,4-Furandimethanol,3,7-Dimethyloctyl isopropyl-phosphono-fluoridate, Stigmasterol and 5 peaks are not identified. Compounds which are found only in Sample-2 shows 41 compounds where 26 compounds are different as compared to Bal moolak 3-Furanmethanol, Propane, 1-(1-methylethoxy)-, 2-Hexene, (E)-, Acetic acid, 1-(2-methyltetrazol-5-yl)ethenyl ester, 2-Furancarboxaldehyde, 5-methyl-, Glutaconic anhydride, Pentanoic acid, 4-oxo-, Furanone, 3-hydroxy-4,5-dimethyl, 4-Methylpiperidine-1-carboxylic acid, phenyl ester, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, Erythritol, 4H-Pyran-4-one, 5-hydroxy-2-(hydroxymethyl)-, 2-Acetonyl-9-[3-deoxy-.beta.-d-ribouranosyl]hypoxanthine, Furancarboxaldehyde, d-Glycero-d-tallo-heptose, 1-Amino-4-methylpiperazine, Nonanoic acid, 6-oxo-, ethyl ester, 3-Cyclopentylpropionic acid, 2-tetrahydrofurylmethyl ester, Isopropylphosphonic acid, dicylcopentyl ester, Butanamide, 2-hydroxy-N,2,3,3-tetramethyl-, Anhydro-.beta.-D-glucopyranose, Diethyl Phthalate, Acetate, [4-hydroxy-4-(1-methylethyl)-5-methyl-2-hexynyl] ester, Borane, silane, Epi-Inosito, Cyclobutanecarboxylic acid, 2-ethylcyclohexyl ester, cis,cis,cis-7,10,13-Hexadecatrienal, Octahydroindene. These peaks are indication of phytoconstituents (compounds) but 6 peaks of Sample 1 (Balmoolak) 7, 10, 14, 16, 33, 35 are not identified.

CONCLUSION

Raphanus sativus is used as medicine for different diseases due to secondary metabolites of methanolic extract of moolak. 15 similar compounds are present in both samples but in different fraction while 23 other compounds are present in sample 1 (balmoolak) and 26 extra compounds in sample 2 (vriddha moolak). This study shows the difference in chemical composition and different medicinal properties (Due to different phytoconstituents) of both stages.

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