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BIOCHEMISTRY

Evaluation of Secondary Metabolites in Wheat Grain (*Triticum* Sp.) Grown in Humid South Eastern Plain Zone of Rajasthan (India)

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ABSTRACT

Cereal crops such as wheat, rice and barley underpin the staple diet for human consumption, globally. In India, wheat is qualitatively a major source of macromolecule, energy and fiber for human community nutrition since long time hence, preferably used as a staple food grain for society and also used as major source of fodder for animal feeding. The health benefits of whole grains are linked to the existence of secondary bioactive metabolites including phenolic acids, flavonoids and phytosterols. Flavonoids have the properties like anti-inflammatory, antioxidant, antiallergic, antithrombotic, antiviral and anticarcinogenic activities. Quercetin is one with an average of daily consumption of 25 mg to 40 mg. Kaempferol used as an antioxidant. Quantification data revealed that the total flavonoid content (free + bound) was observed highest and lowest in durum genotypes of HD 4728 (1.75 mg/gdw) and Raj 6560 (0.92 mg/gdw), respectively and in aestivum genotypes Raj 4037 (1.38 mg/gdw) and HI 1544 (0.72 mg/gdw) gave similar trend. Regular daily intakes of whole grain products are associated with reduced risk of several diseases. The objective of this study was identification and characterization of secondary metabolites i.e. flavonoids in *Triticum* sp.

Highlights

- Quantification study of secondary metabolites (flavonoid compounds) in 2 wheat species, standard samples were analyzed by GC-MS, Flavonoid's have the strongest antioxidant properties.

Keywords: Triticum, Secondary metabolite, Flavonoid, TLC, GC-MS

Wheat is a herbaceous plant and monocotyledonous member of *Gramineae* family. Probably, it's the First plant that is domesticated and cultivated by human. The Indian wheat is predominantly mature and known for creating tasty and quality Chapattis. The abnormal amplify in area, production and productivity of wheat crop, established newer higher records with each passing year. Wheat is a big concerning 29 million hectare acreage of everywhere in the India (J. Kumar *et al*., 2016), used mainly for human consumption to support approximate 35% population of the world (Mohammadi-joo *et al.* 2015) and also provide 20 per cent of the total food calories (Anonymous, 2014).

According to United Nations DESA (Department of Economic and Social Affairs) report, World population is expected to increase from around 7 billion now to around 8.5 billion in the year 2030. In South Asia and continent, 2 out of 5 kids may still starving despite, distinct improvement in per caput food accessibility. Thus, food and nutritional security will continue to remain major challenge for developing countries in the world.

Based on rainfall, soil characterization, temperature *etc.* wheat cultivation is divided in to six mega zones of India (Map-I). The biggest state Rajasthan consisting 10.4 per cent of total geographical region

and 5.67 per cent of total population of the Republic of India (Census, 2011). In Agro climatic zones, India has been portrayed into 126 agro-climatic zones by the Indian Council of Agricultural Research (ICAR), out of that Rajasthan has been bifurcate into ten agro-climatic zones. In agricultural regions of Rajasthan factor's like rainfall, altitude, latitude, temperature, topography, natural vegetation, soils, crops, irrigation availability and livestock are taken into consideration. Humid South Eastern Plain Zone covers the area of Kota, Bundi, Baran, Jhalawar and some parts of Sawai Madhopur districts of Rajasthan state in India.

Generally plant produces two types of metabolites *i.e.* primary metabolites and secondary metabolites. Primary metabolites directly concerned in growth and metabolism of plant. Secondary metabolites are considered as end product of primary metabolites that have no major role in the maintenance of plant, but they are extremely important compounds from the biological point of view. They are the species-specific and are formed only in certain tissue, cell or organ (Pichersky and Gang 2000). Plant secondary metabolites *i.e.* phytochemicals *etc*. are an important sources for pharmaceutical, that contribute medical properties of plant. They provide the flavors, specific odors and colors in plants. They are found in cereals either as free or bound molecules (Dykes and Rooney 2007).

Secondary metabolites includes alkaloids, glycosides, gums, flavonoids, coumarins, polysaccharides, tannins, terpenes, phenols and terpenoids and formed in plants ordinary metabolic processes (Harborne 1973; Okwu 2004). Flavonoids are one of the important group of secondary metabolites, its, water soluble phenolic glycosides imparting colour to flowers and fruits of higher plants. Flavonoids also contribute in the physiological functions such as seed maturation and dormancy (Brenda 1998). These are bioactive compounds with more than 9000 structural variants known (Williams and Grayer, 2004). According to (Peer and Murphy 2007) one of their most important roles is to influence the transport of plant hormone auxin. These types of compounds also appear for play vital roles to defense plants against pathogens and predators (Winkel-Shirley 2002). It also have the properties like anti-inflammatory, antioxidant, antiallergic, antithrombotic, antiviral and anticarcinogenic activities (Middleton and Kandaswami 1993, Williams and Grayer 2004; Garcia – Mediavilla *et al.* 2007; Pandey *et al.* 2007; Kim *et al*. 2008; Singh *et al*. 2008; Sharma and Sarin 2012c; 2012d).

Juice of wheatgrass may be used for increasing production of haemoglobin in blood, improving wound healing, prevention of tooth decay and prevention of bacterial infections. It should conjointly used as removing of significant metals, medicine and cancer-causing agents from the body and also for removing toxins from the liver and blood (MacIntosh 2008). Many more research has been done on secondary metabolites available in wheat grass juice but there are lacks of research in wheat grain. So, this research interest is made on that.

MATERIALS AND METHODS

The experiment was conducted at Dhakarkheri village, Kaithoon road, Kota (Rajasthan) situated in between 25° 11′ N latitude and 75° 54′ E longitudes at 273 m altitude from mean sea level, during two consecutive *rabi* season 2015-16 and 2016-17. In this study seeds of 2 wheat species 10 genotypes (Recommended for the region) in which 5 genotypes each of species *Triticum aestivum* L. (Raj 4037, Raj 4238, GW 322, GW 366, HI 1544) and *Triticum durum* desf. (Raj 6560, MPO 1215, HI 8498, HI 8737, HD 4728) was sown in Randomized Block Design with three replications.

Isolation, Identification and Quantification of Secondary Metabolite (Flavonoids)

Extraction

Seeds of studied wheat genotypes were air dried and grind fine powdered separately. All the genotypes seed samples was extracted separately with 80% methanol on water bath (Subramanian and Nagarajan 1969) for 24 hrs. In the beginning methanol soluble fractions were filtered then concentrated *in vacuo* and aqueous fractions were later fractioned by sequential extraction with petroleum ether (Fr I), diethyl ether (Fr II) and ethyl acetate (Fr III), separately. Every process was repeated three times for complete extraction; fraction I was discarded in each process because it contain fatty substance, whereas fraction II

and fraction III were concentrated and used for evaluation of flavonoids.

Fraction III was hydrolyzed by refluxing with 7% Sulphuric acid (H_2SO_4) (10 mLg^{-1} plant material for 2 hrs.) filtered and filtrate was extracted three times with ethyl acetate. All ethyl acetate layers were pooled separately, neutralized by distilled water with repeated washings and concentrated *in vacuo.* Both, fraction II and III were taken up in a little volume of ethanol (2-5 mL) before chromatographic investigations.

Qualitative procedure

Thin Layer Chromatography (TLC)

20 × 20 cm thin glass plates were coated with 250 mμ thick Silica gel G. Newly prepared gel plates then air dried at room temperature; thereafter these TLC plates were kept at 100°C for ½ hrs to activate and then cooled at room temperature. These silica gel plates may be used for the determination.

Every extracted sample was co-chromatographed with an authentic flavonoid as marker (quercetin and kaempherol). Ready TLC plates were developed in an air tight chromatographic chamber and then saturated with solvent mixture (Benzene: Acetic Acid: Water :: 125:72:3; Wong and Francis, 1968). Developed gel plates were air dried and visualized under UV light by exposure to ammonia fumes. The mouth of a 100 mL containing concentrated NH_4OH was held in contact with each spot for about 5-10 seconds and fluorescent spots corresponding to that of standard markers were marked. Thereafter, gel plates sprayed with 5% $FeCl_3$ and 0.1% alcoholic $AlCl_3$ kept in an I_2 (Iodine) chamber, separately. Colored spots developed to be noted and the Rf value of each spot was then calculated and computed. Many other such type of solvent systems like n-butanol: acetic acid: water :: 4:1:5; tertiary butanol: acetic acid: water :: 3:1:1 were also used for this purpose, but the solvent system benzene: acetic acid: water :: 125:72:3 gave the appropriate and suitable result.

GC-MS Profiling

The extract and standard samples were analyzed by GC-MS (Gas Chromatography and Mass Spectroscopy) of Hewlett-Packard 6890/5973 operating at 1000 ev ionization energy, equipped with using Agilent 7890A/ 5975C GC HP-5. Containing capillary column (phenyl methyl siloxane, 25 m×0.25 mm i.d) with Helium (He) was used as carrier gas with flow of (0.9 mL/min) and split ratio of 1:5. Oven temperature was 100°C (3 min) to 280°C at 1 to 40°C/min; detector temperature 250°C to 280°C. Retention indices were determined by using retention times (RT) of samples that were injected under the same chromatographic conditions. All components of the standard and plant samples were justified by comparison of their mass spectra and retention time with those given in literature and by comparison with the mass spectra of Wiley library or by the published mass spectra.

RESULTS AND DISCUSSION

Spot of flavonoids were observed in sample of wheat species *viz; Triticum aestivum* L. and *Triticum durum* desf. by thin layer chromatography (TLC) plates prepared and sprayed with 5% $FeCl_3$. The R_f value of the spots matched with their authentic standards and then identified as quercetin and Kaempferol. Solvent system Benzene: Acetic Acid: Water (125:72:3) gave best results with R_f values *viz;* quercetin 0.78 and Kaempferol 0.83 (Table 2). Availability of flavonoid compounds was confirmed with thin layer chromatography (TLC) and Gas chromatography and mass spectrometry (GC-MS). The identification and isolation of different flavonoids (Quercetin and Kaempferol) was done by some common measurements *i.e.* mp, mmp, performed in capillaries (Toshniwal freezing Point Apparatus), IR (Infra-red spectrophotometer; Perkin, Elmer 337, Grating Infra-red spectrophotometer) UV (Ultraviolet and visual spectrophotometer; Carl Zeiss, Jena, DDR, VSU-ZP spectrophotometer) analysis along with their several authentic samples. TLC plates proved that two fluorescent spots coinciding are as authentic standard of quercetin and Kaempferol. Further identification was done by R_f values (quercetin 0.78 and Kaempferol 0.83), melting point (Quercetin 309-311°C, Kaempferol 271-273°C) and the colour reaction tests, when sprayed with 5% ethanolic $FeCl_3$ (Quercetin: green-yellow, Kaempferol: yellow-brown). The characteristic IR peaks of isolated flavonoids were found to be super impossible with their respectable standard quercetin and kaempferol.

Table 1: Flavonoid contents (free + bound) in 10 genotypes of *Triticum* species

Sl. No.	Wheat Genotypes	Free flavonoids (mg/gdw)			Bound flavonoids (mg/gdw)			Total Kaempferol (mg/gdw)	Total Quercetin (mg/gdw)	Total flavonoids (free+bound) (mg/gdw)
		Q	K	T	Q	K	T			
1	Raj 4037	0.35	0.76	1.11	0.03	0.24	0.27	1.0	0.38	1.38
2	Raj 4238	0.29	0.65	0.94	0.02	0.12	0.14	0.77	0.31	1.08
3	GW 322	0.13	0.44	0.57	0.09	0.36	0.45	0.8	0.22	1.02
4	GW366	0.18	0.44	0.62	0.14	0.36	0.50	0.8	0.32	1.12
5	HI1544	0.09	0.30	0.39	0.05	0.28	0.33	0.58	0.14	0.72
6	Raj 6560	0.18	0.33	0.51	0.12	0.29	0.41	0.62	0.3	0.92
7	MPO1215	0.22	0.47	0.69	0.06	0.32	0.38	0.79	0.28	1.07
8	HI 8498	0.37	0.78	1.15	0.10	0.35	0.45	1.13	0.47	1.60
9	HI 8737	0.29	0.64	0.93	0.08	0.55	0.63	1.19	0.37	1.56
10	HD 4728	0.43	0.85	1.28	0.11	0.36	0.47	1.21	0.54	1.75

Abbreviations: Q: Quercetin, K: Kaempferol, T: Total.

Table 2: Chromatographic data and colour reaction of the flavonoids isolated from seed of *Triticum* species

Flavonoids	Rf (×100) in BeAW+		Colour reaction		MP°C	UV maximum
	Standard	Seed	Day- light	I_2 vapours		
Quercetin	78	0.77	GN-YW	YW-BN	309-311	258, 373
Kaempferol	83	0.81	GN-YW	YW-BN	271-273	268, 368

Abbreviations: +BeAW = Benzene: Acetic acid: Water (125: 72: 3); BN = Brown; GN = green; YW = yellow.

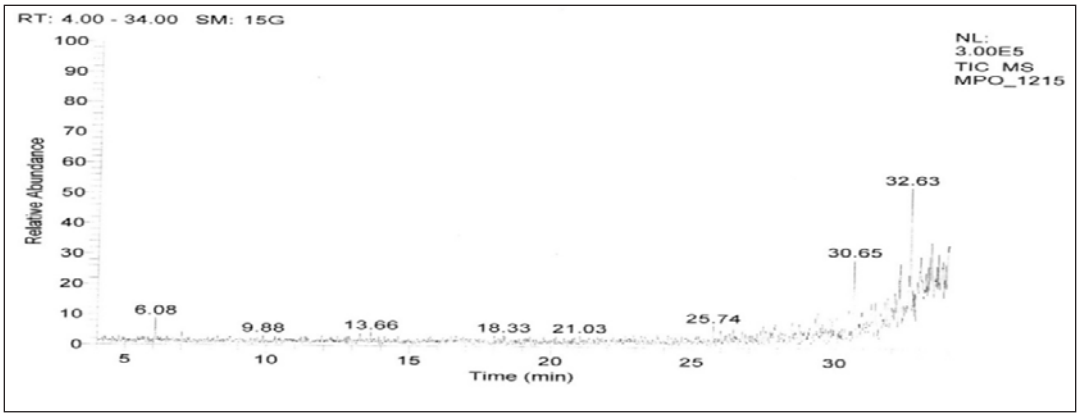

Fig. 1: GC-MS analysis of *Triticum* species genotype MPO 1215

In the most excessive dietary flavonoids quercetin is one with an average of daily consumption of 25 mg to 40 mg (Formica and Regelson 1995). Kaempferol is a secondary substance that is type of flavonoid commonly used in several plants, plant-derived foods and ancient medicines (Calderon-Montano *et al.* 2011). It is used as an antioxidant. Ancient research studies recommend that consuming kaempferol may decrease the risk of many types of cancers but currently, it is under the contemplation for possible cancer treatment.

It was observed that in GC-MS profiling of flavonoids, total 50 compounds each were found for 4 best among 10 studied genotypes of *Triticum* species. The spectra revealed that major compound present in seed of *Triticum* sp. were Butyl Isobutyl Isobutal (9.81%), followed by Phenyltetrafluorophosphorane (7.52%), Oxalic acid, monoamide, n-propyl, tridecyl ester (6.73%), Dichloroacetic acid, 2,2-dimethylpropyl ester (5.02%) and Propane,1-(2,2-dichloro-1-methylcyclopropyl)-2,2-dimethyl (5.02%) in MPO 1215 genotype (Table 3, Fig. 1).

Table 3: Phytocompounds identified by GC-MS in seed extract of *durum* wheat genotype (MPO 1215)

Sl. No.	RT	Compound Name	Molecular Formula	MW	Area (%)
1	6.08	4H-1-Benzopyran-4-one, 3,8-dihydroxy-5,6,7-trimethoxy-2-(4-methoxyphenyl)-	$C_{19}H_{18}O_8$	374	0.83
2	25.74	6-Ethyl-4,5,7,8-tetrathia-2-nonene	$C_7H_{14}S_4$	226	0.61
3	26.44	Cyclohexane, 1,4-dichloro-, cis-	$C_6H_{10}C_{l2}$	152	0.45
4	27.49	2-(tert-Butylsulfonyl)-N'-hydroxyethanimidamide	$C_6H_{14}N_2O_3S$	194	0.69
5	27.90	Isoindole-1,3-dione, 1,3-dihydro-5-(3-aminophenoxy)-2-(4-aminophenyl)-	$C_{20}H_{15}N_3O_3$	345	1.83
6	28.49	Pentane-1,3-diol dipropionate, 2-methyl-	$C_{12}H_{22}O_4$	230	0.66
7	28.74	1-Butanol, 2-methyl-, propanoate	$C_8H_{16}O_2$	144	0.79
8	29.30	Acetic acid, trifluoro-, 2,2-dimethylpropyl ester	$C_7H_{11}F_3O_2$	184	1.22
9	29.39	1,3,2-Dioxathiolane, 4-methyl-, 2-oxide	$C_3H_6O_3S$	122	1.41
10	29.48	Peroxide, dibutyl	$C_8H_{18}O_2$	146	0.36
11	29.55	Cyclopropaneethanol, 2-iodo-	C_5H_9IO	212	0.49
12	29.66	Pentanoic acid, 1,1dimethylpropyl ester	$C_{10}H_{20}O_2$	172	0.67
13	29.84	5-Amino-7-methylaminofurazano[3,4-d]pyrimidine	$C_5H_6N_6O$	166	0.33
14	29.89	Tert-Butyl methyl carbonate	$C_6H_{12}O_3$	132	0.82
15	30.04	Propanamide, 2,3,3,3-tetrafluoro-2-trifluoromethoxy-	$C_4H_2F_7NO_2$	229	1.92
16	30.24	1-(2-Phenylsulfanyl-ethyl)-pyrrolidine	$C_{12}H_{17}NS$	207	0.35
17	30.48	4-((5-Ethenyl-1-azabicyclo(2,2,2)octan-2-yl)oxymethyl)-6-methoxy-quinoline	$C_{20}H_{24}N_2O_2$	324	2.31
18	30.65	Oxalic acid, butyl cyclobutyl ester	$C_{10}H_{16}O_4$	200	4.84
19	30.91	Pentane, 1bromo3,4dimethyl	$C_7H_{15}Br$	178	1.10
20	30.95	Dichloroacetic acid, 2,2dimethylpropyl ester	$C_7H_{12}C_{l2}O_2$	198	1.02
21	31.26	Carbonic acid, neopentyl cyclohexylmethyl ester	$C_{13}H_{24}O_3$	228	1.28
22	31.40	Trifluoroacetyl-di-tbutylphosphine	$C_{10}H_{18}F_3OP$	242	2.53
23	31.59	Sulfurous acid, isobutyl pentyl ester	$C_9H_{20}O_3S$	208	1.44
24	31.68	5-Hepten-3-one, 5-ethyl-2-methyl-	$C_{10}H_{18}O$	154	0.86
25	31.74	2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	$C_{10}H_{22}O_3S_2$	254	2.19
26	31.83	1nButoxy2,3dimethyldiaziridine	$C_8H_{17}NO$	143	1.86
27	31.98	2-Hexene,1-(pentyloxy)-,(E)-	$C_{11}H_{22}O$	170	1.25
28	32.04	2-Hexenoic acid, 4-methylphenyl ester	$C_{13}H_{16}O_2$	204	0.96
29	32.09	2,4Dimethylpentan3yl isobutyl carbonate	$C_{11}H_{22}O_3$	202	1.83
30	32.15	Ethane, 1,2-dicyclopropyl-	C_8H_{14}	110	1.99
31	32.22	3-Methylbutyl N-(heptafluorobutyryl)isoleucinate	$C_{15}H_{22}F_7NO_3$	397	3.09
32	32.27	Sulfurous acid, butyl hexyl ester	$C_{10}H_{22}O_3S$	222	4.66
33	32.39	Peroxide, dibutyl	$C_8H_{18}O_2$	146	0.50
34	32.63	Butyl Isobutyl Isobutal	$C_{12}H_{26}O_2$	202	9.81
35	32.71	Cis-1-methyl-3-n-nonylcyclohexane	$C_{16}H_{32}$	224	1.25
36	32.75	Acetic acid, trifluoro-, 2,2-dimethylpropyl ester	$C_7H_{11}F_3O_2$	184	1.00
37	32.80	2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	$C_{10}H_{22}O_3S_2$	254	1.31
38	32.88	Dichloroacetic acid, 2,2-dimethylpropyl ester	$C_7H_{12}C_{l2}O_2$	198	5.02
39	32.98	Phenyltetrafluorophosphorane	$C_6H_5F_4P$	184	7.52
40	33.12	1-Cyclopentyl-2,2-dimethyl-1-propanol	$C_{10}H_{20}O$,	156	0.43
41	33.17	6-Chloro-2,2,9,9-tetramethyl-3,7-decadiyn5-ol	$C_{14}H_{21}C_{lO}$	240	1.01
42	33.25	3-Methyl-1-[(1H)-1,2,4-triazol-1-yl]butan-2-one	$C_7H_{11}N_3O$	153	1.26
43	33.30	Oxalic acid, ethyl neopentyl ester	$C_9H_{16}O_4$	188	2.36
44	33.37	2,2-Dimethyl-propyl 2,2-dimethyl-propane-thiosulfinate	$C_{10}H_{22}OS_2$	222	4.22
45	33.57	2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	$C_{10}H_{22}O_3S_2$	254	1.34
46	33.62	Oxalic acid, allyl heptyl ester	$C_{12}H_{20}O_4$	228	2.41
47	33.79	Propane, 1-(2,2-dichloro-1-methylcyclopropyl)-2,2-dimethyl-	$C_9H_{16}C_{l2}$	194	5.02
48	33.83	2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	$C_{10}H_{22}O_3S_2$	254	0.75
49	33.88	Dichloroacetic acid, 2,2dimethylpropyl ester	$C_7H_{12}C_{l2}O_2$	198	1.41
50	33.95	Oxalic acid, monoamide, n-propyl, tridecyl ester	$C_{18}H_{35}NO_3$	313	6.73

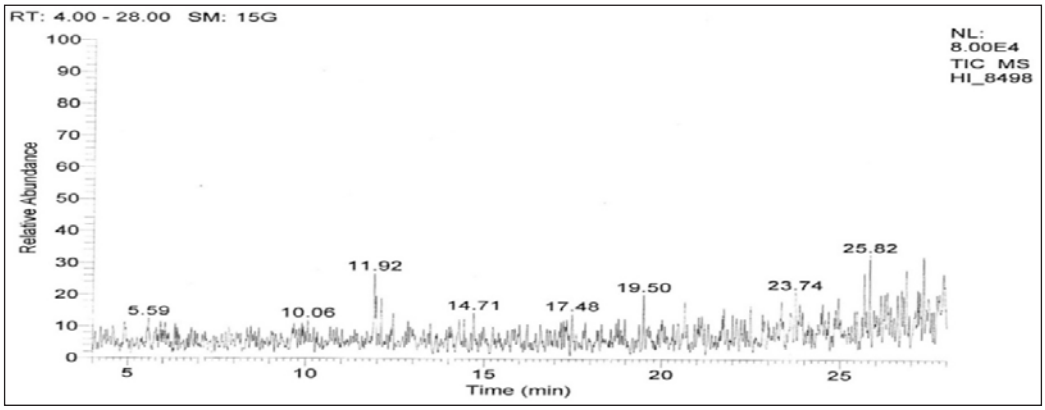

Fig. 2: GC-MS analysis of *Triticum* species genotype HI 8498

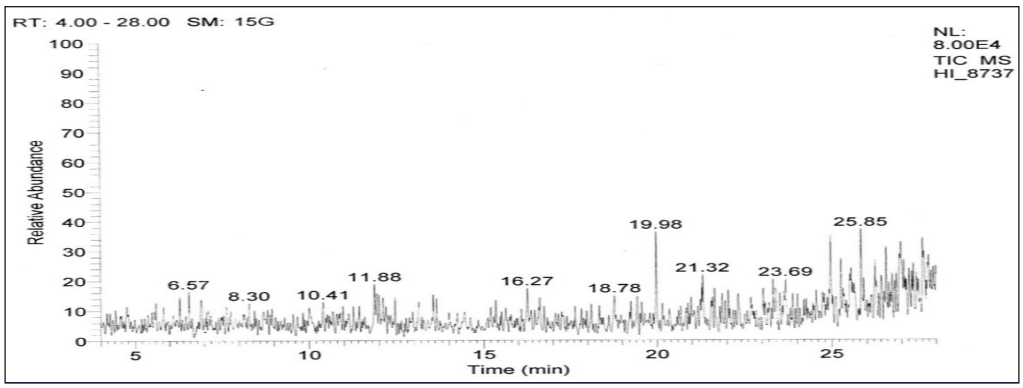

Fig. 3: GC-MS analysis of *Triticum* species genotype HI 8737

In HI 8498 genotype maximum area (6.70%) was observed by Isobutyl pentyl disulfide followed by Tert-Butyl cyclopropylmethyl sulfoxide (6.29%), Conessine (5.18%) and 3nPentylthiolane, S, Sdioxide (5.05 %) (Table 4, Fig. 2).

Maximum area (10.48%) was observed by Cyclohexanemethyl propanoate followed by Isobutyraldehyde, bis (2-methylallyl) acetal (6.37%), 4-Trifluoroacetoxyoctane (4.97%) and 1-Vinylimidazole, 4-nitro-(4.26%) in HI 8737 genotype (Table 5, Fig. 3).

Compound found with maximum area (7.14%) in Dimethylchlorsilyl tert-buthyl peroxide followed by 1,1-Dichloro-1-silacyclohexadiene-2,5 (7.06%), 1,2,4,5-Tetrazine,3,6-diethyl (6.80%) and Acetic acid, (2,4,5-trichlorophenoxy)-, isooctyl ester (6.71%) in genotype HD 4728 (Table 6, Fig. 4).

Quantification data revealed that the total flavonoid content (free + bound) was observed highest and lowest in *durum* genotypes of HD 4728 (1.75 mg/gdw) and Raj 6560 (0.92 mg/gdw), respectively. But, when compared to the *aestivum* species, it was noticed that genotypes Raj 4037 (1.38 mg/gdw) and HI 1544 (0.72 mg/gdw) gave similar trend. In 10 genotypes of 2 wheat species, it was observed that total flavonoids in their bound form was highest in seed of *durum* genotype HI 8737 (0.63 mg/gdw) and lowest in seed of *aestivum* genotype Raj 4238 (0.14 mg/gdw). The total flavonoids content in their free form was highest in *durum* genotype HD 4728 (1.28 mg/gdw) and lowest in seed of *aestivum* genotypes HI 1544 (0.39 mg/gdw). *Durum* genotype HD 4728 gave maximum kaempherol (1.21 mg/gdw) and quercetin (0.54 mg/gdw) content and minimum value was given by *aestivum* genotype HI 1544 (0.58 & 0.14 mg/gdw), respectively (Table 1).

Moreover, in plant secondary metabolites occupied chemical and pharmaceutical properties fascinating for health of human being. Presently different kind of compounds belonging to the terpenoids, alkaloids and flavonoids used as medication or as dietary supplements to prevent human from various unknown diseases (Raskin *et al.* 2002) and in particular a few of these compounds may be efficient for curing various kinds of cancer (Watson *et al.* 2001; Reddy *et al.* 2003).

Table 4: Phytocompounds identified by GC-MS in seed extract of *durum* wheat genotype (HI 8498)

Sl. No.	RT	Compound Name	Molecular Formula	MW	Area (%)
1	11.92	Phenyltetrafluorophosphorane	$C_6H_5F_4P$	184	3.12
2	11.96	2,4-Pentadienoic acid, 1-cyclopenten-3-on-1yl ester	$C_{10}H_{10}O_3$	178	2.28
3	12.11	1,3-Benzenediol, O-(2- ethoxyethoxycarbonyl)-	$C_{10}H_{12}O_5$	212	2.17
4	12.44	Butyric acid, 3amino4methoxy	$C_5H_{11}NO_2S$	149	1.06
5	14.44	20-Carboethoxy-20-demethylvincadifformine	$C_{23}H_{28}N_2O_4$	396	1.10
6	17.48	1-Benzylcyclopentanol-1	$C_{12}H_{16}O$	176	0.94
7	18.80	1,2,3-Oxadiazol-3-ium, 5-trifluoroacetylamidato-3-methyl	$C_5H_4F_3N_3O_2$	195	0.80
8	19.50	Cholestan-6-one, 3-(2-hydroxypropoxy)-cyclic1,2-propanediyl acetal, (3á,5à)-	$C_{33}H_{58}O_4$	518	2.40
9	20.66	4Butylbenzoic acid, octadecyl ester	$C_{27}H_{46}O_2$	402	1.39
10	21.05	Benzene, tert-butyl-	$C_{10}H_{14}$	134	0.80
11	21.14	Octane, 1,1'-[ethylidenebis(oxy)]bis-	$C_{18}H_{38}O_2$	286	0.84
12	21.22	Hydroxyurea, N,N,N',O-tetramethyl-	$C_5H_{12}N_2O_2$	132	0.84
13	21.71	Butyric acid, crotyl ester	$C_8H_{14}O_2$	142	0.82
14	21.74	3Ethoxycarbonylquinoxaline 1oxide	$C_{11}H_{10}N_2O_3$	218	1.53
15	21.99	Succinic acid, 4methoxyphenyl nonyl ester	$C_{20}H_{30}O_5$	350	1.13
16	22.33	Azobenzene	$C_{12}H_{10}N_2$	182	0.94
17	22.49	Benzenesulfonamide, N,4dimethyl	$C_8H_{11}NO_2S$	185	1.49
18	22.82	6Uracilsulfonamide	$C_4H_5N_3O_4S$	191	1.09
19	22.98	Benzenepentanamine	$C_{11}H_{17}N$	163	0.97
20	23.36	5-Chlorovaleramide, N-(2-fluorophenyl)-	$C_{11}H_{13}ClFNO$	229	1.74
21	23.73	1,3-Benzenediol, o-acryloyl-o'-heptafluorobutyryl-	$C_{13}H_7F_7O_4$	360	4.40
22	23.86	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	1.13
23	24.50	Benzenemethanol, 2-chloro-à-[[(1-methylethyl)amino]methyl]-	$C_{11}H_{16}ClNO$	213	1.53
24	24.67	4-Spirohexanone-5,5-dichloro-	$C_6H_6C_{l2}O$	164	1.11
25	24.87	2H-Pyran,2-(butylthio)tetrahydro-	$C_9H_{18}OS$	174	0.88
26	24.94	Propanamide, N-(1-naphthyl)-2,2-dimethyl-	$C_{15}H_{17}NO$	227	1.76
27	25.41	Trifluoromethyl t-butyl sulfide	$C_5H_9F_3S$	158	1.23
28	25.46	Imidazole, 5tbuyloxycarbonylamino4fluoro	$C_8H_{12}FN_3O_2$	201	2.00
29	25.61	Acrylic acid, 2,4dichloronaphthyl1ester	$C_{13}H_8C_{l2}O_2$	266	0.98
30	25.67	Decane, 2,2,9trimethyl	$C_{13}H_{28}$	184	3.76
31	25.73	Fumaric acid, monoamide, N,N-dimethyl-, 4-isopropoxyphenyl ester	$C_{15}H_{19}NO_4$	277	0.96
32	25.82	3nPentylthiolane, S,Sdioxide	$C_9H_{18}O_2S$	190	5.05
33	25.94	Oxalic acid, ethyl isobutyl ester	$C_8H_{14}O_4$	174	0.84
34	26.13	Butanoic acid, 2-oxo	$C_4H_6O_3$	102	2.55
35	26.24	1,2,4Benzenetriol, triacetate	$C_{12}H_{12}O_6$	252	1.74
36	26.31	2,3Dimethyl-5-(2,6,10-trimethylundecyl) thiophene	$C_{20}H_{36}S$	308	1.76
37	26.41	2-Furancarboxylic acid, 3,5-difluorophenyl ester	$C_{11}H_6F_2O_3$	224	0.82
38	26.61	Carbonic acid, bis(2-methylpropyl) ester	$C_9H_{18}O_3$	174	3.03
39	26.72	3-Methylbut-2-enoic acid, 2-diethylaminoethyl ester	$C_{11}H_{21}NO_2$	199	2.65
40	26.78	2,7-Dimethyl-2,6-octadien-4-ol	$C_{10}H_{18}O$	154	1.92
41	26.85	Conessine	$C_{24}H_{40}N_2$	356	5.18

42	26.93	1,2Cyclohexanedimethanol	$C_8H_{16}O_2$	144	0.86
43	27.17	6-Thiopyrazolo[3,4-d]pyrimidin-4,6(5H,7H)-dione-3-carbo xamide	$C_6H_5N_5O_2S$	211	2.11
44	27.23	4-Ethenylhexadecyloxybenzene	$C_{24}H_{40}O$	344	2.19
45	27.33	Tert-Butyl cyclopropylmethyl sulfoxide	$C_8H_{16}OS$	160	6.29
46	27.47	Urea, N,N′-di-2-propenyl-	$C_7H_{12}N_2O$	140	2.69
47	27.63	2Ethylbutyl propionate	$C_9H_{18}O_2$	158	1.12
48	27.72	2-Butene, 1-bromo-	C_4H_7Br	134	1.97
49	27.79	Propanoic acid, 2,2-dimethyl-, 2-(1,1-dimethylethyl)-4-methylphenyl ester	$C_{16}H_{24}O_2$	248	3.36
50	27.91	Isobutyl pentyl disulfide	$C_9H_{20}S_2$	192	6.70

Table 5: Phytocompounds identified by GC-MS in seed extract of *durum* wheat genotype (HI 8737)

Sl. No.	RT	Compound Name	Molecular Formula	MW	Area (%)
1	5.61	Isonipecotic acid, N-(4-butylbenzoyl)-, butyl ester	$C_{21}H_{31}NO_3$	345	0.58
2	6.30	1H-Pyrrolizine-1-methanol, hexahydro-7-hydroxy, [1S-(1à,7à,7aá)]-	$C_8H_{15}NO_2$	157	0.89
3	6.57	1,2-Ethanediamine, N,N-dimethyl-	$C_4H_{12}N_2$	88	1.34
4	11.88	6-Ethoxycarbonyl-7-oxo-1,7-dihydro-[1,2,4]triazolo(4,3-b)[1,2,4]-triazine	$C_7H_7N_5O_3$	209	2.02
5	11.94	Furfuryl sulfide	$C_{10}H_{10}O_2S$	194	2.07
6	12.00	Oxalic acid, cyclohexylmethyl undecyl ester	$C_{20}H_{36}O_4$	340	1.08
7	12.46	5-Pyrimidinol, 2-methyl-	$C_5H_6N_2O$	110	1.11
8	13.55	But-3-enyl (E)-2-methylbut-2-enoate	$C_9H_{14}O_2$	154	0.97
9	13.64	Thiophen-2-methylamine, N,N-diheptyl-	$C_{19}H_{35}NS$	309	0.99
10	16.27	Trifluoromethyl tbutyl disulfide	$C_5H_9F_3S_2$	190	1.31
11	16.62	Isophthalic acid, 2-fluorophenyl isobutyl ester	$C_{18}H_{17}FO_4$	316	0.76
12	18.78	1,1Dimethoxypent4en3one	$C_7H_{12}O_3$	144	0.74
13	19.98	Sedoheptulosan tetrabenzoate	$C_{35}H_{28}O_{10}$	608	3.70
14	20.89	Fumaric monoamide, N-(2-bromophenyl)-, nonyl ester	$C_{19}H_{26}BrNO_3$	395	0.79
15	21.32	2,6-Octadiene-4,5-diol, 3,6-dimethyl-	$C_{10}H_{18}O_2$	170	1.79
16	21.66	1,1,1,3,3-Pentafluoroacetone, fluoroimine	C_3HF_6N	165	1.46
17	22.04	Silane, 2-butenyltrichloro-, (Z)-	$C_4H_7Cl_3Si$	188	1.25
18	23.04	Propanal, butylhydrazone	$C_7H_{16}N_2$	128	1.51
19	23.32	Chromium,[(1,2,3,4ü)-1,3-cycloheptadiene](ü7-cycloheptatrienylium)-	$C_{14}H_{17}Cr$	237	1.63
20	23.39	Thiepane, 1,1dioxide	$C_6H_{12}O_2S$	148	1.97
21	23.52	Sydnone, 3(1,1-dimethylethyl)-	$C_6H_{10}N_2O_2$	142	1.65
22	23.69	Benzenesulfonic acid, methyl ester	$C_7H_8O_3S$	172	1.68
23	23.88	2,5Difluorobenzoic acid, 4cyanophenyl ester	$C_{14}H_7F_2NO_2$	259	1.02
24	24.02	2,4(3H,5H)-Furandione, 3,5-dimethyl-	$C_6H_8O_3$	128	0.83
25	24.55	Trifluoromethyldifluorophosphine	CF_5P	138	0.70
26	24.97	1-Vinylimidazole, 4-nitro-	$C_5H_5N_3O_2$	139	4.26
27	25.26	4-(Iodomethyl)-1-azabicyclo[2.2.2]octane	$C_8H_{14}IN$	251	2.71
28	25.52	Undecane, 6-methyl-	$C_{12}H_{26}$	170	1.56
29	25.56	3-Buten-1-ol, propanoate	$C_7H_{12}O_2$	128	2.48
30	25.85	4-Trifluoroacetoxyoctane	$C_{10}H_{17}F_3O_2$	226	4.97

31	25.92	2,6Octadiene	C_8H_{14}	110	1.24
32	26.25	2,2-Dimethylnon-5-en-3-one	$C_{11}H_{20}O$	168	3.08
33	26.42	Spiropentane	C_5H_8	68	3.74
34	26.50	4-(2-Dimethylaminoethoxy) benzonitrile	$C_{11}H_{14}N_2O$	190	1.40
35	26.56	4Octene, 2,3,6,7tetramethyl	$C_{12}H_{24}$	168	3.27
36	26.64	Oxalic acid, neopentyl propyl ester	$C_{10}H_{18}O_4$	202	1.34
37	26.73	1,3Benzodioxole, 2hexyloxy	$C_{13}H_{18}O_3$	222	1.60
38	26.81	Butanimidamide, N-(1-chloro-2-methyl-1-butenyl)-2-methyl-	$C_{10}H_{19}ClN_2$	202	1.50
39	26.86	3-Iodopropanesulfonic acid, methyl ester	$C_4H_9IO_3S$	264	1.46
40	26.99	Cyclohexanemethyl propanoate	$C_{10}H_{18}O_2$	170	10.48
41	27.07	2-Furancarboxylic acid, 2-formyl-4,6-dichlorophenyl ester	$C_{12}H_6C_{l2}O_4$	284	1.75
42	27.22	1,3-Dinitro-2-imidazolidinone	$C_3H_4N_4O_5$	176	1.46
43	27.30	2-Butanone,1-(2-furanyl)-3methyl-	$C_9H_{12}O_2$	152	1.24
44	27.35	Propargyl alcohol, trifluoroacetate	$C_5H_3F_3O_2$	152	1.61
45	27.44	4Nonene,2,3,3trimethyl,(E)	$C_{12}H_{24}$	168	0.94
46	27.61	Isobutyraldehyde, bis(2-methylallyl) acetal	$C_{12}H_{22}O_2$	198	6.37
47	27.78	1,3-Benzenediol, O-cyclopropanecarbonyl-O′-pivaloyl-	$C_{15}H_{18}O_4$	262	4.00
48	27.86	Butanimidamide, N-(1-chloro-2-methyl-1-butenyl)-2-methyl-	$C_{10}H_{19}ClN_2$	202	0.92
49	27.92	Bicyclo[3.2.1]octane2,3,4trione	$C_8H_8O_3$	152	1.10
50	28.00	(CH3)2NCH2Si(CH3)3	$C_6H_{17}NSi$	131	1.63

Table 6: Phytocompounds identified by GC-MS in seed extract of *durum* wheat genotype HD 4728

Sl. No.	RT	Compound Name	Molecular Formula	MW	Area (%)
1	14.39	2,6 Difluorobenzoic acid, phenyl ester	$C_{13}H_8F_2O_2$	234	0.37
2	20.10	Ethyl n-butyl disulphide	$C_6H_{14}S_2$	150	0.64
3	22.51	4-Allyl-1,6-heptadiene-4-ol	$C_{10}H_{16}O$	152	0.49
4	22.62	Butylphosphonic acid, 4chlorophenyl propyl ester	$C_{13}H_{20}C_lO_3P$	290	0.82
5	23.97	Borinic acid, diethyl-, 1-phenyl-1-propenyl ester	$C_{13}H_{19}BO$	202	0.40
6	26.26	(Phenylthio)acetic acid, propyl ester	$C_{11}H_{14}O_2S$	210	0.41
7	27.31	Hexafluoromethanediamine	CF_6N_2	154	0.51
8	27.87	Peroxide, dibutyl	$C_8H_{18}O_2$	146	0.54
9	27.97	2Oxovaleric acid, methyl ester	$C_6H_{10}O_3$	130	0.68
10	28.09	Dimethylphosphinic azide	$C_2H_6N_3OP$	119	1.84
11	29.10	Furmecyclox	$C_{14}H_{21}NO_3$	251	0.56
12	29.34	Propane, 3-chloro-1,1,1-trifluoro	$C_3H_4ClF_3$	132	0.86
13	29. 41	4Fluorophenyl 2thiophenecarboxylate	$C_{11}H_7FO_2S$	222	1.16
14	29.45	2Methyl-4-bromo-1-butene	C_5H_9Br	148	1.39
15	30.07	Peroxide, dibutyl	$C_8H_{18}O_2$	146	1.16
16	30.12	N-Methyl-2-isopropoxycarbonylazetidine	$C_8H_{15}NO_2$	157	0.62
17	30.19	2-Buten-1-ol, propanoate	$C_7H_{12}O_2$	128	0.81
18	30.30	Propane-1,1-diol dipropanoate	$C_9H_{16}O_4$	188	1.76
19	30.53	Tetrahydrofuran-2-carboxylic acid, dibenzofuran-3-ylamide	$C_{17}H_{15}NO_3$	281	0.90
20	30.60	3-Pentanone, 2-methyl-	$C_6H_{12}O$	100	0.55
21	30.95	Glyoxylic acid, O-penta fluorobenzyloxime, trimethylsilyl ester	$C_{12}H_{12}F_5NO_3Si$	341	0.46
22	31.18	2Propenoic acid, 2methyl, 4formyl2methoxyphenyl ester	$C_{12}H_{12}O_4$	220	1.27
23	31.34	Oxalic acid, neopentyl pentyl ester	$C_{12}H_{22}O_4$	230	0.94

24	31.43	2-(1-Methylcyclohexyloxy)-tetrahydropyran	$C_{12}H_{22}O_2$	198	1.40
25	31.57	Propanamide, 2,2-dimethyl-N (2,4,6-tribromophenyl)-	$C_{11}H_{12}Br_3NO$	411	1.55
26	31.71	Methane, bis[4-(2,2dimethylpropanamido) phenyl]-	$C_{23}H_{30}N_2O_2$	366	1.40
27	31.79	Butanoic acid, heptafluoro-, 1-(butoxycarbonyl)propyl ester	$C_{12}H_{15}F_7O_4$	356	3.25
28	31.85	Benzenamine, 4chloro-N-[(4nitrophenyl) methylene]-,N-oxide	$C_{13}H_9ClN_2O_3$	276	0.58
29	31.89	Benzaldehyde, 3-benzyloxy-2-fluoro-	$C_{14}H_{11}FO_2$	230	1.09
30	31.96	4-Hydroxybutyl acrylate	$C_7H_{12}O_3$	144	0.54
31	32.08	2-Pentanone, 1(2-furanyl)-	$C_9H_{12}O_2$	152	2.02
32	32.12	2,2-Dimethylpropanoic acid, cyclobutyl ester	$C_9H_{16}O_2$	156	2.82
33	32.28	4,8-Dioxatricyclo[5.1.0.0(3,5)]octane, 1-methyl-5-(1-methylethyl)-,(1à,3á,5á,7à)-	$C_{10}H_{16}O_2$	168	6.30
34	32.46	2-Ethylthiolane, S,S-dioxide	$C_6H_{12}O_2S$	148	1.19
35	32.53	2,2-Dimethyl-propyl 2,2-dimethyl-propane-thiosulfinate	$C_{10}H_{22}OS_2$	222	1.12
36	32.60	3-Butenoic acid, ethyl ester	$C_6H_{10}O_2$	114	1.67
37	32.68	1-Phenyl-1-nonanol	$C_{15}H_{24}O$	220	5.59
38	32.88	Octane, 2,3,6-trimethy-1-	$C_{11}H_{24}$	156	3.38
39	32.95	1,2-Benzenediol, O-acryloy-1-	$C_9H_8O_3$	164	2.20
40	33.01	Oxalic acid, isobutyl propyl ester	$C_9H_{16}O_4$	188	2.38
41	33.07	Acetic acid, (2,4,5-trichlorophenoxy)-, isooctyl ester	$C_{16}H_{21}Cl_3O_3$	366	6.71
42	33.14	cis2,4Dimethylthiane, S,Sdioxide	$C_7H_{14}O_2S$	162	2.24
43	33.26	Chloroacetic acid, 2,2-dimethylpropyl ester	$C_7H_{13}ClO_2$	164	1.24
44	33.37	1,3,7-Nonatriene-1,1-dicarbonitrile, 4,8-dimethyl-, (E)-	$C_{13}H_{16}N_2$	200	2.80
45	33.46	1,2:3,4-Bis-O-isopropylidene-d-galactopyranose sulfamate	$C_{12}H_{21}NO_8S$	339	3.23
46	33.57	Dimethylchlorsilyl tert-buthyl peroxide	$C_6H_{15}ClO_2Si$	182	7.14
47	33.66	Cis-2-Nitro-4-t-butylcyclohexanone	$C_{10}H_{17}NO_3$	199	2.50
48	33.74	1,2,4,5-Tetrazine,3,6-diethyl-	$C_6H_{10}N_4$	138	6.80
49	33.85	Oxalic acid, cyclobutyl isohexyl ester	$C_{12}H_{20}O_4$	228	2.67
50	33.98	1,1-Dichloro-1-silacyclohexadiene-2,5	$C_5H_6Cl_2Si$	164	7.06

RT- Retantion Time, MW- Molecular weight.

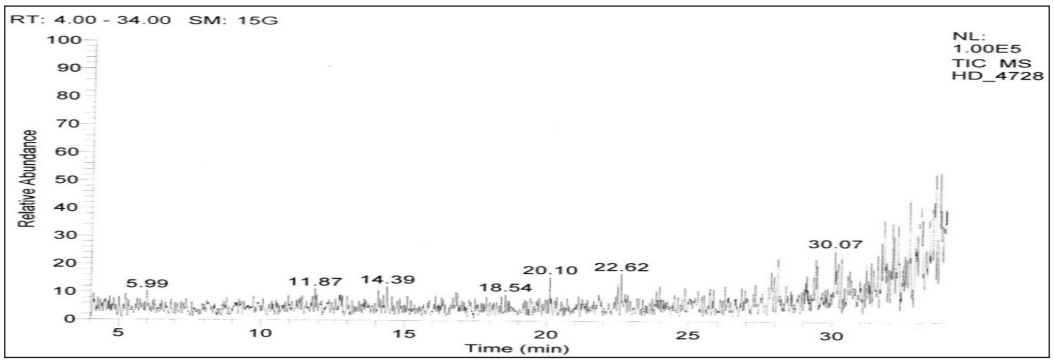

Fig. 4: GC-MS analysis of *Triticum* species genotype HD 4728

In the present investigation, flavonoid has been extracted from seed of *Triticum sp*. The isolated compounds were identified and confirmed by using the techniques like chromatography and spectroscopy. In various kinds of phytonutrients, flavonoids are the strongest antioxidant with having different helpful properties *i.e.* anti inflammatory and immunity. Flavonoids rich diets are associated with cancer and cardio vascular disease prevention. Although, this is not yet much determined that flavonoid are main factor for this. Flavonoids are a part of polyphenol class of phytonutrients. There are various significant sub-groups of flavonoids. Some important are including anthocynidins, flavanols, flavones, flavonols, flavonones and isoflavones. It is consumed to reduce the risk of heart related

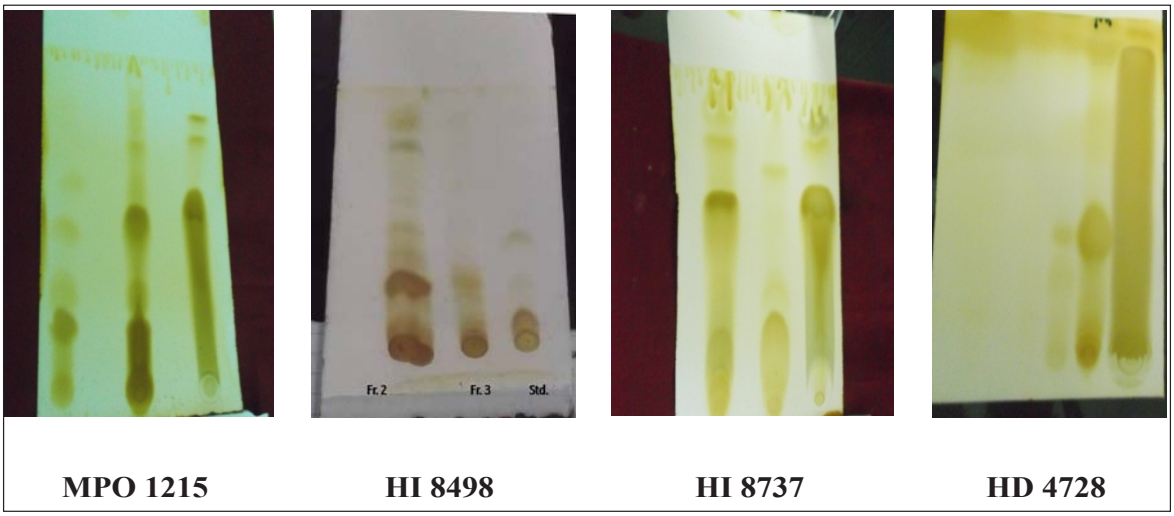

Fig. 5: TLC plates of *Triticum* genotypes

diseases in the body (Urquiaga and Leighton 2000). As anti-cancer activity treatment, they inhibit the initiation, promotion and progression of the several kinds of tumors (Urquiaga and Leighton 2000; Okwu 2004). Flavonoid compounds seem to have significant characters to stop against pathogens, predators and contribute to physiological activities like seed maturation and dormancy (Winkel-Shirley 2002).

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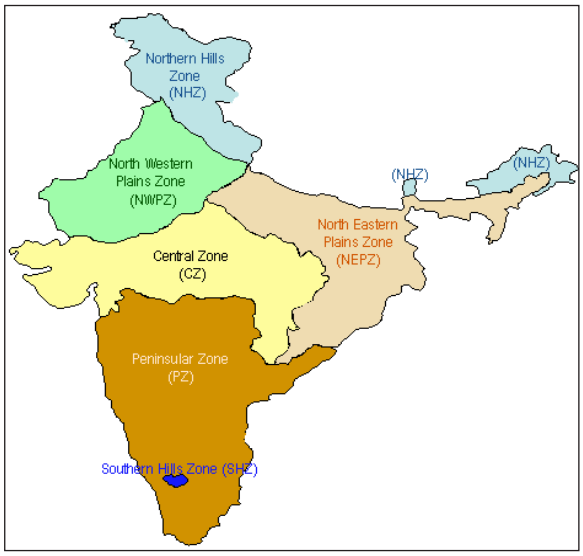

Map I: Wheat growing mega zones of India

Source: *http://www.krishisewa.com/agroclimatic-zones/wheat-growing-zones.html*

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