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Phytochemical evaluation of Kodo (*Paspalum scrobiculatum* L.) bran

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Abstract
The physical properties of Kodo bran and defatted Kodo bran, i.e., bulk density, true density, porosity, and angle of repose, were found to be 436.9 and 518.3 kg/m^3, 1006.4 and 1008.3 kg/m^3, and 56.6 and 48.6%, respectively. The coefficient of friction of Kodo bran and defatted Kodo bran was observed on three surfaces: aluminium sheet, G.I. and an MS plate value varying from 0.932 to 1.305. The highest value of the coefficient was observed in the G.I. plate. The chemical properties of bran show significant increases in all parameters after the extraction of oil. The Kodo bran was found to have a high content of fat, protein and carbohydrates. The total phenolic content, total flavonoid content, total tannin content and total saponin content in Kodo bran and defatted Kodo bran were found to be 510 mg GAE/100 g and 618 mg GAE/100 g, 347 mg QE/100 g and 355 mg QE/100 g, 68 mg TAE/100 g and 79 mg TAE/100 g and 92 mg SE/100 g and 43 mg SE/100 g, respectively.

Keywords: Phenolic, flavonoids, tannin, saponin

Introduction
Millets are regarded as substitutes for traditional cereals because the world's population believes that cereals are insufficient to meet global food demand, and millets are regarded as a reliable source of nutrition (Kumar *et al*., 2018) [29]. It is more affordable than other famous millets and grains. *Paspalum scrobiculatum* is a small seeded cereal grain and is amongst one of the oldest cultivated crops in India. Kodo millet (*Paspalum scrobiculatum* L.) is native to India and is said to have been domesticated thousands of years ago (Khobragade, 2020) [11]. Millets are rich in dietary fibre, iron, calcium, and B-vitamins and particularly low in phytic acid. Some of them exceed the average protein, fat and mineral content (Gopalan and Shastri, 2009). Additionally, it has higher percentages of inaccessible carbs (Malathi *et al*., 2012) [16].
This grain's aqueous and ethanolic extracts led to a dose-dependent decrease in fasting blood glucose (FBG) and a notable rise in serum insulin levels. This suggests that P. scrobiculatum has potent anti-diabetic properties (Jain *et al*., 2010) [30].
It cultivated on Deccan plateau in India consider as category of small millet. Gujarat, Chhattisgarh, Eastern Madhya Pradesh, Karnataka and Tamil Nadu are some state where it is typically grown in India (Kumar *et al*., 2016) [13]. The term "millet" refers to a group of "small-seeded annual grasses" that are typically grown as a staple crop on marginal lands in arid and semi-arid climates. Kodo millet is grown over 2.44 lakh ha in India, with a productivity of 312 kg/ha and a total production of roughly 0.73 lakh tonnes (Chandra *et al*., 2021) [5].
Overall production in Chhattisgarh is approximately 0.17 lakh tonnes (Suresh *et al*., 2022) [28]. Kodo millet has a high moisture content of 14% and is a good source of fibre (6.39±0.35%), protein (9.83±0.43%), fat (2.65±0.11%) and carbohydrates (65.32±0.99%) (Reddy *et al*., 2019) [24]. The grains are rich in vitamins (thiamine 0.3 mg, riboflavin 0.09 mg and niacin 0.2 mg), as well as minerals (calcium 27 mg, phosphorus 188 mg, iron 0.5 mg and zinc 0.5 mg) (Muragod *et al*., 2019) [17]. Kodo millet has an ethanolic extract with a TPC of 175.94±1.018 mg GAE/g extract and a TFC of TFC 116.48±1.57 mg RE/g extract (Shabeer, 2018) [26].
Bran, a by-product of milling, is frequently used for the animal feed or thrown away as waste. In recent years, a large number of studies have found that millet bran contains a lot of lipids. Due to RBO's growing significance in recent years for its health advantages, it has achieved widespread acceptance.

Due to its distinctive qualities and therapeutic significance, it has found applications in the food, pharmaceutical, cosmetic, and chemical industries (Garba *et al*., 2017) [6]. Numerous waste products, including gum sludge, wax sludge, deodorizer distillate (DOD) and fatty acid distillate, soapstock are building up as more enterprises produce edible-grade oil from rice bran (Krishna *et al*., 2013) [12].

Materials and Methods

Isolation of Kodo Bran (KB)

The bran Sample was obtained from the PHET laboratory, SV CAET & RS, IGKV, Raipur, India). Kodo millet was cleaned properly. Kodo millet was dehusked by using grain dehusker (SV CAET & RS, Raipur, India) and grain polisher (SV CAET & RS, IGKV, Raipur) was used for separate the bran. The bran was separate using gravity sieve shaker then store in desiccator.

Physico-chemical Properties of KB

Physical Properties of KB

The physical properties like bulk density, true density and porosity was determined by follow by Kumar *et al.* (2016) [13]. The angle of repose determines the maximum angle of the pile of bran in the horizontal plane by Patel *et al.* (2021) [20] with some modification like cone was formed above the table or plane.

Coefficient of friction

The coefficient of friction was computed by using various surfaces (aluminum sheet, and G.I. and MS plate) that were placed one by one on a moveable plane. Kodo bran was placed over the surface. The movable plane was slowly lifted to a position whereby the Kodo bran had just begun to slide down to the plane. The plane existed at this position and the angle of friction was noted. The coefficient of friction was calculated by the following formula:

The coefficient of friction $\mu = \tan \theta$

Where, μ = Coefficient of static friction; and θ = Angle of static friction.

Chemical Properties of KB

Moisture content, ash content, oil content, protein content and fibre content were estimated as per AOAC, 2005 [1]. The total carbohydrate was estimated by using AOAC, 2000 [2].

Evaluation of Phytochemical Composition and Total Antioxidant Capacity of KB

Total Phenolic Content (TPC)

Total phenolic content was determined spectrophotometrically according to the Folin-Ciocalteu method (Mahalle *et al*., 2012) [15]. For the preparation of standard gallic acid was used having concentration of 5-25 µg/ml. The TPC was expressed in terms of mg of gallic acid equivalent per 100 gram of sample (mg GAE/ 100 g).

Total Flavonoids Content (TFC)

The total flavonoid content was determined spectrophotometrically according to the Aluminum chloride method (Mahalle *et al*., 2012) [15]. The flavonoids content was expressed in terms of mg of quercetin equivalent per 100 gram of sample (mg QE/100 g).

Total Tannin Content (TTC)

The total tannin content was determined spectrophotometrically according to the Folin-Ciocalteu method (Chandran *et al*., 2016) [5]. 80% ethanolic extract (semi-solid) was used for test. The tannin content was expressed in terms of mg of tannic acid equivalents per 100 gram of sample (mg TAE/100 g).

Total Saponin Content (TSC)

The total saponin content was determined spectrophotometrically using saponin as standard (Jain *et al*., 2017 [8] with some modification. The saponin content was expressed in terms of mg of saponin equivalent per 100 gram of sample (mg SE/100 g).

Total Antioxidant Capacity (TAC)

The antioxidant capacity of extracts during TAC assay was determined by following the method as described by Punia *et al*., (2021) [31]. KB and DKB extracts were analyzed for antioxidant capacity using sodium hydrogen orthophosphate (28 mM); conc. H_2SO_4 (0.6 M) and ammonium molybdate (4 mM) at 95 °C for 90 min.

Results and Discussion

Physico-chemical Properties of KB

The values (Table 1) of bulk density, true density, porosity and angle of repose of Kodo bran and defatted Kodo bran were found to be 436.9 kg/m³and 518.3 kg/m³, 1006.4 kg/m³ and 1008.3 kg/m³, 56.9% and 48.6% and 38.7 and 39.1, respectively. Khobragade *et al*., 2020 [11], shown the Kodo bran physical properties like bulk density, true density and porosity are found to be similar results. The coefficient of friction (Table 2) of bran and defatted bran range 0.932 to 1.305 minimum value in MS plate and highest value in GI sheet. Angle of repose and coefficient of friction of Kodo bran was similar result as rice bran by Bhattacharya and Syed (2015) [4]. The results of the proximate analysis experiment (Table 3) moisture content (db%) of Kodo bran and defatted Kodo bran was 5.75% and 5.66% similar results as rice bran by Ghodrata *et al.* (2015) [7]. The protein content (Table 1) obtained was found to be 10.07% and 10.81%, similar results as rice bran by Ghodrata *et al.* (2015) [7], foxtail millet bran by Liang *et al.* (2020) [14], and Kodo bran by Onipe *et al.* (2015) [18]. The ash content of KB and DKB sample was found 6.20% and 6.62%, similar result as wheat bran by Ghodrata *et al.* (2015) [7]. The vales of crude fibre and carbohydrate of KB and DKB were found to be 3.32% and 6.65% and 61.03% and 70.48%, respectively shown similar result reported by Reddy *et al.* (2019) [24] and Onipe *et al*. (2015) [18] of wheat bran for carbohydrate. The oil content in Kodo bran was found to be 13.95% (Table 1). Khobragade, (2020) [11] shown similar result for KB and Ruen-ngam *et al*. (2018) [25] reported that four varieties of rice bran and the oil content ranged from 10.42±01 to 20.76±0.47 g/100 g. The result showed that oil content varied from variety to variety. The proximate analysis of KB and DKB showed increases in protein, ash and carbohydrate after oil extraction the same result was obtain in rice bran by Sirikul *et al.* (2009) [27].

Table 1: Physical properties of bran

Parameter	Kodo bran	Defatted Kodo bran
Bulk density (kg/m$^{3)}$	436.9	518.3
True density (kg/m$^{3)}$	1006.4	1008.3
Porosity (%)	56.6	48.6
Angle of repose	34.6	35.1

Table 2: Coefficient of friction of bran

Sheet	Kodo bran	Defatted Kodo bran
S1	1.305	1.305
S2	0.932	0.932
S3	1.065	1.065

Table 3: Chemical analysis of bran

Nutrient	Kodo bran	Defatted Kodo bran
Moisture content (db %)	5.75	5.66
Ash content	6.20	6.62
Oil content	13.95	0.00
Protein content	10.07	10.81
Crude fibre content	3.32	6.65
Total carbohydrate	61.03	70.48

Evaluation of Phytochemical Composition and Total Antioxidant Capacity of KB

Effect of oil extraction on phytochemical composition in bran

In the present investigation, phytochemical composition was determined by using regression equations. The values of phytochemical composition were shown in Table 4. The phenolic content was determined using the regression equation for the Gallic acid calibration curve ($y = 0.0042x + 0.0191$ with $R^2 = 0.9787$) and it was represented in GAE as milligrams per gram of extract (mg GAE/100g). The total phenolic content of Kodo bran samples was 510 mg GAE/100 g sample in bran and 618 mg GAE/100 g sample in defatted bran. The flavonoid content was determined using the regression equation for the Quercetin calibration curve ($y = 0.0064x + 0.0035$ and $R^2 = 0.9953$), and it was represented in QE as milligrams per gram of extract (mg QE/100g). The total flavonoid content of Kodo bran samples was 347 mg QE/100 g sample in bran and 355 mg QE/100 g sample in defatted bran. Pradeep and Yadahally, (2017) [23] reported TPC and TFC values are increase in bran as compare to grain. The values TPC and TFC in KB and DKB found was higher value then Bhatia *et al*., (2010) [3] because the value was obtain from millet. The tannin content was determined using the regression equation for the Tannic acid calibration curve ($y = 0.0094x + 0.0545$ and $R^2 = 0.9915$), and it was represented in TAE as milligrams per 100 gram of bran sample (mg TAE/100 g sample). The values of total tannin content of Kodo samples were 68 mg TAE/100g sample in Kodo bran and 79 mg TAE/100g sample. Patil *et al.* 2020 reported near similar value. Total saponin content in Kodo bran was determined by using a regression equation for the saponin calibration curve ($y = 0.0128x - 0.0998$ with $R^2 = 0.9731$), and it was represented in SE as milligrams per 100 gram of sample (mg SE/100g sample). The values of total saponin content of fatted and defatted Kodo bran were found to be 92 and 43 mg SE/100 g, respectively. The values found of TSC in KB less than to values obtain by Panwar *et al.* (2016) [19] for barnyard millet and finger millet. Sirikul *et al.* (2009) [27] reported that the value of TPC and antioxidant activity were increase after the extraction process which was similar in this report for TPC, TFC and TTC where DKB shown higher value then KB.

Table 4: Phytochemical composition of bran

Nutrient	Kodo bran	Defatted Kodo bran
TPC (mg GAE/100g)	510	618
TFC (mg QE/100g)	347	355
TTC (mg TAE/100g)	68	79
TSC (mg SE/100g)	92	43

Effect of oil extraction of antioxidant capacity in bran

Total antioxidant capacity of bran samples was analysis in different concentrations of the ethanolic extract of the Kodo bran and defatted Kodo bran samples ranging from 0µg to 1000 µg were used to see the effect of the total antioxidant capacity. The result shows a significant increase in antioxidant activity by increasing the concentration of sample in the solution. The defatted Kodo bran by n-hexane shows more antioxidant activity than Kodo bran but less than the ascorbic acid. Ascorbic acid was taken available in the market to compare the value of bran's total antioxidant capacity.

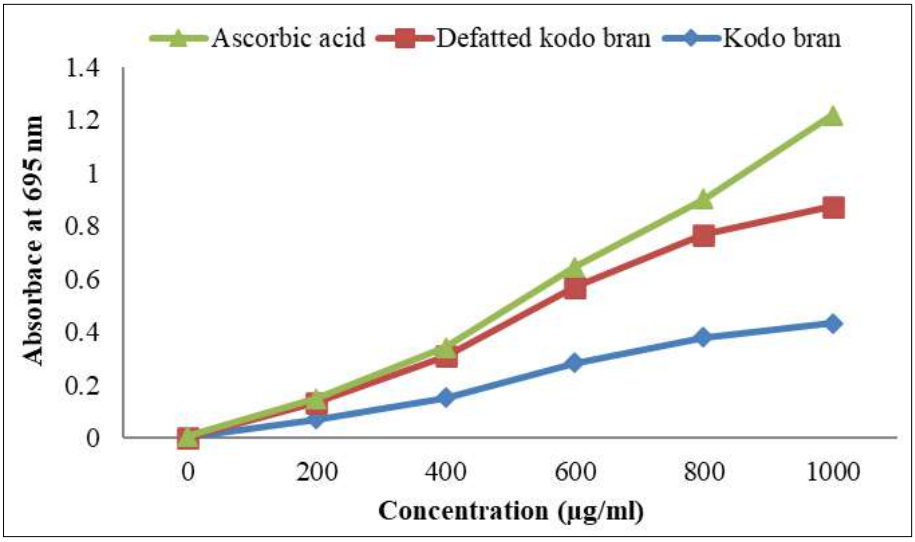

Fig 1: Total antioxidant activity of bran samples

Conclusion

The study focused on Kodo bran and defatted Kodo bran, which are by-products of polishing and oil extraction, respectively. In both samples, phytochemical compounds are present in high amounts. This study shows that bran contains high protein levels of 10.07% and 10.81% in bran and defatted Kodo bran, respectively, which indicates that significant increases in protein. The fibre carbohydrate,

protein and ash all chemical properties are increase in defatted Kodo bran. The phytochemicals and total antioxidant capacity of Kodo bran were also changed in the result. Phytochemicals like phenolic, flavonoids and tannin content in Kodo bran were increased in defatted Kodo bran. Saponin content was decreased after the oil extraction process because it is soluble in n-hexane. The Kodo bran shows the significant increases in total antioxidant capacity after the extraction process.

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