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

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

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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, vanillin 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 fast- ing 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 methanol 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-PoIQ 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. Phenolic compounds were analyzed by TLC and Lecithins were visualized by spraying the developed chromatograms by Dragendorf’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-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 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: vanillin acid, syringic acid, cis- ferulic acid, p-hydroxy benzoic acid and melilotic acid. Total phenols were 1.120 mg/g in terms of galactotannins. 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.
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 cephalins, two bands of lecithin and a single band of galactocephalin.

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 on 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 enlisted 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 H2O2 (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 (Vessali et al., 2003), 3) partially preventing degeneration of β-cells (Coskun et al., 2005), 4) alleviate diabetic symptoms in streptozotocin-induced diabetes (Jung et al., 2011) 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., 1992), 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, cia-ferulic acid, p-hydroxy benzoic acid and mellitic 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 in STZ-induced diabetic mice. In KK-A^y mice 0.05% FA suppressed effectively blood glucose levels in STZ-induced diabetic mice. 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 germ is removed during processing, while oil in Kodo millet is available with the flour itself.

The presence of good amounts of phospholipids consisting cephalins, 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 pheno- nolic 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., 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).

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