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Detection and Assessment of Nutraceuticals in Methanolic Extract of Finger (*Eleusine coracana*) and Barnyard Millet (*Echinochloa frumentacea*)

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The investigation was carried out to determine the chemical components from methanolic extract of finger millet and barnyard millet genotypes using gas-chromatography mass spectrometry (GC-MS). GC-MS analysis of the methanolic extract led to identification of more than 13 compounds in both the crops together. The major compounds in both millets were found to be (*Z,Z*)-9,12-octadecadienoic acid (26.80 %), (*E*)-9-octadecenoic acid (17.37 %), caprolactam (6.69 %) and tetradecanoic acid (6.41 %). Moreover, the barnyard millet genotype VL 232 also showed γ-sitosterol, stigmasterols and campesterols in small percentage (1.49, 0.46 and 0.56 %). Oleic acid (2.47 %) was also present in small amount in finger millet. The detection of these compounds in both small millets highlights the importance of these under-utilized crops in terms of their nutritive value. The results inferred that both finger and barnyard millet extract contain variable patterns of bioactive compounds and could be used as natural antioxidant source for medicinal purposes.

Keywords: Barnyard millet, Finger millet, GC-MS analysis, Methanolic extract.

INTRODUCTION

Millets are designated as 'nutritious millets' and they deserve to be reclassified so, because of its nutritional properties. They also possess antioxidant properties as they contain phenolic compounds [1-3]. In spite of these, the potential of millets is not fully exploited. Minor millets are a group of grassy plants with short slender culm and small grains, possessing remarkable ability to survive under severe drought conditions. Indian barnyard millet (*Echinochloa frumentacea*) is one of the hardiest millets, which is locally known by several names *viz*., Madira, Jhangora, Ooda, Oadalu, Sawan, Sanwa and Sanwank. Similarly, finger millet (*Eleusine coracana*) commonly known as 'Ragi' is one of the important minor millets of the Indian subcontinent. Nutritionally too, both the crops are considered very important for their potential health benefits and providing nutritional security. They are fair source of protein, which is highly digestible, micronutrients (mainly calcium, iron & zinc) and are excellent source of dietary fibre with good amounts of soluble and insoluble fractions [4,5]. The carbohydrate content is low and slowly digestible, which makes small millets a nature's gift for the modern humanity engaged in sedentary activities. Based on traditional information, the iron and zinc fortified flour of coarse and pseudocereals would serve to enhance nutritional security of populations dependent on these crops as their staple food [6]. Phytochemicals in millets are the most important compounds because of their nutraceutical potentials such as antioxidant, anti-inflammatory, anticarcinogenic, antimicrobial, antidiarrheal, antiulcer and anti-cardiovascular properties [7,8].

Many plants emit substantial amounts of phytogenic volatile organic compounds which include alkanes, alkenes, alcohols, aldehydes, ethers, esters and carboxylic acids [9,10]. The biological activity of volatile compounds is dependent on the synergistic or additive effects of the constituent types present at different concentrations. All volatile organic compounds emitted from plants can originate from biogenic and or anthropogenic sources.

The present work aimed to compare the phytochemicals of two aforementioned small millets through GC-MS assay of methanolic extract by Soxhlet extraction. This research explores the possible medicinal values of the grains of finger and barnyard millet through identification of essential compounds present in the crops.

EXPERIMENTAL

Pure clean seeds of two finger millet released varieties namely VL Mandua 347 and VL Mandua 315 and barnyard millet varieties/genotypes *viz*., VL Madira 29 and VL 232 of ICAR-Vivekananda Institute of Hill Agriculture, Almora, India were used in the analysis.

Preparation of extract: 40 g finely ground seeds of finger millet and barnyard millet genotypes were soaked in 100 mL of 80 % methanol overnight and then filtered through Whatmann filter paper No. 41. The filtrate was concentrated by removing methanol using rotary vapour at 68.7 °C until the final volume of 1 mL.

Gas chromatography mass spectrometric assay (GC-MS): The obtained extracts were analyzed for gas-chromatography mass spectrometry to identify the number of compounds. Agilent 6890 gas chromatograph and 5975B mass spectrometer in trace ion detection mode with a programmable temperature vapourizer injector (PTV) was used to characterize secondary metabolites and oleo/aromatic compounds. The chromatographic separation was done on a capillary column of fused silica HP-5ms (0.25 mm × 30 m× 0.25 µm film thickness). 1 µL of the extract was injected in the split mode (1:50) by empty baffled liner at 280 °C (Agilent#5183-2037). The oven programming was set according to Medini *et al*. [11]. Elements were detected in EI mode and scan range was (*m/z* 40-1050). All the mass spectra of the identified peaks were compared with the spectra from the NIST'05, WILEY spectral library and F.A.M.E mix (C8:C24). The results (quality match > 90 %) for individual compounds were only reported as their percentage of the total area of peaks in the total ion chromatogram. Total GC running time was 45 min.

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) which consists of more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known component inherent in the NIST library.

RESULTS AND DISCUSSION

All compounds identified by GC-MS were grouped according to their chemical nature and are presented in Tables 1-4. Data showed the presence of non-polar phyto-components such as aromatic phenolic compounds, fatty acids, sterols, alcohols, aldehydes, alkenes.

Barnyard millet: The major compounds in barnyard millet genotype VL Madira 29 were benzoic acid, 4-ethoxy-ethyl ester (6.0 ± 0.22 %), 9H-fluoren-9-one (4.37 ± 0.12 %), eicosane (2.83 ± 0.54), xanthone (1.20 ± 0.20 %). However, in VL 232, the major compounds were caprolactam (6.69 ± 0.15 %), (*Z,Z*)-9,12-octadecadienoic acid (26.80 ± 0.34 %), tetradecanoic acid (6.41 ± 0.31 %), campesterol (0.56 ± 0.28 %), stigmasterol (0.46 ± 0.35 %), γ-sitosterol (1.49 ± 0.21 %), (*Z*)-9,17-octadecadienal, (3.97 ± 0.06 %) 4-((1*E*)-3-hydroxy-

TABLE-1
GC-MS PROFILING OF VL 29

RT ± 0.5 (min)	Components	Area (%) (SD)
9.996	4-Ethoxy benzoic acid, ethyl ester	6.00 ± 0.22
11.874	Eicosane	2.83 ± 0.54
14.984	(*Z*)-6-Tridecene	0.59 ± 0.16
15.062	(*E*)-5-Eicosene	0.91 ± 0.30
15.862	9*H*-Fluoren-9-one	4.37 ± 0.12
15.984	9*H*-Fluoren-9-one	2.36 ± 0.62
17.328	1-Decanol, 2-hexyl-Oxalic acid, cyclobutyl heptadecyl ester	1.07 ± 0.18
17.884	Xanthone	1.20 ± 0.20

TABLE-2
GC-MS PROFILING OF VL 232

RT ± 0.5 (min)	Components	Area (%)
10.130	5(hydroxymethyl)-2-Furancarboxaldehyde,	0.42 ± 0.22
10.608	Caprolactam	6.69 ± 0.15
11.418	2-Methoxy-4-vinylphenol	0.22 ± 0.13
12.474	1-Dodecene	0.05 ± 0.62
12.663	3-Hydroxy-4-methoxy benzaldehyde	0.07 ± 0.23
14.118	2,4-*bis*(1,1-dimethylethyl) phenol	0.40 ± 0.20
14.751	3-Hydroxy-4-methoxybenzoic acid	0.06 ± 0.15
15.062	*n*-Heptadecyl ester trifluoroacetic acid	0.18 ± 0.32
15.518	3-Deoxy-D-mannoic lactone	0.33 ± 0.54
16.828	4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol	0.55 ± 0.26
18.706	Methyl ester hexadecanoic acid	0.21 ± 0.20
19.106	Tetradecanoic acid	6.41 ± 0.31
20.061	4-Quinolinol	0.12 ± 0.20
20.228	1-Heptadecanol	0.48 ± 0.15
20.350	Methyl ester 9,12-octadecadienoic acid, (linoleic acid)	0.85 ± 0.16
20.816	(*Z,Z*)-9,12-Octadecadienoic acid	26.80 ± 0.34
22.061	1,8-Diazacyclotetradecane-2,9-dione	1.33 ± 0.23
23.027	(*Z*)-13-Octadecenal	0.17 ± 0.52
23.571	2(1*H*)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a.alp	0.15 ± 0.13
23.871	2-Hydroxy-1-(hydroxymethyl)ethyl ester hexadecanoic acid	1.36 ± 0.10
25.260	(*Z*)-9,17-Octadecadienal	3.97 ± 0.06
31.958	Campesterol	0.56 ± 0.28
32.592	Stigmasterol	0.46 ± 0.35
33.836	γ-Sitosterol	1.49 ± 0.21

TABLE-3
GC-MS PROFILING OF VL347

RT ± 0.5 (min)	Components	Area (%)
10.474	Caprolactam	3.66 ± 0.26
12.474	1-Nonene	0.08 ± 0.36
14.107	2,4-*bis*(1,1-dimethylethyl) phenol	0.30 ± 0.12
17.328	*n*-Heptadecyl ester trifluoroacetic acid	0.07 ± 0.25
18.706	Methyl ester hexadecanoic acid	0.15 ± 0.61
19.072	Tetradecanoic acid	5.65 ± 0.20
20.216	(*Z*)-7-Hexadecene	0.66 ± 0.14
20.339	Methyl ester 9,12-octadecadienoic acid, (linoleic acid)	0.19 ± 0.51
20.394	Methyl ester (*Z*)-9-octadecenoic acid	0.34 ± 0.23
20.783	(*E*)-9-Octadecenoic acid	20.04 ± 0.21
20.938	Oleic acid	2.47 ± 0.16
21.127	(*Z*)-9,17-Octadecadienal	1.40 ± 0.14
22.027	1,8-Diazacyclotetradecane-2,9-dione	1.14 ± 0.23
25.249	(*Z*)-13-Octadecenal	1.32 ± 0.18

TABLE-4
GC-MS PROFILING OF VL315

RT ± 0.5 (min)	Components	Area (%)
10.085	5-(Hydroxymethyl)-2-furancarboxaldehyde	0.50 ± 0.25
10.496	Caprolactam	4.45 ± 0.16
12.463	1-Undecanol	0.05 ± 0.14
14.107	2,4-*bis*(1,1-dimethylethyl) phenol	0.31 ± 0.23
17.328	(*E*)-9-Eicosene	0.14 ± 0.19
18.706	Methyl ester, 14-methyl pentadecanoic acid	0.16 ± 0.23
19.083	*n*-Hexadecanoic acid	6.45 ± 0.28
19.361	Pentadecyl ester trichloroacetic acid	0.44 ± 0.21
19.983	Pentadecanoic acid	0.10 ± 0.17
20.216	1-Heptadecanol	0.69 ± 0.19
20.339	Methyl ester 9,12-octadecadienoic acid, (linoleic acid)	0.24 ± 0.25
20.394	Methyl ester 10-octadecenoic acid	0.40 ± 0.14
20.794	(*E*)-9-Octadecenoic acid	20.91 ± 0.40
20.939	Oleic acid	2.92 ± 0.26
22.027	1,8-Diazacyclotetradecane-2,9-dione	1.00 ± 0.14
23.460	2,2'-Oxybis[N,N-dimethyl ethanamine	0.58 ± 0.16
23.849	2-Hydroxy-1-(hydroxymethyl) ethyl ester hexadecanoic acid	0.46 ± 0.17
25.260	2-Hydroxy ethyl ester (*Z*)-9-octadecenoic acid	1.55 ± 0.52

1-propenyl)-2-methoxyphenol (0.55 ± 0.26 %), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (1.36 ± 0.10 %). The compounds 4-((1*E*)-3-hydroxy-1-propenyl)-2-methoxyphenol and 2-furancarboxaldehyde were also present in VL 232 in good amount.

Finger millet: In finger millet variety VL Mandua 347, caprolactam (3.66 ± 0.26 %), tetradecanoic acid (5.65 ± 0.20 %) oleic acid (2.47 ± 0.16 %), (*E*)-9-octadecenoic acid (20.04 ± 0.21 %), (*Z*)-9,17-octadecadienal (1.40 ± 0.14 %), (*Z*)-7-hexadecene (0.66 ± 0.14 %) were identified as chief compounds. Similarly, (*E*)-9-octadecenoic acid, (20.91 ± 0.40 %) and *n*-hexadecanoic acid (6.45 ± 0.28 %), caprolactam (4.45 ± 0.16 %), oleic acid (2.92 ± 0.26 %),1,8-diazacyclotetradecane-2,9-dion (1.00 ± 0.14 %) were major compounds present in the other finger millet variety *i.e.*, VL Mandua 315 (Fig. 1).

The common compound identified in both crops were, *n*-hexadecanoic acid, tetradecanoic acid, hexadecanoic acid, 9,12-octadecadienoic acid, octadecanoic acid and caprolactum. These compounds have various therapeutic functions to treat various human diseases (Table-5). However, few compounds were present only in barnyard millet not in finger millet and *vice versa*. 9*H*-fluoren-9-one, xanthone, 1-dodecene, 4-quinolinol, deoxy-D-mannoic lactone, 6-tridecene and 2-methoxy-4-vinylphenol were present only in Barnyard millet whereas 1-nonene, 9-dicosene, 2,4-*bis*-2-furancarboxaldehyde phenol, 1-undecanal, 1-heptadecanol and ethanamine were present only in finger millets.

Phytosterols functioning in plants are similar to that of cholesterol in animals. It has been reported as a positive influence for diabetic state by directly lowering fasting blood glucose levels by cortisol inhibition [13]. These are also known for their saturated fat reducing and cholesterol lowering activity and thus may reduce the risk of heart disease [14,15]. There are over 40 phytosterols identified so far, with the most prominent being campesterol, β-sitosterol and γ-sitosterol and stigmasterol. These compounds are present in a variety of plant-based foods, often at low levels.

Till date there are no reports on the presence of caprolactum, sitosterols, phytosterols in the barnyard millet species. Pearson *et al.* [16] reported that caprolactum inhibits the content of antinutritional factors in buckwheat plants. Sitosterols are the most widely distributed of the plant sterols and interferes with the absorption of cholesterol [17]. Similarly, γ-sitosterol has been shown to positively influence on a diabetic

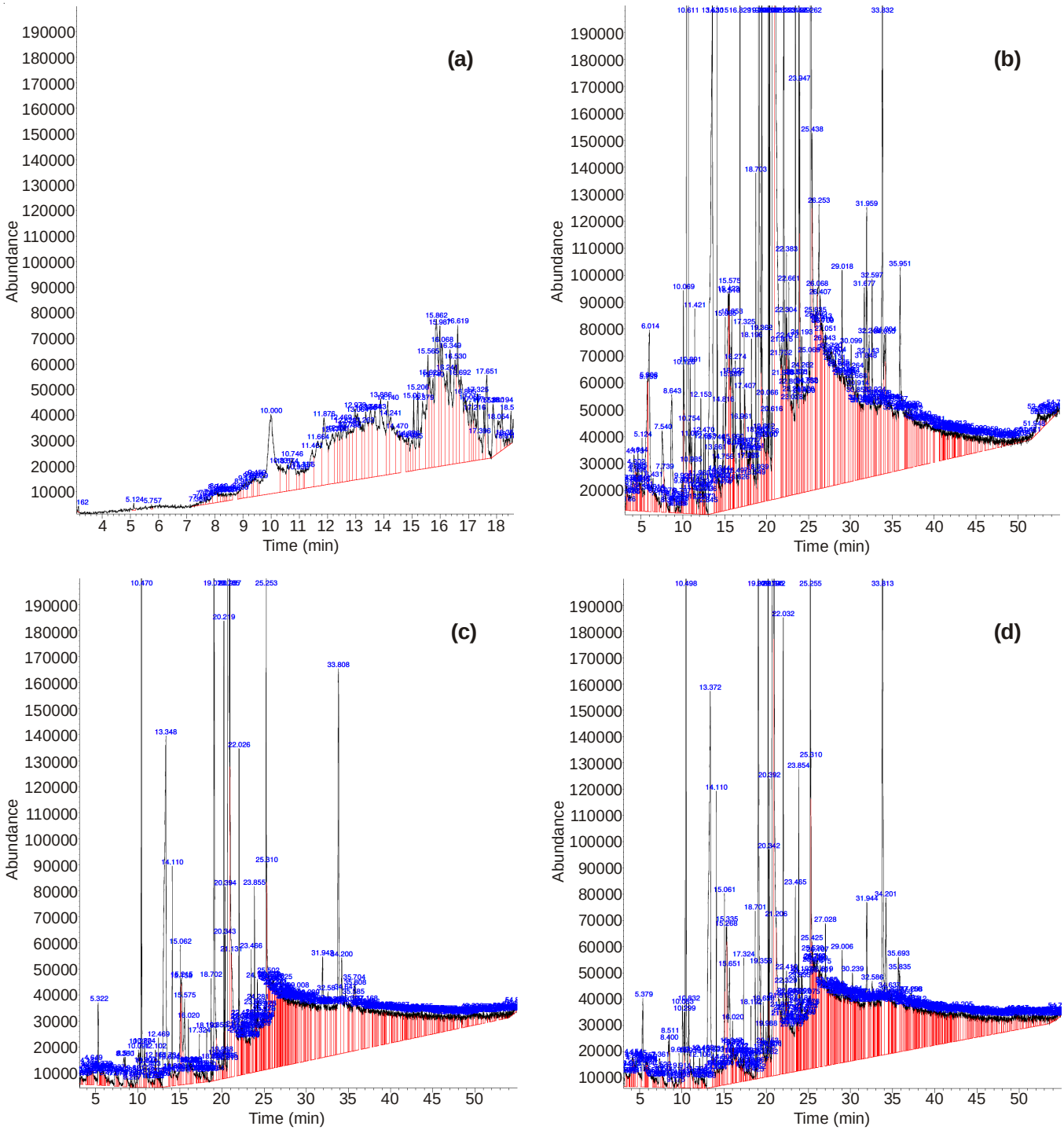

Fig. 1. GC-MS analysis of methanolic extract of seeds of barnyard millet & finger millet (a) Barnyard Millet variety VL 29; (b) Barnyard Millet genotype VL 232; (c) Finger millet variety VL 347; (d) Finger millet variety VL 315

TABLE-5
MAJOR COMPOUNDS WITH THEIR ACTIVITY [Ref. 12]

Name of the compound	m.f.	Nature of the compound	Activity
Pentanoic acid	$C_5H_{10}O_2$	Valeric acid	Flavour
5-(Hydroxymethyl)-2-furancarboxaldehyde	$C_6H_6O_3$	5-(Hydroxymethyl)furfural	Carbon-neutral
Tetradecanoic acid	$C_{14}H_{28}O_2$	Myristic acid	Good immunomodulator, flavour
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Palmitic acid	Antioxidant, hypocholesterolemicne maticide, hemolytic, 5-alpha reductase inhibitor
Octadecanoic acid	$C_{18}H_{36}O_6$	Stearic acid	Anticancer
Methyl ester 9,12-octadecadienoic acid	$C_{20}H_{34}O_2$	Linoleic acid	Antioxidant, hypocholesterolemicne maticide
9-Eicosene	$C_{20}H_{40}$	Long chain fatty acid	Antimicrobial and cytotoxic properties

state by directly lowering fasting blood glucose levels by cortisol inhibition [18]. Campesterol is one of the plant sterols that is added to foods to improve their ability to lower levels of total and low-density lipoprotein (LDL) or 'bad' cholesterol. The presence of campesterol, are well known for their medical, cosmetic and functional food applications and may contribute towards the antimicrobial and antioxidant activities.

Stigmasterol, which is used for the synthesis of progesterone and vitamin D3, is known as "Wulzen factor", a potential anti-inflammatory compound. Its action is mediated by the inhibition of several pro-inflammatory and matrix degradation mediators involved in osteoarthritis-induced cartilage degradation [15]. The interest of adding sterols and stanols to human food for improving health has been discussed earlier [19,20]. Clinical experiments have shown that only high amounts of stanols (about 9 g/day) can decrease serum β-carotene concentrations, without altering those of vitamins A, D and E [21].

The reported saturated fatty acids oleic acid and linoleic acid ((*Z,Z*)-9,12-octadecadienoic acid, methyl ester) are the most common fatty acids found in animals and plants and are primarily used to produce hormone-like substances that regulate a wide range of functions, including blood pressure, blood clotting, blood lipid levels, the immune response and the inflammation response to injury infection [22,23]. In the investigated extracts, linoleic acid (polyunsaturated fatty acid) which belongs to the group of essential fatty acids (EFAs) is in good amount (0.19-0.85 %). VL 232 of barnyard milet showed maximum amount (0.85 %) of linoleic acid. Linoleic acid is also very popular in beauty products as helping in moisture retention, acne reduction and anti-inflammatory. Similarly, finger millet showed substantial amount of oleic acid (2.47-2.92 %) which is not present in two genotypes of barnyard millet. Oleic acid, which is an ω-9 fatty acid as the major fatty acid also is equally important having all the health benefits of linoleic acid. In cases of reduced availability of ω-6-fatty acids, ω-9-fatty acids are converted to ω-6-fatty acids [24]. High amount of unsaturated fatty acids *viz.,* linoleic and oleic have been reported in finger millet earlier by researchers [24,25]. The presence of high amounts of these essential fatty acids suggests that these food ingredients are highly nutritious, due to their ability to reduce serum cholesterol. Similarly, eicosene are reported to have antimicrobial and cytotoxic activity [26,27].

Tetradecanoic acid is also called myristic acid, which constitutes 60-75 % of the fatty acid content. So it is good edible value [28]. In this study, barnyard millet (VL 232) showed high amount of tetradecanoic acid *i.e*., 6.41 % than finger millet (VL 347) which is 5.65 %. Tetradecanoic acid, hexadecanoic acid, octadecanoic acids are among the fatty acids known to have potential antibacterial and antifungal activity [29]. These compounds indicate their potential use for curing various diseases in traditional systems. The presence of these chemical compounds in both finger and barnyard millet demonstrate their potential as therapeutic, functional food and variation for these compounds can be capitalized for development of varieties with enhanced levels of such nutritional compounds.

Conclusion

In the present study, finger millet and barnyard millet were studied to identify the presence of volatile compounds. More than fifteen chemical constituents were identified from the methanolic extract of both finger and barnyard millet by GC-MS. Barnyard millet showed good amount of therapeutic compounds. Further studies are suggested to evaluate bio-activity and toxicity profile through *in vitro* and *in vivo* models.

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