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Evaluation and Identification of Volatile Bio Active Compounds in Methanol Extract of Pearl Millet Genotypes by Gas Chromatography-Mass Spectroscopy

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ABSTRACT

Pearl millet (Pennisetum glaucum) is an important food staples in semi-arid tropics of Asia and Africa. Millet is cereal grains that have prospective to be used as substitute to wheat flour for celiac patients. It is considered as the good source of many important and essential fatty acids. The present study was an effort to explore biochemical composition of five millet varieties with special reference to their volatile profiling. Pearl millet seeds of HHB-67, RHB-177, RHB-173, GHB-558 and GHB-538 were used for identification of volatile compounds in methanolic extract. The GC-MS scan analysis results reveal that linoleic acid was found as major constituents prominently in methanol extract of pearl millet seed. Out of five genotypes RHB-173 contains highest linoleic acid content in methanol extract.

Keywords: *Pearl Millet, GC-MS, linoleic acid*

INTRODUCTION

Millets are a group of cereal crops grown in semi-arid tropics of Asia and Africa for food and staples. The millets include species in several genera, mostly in the sub-family Panicoideae, of the grass family Poaceae. Pearl millet (*Pennisetum glaucum*) is one of the most important drought-resistant crops. Also, millet has resistance to pests and diseases, short growing season, and productivity under drought conditions, compared to major cereals[1]. Millets rank as the sixth most important cereal in the world especially in developed countries where they serve as staple foods for millions of people[2]. It is also known as 'bajra' and is a prominent food of western Rajasthan of India. It is one of the four most important cereals (rice, maize, sorghum and millets) grown in the tropics and is rich in iron and zinc, contains high amount of antioxidants and these nutrients may be beneficial for the overall health and wellbeing. Millet is a primary sources of nutrients e.g. protein, mineral, vitamins and energy[3].

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Pearl millet grain fractions and extracts were found to have antimicrobial activity[4]. Millets contain phenols, phytic acid and tannins which can contribute to antioxidant activity, important in health, ageing and metabolic diseases[5,6]. The main volatile compounds of cereals include alcohols, hydrocarbons, aldehydes, ketones, esters, acids, benzene derivatives, heterocycles and sulphur[7]. While, extensive information is available on proximate composition and mineral accessibility, information on volatile biochemical compounds in pearl millet is scanty.

The present study was conducted at Sanitary and Phytosanitary Laboratory of Agricultural Research Station, Mandor, (Agriculture University Jodhpur) for evaluation and identification of Volatile bioactive compounds present in methanol extract of Pearl millet genotypes prominently grown in western part of the Rajasthan. The seed samples were provided by All India Coordinated Pearl Millet Improvement Project, (Project Coordinating Unit) Mandor, Jodhpur. This is first reported study in pearl millet genotypes HHB-67, RHB-177, RHB-173, GHB-558 and GHB-538.

MATERIALS AND METHODS

Sample Preparation

Five grams of grounded powder was extracted successively with methanol in soxhlet extractor for 16 hours. Green colour residues were obtained after concentrating the extract under reduced pressure using rotary evaporator. The obtained extracts were stored in desiccators for further analysis. The dried sample was re-dissolved in methanol to obtain 10 μg m^{-1} concentrations. Finally, 2 ml of supernatant was taken and filtered through Axiva 0.2 μm nylon syringe filter and transfer to GC vial for analysis[8].

GC-MS conditions

GC-MS analysis was carried out on a Shimadzu GCMS-QP2010 Ultra system. The injector temperature was 280°C. The samples were injected in the split mode with split ratio 1/25. Injection volume was 1 μL. A capillary column Rtx-5MS (5% Diphenyl-95% Dimethyl Polysiloxane), 30 m x 0.25 mm x 0.25μm, was used. Career gas was helium with constant flow of 1.00 ml min^{-1}.

The oven temperature was as follows: initial temperature of 60°C, held for 2 min, increased to 10°C min^{-1} up to 260°C and held for 10 min. The MS ionization potential was 70 eV, and the temperatures were as follows: interface 260°C, Ion source 280°C. Mass scan range 40-550. [9]

RESULTS AND DISCUSSION

GC-MS chromatograms profile of the methanolic extract of pearl millet genotypes showed the number of phytochemical constituents. Eight compounds were identified in all varieties and classified respectively in different chemical classes (Unsaturated fatty acid, aldehydes, organic acids, and amino acids,) (Table-1 & Figure-2). Linoleic acid was found prominently as major constituents in all genotypes followed by n-Hexadecanoic acid.

RHB-173 contains highest linoleic acid content (59.71%) among all genotypes followed by HHB-67 (48.87%), GHB-558 (47.37%), GHB-538 (28.79%) and RHB-177 (10.43%). Linoleic acid is a polyunsaturated omega-6 fatty acid belongs to one of the two families of essential fatty acids, which means that the human body cannot synthesize it from other food components.

Undecane and naphthalene were found in remarkable amount in all genotypes; RHB-177 contains highest naphthalene content (47.83%) among all genotypes. Highest nitroisobutylglycerol content was found in GHB-538 while it was not found in HHB-67 and RHB-177.

n-Hexadecanoic acid was also found most prominently in all genotypes. Highest amount was fond in RHB-177 (18.17%) followed by RHB-173 (16.36%), HHB-67 (15.57%), GHB-558 (13.09%) and GHB-538 (8.07%). Presence of low amount of oleic acid amide was notices only in RHB-177 (5.75%) and RHB-173 (1.30%).

β-Tocopherol was found in trace amount in genotype GHB-558 (1.13%) and GHB-538 (1.30%) while other genotypes not have β-Tocopherol.

β-Sitosterol was found in very remarkable amount in GHB-538 (13.77%) and GHB-558 (8.59%), RHB-173 (3.00%) contains small amount of β-Sitosterol while in HHB-67 and RHB-177 genotypes it was not found. Figure-1 represents the comparative analysis of volatile bioactive compounds in pearl millet genotypes. GC-MS chromatograms of all analyses were shown as figure 3 to figure 7.

A attempt have been made in this study to evaluate the volatile phytochemical constituents of methanolic extract of pearl millet genotypes which will lead to characterization of these genotypes with reference to volatile biochemical constituents.

In literature previously some author were reported volatile profiling of pearl millet. Singh *et al.*[10] reported 1,2 benzenedicarboxylic acid (BDC) as prominent compound in pearl millet.

Chughtai *et al.*[11] Analyzed volatile profile of Pakistan growing pearl millet variety MB-87 and S. Bajra and reported that Aldehydes were the main compounds present in both the varieties.

Liu *et al.*[12] analyzes volatile composition in brown millet, milled millet and millet bran by gas chromatography/mass spectrometry and reported Aldehydes and benzene derivatives were the most numerous among all of the compounds. hexanal, hexadecanoic acid and 2-methylnaphthalene were dominant in the brown millet and mild millet.

In current study Linoleic acid and n-Hexadecanoic acid abundance was found in all genotypes.

Table 1: Volatile Biochemical Composition of Pearl Millet Genotypes

S.No.	Compound Name	R.Time	Mol Weight	Formula	CAS No.	Area %				
						HHB 67	RHB 177	RHB 173	GHB 558	GHB 538
1.	Undecane	6.475	156	$C_{11}H_{24}$	1120-21-4	5.54	10.97	2.60	4.06	3.56
2.	Naphthalene	7.546	128	$C_{10}H_8$	91-20-3	7.68	47.83	8.19	7.31	3.28
3.	Nitroisobutylglycerol	9.991	151	$C_4H_9NO_5$	126-11-4	0.00	0.00	7.29	18.47	35.78
4.	n-Hexadecanoic acid	13.756	256	$C_{16}H_{32}O_2$	57-10-3	15.57	18.17	16.36	13.09	8.07
5.	Linoleic acid	14.895	280	$C_{18}H_{32}O_2$	60-33-3	48.87	10.43	59.71	47.37	28.79
6.	Oleic acid amide	16.233	281	$C_{18}H_{35}NO$	301-02-0	0.00	5.75	1.30	0.00	0.00
7.	β-Tocopherol	21.775	416	$C_{28}H_{48}O_2$	148-03-8	0.00	0.00	0.00	1.13	1.30
8.	β-Sitosterol	26.762	414	$C_{29}H_{50}O$	83-46-5	0.00	0.00	3.00	8.59	13.77

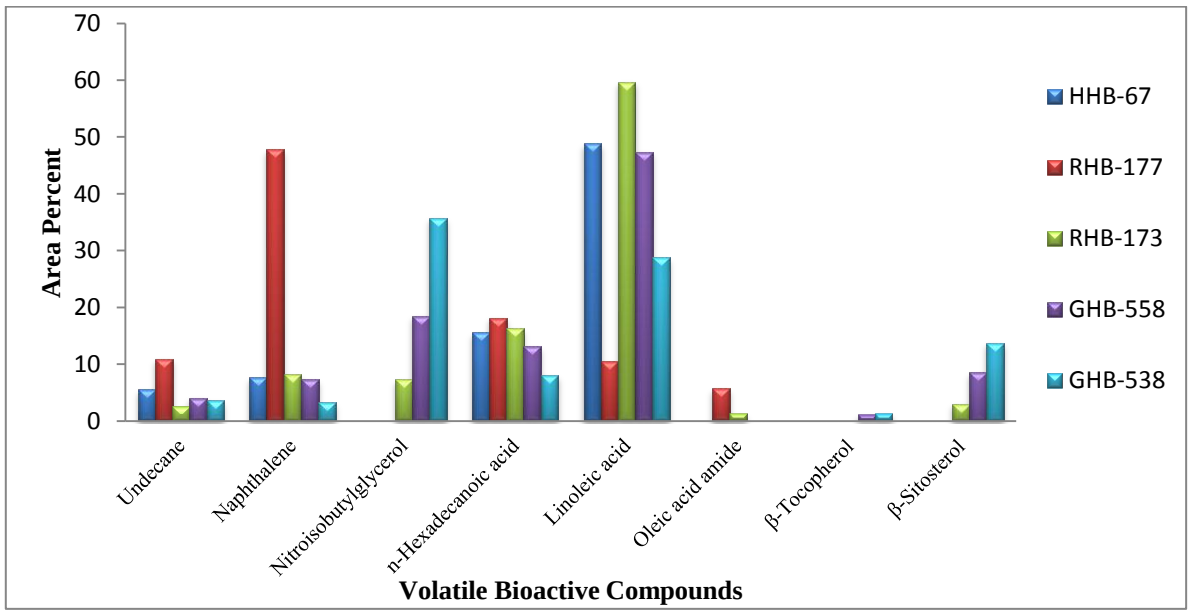
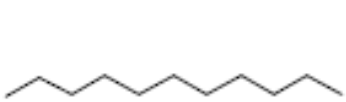
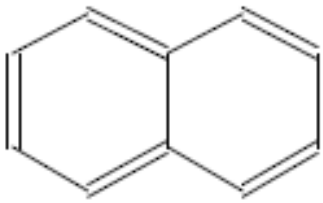


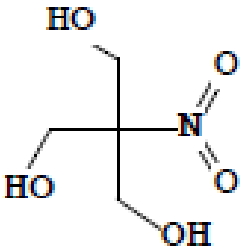
Fig. 1: Comparative Analysis of Volatile Bioactive Compounds in Pearl Millet Genotypes



Undecane

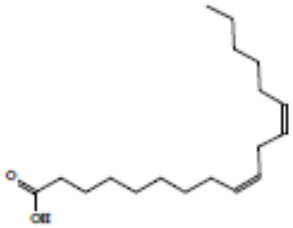


Naphthalene

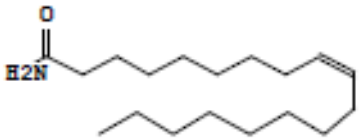


Nitroisobutylglycerol

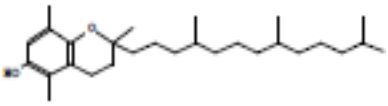
n-Hexadecanoic acid



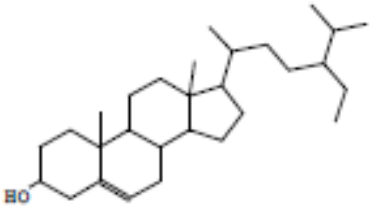
Linoleic acid



Oleic acid amide



β-Tocopherol



β-Sitosterol

Fig. 2: Chemical Structures of Methanolic Extract Constituents of Pearl Millet Genotypes

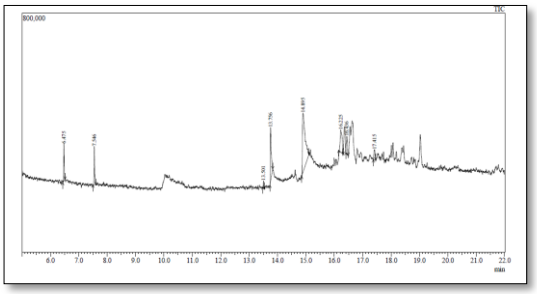


Fig. 3: GC-MS Chromatogram of Pearl millet Genotype HHB-67

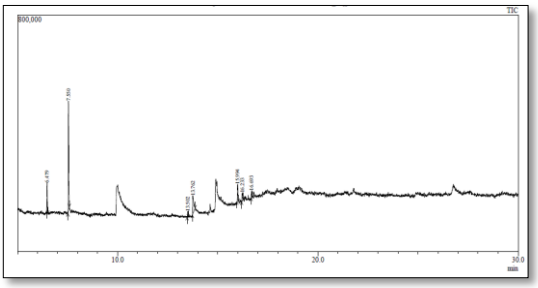


Fig. 4: GC-MS Chromatogram of Pearl millet Genotype RHB 177

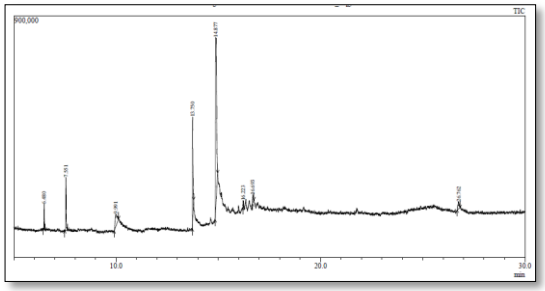


Fig. 5: GC-MS Chromatogram of Pearl millet Genotype RHB 173

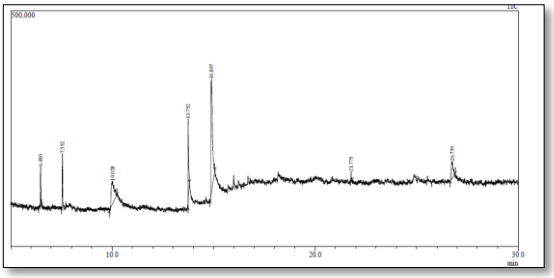


Fig. 6: GC-MS Chromatogram of Pearl millet Genotype GHB 558

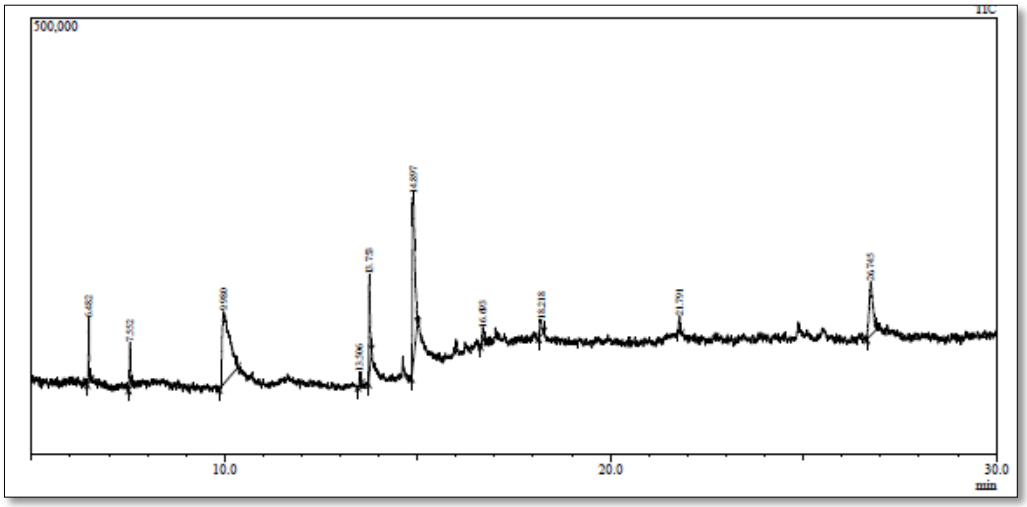


Fig. 7: GC-MS Chromatogram of Pearl millet Genotype GHB 538

CONCLUSION

Pearl millet is one of the most important crops produced prominently from western Rajasthan of India and occupies significant place in Indian agriculture. There were very less literature available on volatile profiling of pear millet. Current study conducted to evaluate the volatile phytochemical constituents in methanolic extract of pearl millet genotypes which will lead to characterization of these genotypes with reference to volatile biochemical constituents. Based on the results of studies carried out, we can observe that millet grains contain many health-promoting components. Research is also needed to determine the bioavailability, metabolism, and health contribution of millet grains and their different fractions in humans. Experimental results reveal that polyunsaturated omega-6 fatty acid was found in most abundant manner

in all genotypes which was categorized in essential fatty acid.

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