Antioxidant potential of green leafy porridges

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(Index words: total polyphenols; Folin Ciocalteau assay; antioxidant potential; ABTS assay; herbal extracts)

Abstract

Objectives To evaluate the antioxidant potential of porridges (*kola kenda*) made incorporating herbs.

Design Experimental study.

Main outcome measures Total phenolic content was estimated by Folin Ciocalteau method (GAEmg/g(FW) and the antioxidant potential by ABTS free radical scavenging activity (TEAC(µg)/100g (FW).

Results Total phenolics and antioxidant potentials of porridges ranged from 5-73 TEAC(µg) /100g and 0.9-34.2 GAEg/100g respectively. Significantly high ($p \leq 0.05$) total phenolic contents were observed in *C. auriculata* ("Ranawara") [342 GAE mg/g (FW)] and in *M. koenigii* ("Karapincha") [199 GAEmg/g(FW)]. The highest antioxidant effect was in *C. auriculata* ("Ranawara") [73 TEAC(µg)/100g(FW)] followed by *M. koenigi* ("Karapincha") [26 TEAC(µg)/100g (FW)] and *C. ternatea* ("Ela Katarolu") [24 TEAC(µg)/100g (FW)]. Lowest antioxidant effect was in porridges made with rice and coconut milk both of which had comparable values. A positive correlation (correlation coefficient > 0.8) between total phenolics and the antioxidant potential was observed for all porridges except for *Asparagus racemosus* (Haathawariya) porridge which had a high antioxidant potential with a low phenolic content.

Conclusions This study provides evidence that green leafy porridges are a good source of antioxidants in addition to their reported low glycaemic indices.

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Introduction

Complications in diabetes are mainly due to the reactive oxygen species (ROS) which initiate glycation reactions in various tissues [1]. In diabetes there is a significant reduction in antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase in islet cells of the pancreas. Therefore pancreatic β-cells are more sensitive to oxidative stress than other tissues. Glycation mediated ROS production reduces insulin gene transcription and also causes apoptosis of β-cells [2]. Antioxidants contribute to reducing micro and macro vascular complications in diabetes, by scavenging free radicals. As much of the antioxidants can be obtained from the diet, consumption of foods with high antioxidant potential is common among the diabetic population.

Green leafy porridge, a food unique to Sri Lanka is made by incorporating rice (white or red), coconut milk and a herbal leaf extract. It is commonly prescribed by indigenous medical practitioners for individuals with diabetes in Sri Lanka and surveys indicate that the water or ethanolic extracts of many of these leaf varieties have antioxidant effects [3].

The main antioxidants present in plants are polyphenols such as flavonoids, tannins, alkaloids and terpenoids. Flavonoids are polyphenolic antioxidant compounds that have anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities [4,5]. Studies have shown that the anti diabetic effects of flavonoids are due to their ability in either reducing glucose absorption, increasing glucose uptake in peripheral tissues or by increasing key enzymes involved in glucose metabolism. Bio-flavonoids also have an insulin mimetic action and insulin secretion stimulatory action [6].

Some plant tannins stimulate insulin secretion by mimicking insulin action and inhibiting α-amylase and α-glucosidase activities [7]. Terpenoids in some plants can modulate activities of ligand-dependent transcription factors [i.e. peroxisome proliferator-activated receptors (PPARs) which are dietary lipid sensors involved in energy homeostasis] and prevent obesity-induced metabolic disorders like type 2 diabetes, hyperlipidemia, insulin resistance, and cardiovascular diseases [8].

Scientific data are scarce regarding the health benefits of consumption of porridges made with herbal leaf extracts. The aim of the present study was to determine the total phenolic content and the antioxidant potential of porridges made by incorporating these extracts.

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Materials and Methods

Materials: A bulk (10 kg) sample of 272 6B red raw rice was obtained from the Department of Agriculture Regional Rice Research and Development Center, Bombuwala, Sri Lanka. The fresh herbal leaves of *Murraya koenigii spreng* ("Karapincha"), *Asparagus racemosus* ("Haathawariya"), *Hemidesmus indicus* ("Iramusu"), *Aegle marmelos* ("Beli"), *Cassia auriculata Linn* ("Ranawara"), *Cardiospermum halicacabum* ("Wel Penela"), *Aerva lanata* ("Polpala"), *Clitoria ternatea Linn*. ("Ela katarolu"), *Scoparia dulcis* ("Wal koththamalli"), *Atlantia zeylanica Linn*. ("Yaki narang"), *Osbeckia octandra* ("Heen bovitiya"), *Cephalandra indica* ("Kowakka") were obtained from the market, Nugegoda, Sri Lanka. Coconuts were purchased from a retail shop in Galle, Sri Lanka.

Porridge preparation: The porridge recipes were standardized following a sensory evaluation by a non trained panel (n=10). When preparing the porridge, coconut milk was obtained from 150g of coconut kernel. Green leaves (40 g) were blended with coconut milk (150 ml) and water (100 ml). The slurry was filtered through a mesh (1mm) and filtrate was obtained (leaf extract).

Rice (25g) was cooked with 250 ml of water and above leaf extract (90 ml) and water (60 ml) were added. All porridges were cooked until the final volume was approximately 300ml (in final porridge leaves: coconut milk: rice = 13:54:25). Coconut milk porridge was made with only rice and coconut milk in 25:54 (w/v) ratio. Rice porridge was prepared with rice and water (25:54) (w/v). Porridges, made according to the above method were blended (Philips, HR-2001, China) and lyophilized for analysis of total polyphenol and antioxidant potential.

Sample preparation: Lyophilized sample (0.5g) was mixed with phosphate saline buffer (pH 7.4: 10 ml) and homogenized. Mixture was filtered (Whatman No 1) and the filtrate was taken to assess the total phenolics and antioxidant potential.

Total polyphenols [9]***:*** Folin Ciocalteau reagent was diluted with deionized water in 1:1 ratio. The above sample filtrate (0.1 ml) was mixed with deionized water (2 ml) and diluted Folin Ciocalteau solution (0.2 ml) and incubated at room temperature for 10 minutes. Na_2CO_3 (1ml) was added and incubated in the dark for 2 hours and absorbance was measured at 765 nm. Results were expressed as Gallic acid equivalents mg/L/10 mg sample (GAE mg/L/10 mg sample).

Antioxidant Potential with ABTS Assay: Free radical scavenging activity was assessed by ABTS cation free radical discoloration assay [10]. ABTS (10 mg) was dissolved in ethanol (200 µl) and phosphate saline buffer (2.6 mL; pH 7.4). The ABTS solution was kept in dark for 12-16 hours for the development of free radicals after adding $K_2S_2O_8$ (0.007M, 2.6 ml) and was diluted with phosphate saline buffer to obtain an absorbance of 0.700±0.02 at 734 nm against a buffer blank. The diluted ABTS solution (2.9 ml), phosphate saline buffer (85µl) and sample filtrate (15 µl) were mixed in a cuvette (3 ml) and the reduction in absorbance measured every 30 seconds up to 6 minutes at 734 nm. Antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC) (Trolox Eq.µ mol/g) and Vitamin C equivalent antioxidant capacity (Vit C Eq.µg/10mg).

Statistical Analysis: The results were expressed as means of six replicates and the significance was analysed using Student T test. Analyses were done using Microsoft Excel 2007.

Results

Table 1 shows the antioxidant potential of green leafy porridges in TEAC µg/100g and Vit C Eq µg/100 g and total phenoilc content in GAE g/ 100 g (FW).

Table 1. Antioxidant potential (TEAC (µg)/100 g, Vit C Eq (µg)/100 g) and total phenols in porridges (FW = fresh weight)

Porridge	*Total phenolic content*		*Antioxidant activity*	
	GAE mg/ g (FW)	*GAE g/ 100g (FW)*	*TEAC(µg)/100g sample (FW)*	*Ascorbic acid Eq(µg)/ 100g (FW)*
Coconut milk porridge (pol kiri kenda)	9[a]	0.9	5[a]	13
Rice porridge (lunu kenda)	9[a]	0.9	5[a]	14
Murraya Koenigii spreng (Karapincha)	199[e]	19.9	26[d]	86
Hemidesmus indicus (Iramusu)	44[b]	4.4	13[c]	40
Aegle marmelos (Beli)	157[f]	15.7	6[a]	18
Cassia auriculata Linn. (Ranawara)	342[h]	34.2	73[e]	239
Clitoria ternatea Linn. (Ela katarolu)	100[g]	10.0	24[d]	77

Continued

Porridge	Total phenolic content		Antioxidant activity	
	GAE mg/ g (FW)	GAE g/ 100g (FW)	TEAC(µg)/100g sample (FW)	Ascorbic acid Eq(µg)/ 100g (FW)
Cardiospermum halicacabum (Wel penela)	47[b]	4.7	8[b]	24
Atlantia zeylanica Linn. (Yaki narang)	45[b]	4.5	11b[c]	33
Cephalandra indica (Kowakka)	45[b]	4.5	12[c]	36
Osbeckia octandra (Heen bovitiya)	41[b]	4.1	9[b]	27
Aerva lanata (Polpala)	19[c]	1.9	5[a]	14
Asparagus racemosus (Haathawaariya)	23[c]	2.3	12[c]	37
Scoparia dulcis (Wal koththamalli)	64[d]	6.4	12[c]	36

n=6; Values are expressed as an average of 6 determinations. Same superscript along a column indicates no significant difference ($p \leq 0.05$).

In all porridges the total phenolic and antioxidant potential were in the ranges of 5 - 73 TEAC (µg)/ 100 g and 0.9 - 34.2 GAE g/ 100 g respectively. Total phenolic content were significantly high ($p \leq 0.05$) in *C. auriculata* and in *M. koenigii* compared to other porridges. The highest antioxidant effect was in *Cassia auriculata* followed by *Murraya koenigi* and *C. ternatea* where the values correlate with the polyphenol content. Lowest antioxidant effect was observed in porridges made with rice and coconut milk which had comparable values.

A positive correlation (correlation coefficient > 0.8) (Figure 1) was observed between the total phenolics and the antioxidant potentials of the herbal leafy porridges. Thus proving that, at least in majority of the porridges, the total phenolics were responsible for the antioxidant effect. Exception to above was observed in *Asparagus racemosus* porridge which had a high antioxidant potential with a low phenolic content. This indicates the presence of compounds in porridges other than polyphenols which are cpable of scavenging free radicals.

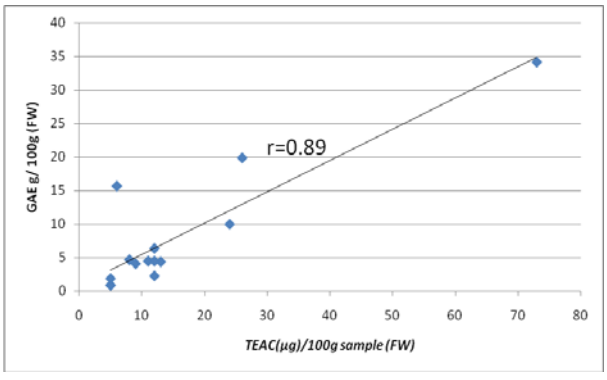

Figure 1. **Correlation between total phenolics and antioxidant potential.**

Discussion

Among the main antioxidants present in plants polyphenols, terpenoids, organic sulphur compounds and long chain alkylphenol derivatives are predominant. Antioxidants reduce oxidative damage to tissues by reacting with free radicals, chelating catalytic pro-oxidant metals and by scavenging ROS [11]. In addition to the antioxidant effects, these phytochemicals also have other functions which contribute to attenuate complications related to diabetes.

In the present study, total phenolic content in porridge made with *Cassia* (342 GAE mg/g) exceeds the phenolics in green or black tea and is comparable to the amount present in *Vitex negundo* L. (Verbenaceae) (363 GAE mg /g), an aromatic shrub used in reducing rheumatic pain and swelling of the joints due to its antioxidant effect [12]. The highest antioxidant activity was also observed in *Cassia* which has proven antioxidants like kaempferol-3-*O*-rutinoside, kaempferol, quercetin and luteolin.

Literature shows that *Tridax procumbens* has beneficial effects on liver antioxidant defense system. However, according to the present study *Murraya* has higher total phenolic content than *Tridax procumbens* (12 GAE mg/g) [13] and the total phenolic content in *Murraya* (19.9 GAE g/100 g) was in the range of the polyphenols in green tea (mean 17.67 GAE g/ 100 g) and black tea (mean 13.02 GAE g/100 g) [14]. The high antioxidant potential elicited by *M. koenigii* in the present study may be due to the phenolic compounds such as mahanimbine, murrayanol, and mahanine [15]. The leaves of *C. ternatea* are reported to contain polyphenolic flavonoids, flavonols (kaempherols, quercetin and myricetin), anthocyanin glycosides, pentacyclic triterpenoids and phytosterols [16]. Therefore the antioxidant potential elicited by *C. ternatea* is significantly higher ($p \leq 0.05$) than all the porridges except for porridges made with *Murraya* and *Cassia*.

Although the total phenolic content is comparatively low, a high antioxidant potential was observed in *A. racemosus*. The antioxidant properties of *A. racemosus* are due to racemofuran, asparagamine *A. racemosol,* asparagamine A (a polycyclic alkaloid with antitumor activity), steroidal saponins (shatavaroside A and B, filiasparoside C, shatavarins VI-X, shatavarin I, IV, V, immunoside and schidigerasaponin D5 and an isoflavone - 8-methoxy-5,6,4'-trihydroxyisoflavone 7-O-beta-D-glucopyranoside [17].

Total phenolic content in *H. indicus*, *C. indica* and *S. dulcis* were 44, 45 and 64 GAE/mg/g (FW) respectively. The antioxidant potentials also varied as 36, 36 and 40 ascorbic acid Eq (µg)/100g (FW) respectively. The well known phenolic compounds such as glycosides, phyto-sterols, saponins, steroidal sapogenins, triterpenoids and tannins in *H. indicus* and flavonoids and diterpenoids in *Scoparia dulcis* which also have insulin stimulatory action on beta cells of pancreas and insulin mimetic action and steroids, saponins, ellagic acid, lignin's, triterpenoids, alkaloids, tannins, flavonoids, glycosides, phenols [20] in *C. indica* might have contributed to the antioxidant potential of these porridges [17-19].

A high phenolic content and a low antioxidant potential were observed for *Aegle* in the present study [15.7 GAE g/100 g and 18 Ascorbic acid Eq (µg)/100 g (FW) respectively]. This is despite the phenolic content of *Aegle* being in the range of green and black tea. The many phenolics in *Aegle* include aegeline, agelinine, rutin, sterol, sitosterol, D-glucoside, marmesinine, lupeol, tannins, phlobatannins, flavonoids, umbelliferone and quercetin. Observed low antioxidant activity could be due to the absence of antioxidant effect in some phenolic compounds or interaction of these with other molecules in the porridge.

A low antioxidant potential as well as a low phenolic content was elicited by *A. lanata* porridge despite having phenolic compounds such as alkaloids, flavonoids (kaempferol, quercetin, isorhamnetin, persinol, persinisides A and B), methyl grevillate, lupeol, lupeol acetate benzoic acid, beta sitosteryl acetate and tannic acid. It is speculated that this could be due to the low extraction of these substances in to the leaf extract when preparing the porridge.

The leaves of *C. halicacabum* also contain a proven antioxidant rutin which animal studies have shown to increase colonic glutathione levels, free radical-scavenging and iron chelating activity while a species belonging to *Osbeckia* had antioxidant compounds such as flavonoids, hydrolyzable tannins, osbeckic acid, a furan-carboxylic acid which also can be present in *O. octandra* due to the similar therapeutic effects of these two plants [3,20]. However, compared to the obtained total phenolic contents in the present study, *C. halicacabum* and *O. octandra* elicited lower antioxidant potentials. These observations could be due to the absence of antioxidant effect in some phenolics of the above leaf varieties.

The lowest total phenolics as well as the antioxidant potentials were observed in rice porridge and coconut milk porridge. This indicates that the main contributor to antioxidant potential in these porridges is the extracts of herbal leaves.

According to the present study, it can be presumed that the total phenolic content in most of the porridges is adequate for beneficial effects against oxidative stress in tissues, although the amount of leaf extracts that is being incorporated into the porridges is inadequate. Due to the presence of antioxidants, phyto-nutrients and reported low glycaemic indices, these porridges could be recommended as good dietary remedies for people with diabetes [21].

In conclusion, porridges made incorporating herbal extracts are a good source of antioxidants due to the presence of phenolic and other free radical scavenging compounds. The study also proves that the preparation method of porridges is not detrimental to the antioxidant compounds.

References

1. Tajiri Y, Moller C, Grill V. Long term effects of amino-guanidine on insulin release and biosynthesis: evidence that the formation of advanced glycosylation end products inhibits B cell function. *Endocrinology* 1997; **138**: 273-80.
2. Kaneto H, Fujii J, Myint T, *et al*. Reducing sugars trigger oxidative modification and apoptosis in pancreatic b-cells by provoking oxidative stress through the glycation reaction. *Biochemical Journal* 1996; **320**: 855-63.
3. Babu KCV, Krishnakumari S. Anti-inflammatory and antioxidant compound, rutin incardiospermum halicacabum leaves. *Ancient Science of Life* 2005; **25**: 7-49.
4. Yamamoto, Gaynor. Therapeutic potential of inhibition of the NF-kB pathway in the treatment of inflammation and cancer. *Journal of Clinical Investigation* 2001; **107**: 135.
5. de Sousa RR, Queiroz KC, Souza AC, *et al*. "Phosphoprotein levels, MAPK activities and NFkappaB expression are affected by fisetin". *Journal of Enzyme Inhibition and Medicinal Chemistry* 2007; **22**: 439-44.
6. Brahmachari G. Bio-flavonoids with promising antidiabetic potentials: A critical survey opportunity. *Challenge and Scope of Natural Products in Medicinal Chemistry* 2011; 187-212.
7. Kunyanga CN, Imungi JK, Okoth M, *et al*. Antioxidant and Antidiabetic Properties of Condensed Tannins in Acetonic Extract of Selected Raw and Processed Indigenous Food Ingredients from Kenya. *Journal of Food Science* 2011; **76**: C560-7.
8. Goto T, Takahashi N, Hirai S, Kawada T. Various Terpenoids Derived from Herbal and Dietary Plants Function as PPAR Modulators and Regulate Carbohydrate and Lipid Metabolism. *PPAR Research* 2010: 9.
9. Waterhouse AL. Determination of total phenolics. Handbook of food analytical chemistry, Unit I 1.1: *Polyphenolics* 2001: 464-5.

10. Shahidi F, Wanasundara PKJPD. Phenolic antioxidant. *Critical Reviews in Food Science and Nutrition* 1992; **32**: 67-103.

11. Zargar M, Azizah AH, Roheeyati AM, Fatimah AB, *et al*. *African Journal of Pharmacy and Pharmacology* 2010; **4**: 123-6.

12. Habila JD, Bello IA, Dzikwi AA, *et al*. Total phenolics and antioxidant activity of Tridax procumbens Linn. *African Journal of Pharmacy and Pharmacology* 2010; **4**: 123-6.

13. Anesini C, Ferraro GE, Fillip R. Total Polyphenol Content and Antioxidant Capacity of Commercially Available Tea (*Camellia sinensis*) in Argentina. *Journal of Agricultural and Food Chemistry* 2008; **56**: 9225-9.

14. Nayak A, Mandal S, Banerji A, Banerji J. Review on chemistry and pharmacology of Murraya koenigii Spreng (Rutaceae). *Journal of Chemical and Pharmaceutical Research* 2010; **2**: 286-99.

15. Mukherjee PK, Kumar V, Houghton PJ. Screening of Indian medicinal plants for acetyl cholinesterase inhibitory activity. *Phytotherapy Research* 2007; **21**: 1142-5.

16. Saxena VK, Chourasia S. A new isoflavone from the roots of Asparagus racemosus. *Fitoterapi* 2001; **72**: 307-9.

17. Murali A, Ashok P, Madhavan V. Antioxidant activity of leaf of Hemidesmus indicus (L.) R. Br. var.pubescens (W. A.) Hk.f. (Periplocaceae)- an in vivo analysis. *Spatula DD* 2011; **1**: 91-100.

18. Latha M, Pari L, Sitasawad S, Bhonde R. Insulin-secretagogue activity and cytoprotective role of the traditional antidiabetic plant *Scoparia dulcis* (Sweet Broomweed). *Life Sciences* 2004; **75**: 2003-14.

19. Rastogi RP, Mehrotra BN. *Compendium of Indian Medicinal Plants* 1998; **1**: 115.

20. Su JD, Osawa T, Kawakishi S, Namiki M. A novel antioxidative synergist isolated from *Osbeckia chinensis* L. *Agricultural and Biological Chemistry* 1987; **51**: 3449-50.

21. Senadheera SPAS, Ekanayake S. Green leafy porridges: How good are they in controlling glycaemic response?, *International Journal of Food Science and Nutrition* 2012. [Internet document] DOI: 10.3109/09637486.2012.710895. Accessed 14/10/2012.

Validity of the Sinhala version of the Centre for Epidemiological Studies Depression Scale (CES-D) in out-patients

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(Index words: depressive disorder, validation studies, CES-D, Sri Lanka)

Abstract

Objectives To translate and validate the Sinhala version of the Centre for Epidemiological Studies Depression scale (CES-D) for diagnosing depression in out-patients.

Design A combined qualitative and quantitative approach was used for the translation of the CES-D. Sample size was calculated to detect a targeted sensitivity and specificity of 85%. The sample consisted of 75 participants diagnosed with major depressive disorder according to DSM IV criteria and 75 gender matched controls. Criterion validity was assessed using receiver operating charact-eristic (ROC) analysis. The Structured Clinical Interview for DSM-IV (SCID-II) conducted by a psychiatrist was used as the gold standard.

Results Mean age of the sample was 33 years. There were 91 females (60.7%). There was significant difference in the mean CES-D scores between cases (13.94) and controls (6.58) [t=14.50, df=148, $p<0.001$]. A score of ≥16 gave a sensitivity of 84% and specificity of 92%. A score of ≥21 gave a sensitivity of 73.3% and specificity of 96%. The Cronbach's alpha was 0.93. The four items that were reverse coded had poor correlation with total scores. The average correlation coefficient for the reverse-scored items was 0.35 and for the rest of the items 0.63. Principal component analysis with oblique rotation identified four factors. Factor 1 corresponds to the "depressed affect" and "somatic complaints" in the original model proposed by Radloff. Factor 2 corresponds to the interpersonal concerns. Factors 3 and 4 loaded the reversed coded items.

Conclusions The Sinhala version of the CES-D is a valid and reliable instrument for diagnosing major depressive disorder.

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