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Phytochemical profiling of the leaves of *Brassica juncea* L. using GC-MS

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Abstract

Brassica juncea L. is a source of several phytochemicals of economic importance. The aim of the present study was to identify active phytochemicals from the leaves of 60 days old plants after extraction with different solvents. Number of phytochemicals detected were 51 (chloroform), 48 (ethyl-acetate), 40 (methanol), 33 (petroleum ether), and 28 (n-hexane). The major compounds identified were benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (22.98%, in methanol), n-eicosane (26.69%, in ethyl acetate), n-pentacosane (50.0%, in chloroform) and n-tetratetracontane (42.47 and 49.19%, in petroleum ether and n-hexane respectively). In all of the extracts, it was observed that compounds which belonging to alkane group dominated the most, followed by carboxylic acids containing fatty acids.

Introduction

Brassica juncea L. commonly known as Indian mustard belongs to family brassicaceae. *B. juncea* is an economically important plant widely used as an oil source, a green vegetable and also having a medicinal value. This species has been described in traditional remedies in the ancient literature (Manohar *et al*., 2009). Indian mustard is consumed as leafy vegetable and is a source of various micronutrients as well as antioxidants, vitamin c and e, β-carotenoids etc. *B. juncea* is believed as eco-friendly source for various nutraceuticals or drugs which are used to prevent and cure of wide range of non-communicable diseases in present time (Kumar *et al.*, 2011). Food preparation of Indian mustard leaves is helpful in lowering the cost for diabetic patients suffering with comorbid anxiety disorders (Thakur *et al*., 2013). Plants of genus *Brassica* are also known for the production of various volatile organic compounds like ketones, aldehydes, esters, alcohols, terpenes and glucosinolates. These volatile compounds help in pollination by attracting various insects and animals, as well as they protect plants from herbivorous attack (Kessler and Baldwin, 2002). *B. juncea* is reported to produce bio-chemicals including glycosides, flavonoids, phenols, sterols, triterpene alocohols, proteins and carbohydrates (Lie *et al*., 2000; Yokozawa *et al*., 2002; Das *et al*., 2009; Jung *et al.*, 2009). Methanolic extracts of *B. juncea* leaves were observed to possess antihyperglycemic activity (Manohar et al., 2009; Rahmatullah et al., 2010; Valavala *et al*., 2011). Dried methanolic extracts were found to reduce diabetes related mental health problem (Kumar *et al*., 2011; Thakur *et al*., 2013). The leaves of *B. juncea* are utilized to produce medicines which act as stimulants, diuretics and expectorants (Farrell *et al*., 1985). Indian mustard is also known for its therapeutically pharmacological uses due to its active bio-constituents (Kumar *et al*., 2011). Glucosinolates and isothiocyanates are reported to be very active in *B. juncea* (Hill *et al*., 1987; McNaughton and Marks, 2003) which act as anti-cancerous and anti-microbial compounds (Luciano and Holley, 2009; Okulicz, 2010; Zhang *et al.*, 2010). Leaves of Indian mustard were also reported to have anti-depressant effects during diabetes (Thakur *et al*., 2014). The presence of different brassinosteroids namely castasterone, teasterone, 24-epibrassinolide and typhasterol have been reported from *B. juncea* (Kanwar *et al*., 2015). 24-epibrassinolide is reported to enhance the phytochemical biosynthesis in *B. juncea* under imidacloprid pesticide stress (Sharma *et al*., 2015a, 2015b). Keeping in view the edible and medicinal value of *B. juncea* L., the present study is focused on the identification of various active bio-chemicals from the leaves of 60 days old plants using GC-MS.

Materials and Methods

Plant material

Seeds of *B. juncea* L. variety RLC-1 were procured from Punjab Agricultural University,

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Table 1. Phytochemical profiling of chloroform extracts of 60 day old leaves of *B. juncea* L. plants by GC-MS

Peak No.	Name of compound	Retention time	Area %
1	2,4-Bis(tert-butyl)-phenol	13.58	0.40
2	2-Methyl-decane	13.66	0.10
3	Hexadecane	14.25	0.10
4	Cyclotetradecane	14.82	0.20
5	Pentadecane	14.95	0.10
6	Myristic acid	16.71	0.20
7	5-Eicosene (E)	17.15	0.50
8	Neophytadiene	17.64	4.20
9	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]	17.75	0.30
10	Dodeca-1, 6-dien-12-ol, 6, 10-dimethyl	17.89	0.60
11	Methyl stearidonate	18.03	0.20
12	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	18.07	1.10
13	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- methyl ester	18.37	0.20
14	9-Octadecenoic acid, 12-(acetyloxy)-methyl ester	18.41	0.20
15	Alpha-linolenic acid	18.49	1.30
16	Oleic acid	18.81	2.00
17	2,6,10,15-Tetramethylheptadecane	19.03	0.20
18	Hexadecanoic acid- ethyl ester	19.11	0.20
19	3-Eicosene, (E)	19.21	0.50
20	n-Eicosane	19.30	0.20
21	Linolenic acid-methyl ester	20.03	0.50
22	trans-Phytol	20.26	1.10
23	Gamolenic acid	20.43	6.80
24	Docosanoic acid	20.70	0.80
25	Tetradecanoic acid-ethyl ester	20.99	0.10
26	Tridecanol, 2-ethyl-2-methyl	21.04	0.20
27	9-Eicosene, (E)	21.09	0.50
28	n-Pentadecane	21.16	0.20
29	n-Hexadecyl iodide	22.54	0.10
30	Hexadecylheptafluorobutyrate	22.81	0.40
31	n-Nonadecane	22.88	0.30
32	Tricosane	23.68	0.10
33	1,2-Benzenedicarboxylic acid-dioctyl ester	23.74	0.10
34	Heptadecyltrifluoroacetate	24.42	0.20
35	2,6,11-Trimethyldodecane	24.60	0.10
36	1-Docosanol	25.19	0.30
37	n-Octacosane	25.38	1.00
38	Tetracosan-1-ol	26.36	0.20
39	n-Tetratetracontane	26.45	0.30
40	1-Octacosanol	27.52	2.60
41	n-Pentacosane	27.82	50.0
42	17-Pentatriacontene	28.68	0.40
43	8-Hexylpentadecane	29.35	0.50
44	Palmitaldehyde	29.75	0.60
45	Tetrapentacontane, 1,54-dibromo	30.66	5.30
46	Stearyl alcohol	31.10	3.70
47	Nonadecylpentafluoropropionate	31.24	3.00
48	n-Hentriacontane	31.37	2.80
49	Tridecanaldehyde	34.55	1.70
50	Cholesteryl	35.06	2.90
51	Propionic acid anhydride	37.48	0.60

Table 2. Phytochemical profiling of ethyl-acetate extracts of 60 day old leaves of *B. juncea* L. plants by GC-MS

Peak No.	Name of compound	Retention time	Area %
1	n-Octyl acetate	6.64	0.15
2	1-Dodecene	8.07	2.21
3	n-Tetradecane	8.36	0.28
4	delta.-Nonalactone	8.74	0.65
5	n-Dodecane	10.51	0.35
6	Linoleic acid	10.75	0.12
7	Cyclopropane, tetramethylpropylidene	11.44	0.16
8	1-Pentadecene	12.05	5.44
9	Pentadecane	12.21	0.80
10	Decanoic acid,-octyl ester	12.41	0.24
11	1,2-Cyclopentanedicarboxylic acid, 4-(1,1-dimethylethyl)-dimethyl ester, (1, alpha, 2, beta, 4, beta)	12.63	0.17
12	2-Cyclopentene-1-undecanoic acid	12.79	0.13
13	2,4-Di-tert-butylphenol	13.58	5.97
14	n-Tridecane	13.65	0.64
15	9-Methyl-Z-10-pentadecen-1-ol	13.75	0.32
16	9-Octadecene (E)	14.83	7.95
17	n-Hexadecane	14.95	0.56
18	Heptadecane	16.14	0.17
19	5-Eicosene, (E)	17.15	8.18
20	1-Chlorooctadecane	17.25	0.18
21	Tetradecanoic acid, 1-methylethyl ester	17.40	0.59
22	1,2-Benzenedicarboxylic acid,-didodecyl ester	17.53	0.24
23	Neophytadiene	17.64	2.89
24	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	17.89	0.43
25	n-Octadecanal	18.07	0.82
26	Phthalic acid- butyl undecyl ester	18.49	2.87
27	Oleic acid	18.82	1.69
28	trans-2-Octenol	18.95	0.69
29	3-Eicosene, (E)	19.22	6.41
30	trans-Phytol	20.25	0.45
31	Gamolenic acid	20.42	2.79
32	Stearic acid	20.70	0.35
33	9-Eicosene, (E)	21.09	3.74
34	Heptadecyltrifluoroacetate	22.80	1.87
35	1,2-Benzenedicarboxylic acid, dioctyl ester	23.74	1.75
36	Octadecyltrifluoroacetate	24.41	0.93
37	n-Tetratetracontane	25.38	0.54
38	2-Hexyl-1-decanol	26.36	0.44
39	Farnesane	26.44	0.34
40	1-Docosanol	27.51	1.15
41	n-Eicosane	27.80	26.69
42	n-Tritetracontane	29.34	0.53
43	Palmitaldehyde	29.74	0.25
44	2L,4L-Dihydroxyeicosane	30.65	1.43
45	Lignocerol	31.08	1.29
46	n-Hexatriacontane	31.38	2.29
47	Myristaldehyde	34.54	0.72
48	Vitamin A aldehyde	35.04	1.15

Ludhiana, India, and were grown in pots.

Sample preparation

For phytochemical profiling, 1 g of 60 days old leaves were separately extracted with 50 ml of methanol, ethyl acetate, chloroform, petroleum ether and n-hexane. The extracts were dried at 50°C using rotary evaporator and the residues were further reconstructed to 2 ml with respective solvents.

Analysis by GC-MS

The extracts of B. juncea L. leaves were analysed using Shimadzu GCMS-QP2010 Plus. Helium gas was used as carrier gas, the injection temperature was set at 280°C, initial column temperature was 70°C, held for 6 minutes before increased to 250°C and then 300°C at the rate of 10°C/min. and was held for 10 minutes respectively. Injection mode set was split with 1 minute sampling time, linear flow control

mode, 110.8 KPa pressure, 38.9 ml/min total flow and 1.71 ml/min column flow, 47.2 cm/sec linear velocity, analytical column used was DB-5ms with 30 m length and 0.25 mm id. Ion source temperature was 250°C and interface temperature was 290°C.

Identification of the phytochemicals

Phytochemicals detected were compared with National Institute of Standard and Technology (NIST08s) and Wiley7 library using mass spectra.

Results and Discussion

GC-MS analysis of 60 days old B. juncea L. leaves by using different solvents resulted in the detection of number of phytochemicals. In chloroform, a maximum number of 51 compounds were detected, followed by 48, 40, 33, and 28 compounds in ethyl-acetate, methanol, petroleum ether and n-hexane extracts respectively. Oleic acid and linolenic acid were found to be present in all the extracts. The main compounds detected in chloroform extract (Table 1) were n-pentacosane (50.0%, RT 27.82), gamolenic acid (6.80%, RT 20.43) and tetrapentacontane, 1, 54-dibromo (5.30%, RT 30.66). The major compounds identified in ethyl-acetate extract (Table 2) were n-eicosane (26.69%, RT 27.80), 5-eicosene (E) (8.18%, RT 17.16) and 9-octadecene (E) (7.95%, RT 18.83). In methanolic extract (Table 3), main phytochemicals detected were benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy, methyl ester (22.98%, RT 18.38), cis,cis,cis-7,10,13-hexadecatrienal (12.42%, RT 20.43) and eicosanoic acid (7.87%, RT 18.83). The major compounds identified in petroleum ether extract (Table 4) were n-tetratetracontane (42.47%, RT 27.81), cis,cis,cis-7,10,13-hexadecatrienal (14.61%, RT 20.46) and pentadecanoic acid (11.24%, RT 18.86). Main compounds detected in n-hexane extracts (Table 5) were n-tetratetracontane (49.19%, RT 27.83), alpha-linolenic acid (12.30%, RT 20.48) and 2L,4L-dihydroxyeicosane (6.22%, RT 30.67).

Most of the detected compounds are biologically active and are known to strengthen the plant defence system as these are part of plant protective compounds like phytoanticipins and phytoprotectants (Hossain *et al.*, 2006; Shah and Hossain, 2014; Hossain and Shah, 2015). Present study gives a base line data of the phytochemicals present in *B. juncea* L. plants.

Conclusion

GC-MS analysis of the leaves of *B. juncea* L. by using different organic solvents resulted in the detection of 132 different compounds. In chloroform extracts, the compounds detected were maximum as compared to the n-hexane extracts where compounds detected were minimum.

Table 3. Phytochemical profiling of methanolic extracts of 60 day old leaves of *B. juncea* L. plants by GC-MS

Peak No.	Name of compound	Retention time	Area %
1	2-Propyl-2-pentenal	4.44	1.28
2	3-n-Butylthiolane	5.85	1.56
3	3,5-Hexadien-2-ol, 2-methyl	8.31	1.56
4	2-Methoxy-4-vinylphenol	10.15	1.04
5	1-Dodecene	12.03	1.01
6	2H-Pyran-2-on, 5,6-dihydro-4-(2,3-dimethyl-2-buten-4-yl)	14.01	0.96
7	1-Pentadecene	14.81	4.07
8	Pentanoic acid-octyl ester	14.94	0.70
9	Gamma-pyronene	15.84	0.66
10	P-T-Amylphenol	15.93	2.37
11	Nonyl-phenol mix of isomers	16.04	1.87
12	4-Nonylphenol	16.13	2.28
13	meso-Hexestrol	16.24	0.32
14	Benzestrol	16.28	0.58
15	Cyclopropanecarboxylic acid, 3-(3-methoxy-2-methyl-3-oxo-1-propenyl)-2,2-dimethyl-, 3-(2-butenyl)-2-methyl-4-oxo-2-cyclopenten	16.34	0.65
16	p-tert-Butylphenol	16.45	2.33
17	Acetic acid, 4-(7-methylydenebicyclo[3.3.1]non-2-en-3-yloxy)-butyl ester	16.53	1.40
18	Oleic acid	16.70	0.59
19	1-Hexadecene	17.14	5.26
20	Neophytadiene	17.63	4.77
21	Oxirane, tetradecyl	17.88	0.75
22	9-Eicosyne	18.06	1.07
23	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- methyl ester	18.38	22.98
24	Linolenic acid-methyl ester	18.48	2.46
25	Eicosanoic acid	18.83	7.87
26	3-Eicosene, (E)	19.21	3.18
27	Gamolenic acid	20.02	0.93
28	Oxirane, hexadecyl	20.25	1.52
29	cis,cis,cis-7,10,13-Hexadecatrienal	20.43	12.42
30	Stearic acid	20.70	2.29
31	1-Docosanol	21.08	1.35
32	Lignocerol	22.80	0.52
33	Linoleic acid	23.55	0.33
34	8-Methyl-6-nonenamide	25.73	1.36
35	Farnesol	26.56	0.33
36	Myristaldehyde	27.50	0.71
37	Sulfurous acid, 2-propyl tridecyl ester	27.74	0.60
38	Oxirane, [(hexadecyloxy)methyl]	30.63	1.17
39	Nonadecanol	31.20	0.85
40	Vitamin A aldehyde	35.03	2.04

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Table 4. Phytochemical profiling of petroleum ether extracts of 60 day old leaves of *B. juncea* L. plants by GC-MS

Peak No.	Name of compound	Retention time	Area %
1	Cyclopentaneundecanoic acid- methyl ester	8.28	0.28
2	Benzenepropanoic acid, alpha-(hydroxyimino	9.59	0.17
3	2,3,5,8-Tetramethyldecane	10.15	0.19
4	1-Undecene, 4-methyl	11.00	0.25
5	2,4-Di-tert-butylphenol	13.57	0.27
6	2-Methyldecane	13.65	0.27
7	Farnesane	14.24	0.18
8	Palmitic acid	14.40	0.24
9	Octadecyl chloride	14.94	0.13
10	Octadecane	16.13	0.12
11	Hexadecyl chloride	16.31	0.12
12	Myristic acid	16.72	0.69
13	n-Eicosanol	17.14	0.08
14	2-Butyloctanol	17.25	0.10
15	Linolenic acid-methyl ester	18.52	3.26
16	Linoleic acid	18.59	1.64
17	Pentadecanoic acid	18.86	11.24
18	Oleic acid	19.61	0.37
19	trans-Phytol	20.26	1.00
20	cis,cis,cis-7,10,13-Hexadecatrienal	20.46	14.61
21	Docosanoic acid	20.71	1.03
22	1,2-Benzenedicarboxylic acid-dinonyl ester	23.74	0.56
23	n-Tritetracontane	25.38	0.90
24	Farnesol	26.56	2.27
25	1-Octacosanol	27.52	3.33
26	n-Tetratetracontane	27.81	42.47
27	Sulfurous acid, 2-propyl tridecyl ester	29.34	0.44
28	Myristaldehyde	29.75	0.94
29	Oxirane, [(hexadecyloxy)methyl]	30.65	3.94
30	Nonadecanol	31.08	2.21
31	Docosylpentafluoropropionate	31.23	0.91
32	11-n-Decyltetracosane	31.37	3.41
33	Palmitaldehyde	34.55	2.38

Table 5. Phytochemical profiling of n-hexane extracts of 60 day old leaves of *B. juncea* L. plants by GC-MS

Peak No.	Name of compound	Retention time	Area %
1	Phenethanol	4.94	0.48
2	n-Undecane	5.28	1.24
3	n-Dodecane	8.36	0.32
4	n-Octadecyl chloride	18.30	0.23
5	Linolenic acid-methyl ester	18.51	1.88
6	Linoleic acid	18.58	0.31
7	Oleic acid	18.85	4.74
8	Octadecane	19.30	0.36
9	trans-Phytol	20.25	1.10
10	Alpha-linolenic acid	20.48	12.30
11	Docosanoic acid	20.71	0.60
12	Heptacosane, 1-chloro	21.16	0.38
13	1-Octadecanesulphonyl chloride	22.04	0.32
14	Sulfurous acid, pentadecyl 2-propyl ester	22.87	0.27
15	1,2-Benzenedicarboxylic acid-ditridecyl ester	23.75	4.19
16	Tritetracontane	25.38	1.04
17	Tetrapentacontan, 1,54-dibromo	26.45	0.46
18	17-Pentatriacontene	27.52	2.34
19	n-Tetratetracontane	27.83	49.19
20	2-Hexyl-1-decanol	28.68	0.20
21	n-Nonadecane	29.35	0.61
22	Palmitaldehyde	29.75	0.90
23	2L,4L-Dihydroxyeicosane	30.67	6.22
24	1-Hentetracontanol	31.08	1.59
25	Tricosylpentafluoropropionate	31.24	1.50
26	n-Eicosane	31.38	3.69
27	Myristaldehyde	34.56	3.10
28	Vitamin A aldehyde	35.05	0.42

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