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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(4): 12-16

www.thepharmajournal.com
Received: 07-02-2017
Accepted: 08-03-2017

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Antioxidant potential of rice bran and vegetable waste powders incorporated extrudates

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Abstract
Now-a-days an increased amount of attention is focused on the development of value added foods that promote well-being and improve health. The increasing of awareness amongst consumers is placing greater demand on functional foods with maximum health benefits. The increased consumption of antioxidants from dietary sources like fruits and vegetables do contribute to better quality of life by delaying the onset and decreasing the risk of degenerative diseases associated with life style changes and aging. The results showed that stabilized rice bran and control with stabilised rice bran extrudate had almost all phytochemicals except phlobatinins and quinines. Total phenolic content showed maximum percent inhibition for stabilised rice bran at 71.51% and for cauliflower trimmings powder at 71.52% in scavenging the free radicals by DPPH method using methanol extracts. The stabilized rice bran extract had significantly higher (p<0.05) antioxidant potential with IC_{50} of 6.94 mg/ml.

Keywords: Antioxidants, phytochemicals, total phenolic content, stabilized rice bran, cauliflower trimmings, beetroot pomace.

Introduction
Plants are good sources of antioxidants like polyphenols, carotenoids, tocopherols, glutathione and ascorbic acid that fight hazardous oxidative damage of cells which otherwise cause pathogenesis and aging [1, 2].
Rice (*Oryza sativa*) has been part of human diet for almost 5,000 years [3] and is ranked as world's number one food crop [4]. Rice flour is an attractive ingredient in the extrusion industry as it has bland taste, attractive white color, hypoallergenicity, easy to digest along with low sodium, fat and protein content [5]. Rice bran is the by-product of milling paddy grains accounting to 12-20% total kernel weight and is a rich source of bioactive phyto-chemicals like γ- oryzanol, tocopherols and tocotrienols that possess health benefits and antioxidant properties [6].
Diets with fruit and vegetable servings provide defence against cancers and cardio-vascular diseases as these people contain high plasma antioxidant levels [7]. There are many types of antioxidants in foods that are immeasurable. Hence the measurement and compilation of total antioxidant potential of foods like fruits, vegetables, spices, herbs and their products has vast research potential [8].
Free radicals generated in human body causes oxidative damage to lipid, protein and nucleic acid cell walls that promote the development of degenerative diseases. Phenols, flavanoids, terpenoids and other phytochemicals from plant sources are capable of neutralizing these free radicals and thus help in preventing the degenarative diseases [9].
Now-a-days, there is an increasing interest to replace the synthetic antioxidants with natural ones from food sources. The antioxidant components obtained from discards and by-products of food industry ensure protection against oxidative damage in living organisms by scavenging the free radicals and increase the shelf life of foods by retarding lipid peroxidation [10].

Materials and Methods
Selection of best extrudates
Rice flour and refined wheat in 2:3 ratio was taken to which 15% stabilized rice bran was added and extrudates prepared [11]. To these extrudates, beetroot pomace powder (BPP) at 7.5, 10, 12.5 and 15% or cauliflower trimming powder (CTP) at 5, 10, 15 and 20 % were incorporated to improve the antioxidant potential. The most accepted was 7.5% BPP and CTP of 5% and these were selected by sensory evaluation using 9 point hedonic scale with 15 semi trained panelists [12].

Preparation of stock solution

2.0 g of each extrudates or vegetable waste powders or stabilized rice bran were taken, ground in methanol and left for 24 hrs. Next, the ground macerates were subjected to centrifugation at 3000 rpm for 10 min, filtered and stored at 4 °C till use.

Preliminary phytochemicals analysis

The extrudates or vegetable waste powders or stabilized rice bran extracts were tested for preliminary phytochemicals like carbohydrate, alkaloids, proteins, amino acids, flavanoids, fixed oils, terpenoids, cardiac glycosides, steroids, tannins, phlobatins, phenols and quinones [13].

Total phenolic compounds

It was determined as per the modified method of using Folin – Ciocalteau reagent [14]. The total phenol content in the extracts were expressed as µg of pyrocatechol equivalent using the formula given below:

Absorbance = 0.0021 X total phenols (µg pyrocatechol) - 0.00929

Free radical scavenging assay

The antioxidant potential was determined by the free radical scavenging assay using 1-diphenyl-2- picrylhydrazil (DPPH) [15].

Result and Discussion

Preliminary phytochemical screening

The methanol extract of control extrudate contained carbohydrates, proteins, amino acids, flavonoid, fixed oils and fats, terpenoids, cardiac glycosides and steroids. In stabilized rice bran powder and CBP extrudate had almost all phytochemicals were present except phlobatinins and quinones, where as CTP extrudate and BPP extrudates lacked terpenoids, cardiac glycosides only. The CTP and BPP powders lacked carbohydrates, alkaloids (Mayer's test), phlobatinins, quinones, fixed oils and fats. Terpenoids were present in BPP and absent in CTP as shown in Table 1.

The health promoting phytochemicals include total phenolic, total flavanoids and glucosinolates in white cauliflower by-products powders as antioxidants [16]. Rice bran is a rich source of phytochemicals that worked both synergistically and in parallel with each other to promote health and fight disease. Rice bran is much richer in phytonutrients and antioxidants than are corn, wheat or oat bran [17].

Cereal grains contain phytochemicals like flavonoids, phenolic acids, tocopherols, lignans, phytosterols and carotenoids. Phenolic acids are the most complex class of phytochemicals found abundantly in the bran of cereal grains and they are derivatives of cinnamic and benzoic acid. The cinnamic acid class of phenolic compounds includes ferulic acid, sinapic and p-coumaric acid [18].

Table 1: Phytochemical screening of extrudates and prepared samples

S. No.	Phytochemicals	Name of the test	Extrudates				Stabilized rice bran	CTP	BPP
			Control	CRB	CTP	BPP			
1	Carbohydrate	Molisch test	+	+	+	+	+	-	-
2	Alkaloids	Mayer's test	-	+	+	+	+	-	-
		Wagner's test	-	+	+	+	+	+	+
		Hager's test	-	+	+	+	+	+	+
3	Proteins	Kjeldhal method	+	+	+	+	+	+	+
4	Amino acids	Ninhydrin test	+	+	+	+	+	+	+
5	Flavanoids	With ammonia solution	+	+	+	+	+	+	+
6	Fixed oils and fats	Foam test	+	+	+	+	+	-	-
7	Terpenoids	-	+	+	-	+	+	-	+
8	Cardiac glycosides	-	+	+	+	-	+	+	-
9	Steroids	Liebermann- Buchard test	+	+	+	+	+	+	+
10	Saponins	Foam test	-	+	+	+	+	-	-
11	Tannins	$FeCl_3$ test	-	+	+	+	+	+	+
12	Phlobatinins	With HCl	-	-	-	-	-	-	-
13	Phenols	Ferric chloride test	-	+	+	+	+	+	+
		Liebermann's test	-	+	+	+	+	+	+
14	Quinones	With conc. HCl	-	-	-	-	-	-	-

Note: All screening tests were carried out in triplicates.
Control: Rice flour + refined wheat flour extrudate
CRB: Control + stabilized rice bran extrudate
CTP: Cauliflower trimmings powder incorporated CRB extrudate
BPP: Beetroot pomace powder incorporated CRB extrudate
CTP: Cauliflower trimmings powder
BPP: Beetroot pomace powder

Total phenolic content

Plants contain safe natural antioxidants that have immense use in food industry and the majority of antioxidants isolated were polyphenols. These polyphenols have functional and nutritional values of vegetable proteins along with contributing to the sensory and organoleptic properties of fruits and vegetables [19].

In this study the maximum inhibition was observed for stabilized rice bran (71.51%) and CTP (71.52%) with the lowest inhibition for control extrudates (55.97%). The IC_{50} value for control extrudate was 27.68, stabilized rice bran incorporated extrudate 23.99, CTP extrudate 22.34, BPP extrudate was 25.79, stabilized rice bran 18.79, CTP 22.01 and BPP 22.31 mg/ml. The lowest IC_{50} value indicates the highest phenolic inhibition as shown in Table 2.

Table 2: Phenolic activity of extrudates and prepared samples

S. No.	Parameter	Extrudates				Stabilized rice bran	CTP	BPP
		Control	CRB	CTP	BPP			
1.	IC_{50} (mg/ml)	26.01± 1.27	23.93±0.06	22.34± 0.51	26.02±1.30	18.79±0.28	22.0± 0.53	22.31±0.53
2.	Mean	32.69	36.01	37.50	36.96	42.30	40.88	41.27
3.	S.E	0.2347	0.0228	0.3376	0.2255	0.1882	0.3272	0.2423
4.	C.D	0.5231	0.0509	0.7523	0.5025	0.4193	0.7291	0.5399
5.	C.V (%)	0.87	0.078	1.10	0.74	0.54	0.98	0.71

Note: Values expressed are mean ± standard deviation of triplicates.
Units expressed as mg/ml.
Means within the same column followed by a common letter do not significantly differ at $p \leq 0.05$.
Control: Rice flour + refined wheat flour
CRB: Control + stabilised rice bran
BPP: Beetroot pomace powder incorporated CRB extrudate
CTP: Cauliflower trimmings powder incorporated CRB extrudate
BPP: Beetroot pomace powder
CTP: Cauliflower trimmings powder

The percentage increase in total phenol content of extrudates, powders and stabilized rice bran was shown in Figure 1.

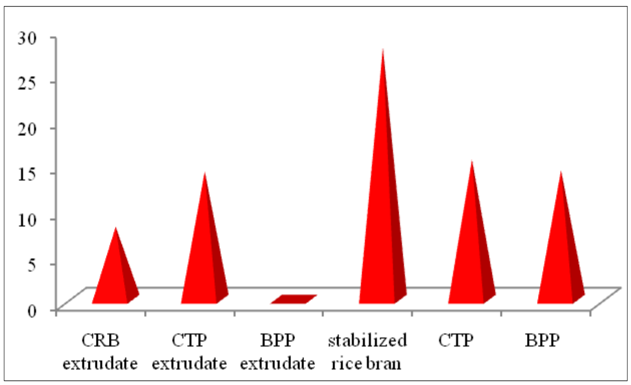

Fig 1: Total phenolic content of extrudates, vegetable waste powders and stabilized rice bran

The highest increase of 27.75% was seen in stabilized rice bran followed by CTP, BPP and extrudates. The BPP extrudates showed a slight decrease in the phenolic content. The CTP incorporated extrudate had higher phenolic content than the stabilized rice bran incorporated extrudate.

Free radical scavenging assay by DPPH method

In the present study, the antioxidant potential of crude methanol extracts was estimated with *in-vitro* antioxidant models in comparison with ascorbic acid, a known antioxidant because of its ability to impair the formation of free radicals in the intracellular substances throughout the body.

The increased consumption of antioxidants from dietary sources contributes to the betterment of life by subsiding the onset and reducing the risk of degenerative diseases due to life style changes and aging. This free radical DPPH method is an easy, stable, rapid and sensitive way to determine the antioxidant potential of plant extracts. The antioxidants present in the methanolic extracts react with DPPH, a known free radical that was converted to 1, 1 – diphenyl – 2 – picryl hydrazine. The degree of discoloration indicates the radical scavenging potential of the antioxidant components or reference antioxidant [20]. The percentage radical scavenging effect and IC_{50} of the methanol extract in various concentrations were summarized in Figure 2.

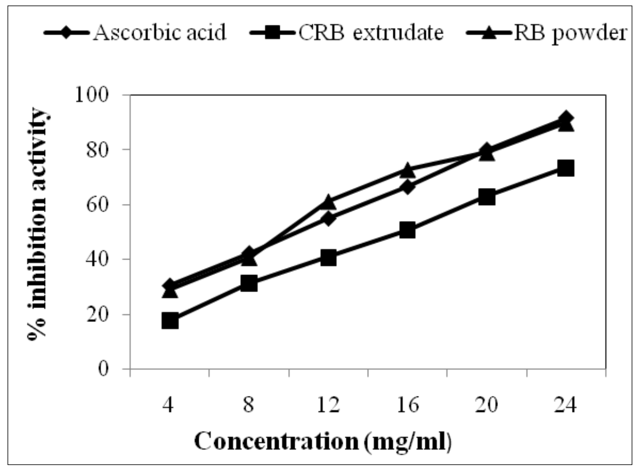

Fig 2a: CRB and it's extrudates

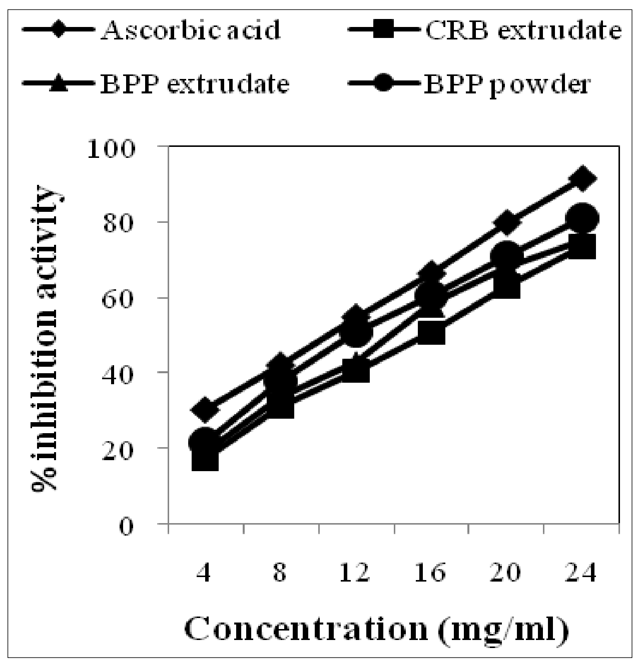

Fig 2b: BPP and it's extrudates

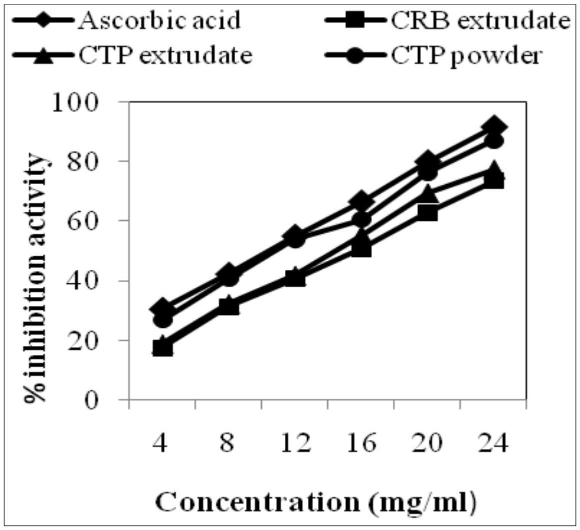

Fig 2c: CTP and it's extrudates

The extrudates incorporated with stabilized rice bran, cauliflower trimmings and beetroot pomace powders showed a dose dependent radical scavenging effect. The most prominent scavenging effect was for stabilized rice bran with 89.97% which was comparable with standard ascorbic acid activity at 91.61%. IC_{50} was obtained by interpreting the linear regression curves from the data obtained at various concentrations. The IC_{50} were 6.94, 7.44 and 9.16 mg/ml for stabilized rice bran, CTP and BPP powders. The extrudates of control, CRB, CTP and BPP had IC_{50} of 13.22, 11.41, 10.53 and 10.49 mg/ml respectively. The increasing order of inhibition was CRB < CTP < BPP < BPP extrudate < CTP extrudate < CRB extrudate and control extrudate as shown in Table 3. It can be concluded that stabilized rice bran had the highest potency in comparison with all other extrudates where as the least was seen for control extrudate.

Table 3: Antioxidant activity of extrudates and prepared samples

S. No.	Parameter	Extrudates				Stabilized rice bran powder	CTP	BPP	Ascorbic acid
		Control	CRB	CTP	BPP				
1.	IC_{50}	13.22 ± 0.02	11.41± 0.06	10.53± 0.14	10.49±0.10	6.94± 0.01	7.44± 0.02	9.16± 0.06	6.58± 0.01
2.	Mean	37.97	46.12	49.28	49.47	62.10	57.499	53.95	60.92
3.	S.E	0.1856	0.1339	0.2683	0.1930	0.0203	0.0211	0.0732	0.0173
4.	C.D	0.4136	0.2985	0.5980	0.4300	0.0453	0.0471	0.1631	0.0385
5.	C.V (%)	0.59	0.35	0.66	0.47	0.040	0.04	0.16	0.035

Note: Values expressed are mean ± standard deviation of triplicates.
Units expressed as mg/ml.
Means within the same column followed by a common letter do not significantly differ at $p \leq 0.05$.
Control: Rice flour + refined wheat flour
CRB: Control + rice bran
BPP: Beetroot pomace powder incorporated CRB extrudate
CTP: Cauliflower trimmings powder incorporated CRB extrudate
BPP: Beetroot pomace powder
CTP: Cauliflower trimmings powder

The free radical scavenging activity was best in methanol extract in comparison with ethanol and aqueous extracts of beetroot pulp waste sample. The IC_{50} of beetroot pulp extracts were 10.24 mg/ml for methanol, 33.64 mg/ml for ethanol and 23.48 mg/ml for aqueous extracts [21].

The rice bran in three different extraction solvents namely methanol, ethanol and ethyl acetate were used to evaluate the antioxidant phyto-compounds. The methanol extract produced a significantly greater yield than ethanol and ethyl acetate. The presence of tocotrienols or synergistic effect of tocopherols and tocotrienols were the reason for strong antioxidant activity [22].

The non-starch polysaccharides of plant origin present in rice bran possess antioxidant properties which can be explored for their potential and undiscovered antioxidant activity. These rice bran polysaccharide fractions provide protection against lipid peroxidation, superoxide and hydroxyl radicals due to high reducing power and by chelating ferrous ions. This provides the scope to use rice bran dietary fibres with high antioxidant activity as an essential ingredient that helps to stabilize fatty foods, which in-turn provide oxidative stability and improves their shelf life [23].

The extracts of beet root pomace in ethanol, acetone and water showed total phenolic content from 316.30 to 564.50 mg GAE (gallic acid equivalent) / g, flavanoid content from 316.30 to 564.50 mg RE (rutin equivalent) / g, betacyanins from 18.78 to 24.18 mg / g and betaxanthins from 11.19 to 22.90 mg / g on dry basis. The DPPH free radical scavenging activity of the extracts has EC_{50} from 0.133 to 0.275 mg / ml. There was significant correlation between phyto – chemical components and scavenging activity [24].

Fresh cauliflower green leaves powder incorporated to pancake, dhokla and idli at 2.0 and 5.0 g of powder per serving had moisture content of 3.4%, protein 21.6%, crude fiber 10.23% and iron 62.0 mg/100 g sample. The high iron content of powder can be used to supplement it in low cost iron rich recipes [25].

Conclusion

The present investigation was undertaken to comparatively assess the antioxidant potential of powders and extrudate extracts. It can be conclude that stabilized rice bran had the highest potency in comparison with the powders and extrudates where as the least was seen for control extrudate without any value addition.

Acknowledgement

The authors thank Professor Jayashankar Telangana State Agricultural University for facilitating the completion of research work.

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