RESEARCH PAPER | Chemistry | Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

Antidiabetic Principles, Phospholipids And Fixed Oil of Kodo Millet (*Paspalum scrobiculatum* Linn.)

KEYWORDS: Kodo Millet, Paspalum scrobiculatum, Antidiabetic activity, Quercetin, Ferulic acid, Antioxidant principles

Kiran P.	Denni. M	M. Daniel
Department of Botany, Faculty of Science, M. S. University, Vadodara, 390002	Department of Chemistry, Faculty of Science, M. S. University, Vadodara,390002	Dr. Daniel's Laboratories, Rutu Platina, Opp. Hans Farm, Manjalpur, Vadodara, 390011.

ABSTRACT *Kodo millet (Paspalum scrobiculatum Linn.), is an extremely drought and salt resistant wild millet attributed with a number of medicinal properties such as antidiabetic, tranquilising, hypolipidaemic, anti-rheumatic and wound-healing. Though the amount of carbohydrates, protein, oil and fiber contents are known, a detailed study on the medicinal principles are lacking. In our work, this millet is found to contain 1.120mg/g. phenolics consisting of a flavonol, quercetin and phenolic acids such as cis- ferulic acid, vanillic acid, syringic acid, p-hydroxy benzoic acid and melilotic acid. The total antioxidant potential was found to be– IC50 = 31.5 ± 0.03 mg/ml in terms of ascorbic acid and gallotannins. . The oil consisted of oleic acid (40.7%), stearic acid (37.5%) and palmitic acid (19.5%). Phospholipids present in the grain were 0.24%, consisting of four bands of cephaelins, two bands of lecithin and a single band of galactolipid. The role of quercetin and most of the phenolic acids in the medicinal properties listed especially of antidiabetic activity is discussed and the need to bring this grain in the mainstream agriculture is emphasised.*

Introduction.

Kodo millet (*Paspalum scrobiculatum* Linn.), is a wild cereal which yields a white- husked grain and cooked as rice. It is cultivated in many countries, both for grain and for fodder. In India this is grown in pockets in Andhra Pradesh, Tamil Nadu, Orissa, Bihar, MP, Maharashtra and Gujarat. Since both the leaves and stalks are violet in color, the fields possess a characteristic violet look. It is extremely drought and salt resistant and this is grown in saline soils also. The grain is very coarse with a horny seed coat which is removed before cooking. The grains are reported to contain 74% carbohydrates, 11.5% protein, 1.3% fat and 10.4% fiber (Leder, 2004).

A number of medicinal properties such as antidiabetic, tranquilising, antirheumatic and wound-healing are attributed to this grain. It is recommended as food for diabetic patients (Murty and Subramanyam, 1989). Aqueous and ethanolic extracts of this grain produced a dose-dependent fall in fasting blood glucose (FBG) and a significant increase in serum insulin level. This indicates that P. scrobiculatum possesses significant antidiabetic activity. (Jain et al., 2010). Tranquilizing action of the dried ethanol extract of the husk of this grain was proved by Bhide (1962). A study on inhibition of collagen glycation and crosslinking in vitro by methanolic extracts lead to the conclusion that Kodo millet can be used in the treatment of skin wounds (Hegde et al., 2005).

All the above data show that it is mainly the alcoholic extract which is pharmacologically active. But the chemical composition of this extract is not elucidated. Though fats are reported from this cereal, no data on the quality of the fat and phospholipids are available in literature. Therefore, in the present work an attempt to generate these parameters so that a true assessment on the quality of the grain can be accomplished. In addition the total phenols as well as the antioxidant potential also are calculated, which will lead to a better understanding of the properties of this grain.

Materials 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 and 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. Galacto- and glucolipids were located with the help of sugar reagents like anisaldehyde-sulphuric acid 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 the identities are confirmed by co-chromatography with authentic samples. The identification of phenolic acids was done following Ibrahim & Towers (1960) and co-chromatography with standard compounds. The total antioxidant activity was measured using the well-known DPPH method (Siddique et al. 2010).

Results

The alcoholic extract of Kodri, which is responsible for the antidiabetic property, is found to possess both flavonoids and phenolic acids. Quercetin was the flavonol present. The phenolic acids located were five, viz: vanillic acid, syringic acid, cis- ferulic acid, p-hydroxy benzoic acid and melilotic acid. Total phenols were 1.120 mg/g in terms of gallotannins. The total antioxidant potential was – IC50 = 31.5 ± 0.03 mg/ml in terms of ascorbic acid and gallotannins.

The grains of kodo millet yielded 0.856% of a clear yellow fatty oil. It gave a saponification value of 294.4. On analysis by GCMS, the oil was found to contain esters of four major fatty acids, i.e. oleic acid, stearic acid, palmitic acid

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

and linoleic acid. Saturated fatty acids were more amounting to 57% consisting of stearic acid (37.5%) and palmitic acid (19.5%). Though oleic acid was maximum amounting to 40.7%, the other unsaturated acid, linoleic was only 1.57%. Phospholipids present in the grain was 0.24%, consisting of four bands of cephaelins, two bands of lecithin and a single band of galactolipid.

Discussion.

The data on the phytochemicals of kodo millet substantiates the antidiabetic property exhibited by this grain. Quercetin, the flavonol present in this millet, is known to possess a large number of pharmacological properties including antidiabetic action. In a recent review, entitled " Beneficial effects of quercetin on obesity and diabetes" Aguirre et al., (2011), enlist all the researches conducted all over the world and emphasises that in animal models and cell cultures quercetin is proved to be antidiabetic in nature. In vitro studies proved that quercetin can 1) reduce intestinal glucose absorption at the level of glucose transporters (Kwon et al., 2007), 2) block tyrosine kinase (Elberg et al., 1995), 3) potentiate both glucose and glibeclamide induced insulin secretion, and protect β cells from oxidative damage induced by H^2O^2 (Youl et al.,2010), 4) inhibit glucose uptake (Strobel et al., 2005) and 5) improve glucose homeostasis (Torres-Piedra et al., 2010). In vivo studies gives more definite roles for quercetin such as 1) inhibition of small intestine maltase (Kim et al., 2011), 2) increased glucokinase activity and an increase in the number of pancreatic islets (Vessal et al., 2003), 3) partially preventing degeneration of β-cells (Coskun et al., 2005), 4) alleviate diabetic symptoms and liver injury (Kobori et al., 2009) and 5) improve insulin sensitivity (Wein et al., 2010). It is also revealed that quercetin rich food is more effective than pure quercetin in controlling diabetes (Jung et al., 2011).

Another important role of quercetin that is being followed up of late is its role in obesity (Aguirre et al., 2011). It is proved to reduce triacylglycerol content, inhibition of lipogenesis (Ahn et al., 2008), inhibit lipoprotein lipase (Motoyashiki et al.,1996), activate lipase and thus increase lipolysis (Kuppusamy and Das, 1992),increasing apoptosis (Hsu and Yen, 2006), reduce body weight (Rivera et al., 2008) and decrease oxidative stress (Kobori et al., 2011).

Out of the five phenolic acids located in Kodo millet, i.e. vanillic acid, syringic acid, cis- ferulic acid, p-hydroxy benzoic acid and melilotic acid, all except the last one, are found recently to possess antidiabetic properties and ferulic acid is found to be most active. Ferulic acid is found to exert protective and therapeutic effects on diabetic nephropathy by reducing oxidative stress and inflammation (Choi et al., 2011). Supplementation of this phenolic acid to the in the food of diabetic rats resulted in a decrease in the levels of glucose, TBARS, hydroperoxides, FFA and an increase in reduced glutathione (GSH). FA also resulted in increased activities of SOD, CAT, GPx and expansion of pancreatic islets. The effect was much pronounced with lower dose treatment. Thus it is proved that administration of ferulic acid helps in enhancing the antioxidant capacity of these diabetic animals by neutralizing the free radicals formed thereby reducing the intensity of diabetes (Balasubashini et al., 2004). Addition of ferulic acid at 0.01% and 0.1% of basal diet showed to suppress significantly blood glucose levels in STZ-induced diabetic mice. In KK-A^{y} mice 0.05% FA suppressed effectively blood glucose levels. These findings suggest that dietary ferulic acid is useful in alleviating oxidative stress and attenuating the hyperglycemic response associated with diabetes (Ohnishi et al., 2004). This compound possesses antioxidant properties that make it an important anti-aging supplement, and they also contribute to its other potential uses. These include applications in cancer, neuroprotection, bone degeneration, menopause, immunity, and (perhaps) athletic performance. In addition it has a cardioprotective effect via increasing SOD activity and NO levels in plasma and myocardium, inhibiting oxidative stress in plasma and myocardium, and inhibiting the expression of CTGF in myocardium in diabetes rats (Xu et al., 2012).

Recently, vanillic acid is established to contribute to the prevention of the development of diabetic neuropathy by blocking the methylglyoxal-mediated intracellular glycation system (Huang et al., 2008). Syringic acid as well as vanillic acid increased cell viability and decreased apoptosis of cells, among other effects when exposed to methylglyoxal. They were found to be the most inhibitory of the p38 MAPK pathway that leads to apoptosis of the Schwann cells. Hypoglycemic activity of ρ-hydroxybenzoic acid, was proved when activity -guided fraction from *Pandanus odorus* Ridl. (Thai name: Toei-hom, Pandanaceae), containing this compound showed a hypoglycemic effect in normal rats after the oral administration of 5 mg/kg. Additionally, the compound increased serum insulin levels and liver glycogen content in normal rats (Peungvicha, et al., 1998). All the above mentioned phenolics are 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 amount of fatty oil in this grain (0.856%), though not high, is available to the consumer because the grain is not highly polished. A high amount of oleic acid, which is an omega-9 fatty acid, is good because it gets converted to linoleic acid which is easily converted to n-6 eicosanoids, n-6 prostaglandin and n-6 leucotriene hormones (Bergstrom, 1964). 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 poor wound healing. Therefore, the consumption this millet, containing oil, will yield the same advantages to the consumer. Though the major cereals such as rice wheat and corn contain oils, they are not available to people since the bran of rice and wheat and corn germs are removed during processing, while oil in Kodo millet is available with the flour itself.

The presence of good amounts of phospholipids consisting cephaelins, lecithins and galactolipids, also offer many advantages. Phospholipids of other cereals like rice, wheat, corn, etc., similar to oils, 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, behavioural 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).

Conclusions

Since, the latest researches emphasise the antidiabetic and hypolipidaemic activities of quercetin and most of the phenolic acids, these medicinal properties of the grain can be attributed to these compounds. In addition, the presence of phospholipids, fibre contents, low oil content etc. make this grain a true "Nutraceutical". Kodo millet, being an extremely drought and salt resistant, can be cultivated in saline areas and non-irrigational lands. The fact that it is grown in poor gravely or stony soils, where other cereals do not succeed, adds to its acceptability as a cereal.

Acknowledgements.

The authors gratefully acknowledge the help rendered by Dr. S. Maiti, Director, DMAPR, Anand and Dr. V. Rana in getting the analysis of the fatty acids in the oil done.

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

REFERENCE

Adams, R. P. (2007). Identification of essential oil components by gaschromatography/mass spectrometry. Allured Publishing Co. Illinois,USA. | Ahn, J., Lee, H. Suna, K, and Ha, T. (2008). Anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways. Biochem. Biophys. Res. 373: 545-9. | | Aguirre, L., Arias, N., Teresa, M. M., and Portillo, M.P. (2011) Beneficial Effects of Quercetin on Obesity and Diabetes. The Open Nutraceuticals Journal, 4, 189-198 | | Balasubashini, M., Rukkumani, R., and Menon, V.P. (2004) Ferulic acid alleviates lipid peroxidation in diabetic rats, Phytotherapy Research 18 (4): 310–314. | | Bergstrom, S. Danielson, H., Klenberg D, and Samuelsson B (1964) The enzymic conversion of essential fatty acids into prostaglandins. J Biol Chem 239(11): 4006–4008. | | Bhide, N.K., (1962) Pharmacological study and fractionation of Paspalum scrobiculatum extract . Br J Pharmacol Chemother. 18(1): 7–18. | Choi, R., Kim, B.H., Naowaboot, J., Yang, Y. C. and Chung, C.H. (2011) Effects of ferulic acid on diabetic nephropathy in a rat model of type 2 diabetes Exp Mol Med. 43(12): 676–683. | Coskun, O., Kanter, M., Korkmaz, A., Oter, S. (2005) Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β- cell damage in rat páncreas. Pharmacol Res. 51: 117-23. | | De Caterina, R, Zampolli, A. and Del Turco, S. (2006) Nutritional mechanisms that influence cardiovascular disease. Am J Clin Nutr. 83(2): 421S-426S. | | Kobori, M., Masumoto, S., Akimoto, Y. and Takahashi, Y. (2009) Dietary quer-cetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. Mol Nutr Food Res. 53: 859-68. | | Elberg, G., Jinping, L., Leibovitch, A. and Shechter, Y. (1995) Non-receptor cytosolic protein tyrosine kinases from various rat tissues. Biochim et Biophy Acta. 1269: 299-306. | | Hegde, P.S., • Chandrakasan, G. and Chandra,T. (2002) Inhibition of collagen glycation and crosslinking in vitro by methanolic extracts of Finger millet (Eleusine coracana) and Kodo millet (Paspalum scrobiculatum). Jour. Nutr. Biochem. 13(9): 517-521, | Hegde, P.S., Anitha B. and H.S. Chandra (2005) In vivo effect of whole grain flour of finger millet (Elusine coracana) and kodo millet (Paspalum scrobiculatum) on rat dermal wound healing. Indian J Exp Biol . 43: 259-263. | Hsu, C.L .and Yen G.C., (2006) Induction of cell apoptosis in 3T3-L1 pre-adipocytes by flavonoids is associated with their antioxidant activity. Mol Nutr Food Res. 50: 1072-9. | | Huang, S., C. Hsu, H. Chuang, P. Shih, C. and Wu, Yen, G. (2008) Inhibitory effect of vanillic acid on methylglyoxal-mediated glycation in apoptotic Neuro-2A cells NeuroToxicology, 29(6): 1016–1022 | | Jain, S., Bhatia, G., Barik R, Kumar P, Jain A. and Dixit, V.K., (2010). Antidiabetic activity of Paspalum scrobiculatum Linn. in alloxan induced diabetic rats. J Ethnopharmacol. 127(2):325-8. | Jung, J.Y., Lim, Y. and Moon, M.S., (2011) Onion peel extracts ameliorate hyperglycemia and insulin resistance in high fat diet/ streptozotocin-induced diabetic rats. Nutr Metab. 8: 18. | Ibrahim, R.K. and Towers, G.H.N. (1960) The identification by paper chromatography of plant phenolic acids. Arch. Biochem. Biophys. 87: 125-128. | Kim, J.H., Kang, M.J. and Choi, H.N. (2011) Quercetin attenuates fasting and post- pra-ndial hyperglycemia in animal models of diabetes mellitus. Nutr Res Pract 5(2): 107-11. | | Kobori, M., Masumoto, S., Akimoto, Y. and Takahashi.Y., (2009) Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. Mol. Nutr. Food Res. 53: 859-68. | | Kuppusamy, U.R. and Das, N.P., (1992) Effects of flavonoids on cyclic AMP phosphodiesterase and lipid mobilisation in rat adipocytes. Bio-chem Pharma. 44: 1307-15. | | Kwon, O., Eck, P, and Chen, S., (2007). Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. FASEB J. 21: 366-77. | Leder, I. (2004) Sorghum and millets, In Cultivated plants, Primarily as Food Sources, (Ed. G.Fuleky) Encylopedia of Life Support System. EOLSS Publishers, Oxford. | | Mabry TJ, Markham H and Mabry.H (1970) The systematic identification of flavonoids. Springer – Verlag, Berlin. | Motoyashiki, T., Morita, T., and Ueki,H., (1996). Involvement of the rapid increase in cAMP content in the Vanadate-Stimulated Release of Lipoprotein Lipase activity from a rat fat pad. Bio. Pharm. Bull. 19: 1412- 6. | | Murty, A.V.S. and NSA, Subramanyam, N.S.A (1989) Textbook of Economic Botany. Wiley Eastern Limited, New Delhi. | Ohnishi, M., T. Matuo, T. Tsuno, A. Hosoda, E. Nomura, H. Taniguchi, H. Sasaki and Morishita,H. (2004). Antioxidant activity and hypoglycemic effect of ferulic acid in STZ-induced diabetic mice and KK-A^{y} mice, BioFactors, 21(1-4): 315-319. | Peungvicha, P., R. Temsiririrkkul, J. Prasain,Y. Tezuka ,S. Kadota, S. S Thirawarapan, and Watanabe, H. (1998). 4-Hydroxybenzoic acid: a hypoglycemic constituent of aqueous extract of Pandanus odorus root. Jour. Ethnopharmac. 62(1): 79–84 | Rivera, L., Morón, R. and Sánchez , M., (2008). Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese zucker rats. Obesity. 16: 2081-7. | Siddique, N.A., Mujeeb, M., Najmi, A.K. 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. | Singleton, V.L, Rudolf ,O. and Rosa, M.L., (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. | Strobel, P., Allard, C, and Pérez-Acle, T., (2005) Myricetin, quercetin and catechin-gallate inhibit glucose uptake in isolated rat adipocytes. Biochem J. 386: 471-8. | | Torres-Piedra, M., Ortiz-Andrade, R, and Villalobos-Molina,R . (2010) A comparative study of flavonoid analogues on streptozotocine-nicotinamide induced diabetic rats: Quercetin as a potential antidiabetic agent acting via 11b–Hydroxysteroid dehydrogenase type 1 inhibition. Eur J Med Chem . 45: 2606-12. | | Vessal, M., Hemmati, M. and . Vasei.M., (2003) Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. Comp Biochem Physiol C: Comp Pharmacol Toxicol 135: 357-64. | Wein, S., Behm, N. and Petersen, R.K.,(2010) Quercetin enhances adi-ponectin secretion by a PPARβ independent mechanism. Eur J Pharm Sci. 41: 16-22. | | Xu, X, Xiao H, Zhao J and Zhao T. (2012) Cardioprotective Effect of Sodium Ferulate in Diabetic Rats. Int J Med Sci. 9(4):291-300. | | Youl E, Bardy G, and Magous R, (2010). Quercetin potentiates insulin secretion and protects INS-1 pancreatic b-cells against oxidative damage via the ERK1/2 pathway. BJP 161(4): 799-814.