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Phytochemicals with Health Benefits–Contribution from Brown and White Finger Millet (*Eleusine coracana*) Varieties

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ABSTRACT Bearing in mind that millet market is currently growing, comparison of phytochemicals in two finger millet varieties with different seed coat colours (brown and white) has been carried out. The phytochemicals content was higher in the brown variety when compared to the white variety. Gas chromatography coupled with mass spectrometry (GCMS) analysis revealed the presence of propanamide, 2-butenedioic acid (E)-, diethyl ester, 2-amino-1-(o-hydroxyphenyl) propane, oleic acid, propan-1-one, 2-amino-1-piperidin-1-yl-, hexanedioic acid, bis (2-ethylhexyl) ester, guanidine, N,N,N',N'-tetramethyl-, limonene, phthalic acid, 2-cyclohexylethyl butyl ester, actinobolin, n-hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z)-, cyanoacetyl urea, tetracosane, fluoxetine and arginine in the finger millet grains. Phytochemicals are known to have antioxidant activity, and analysis by DPPH assay showed 85.77 percent in the brown variety and 62.18 percent in the white variety. Further studies are needed to isolate active compounds of the extract and correlate structural function relationships in order to propose their exact mechanism of action in various disorders.

INTRODUCTION

Since earlier times, natural products have been used for maintaining health, with more intensive studies for natural therapies especially in the last decade. Traditional medicine is used by almost eighty percent of the population (Eloff 1998). Currently, there is an increased interest among consumers regarding the role of food in maintaining and improving human well-being. Foods are also known to contain bioactive components with therapeutic effects. Plants extracts are sources of antihypertensive, hypoglycaemic, hypolipidaemic, anti-inflammatory and antibiotic compounds. Therefore, such foods need be investigated to better understand their properties and efficiency.

Millets are important components of a healthy diet, and their daily consumption could prevent cardiovascular diseases and certain cancers (Bhupathiraju and Tucker 2011). These beneficial effects of millets have been attributed to non-essential food constituents, known as phytochemicals or bioactive compounds, which have a relevant bioactivity when they are frequently consumed as a part of a regular diet (Mudgal et al. 2010). Millets are ranked sixth in the world for cereal production, and they are the major food source for people living with economically disadvantaged status in Africa and Asia (Kumari et al. 2017).

Finger millet, commonly known as *Ragi*, belongs to the family *Poaceae* (Dida et al. 2006). The plant is a folk remedy for leprosy, liver disease, measles, pleurisy, pneumonia, and small pox. The seed is used as an astringent and has a cooling effect. It is used for fevers, biliousness and hepatitis. The leaf juice has been given to women in childbirth, and the plant is effective as a diaphoretic, diuretic, and vermifuge. The agronomic advantages include their ability to thrive in diverse and adverse environmental conditions, easy to cultivate, and higher yield with good storability (Udeh et al. 2017). It is a potentially tremendous but under-explored source of nu-

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traceutical properties compared to other regularly consumed cereals (Singh et al. 2018).

Nutraceutical properties with higher antioxidant potential of identified genotypes can be suitably deployed for nutritional security, particularly in developing countries (Sharma et al. 2018a). It is essential to identify native crops that are highly adaptive to local climates (Gupta et al. 2017). In this sense, the practical advantages and convenience that the millets offers the consumers renders this market as one of the rapidly growing sector in food industry as well as gaining popularity worldwide (Ragaert et al. 2004). Therefore, it is essential to investigate the presence of important phytochemicals.

Objectives

There are several studies that evaluate the phenolic acids, vitamin C and carotenoids in major millets such as sorghum, pearl millet and maize (Alasalvar et al. 2005; Simões et al. 2011) or lettuce (Martínez-Sánchez et al. 2012). However, there are few studies regarding phytochemicals in finger millets. Nonetheless, there are less data on the specific compounds present in finger millet, and therefore, the present study was intended to carry out the identification of bioactive compounds in finger millet grains with two different seed coat colours by gas chromatography and mass spectroscopy (GC-MS).

METHODOLOGY

Collection of Plant Material

Two varieties of finger millet grains, based on their seed coat colour, were used in this study. The CO13 variety (brown) was procured from Tamilnadu Agriculture University, Coimbatore and the OUAT2 variety (white) was from Orissa University of Agriculture and Technology. The grains were thoroughly washed with tap water, dried in a hot-air oven at 60°C, cooled to room temperature and ground into fine powder. They were transferred to airtight polyethylene zipper bags, labelled and stored for further use.

Preparation of Extracts

The powdered grains (5 g) were mixed with 20 ml of methanol in closed opaque glass bottles and left for 24 h at 40°C. The supernatant was separated by centrifugation at 3500 rpm for 10 min followed by addition of 20 ml of chloroform to the dry residue and kept for incubation as mentioned above. The extraction procedure was repeated with hexane. Each of the solvent extracts were evaporated separately in a dry bath at 55°C, and the final residue was dissolved in double distilled water, pooled and stored at 4°C until analysis.

GC-MS Analysis

The sample (1 μL) was injected in gas chromatography (GC) fitted with a split injector and coupled with a turbo-mass spectrometric (MS) selective detector system. The MS was operated at 70 eV in electron ionization mode with Helium as the carrier gas at a flow rate of 1.2 ml min^{-1}. The analytical capillary column connected to the system was Rtx-5 (length: 60 m, internal diameter: 0.25 mm, thickness: 0.25 μm). The head pressure was adjusted to 196.6 kPa. Temperature was programmed from 100 to 200°C at 10°C per min and from 200 to 300°C at 15°C per min. The withhold time was 5 and 22 min. A solvent delay of 6 min was selected. The injector temperature was set at 260°C. The interface was maintained at 280°C. MS was operated in from 40 to 600 m/z in the full scan mode. The researchers identified the compounds in comparison with the mass spectra from the library of the National Institute of Standard and Technology (NIST), USA/Wiley.

Antioxidant Activity

The methanolic extract of the samples obtained for measuring total polyphenols was taken for the DPPH assay. The antioxidant activity of the extracts was measured in terms of their ability to reduce the DPPH free radical and subsequently decrease in the absorbance and expressed as a percent of inhibition using Trolox as a reference standard (Blois 1958).

RESULTS

The bioactive compounds with their concentration (peak area %) and retention time (RT) are presented in Table 1 and Table 2. Figures 1 and 2 represent the chromatograms for the two millet varieties. These confirm the presence of 18 phy-

Table 1: GC-MS spectral analysis of CO13 finger millet variety

Area (%)	*Compound*	*Retention time*
0.46	Guanidine, N,N,N’,N’-tetramethyl-	7.816
1.75	Oxalic acid, cyclohexyl hexyl ester	8.633
0.77	Limonene	9.736
1.21	Phthalic acid, 2-cyclohexylethyl butyl ester	23.554
4.27	Propanamide	25.443
1.24	Actinobolin	25.555
47.74	2-Butenedioic acid (E)-, diethyl ester	27.189
1.56	2H-Azepin-2-one, hexahydro-1-methyl-	27.342
2.01	2-(E)-Pentenoic acid, (4S)-4-[(t-butoxycarbonyl-(R)-alanyl)amino]-,ethyl ester	27.424
2.68	2-Amino-1-(o-hydroxyphenyl)propane	28.537
25.21	Oleic Acid	28.65
2.45	Propan-1-one, 2-amino-1-piperidin-1-yl-	28.874
1.99	3-(E)-Hexen-2-one, (5S)-5-[(t-butoxycarbonyl-(S)-alanyl)amino]-	29.068
0.18	Acetamide, 2,2,2-trichloro-	29.16
3.77	Hexanedioic acid, bis (2-ethylhexyl) ester	32.868
1.12	2,3-Dimethoxyamphetamine	34.593
1.17	Octodrine	34.941
0.43	Methylpent-4-enylamine	35.38

Table 2: GC-MS spectral analysis of OUAT2 finger millet variety

Area (%)	*Compound*	*Retention time*
0.3	Ethene, ethoxy-	7.805
0.86	Oxalic acid, cyclohexyl dodecyl ester	8.622
0.72	Phthalic acid, isobutyl nonyl ester	23.553
8.13	n-Hexadecanoic acid	25.443
0.61	Propanamide	25.678
24.1	2-Butenedioic acid (E)-, diethyl ester	27.189
0.16	2H-Azepin-2-one, hexahydro-1-methyl-	27.363
6.69	9,12-Octadecadienoic acid (Z,Z)-	28.547
34.86	Oleic Acid	28.67
2.88	Cyanoacetyl urea	28.874
5.54	4-Fluorohistamine	29.078
0.49	Fluoxetine	31.438
2.15	Hexanedioic acid, bis (2-ethylhexyl) ester	32.877
1.1	Benzene, 4-methyl-1,2-dinitro-	33.072
1.11	3,3-Dimethyl-4-methylamino-butan-2-one	33.48
0.82	Benzenemethanol, .alpha.-(1-aminoethyl)-, (R^*,R^*)-(.+/-.)-	34.215
0.48	Arginine	34.369
0.59	Benzenepropanamine, .alpha.-methyl	34.481
2.71	Tetracosane	34.593
0.29	1-Octadecanamine, N-methyl-	34.93
5.1	No matches found	35.124
0.3	3-Propoxyamphetamine	35.349

tochemicals in the CO13 variety and 22 in the OUAT2 variety. Propanamide; 2-Butenedioic acid (E)-, diethyl ester; 2H-Azepin-2-one, hexahydro-1-methyl-; Oleic acid and Hexanedioic acid, bis (2-ethylhexyl) ester were found to be common in both varieties of finger millets.

The percentage content of compounds, namely, propanamide (RT 25.443); 2-Butenedioic acid (E)-, diethyl ester (RT 27.189); 2-(E)-Pentenoic acid, (4S)-4-[(t-butoxycarbonyl-(R)-alanyl)amino]-, ethyl ester (RT 27.424); 2-Amino-1-(o-hydroxyphenyl)propane (RT 28.537); oleic acid (RT 28.65); propan-1-one, 2-amino-1-piperidin-1-yl- (RT 28.874); and hexanedioic acid, bis (2-ethylhexyl) ester (RT 32.868) in the CO13 finger millet variety were found to be 4.27, 47.74, 2.01, 2.68, 25.21, 2.45 and 3.77 percent respectively. Octodrine; 2,3-Dimethoxyamphetamine; Methylpent-4-enylamine; and Acetamide, 2,2,2-trichloro- were less in the range of 1.17-0.18 percent. Some other constituents of significance were guanidine, N,N,N’,N’-tetramethyl-, li-

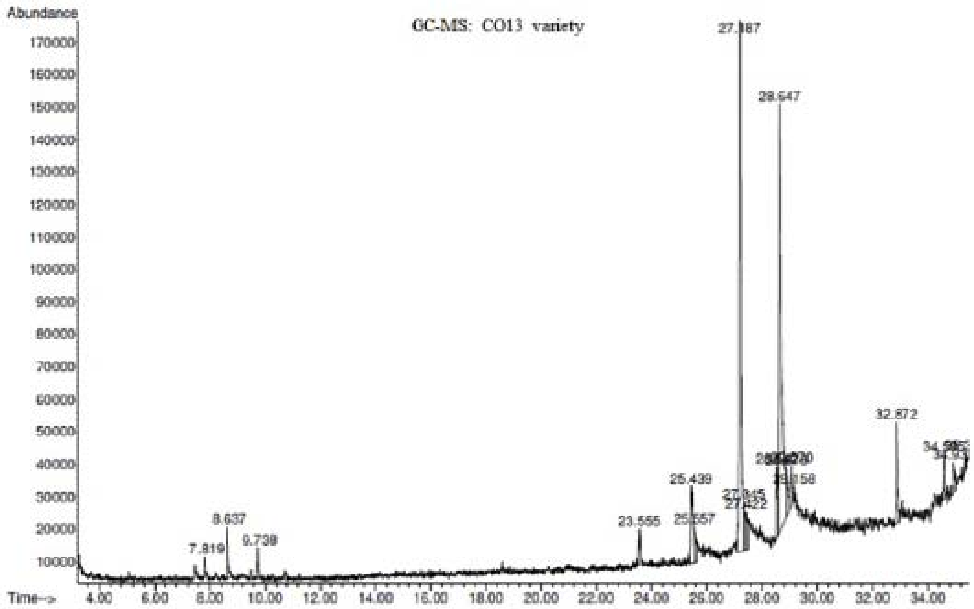

Fig. 1. GC-MS chromatogram of CO13 finger millet variety

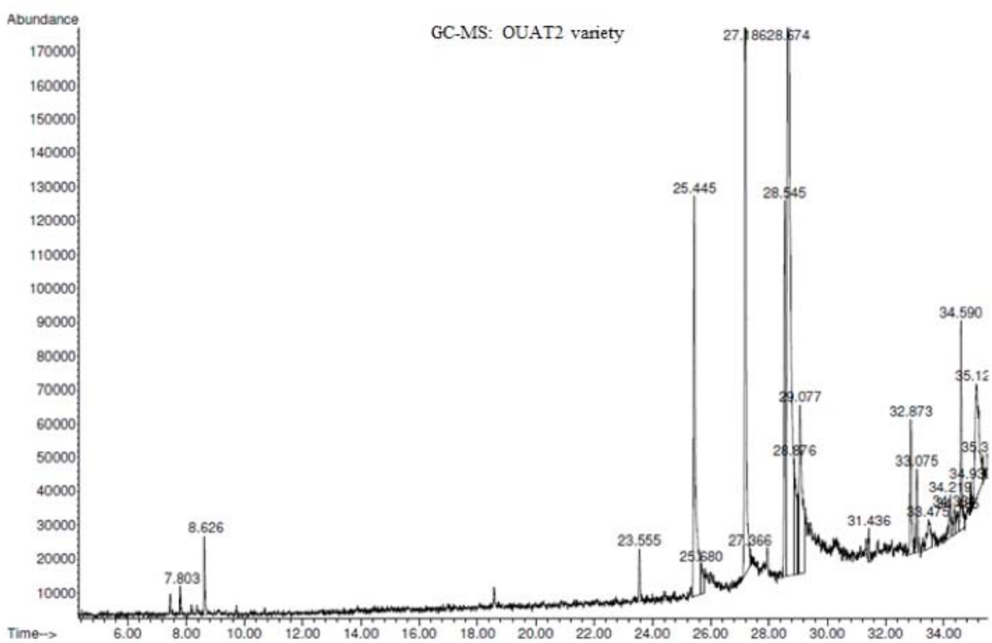

Fig. 2. GC-MS chromatogram of OUAT2 finger millet variety

monene, phthalic acid, 2-cyclohexylethyl butyl ester, and actinobolin.

In the OUAT2 finger millet variety, the bioactive components were 8.13, 24.1, 6.69, 34.86, 2.88, 5.54, 2.15 and 2.71 percent of n-hexadecanoic acid (RT 25.443); 2-Butenedioic acid (E)-, diethyl ester (RT 27.189); 9,12-octadecadienoic acid (Z,Z)- (RT 28.547); oleic acid (RT 28.67); cyanoacetyl urea (RT 28.874); 4-Fluorohistamine (RT 29.078); hexanedioic acid, bis(2-ethylhexyl) ester (RT 32.877); and tetracosane (RT 34.593), respectively. In addition to the above compounds, fluoxetine and arginine were also found. Besides 5.1 percent of an unidentified compound at RT 35.349, 3,3-Dimethyl-4-methylamino-butan-2-one; Benzene, 4-methyl-1,2-dinitro-; Benzenemethanol, .alpha.-(1-aminoethyl)-, (R^*,R^*)-(.+/-.)-; Phthalic acid, isobutyl nonyl ester; Benzenepropanamine, .alpha.-methyl; Ethene, ethoxy-; 3-Propoxyamphetamine; 1-Octadecanamine, N-methyl- were less in the range of 1.11-0.29 percent.

Both CO13 and OUAT2 had 1.75 and 0.86 percent of oxalic acid, respectively. The observed oxalic acid was present in the form of cyclohexyl hexyl ester in both the varieties. Among the phytochemicals, 3-(E)-Hexen-2-one, (5S)-5-[(t-butoxycarbonyl-(S)-alanyl)amino]- was present only in the CO13 (brown) variety. The antioxidant activity was high (85.77%) in the brown variety compared to the white (62.18%). Except for oleic acid, all other components were significantly higher in the brown variety compared to the white variety. It may therefore be inferred that the other bioactive compounds are probably associated with the seed coat of the brown variety.

DISCUSSION

Due to the presence of the aforementioned compounds in the finger millet grains, it can be used in food, pharmaceutical and industrial applications. Viswanath et al. (2009) reported antioxidant activity of approximately twenty-seven percent in the GPU-28 variety. The higher DDPH scavenging property observed may contribute to the nutraceutical properties of the millet. Since antioxidant activity of a compound is generally dose-dependent, a substantial quantity of polyphenols found in finger millet, particularly in the brown variety, can be considered as a rich source of antioxidants. Adequate antioxidant supply is important to protect the brain from oxidative and nitrosative stress (Hithamani and Srinivasan 2017). Tyl et al. (2018) also observed varietal difference in the antioxidant activity of proso millet. Barnyard grass grains (*Echinochloa crus-galli*) had 22.88 percent oleic acid as per the research work of Prietto et al. (2017), which is lower than both types of finger millet reported in researchers' study.

The 2-Butenedioic acid (E)-, diethyl ester (Diethyl fumarate) has immune-modulatory properties and psoriasis treatment with fumaric acid, and its esters have been reported by Gold et al. (2012). Linoleic acid (9,12-octadecadienoic acid (Z,Z)-) found in the white variety, is a polyunsaturated omega-6 fatty acid. A diet deficient in linoleate (the salt form of the acid) causes skin scaling, hair loss and reduced wound healing in rats (Ruthig and Meckling-Gill 1999). It has become widespread in the beauty products industry owing to the favourable aspects (acne reductive, anti-inflammatory, moisture retentive) of linoleic acid on the skin. It can be used to show the antioxidant effect of natural phenols. Previous reports showed biological activities of finger millet such as antiproliferative potential, DNA scission inhibitory activity (Chandrasekara and Shahidi 2011), amelioration of hyperglycaemia (Shobana et al. 2010) and inhibition of aldose reductase from cataracted eye lenses (Chethan et al. 2008). CO13 and OUAT2 had higher levels of oleic acid, *n*-Hexadecanoic acid (palmitic acid), and 9,12-Octadecadienoic acid methyl ester (linoleic acid) than seeds (16.05-8.77%) and leaves (13.99-2.91%) of mustard (*Brassica juncea* L.) (Sharma et al. 2018b).

Singh and Sarita (2016) reported that the finger millet fat consists of oleic acid, linoleic acid, palmitic acid and traces of linolenic acid. The oleic acid that is present in high amounts in both varieties is a mono-unsaturated omega-9 fatty acid with documented health benefits such as hypotensive (Terés et al. 2008) and prevention of breast cancer (Martin-Moreno et al. 1994). These and other bioactive components make finger millet a functional food with great potential for therapeutic effects. The chemical features of these bioactive components differ considerably among species and varieties underscoring the need for their detailed study, particularly in these neglected crops. These compounds possess antioxidant (Kim et al. 2004), anti-inflammatory (González-Gallego et al. 2010; Vincent et al. 2010), lipid profile modification (Perez-Vizcaino and

Duarte 2010; Wang et al. 2011) and antitumor effects (Pietta et al. 2003; Collins 2005; Stan et al. 2008). Natural antioxidants such as flavonoids, polyphenols and tannins are found in this plant.

CONCLUSION

In conclusion, both finger millet varieties contain variable patterns of bioactive compounds that could be used as a natural antioxidant source for medicinal purposes. In particular, the brown finger millet variety with high amounts of bioactive components may contribute to the health value of the diet and make the food a functional one with advantages for the consumer.

RECOMMENDATIONS

In addition to calcium, iron, amino acids such as methionine, and slowly digestible starch, finger millet is a rich source of these phytochemicals. Unlike wheat, it does not contain gluten. It is also low in fat, and easily digestible. Due to these characteristics, finger millet is often termed as a "super cereal". Apart from the agronomic factors, these phytochemicals are important from the view of consumer acceptance.

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