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

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

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, Maharashtra and Gujarat. Since both the seeds 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-PoQ 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 cephalins 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-Ciocalteau 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 potential 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. Antioxidant activity was measured using the well-known DPPH method (Siddique et al. 2010).

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 major fatty acids, i.e. oleic acid, stearic acid, palmitic acid and a number of minor fatty acids. The total saponification value was found to be: IC50 = 31.5 ± 0.03 mg/ml in terms of ascorbic acid and gallotannins. The oil 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).
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 ferulic acid and diabetes” Aguirre et al. (2011), enlist all the researches conducted all over the world and emphasizes 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 the level of glucose transporters (Kwon et al., 2007), 2) block that quercetin can 1) reduce intestinal glucose absorption at 0.01% and 0.1% of basal diet showed to suppress significantly blood glucose levels in STZ-induced diabetic mice. In vivo studies give 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 (Vessali et al., 2003), 3) partially preventing the degeneration of β-cells (Coskun et al., 2005), 4) alleviate diabetic symptoms and injury induced by H2O2 (Youl et al., 2010), 4) inhibit glucose uptake (Strobel et al., 2005) 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 is that 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 mellite is acid, all except the last one, are found recently to have 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 regeneration, 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 p-hydroxybenzoic acid, was proved when activity-guided fraction from Pandanus odorus Ridl. (Thai name: Toei-horn, 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 artherosclerosis (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 betoic 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, gravelly 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. M. Maiti, Director, DMAPR, Anand and Dr. V. Rana in getting the analysis of the fatty acids in the oil done.
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