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Polyphenols, phospholipids and fixed oil composition of pearl millet [*Pennisetum glaucum* (L.) R. Br.]

M. Daniel[1]*, Mammen Denni[2] and Dipa Chauhan[1]
1, Phytochemistry Laboratory, Department of Botany, Faculty of Science,
The Maharaja Sayajirao University of Baroda, Vadodara, (Gujrat) - India
2, Department of Chemistry, Faculty of Science,
The Maharaja Sayajirao University of Baroda, Vadodara, (Gujrat) - India

Abstract

Not much is known on the polyphenols, phospholipids and fixed oil composition of Pearl millet [Bajra-*Pennisetum glaucum* (L.) R. Br.], the poor men's staple food in India and Africa. The present study revealed the presence of 0.9% dry wt. of flavones consisting of tricin, 7-OMe luteolin and acacetin; 0.75% phospholipids containing both lecithins and cephaelins and 5.4% fatty oil composed of principally linoleic acid (45.55%), oleic acid (28.54%), palmitic acid (20.6%) and linolenic acid (2%). The phenolic acids identified were vanillic, syringic, ferulic, p-hydroxy benzoic acid and *cis/trans* p-coumaric acids and the total phenols were found to be 4.08 µg/g. The total antioxidant activity of the grain is found to be Ic_{50} 1.33± 0.03 mg/ml. The significance of these compounds in the wider acceptability of this grain is discussed.

Key-Words: Pearl millet. *Pennisetum glaucum,* Flavones, Polyphenols, Phospholipids, Fixed oil, Linoleic acid, Oleic acid, Phenolic acids, Antioxidant potential.

Introduction

Bajra, the poor men's staple food in India and Africa is grown in arid and semi arid regions of both these countries. Though it was originated in Sahel region of Africa, it has been cultivated both in Africa and India since prehistoric times. In India this crop is grown over 12 million hectares (amounting to 11% of the total cereal production in the country) and the total output is about 5 million tones (Murty and Subramanyam, 1989) . The grains are known to contain about 12 % proteins, 5 % ether extractives (including fats) and 67 % carbohydrates. It is said to be a rich source of minerals and vitamins of the B group (Leder, 2004)

As a major millet crop, pearl millet is extensively studied for its carbohydrates, proteins, vitamins and minerals. Though, the fat content is said to be high, not much is known on the fatty acid composition or the values of the oils, nor attention is given to the advantages they impart. There are no data on the phospholipids in the grain also.

*** Corresponding Author**
E.mail: mdanieluni@gmail.com
Mob. +91- 9727075597

Unfortunately the minor components such as phenolics or flavonoids are considered as anti-nutritional factors and thus ignored. But now there is a resurgence of interest in these compounds as they are found to impart a number of health benefits to the consumer (Dykes and Rooney, 2007). Looking into the vacuum existing in our knowledge on the minor components of this grain, the present study is undertaken. Here, an attempt is made to find out (1) the phenolic components including the flavonoids and phenolic acids, (2) the phospholipids, and (3) the fatty acid composition of the oil. The total antioxidant potential also is assessed.

Material and Methods

The seeds were obtained from Gujarat State Seeds Corporation, Vadodara. The powdered grain was extracted with petroleum ether and the total ether solubles quantified. The saponification value of the oil is estimated using standard methods. The GC-MS analysis of the oil was done at DMAPR, Anand. The instrumental conditions were the following: The Equipment was Focus-PolQ GC/MS (Thermo); Column: ZB-5 capillary column (30 m×0.25 mm×0.25 mm); Oven temperature: 80°C for 5 min, then increased 3°C/min to 220°C and held for 5.0min.; Injector Temperature: 230°C, Carrier gas: Helium

(1mL/min). The injection volume was 0.5ul and EI-MS: 70 eV in the range *m/z* 30-400. Individual compounds were identified as methyl ester by comparing their mass spectrum with library (NIST) and literature (Adams 2007).

The phospholipids and phenolics were extracted from defatted material using methanol in a Soxhlet's extractor. The methanol extract was concentrated and on addition of acetone to this concentrated extract, phospholipids precipitated which were filtered and quantified. The acetone solubles remaining after the separation of phospholipids contained phenolics including flavonoids. Phospholipids were analyzed by TLC (Silica gel) using the solvent systems acetone: benzene: water (91:30:8,v/v/v) and chloroform: methanol: acetic acid: water (170:30:20:7,v/v/v/v). Lecithins were visualized by spraying the developed chromatograms by Dragendorff's reagent (to locate choline-containing lipids) and the cephaelins were located by Ninhydrin reagent. Total phenols were estimated by Folin-Ciocaulteu method (Singleton et al. 1999). Flavonoids were analyzed by standard methods prescribed by Mabry and co-workers (1970) and by co-chromatography with standard compounds. The identification of phenolic acids was done following Ibrahim & Towers (1960). The Total antioxidant activity was measured using the well-known DPPH method (Siddique et al. 2010).

Results and Discussion

Pearl millet was found to be rich in phenolics. The total phenolics in terms of gallotannins were found to be 4.08 mg/1g. The flavonoids identified were three in number, tricin, 7-OMe luteolin, and acacetin. However, glucosyl orientin and glucosyl vitexin as reported by Reichert (1979) were found to be absent in the samples. The phenolic acids located were vanillic, syringic, ferulic p-hydroxy benzoic acid, *cis/trans* p-coumaric acids. The total antioxidants potential Ic_{50}1.33$\pm$ 0.03 mg/ml calculated in terms of ascorbic acid equivalents.

The bajra grain was found to yield 5.23% of golden yellow fatty oil. The saponification value of this oil is 188.8. On GC analysis it yielded three fatty acids as major components. Alpha-Linoleic acid amounted to 45.6% followed by Oleic acid (28.5%) and Palmitic acid (20.6%). Linolenic and stearic acid were the minor fatty acids amounting to 2.1 % and 1.5 % respectively.

The total phospholipid yield was 0.75%. Both lecithins and cephaelins were located in chromatograms. There were three bands found on the TLC for Cephalins, which were ninhydrin positive. Lecithins were represented by only one band.

The present study reveals a number of interesting and useful facts about bajra which was underestimated and used as the food of the underprivileged. The phenolic content especially of flavonoids is very high. The importance of flavonoids in staple foods like Pearl millet need not be explained here in detail. The flavonoids are known to have a positive role in strengthening the capillary walls, thinning blood by reducing the agglutination of RBCs and even preventing cancer. The anti-inflammatory properties of flavonoids also are well known. According to Duke's data base, tricin is found anticarcinogenic, antileukaemic, antioxidant, antitumor and estrogenic. It is considered as a cancer chemopreventive agent of great potential. It's potent interference with the growth of human-derived malignant MDAMB- 468 breast cancer cells and cell cycle-arresting properties in human MDA-MB-468 breast cancer cells *in vitro* are proved by Hudson et al. (2000) and Cai et al. (2004) respectively. Cai et al. (2005) and Al-Fayez et al. (2006) suggest that the proven ability of tricin to inhibit cyclooxygenase enzymes and attenuation modulation of cyclooxygenase- mediated prostaglandin production may contribute to its chemopreventive efficacy on intestinal carcinogenesis and prostatic cancer. Luteolin and its derivatives are known to inhibit tumor necrosis factors (Kim et al. 2011).

Acacetin is anti-aflatoxin, antimalarial, hepatoprotective, anticancer and antihistaminic. It possesses anti-peroxidative, anti-inflammatory and anti-HIV properties. A number of studies have shown that acacetin induces apoptosis in human lung, human prostrate and human breast cancer cells. Therefore the presence of a good amount of flavonoids imparts all these benefits to the poor men who use this millet as their staple diet.

Phenolic acids are also known to possess profound curative properties (Duke's database). All the phenolic acids identified *i.e., p*-hydroxy benzoic acid (antibacterial, anti-sickling, immunosuppressant, cancer preventive and fungistat), ferulic acid is (analgesic, antiallergic, anti-inflammatory, hepatoprotective and antihepatotoxic, immunostimulant, antiallergic, arteriodilato and acts against cancer in the colon, liver and skin), *p*-coumaric acid (antibacterial, antiseptic, antitumour. The compound is also antiseptic, antioxidant and fungicidal), vanillic acid, (anthelmintic, anti-fatigue, anti-inflammatory, antilaeukemic, antiseptic and anti-sickling) and syringic acid (antioxidant, anti-peroxidant and anti-radicular) are known to have distinct pharmacological properties.

Both flavonoids and phenolic acids are known to be highly active antioxidants. The role of antioxidants in human diet is being increasingly felt these days. Since it is understood that all the chronic diseases like diabetes, cancer, stroke, atherosclerosis etc are caused either by the reduced levels of antioxidants in the body or the increased levels of free radicals, "The Anti-oxidant Therapy" in which antioxidant supplements are given regularly are resorted to.

Bajra is rich in oil and this adds to nutritive potential. The high amounts of oils (above 5%), rich in linoleic acid (45%) which is an omega 6-fatty acid, is highly significant because this acid is easily converted to n-6 eicosanoids, n-6 prostaglandin and n-6 leucotriene hormones. This provides targets for drug development in arthrosclerosis (B.P), asthma, arthritis, immunity development etc. Linoleic acid is also very popular in beauty products as helping in moisture retention, acne reduction, and anti-inflammatory. Lack of linoleic acid causes dry hair, hair loss, and wound healing. Therefore, the consumption this millet containing oil will yield the same advantages to the consumer. Oleic acid, which is an omega-9 fatty acid, also is equally important having all the health benefits of linoleic acid. In cases of reduced availability of omega-6-fatty acids, omega-9-fatty acids are converted to omega-6-fatty acids. Linolenic acid, the third fatty acid present, is an essential fatty acid. This acid is useful in conditions of rheumatoid arthritis, cardiac arrhythmias, depression and reduces the risk of ischemic and thrombotic stroke. Omega-3 fatty acids are found to reduce prostate tumor growth, slow histopathological progression, and increase survival in mice (Bergstrom et al. 1964).

Due to higher amount of linoleic acid, bajra oil is comparable to sesame oil. Rice bran oil and wheat germ oil also contain comparatively higher amount of this acid, but they are not available to the consumer since the bran of rice and wheat germ are removed during processing, while, linoleic acid in bajra is available with the flour itself.

The high amount of phospholipids consisting both lecithins and cephalins, also offer many advantages. Phospholipids of other cereals like rice, wheat, corn, etc. are not available to the consumer because they are removed dissolved in oils. These compounds are having great role in general metabolism, being concentrated in brain are useful in brain function, behavioral disorders and stress. They help in regeneration of membranes and protect liver, lungs, kidneys, and gastrointestinal tract. These compounds are known to enhance the bioavailability of other nutrients and medicines (De Caterina et al. 2006).

Conclusion

The identification of good amount of phenolics, flavonoids, phospholipids and linoleic acid in Bajra elevates the potential of this crop and makes one of the best food grains in the world. Other than the nutrients, this crop possesses a large number of medicinal properties such as cancer chemoprotection, which are not available in other grains like rice and wheat. This data will favor the wider acceptability of bajra when compared to the other staple foods. The hardiness of Bajra plants as evidenced by their growth in water and nutrient deficient soil makes this crop desirable to the farmers who will be able to cultivate this crop in dry regions and poor regions. This situation is clear when the common crops like rice and wheat are compared which need great care in terms of fertilizer, water and pests.

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References

1. Adams, R. P. (2007). Identification of essential oil components by gaschromatography/mass spectrometry. Allured Publishing Co., Carol Stream, Illinois, USA.
2. Al-Fayez M, Cai H, Tunstall R, Steward, WP, Gescher, AJ (2006) Differential modulation of cyclooxygenase-mediated prostaglandin production by the putative cancer chemopreventive flavonoids tricin, apigenin and quercetin. Cancer Chemother.Pharmacol., 58, 816–825.
3. Bergstrom S, Danielson H, Klenberg D, and Samuelsson B (1964). "The Enzymatic Conversion of Essential fatty Acids into Prostaglandins". J Biol Chem **239** (11): PC4006–PC4008.
4. Cai, H.; Hudson, E. A.; Mann, P.; Verschoyle, R. D.; Greaves,P.; Manson, M. M.; Steward, W. P.; Gescher, A. J. (2004) Growthinhibitory and cell cycle-arresting properties of the rice bran constituent tricin in human-derived breast cancer cells in vitro and in nude mice in vivo. Br. J. Cancer, 91, 1364–1371.
5. Cai H, Al-Fayez M, Tunstall RG, Platton S, Greaves P, Steward WP, Gescher AJ (2005) The rice bran constituent tricin potently inhibits cyclooxygenase enzymes and interferes withintestinal carcinogenesis in *ApcMin* mice. Mol. Cancer Ther., 4, 1287–1292.

6. De Caterina R,Zampolli A, Del Turco S (2006) Nutritional mechanisms that influence cardiovascular disease. Am J Clin Nutr. 83(2): 421S-426S.
7. Duke J (2011) Phytochemical and Ethnobotanical databases. www.ars-grin.gov/duke Accessed on 5 June 2011.
8. Dykes L and Rooney LW (2007) Phenolic compounds in Cereal Grains and their Health Benefits. Cereal Foods World 52(3): 105-111.
9. Hudson EA, Dinh PA, Kokubun T, Simmonds MSJ, Gescher A (2000) Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. Cancer Epidemiol. Biomarkers PreV. 9, 1163–1170.
10. Ibrahim RK and Towers GHN (1960) The identification by paper chromatography of plant phenolic acids. Arch. Biochem. Biophys. 87: 125-128.
11. Kim JE, Son JE, Jang YJ, Lee DE, Kang NJ, Jung SK, Heo Y, Lee K and Lee HJ (2011) Luteolin, a novel natural inhibitor of TPL2 kinase, inhibits tumor necrosis factor-α-induced cyclooxygenase-2 expression in JB6 mouse epidermis cells, J. Pharmacol Exp. Ther. 337: 3, 111-120.
12. der I (2004) Sorghum and millets, In Cultivated plants, Primarily as Food Sources, (Ed. G.Fuleky) Encylopedia of Life Support System. EOLSS Publishers, Oxford.
13. Mabry TJ, Markham H and Mabry H (1970) The systematic identification of flavonoids. Springer – Verlag, Berlin.
14. Murty AVSSS and Subramanyam NS(1989) A Textbook of Economic Botany. Wiley Eastern Limited. New Delhi. 110
15. Reichert RD (1979) The pH-sensitive pigments in Pearl millet. Cereal Chem.56:291
16. Siddique NA, Mujeeb M, Najmi AK and Akram M (2010) Evaluation of antioxidant activity, quantitative estimation of phenols and flavonols in different parts of *Aegle marmelos.* Afr. J. Plant Sci. 4(1): 1-5.
17. Singleton VL, Rudolf O, Rosa ML (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent.Methods in Enymology 299:152-178. Academic press London.


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