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Phenolic content and antioxidant activity of selected Ugandan traditional medicinal foods

ANDABATI Brain and MUYONGA John*

School of Food Technology, Nutrition and Bioengineering, Makerere University, P.O. Box 7062, Kampala, Uganda.

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Twenty one (21) traditional food plants recognized as medicinal by communities in Kamuli and Gulu districts in Uganda were identified and analyzed for their phytochemical content and antioxidant activity. The total phenolic content (2.6 ± 0.1 to 184.2 ± 6.4 mg GAE gDW $^{-1}$) and flavonoid content (0.3 ± 0.1 to 162.2 ± 3.5 mg CE gDW $^{-1}$) as well as antioxidant activity (0.1 ± 0.1 to 57.8 ± 0.5 mg VCE gDW $^{-1}$) varied widely among the food plants. The seeds of *Tamarindus indica* L. and leaves of *Ipomea eriocarpa* R.Br., *Corchorus trilocularis* L., *Ocimum suave* L., *Corchorus olitorius* L., *Acalypha bipartita* Müll.Arg., and *Hibiscus acetosella* Welw. ex Hiern showed the highest phenolic contents and antioxidant activities. Juice prepared from tamarind pulp alone or mixed with tamarind seed powder (0.5, 1 and 3%) and analyzed for sensory acceptability, phenolic content and anti-oxidant activity were found to be highly acceptable, recording an overall hedonic acceptability scores of 7.40 ± 0.4 , 6.65 ± 0.3 , 7.01 ± 0.1 and 5.34 ± 0.2 , respectively on a nine-point scale for juice containing 0, 0.5, 1 and 3% seed powder. Incorporation of tamarind seed powder into tamarind pulp juice resulted in a dose dependent increase in antioxidant activity from 8.5 ± 0.2 mg VCE 100 ml $^{-1}$ for the control to 12.05 ± 0.3 , 15.33 ± 0.7 and 17.22 ± 0.7 mg VCE 100 ml $^{-1}$ for tamarind juice containing 0.5, 1 and 3% seed powder, respectively. A similar trend was recorded for the total phenolic and flavonoids.

Key words: Medicinal foods, nutraceuticals, antioxidant activity, phenolics, flavonoids.

INTRODUCTION

The consumption of significant amounts of certain particular foods, especially those of plant origin, including fruits, vegetables and whole grain cereals is associated with reduced risk of developing chronic degenerative diseases, such as cardiovascular diseases, cancers and diabetes mellitus (Espín et al., 2007). The health benefits of consuming such foods are ascribed partly to bioactive compounds found in the foods. For example, lycopene in

tomatoes may protect against prostate cancers (Kucuk et al., 2002) and carotenoids in the various fruits and vegetables may also reduce the risk of prostate cancer (Jian et al., 2005) while flavonoids in tea act against cardiovascular diseases (Kris-Etherton and Keen, 2002). The phenolic and polyphenolic compounds comprise of important group of health promoting bioactive compounds, their protective effects are mainly attributed to

*Corresponding author. E-mail: hmuyonga@yahoo.com

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Figure 1. Map of Uganda showing the geographic location of Gulu and Kamuli districts, where a study was conducted to identify foods traditionally considered having medicinal properties. Source: http://en.wikipedia.org/wiki/Gulu_District; http://en.wikipedia.org/wiki/Kamuli_District.

their antioxidant activity (Shahidi, 1997).

Several traditional foods from different communities have been analyzed for presence of relevant bioactive compounds and biological activities (Buřičová and Réblová, 2008; D'Antuono et al., 2010; Heinrich et al., 2005, 2006) and such investigations have provided scientific evidence and validation for health claims of the different traditions. In Uganda, many foods are traditionally believed to provide medicinal benefits (Rubaihayo, 2002; Tabuti and Van Damme, 2012). However, there is inadequate scientific evidence for the ascribed medicinal properties of these traditional foods. Thus, there is need to validate the nutraceutical properties of traditional foods, given the rapidly increasing burden of chronic degenerative diseases in the country. It is anticipated that non-contagious chronic diseases will overtake infectious, maternal and nutritional diseases as the most common cause of death in Uganda by the year 2030 (WHO, 2011a, b).

In view of the above, this study was undertaken to analyze the phenolic content and antioxidant activity of selected traditional foods considered by local communities in Uganda to have health benefits. In addition, the potential of using certain traditional food additives in enhancing the natural antioxidant activity of foods was evaluated.

MATERIALS AND METHODS

Study area

A study to identify foods traditionally considered to have medicinal properties was conducted among the rural communities in Gulu and Kamuli districts, Uganda. Geographically, Gulu district is situated in the northern part of Uganda ($02^{\circ} 45'N$, $32^{\circ} 00'E$) (Figure 1), and is home to the ethnic Acholi tribe who are estimated to be approximately 1.1 million people, representing 4.8% of the population of Uganda (UBS, 2002). Kamuli district on the other hand, is located in the eastern part of Uganda ($00^{\circ} 55'N$, $33^{\circ} 06'E$), with the Basoga tribe as the main ethnic group. The Basoga are the third largest ethnic group in Uganda with population of 2.1 million people, representing about 8.6% of Uganda's population (UBS, 2002). The communities found in these two districts are mainly of rural setting (Lamordea et al., 2010), and consume rich diversity of foods originating from local plant or animal sources through gathering or harvesting (Oryema et al., 2013; Tabuti, 2007).

Data collection

A survey was conducted to identify foods considered to have medicinal value in Gulu and Kamuli districts in Uganda. Ethnobotanical information was obtained from informants through key informant interviews and focus group discussions. A total of 51 informants (27 in Gulu district and 24 in Kamuli district), consisting of traditional healers, health workers and elderly persons who are natives of the area were involved. Semi-structured questionnaires were used to collect information on local food plants considered to

have medicinal value. Samples of identified foods reported to have medicinal value were collected and transported to the herbarium at Makerere University for identification.

Determination of the phenolic content and antioxidant activity of the identified foods

Plant material

The food plant materials collected were analyzed for total phenolic and flavonoid contents including total antioxidant activity.

Sample preparation

Different parts of the food plant (leaves and seeds) were cleaned, dried in shade at ambient temperature of about 25°C, were ground into fine powder using a stainless steel blender 7011S (Waring Commercial, Torrington, CT, USA) and sieved through a 500 µm sieve. The fine powder obtained was stored in airtight plastic containers at 4°C until further analysis, within 30 days.

Extraction of phenolic compounds

The extraction of phenolic compounds was done according to the method described by Makkar (2000) with minor modifications. Briefly, a small quantity (0.1 g) of the powdered sample was extracted in 5 ml of solvent mixture methanol : water (50:50 v/v) for 30 min at room temperature, with intermittent shaking. The extract was cooled in a refrigerator at 4°C for 10 min and then centrifuged at 3000 xg for 10 min using a centrifuge (Fisher Scientific 225, Pittsburgh, PA, USA). The supernatant was recovered and the pellet re-extracted for 45 min under the same conditions until a second supernatant was obtained. Finally, the two supernatants were pooled together and used in the determination of total phenolic and flavonoid contents and total antioxidant activity.

Determination of total phenolic content

The total phenolic content (TPC) was determined using spectrophotometry following Makkar (2000) method. To 100 µl of sample, 400 µl of distilled water was added to make the total volume to 0.5 ml. This was followed by addition of 0.25 ml of 1 N Folin-Ciocalteu reagent and 1.25 ml of 20% sodium carbonate solution. After 40 min incubation at room temperature, absorbance was read at 725 nm on a GENESYS spectrophotometer 10UV (Thermo Electron Corporation, Madison, WI, USA) against a blank solution containing methanol instead of the sample. A calibration curve was constructed within the concentration range 0.025 - 0.225 mg ml⁻¹ ($R^2 = 0.999$). Mean values were calculated from three parallel analyses. The total phenolic contents were expressed in milligram gallic acid equivalents per gram of dry weight (mg GAE gDW⁻¹) of plant material.

Determination of total flavonoid content

The total flavonoid content (TFC) was measured using the colorimetric assay procedure reported by Muanda et al. (2011). Briefly, a 250 µl of a standard solution of catechin at different concentrations was added to 10 ml volumetric flask containing 1 ml of double distilled water (ddH₂O). At zero min, 75 µl of 5% NaNO₂ solution was added to the flask. After 5 min, 75 µl of 10% AlCl₃ solution was added. After 6 min, 500 µl solution of 1 N NaOH was added to the mixture. Immediately after diluting the solution by addi-

tion of 2.5 ml ddH₂O and thoroughly mixing, the absorbance of the pink solution mixture was measured at 510 nm in a GENESYS spectrophotometer 10UV (Thermo Electron Corporation, Madison, WI, USA) against a blank solution containing distilled water instead of sample. A calibration curve was constructed within the concentration range 0.025 - 0.225 mg ml⁻¹ ($R^2 = 0.999$). Mean values were calculated from three parallel analyses. The total flavonoid contents of the samples were expressed as milligram catechin equivalents per gram dry weight (mg CE gDW⁻¹) of plant material.

Determination of total antioxidant activity

The total antioxidant activity (TAC) of the methanol extracts was estimated using the 1, 1-diphenyl-2-pycrylhydrazyl (DPPH) free radical scavenging assay (Kim et al., 2002). Briefly, to a 2.95 ml solution of freshly prepared absolute methanol, 100 µM solution of DPPH, 50 µl of the sample extract or control (50% [v/v] methanol) were added.

The mixture was shaken and allowed to stand in the dark at room temperature for 30 min. The absorbance of the resulting solution was measured at 517 nm in a GENESYS spectrophotometer 10UV (Thermo Electron Corporation, Madison, WI, USA) against a blank solution of absolute methanol. The free radical scavenging activity was calculated as:

$$\text{Scavenging activity (\%)} = \left[1 - \left(\frac{\text{absorbance of sample}}{\text{absorbance of control}} \right) \right] \times 100$$

A standard solution of ascorbic acid was run using several concentrations ranging from 0.002 to 0.1 mg ml⁻¹. A standard calibration curve of ascorbic acid was then prepared by plotting the percentage of free radical scavenging activity against concentration ($R^2 = 0.999$). The final value was expressed as milligram ascorbic acid equivalents per gram dry weight (mg VCE gDW⁻¹).

Production of juice enriched with *Tamarindus indica* L. seed powder

Preparation of tamarind seed powder (TSP)

Fruits of *T. indica* L. were purchased from a local market and transported to the Food Chemistry laboratory at Makerere University. The tamarind fruits were soaked in cold water (seed to water ratio of 1:2 (w/v) and the seeds removed manually from the pulp. The seeds were thereafter autoclaved at 121°C for 30 min to remove astringency (Legesse and Emire, 2012). The seeds were dried under shade at ambient temperature of about 25°C, milled into fine flour using the WonderMill grain mill (Grote Molen Inc, Pocatello, ID, USA), sieved through a 200 µm sieve size and stored in airtight plastic containers at 4°C until further use.

Preparation of mixture of tamarind pulp and seed powder juice

Tamarind pulp juice was prepared by first dissolving 295 g of tamarind fruit into one litre of cold water while stirring for about 30 min and discarding all the seeds and fibres. The resulting pulp juice (about 900 ml) was filtered through cheese cloth to obtain a volume of 600 ml of juice. The volume of juice was then diluted with an equal volume of water and 120 g of sugar added to sweeten the taste. Thereafter, the sweetened juice was further divided into four equal portions of 300 ml each. A preparation of tamarind seed powder (TSP) was added to levels of 0.5, 1 and 3% (w/v) to each of the respective first, second and third portions of the 300 ml of the sweetened juice. All the three portions were heated to boiling point to facilitate extraction of phenolic compounds from the seed powder

and the mixture was filtered through cheese cloth to obtain the final tamarind pulp and seed powder juice. The remaining fourth 300 ml portion of the sweetened juice containing no tamarind seed powder (0% TSP) was used as a control.

Sensory evaluation of the tamarind pulp and seed powder juice

The prepared tamarind pulp and seed juice was evaluated by a panel of 30 randomly selected judges consisting of students of the Department of Food Technology and Nutrition, Makerere University Kampala. The sensory attributes evaluated included color, taste, odor, consistency and general acceptability. A nine-point hedonic scale (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely) was used (Larmond, 1977). The sensory assessors were presented with 30 ml of each sample at room temperature under normal lighting conditions. Water was provided to the assessors for mouth or palate cleansing between each sample.

Analysis of phytochemicals in the prepared tamarind pulp and seed powder juice

Determination of total phenolic content

The phenolic content in the tamarind pulp and seed juice was estimated by Folin-Ciocalteu method as described by Thimmaiah (1999). Briefly, an aliquot (0.5 ml) of each juice was mixed with 2.5 ml of distilled water. To this, 0.5 ml of 1.0 N Folin-Ciocalteu reagent was added and incubated for 3 min. To each of the tube, 2 ml of 20% sodium carbonate solution was added and the tubes incubated in a boiling water bath for 1 min. The test tubes were cooled and the absorbance of each reaction mixture was read at 650 nm in a GENESYS spectrophotometer 10UV (Thermo Electron Corporation, Madison, WI, USA). A standard calibration curve of different concentrations of gallic acid ($0.025 - 0.2 \text{ mg ml}^{-1}$) against 100 ml of juice were plotted ($R^2 = 0.999$). The total phenolic content was expressed in milligram gallic acid equivalents per 100 ml of juice (mg GAE 100 ml^{-1}).

Determination of total flavonoid content

The total flavonoid content was determined using the method of Zhishen et al. (1999). In brief, 0.5 ml of standard solution of catechin or the sample diluted (1:2 with water) was mixed with 2 ml of deionized water and with 0.15 ml of 5% sodium nitrite. After incubation for 5 min at room temperature, 0.15 ml of 10% aluminum chloride was added, and after another 6 min, 1 ml of 1M sodium hydroxide solution was added. The total volume of the solution was adjusted to 5 ml with deionized and the absorbance was read at 510 nm in a GENESYS spectrophotometer 10UV (Thermo Electron Corporation, Madison, WI, USA). A standard calibration curve of different concentrations of catechin ($0.025-0.2 \text{ mg ml}^{-1}$) versus 100 ml of juice was plotted ($R^2 = 0.999$). Total flavonoid content was expressed in milligram catechin equivalents per 100 ml of juice (mg CE 100 ml^{-1}).

Determination of total antioxidant activity

The antioxidant activity of the prepared juice sample was estimated using the 1,1-diphenyl-2-pycrylhydrazyl (DPPH) free radical scavenging assay as described by Shimada et al. (1992). In brief, to 2.9 ml of freshly prepared 100 μM of DPPH in 80% ethanol solution, 0.5 ml of the juice sample or control solution (DPPH alone)

was added. The mixture was shaken and allowed to stand in the dark at room temperature for 30 min. The absorbance of the resulting solution was measured at 515 nm using a GENESYS UV-vis spectrophotometer 10UV (Thermo Electron Corporation, USA) against a blank solution containing only 80% ethanol. The free radical scavenging activity was calculated as:

$$\text{Scavenging activity (\%)} = [1 - \left(\frac{\text{absorbance of sample}}{\text{absorbance of control}} \right)] \times 100$$

A standard solution of ascorbic acid or vitamin C was similarly run using different concentrations ranging from 0.002 to 0.1 mg ml^{-1} . A calibration curve of the percentage free radical scavenging activity of ascorbic acid versus its concentration per 100 ml of juice was then prepared ($R^2 = 0.999$). The final result was expressed as milligram vitamin C equivalents per 100 ml of juice (mg VCE 100 ml^{-1}).

Statistical analysis

Data from three independent experiments were subjected to analysis of variance (ANOVA) at $\alpha = 0.05$ and means were separated using Turkey's post hoc test. Regression analysis was performed to indicate the relationship between total phenolic or flavonoid contents and antioxidant activity. Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS), version 16 (SPSS Inc, Chicago, IL, USA).

RESULTS AND DISCUSSIONS

Phytochemical content and antioxidant activity of food materials

Total phenolic content

The total content of phenolics of the different foods ranged from 2.6 to 184.2 mg GAE g DW^{-1} and was highest for *T. indica* L. seeds and lowest for *Amaranthus hypochondriacus* L. (Table 1). Based on the classification of Lai and Lim (2011), samples with $\text{TPC} > 20 \text{ mg GAE g DW}^{-1}$ are classified as very high, those with $\text{TPC} 10-20 \text{ mg GAE g DW}^{-1}$ are considered high, those with $\text{TPC} 5-10 \text{ mg GAE g DW}^{-1}$ are moderate and samples with $\text{TPC} < 5 \text{ mg GAE g DW}^{-1}$ are considered low. All the foods regarded in the communities studied as having medicinal value showed very high TPCs ($> 20 \text{ mg GAE g DW}^{-1}$), except *Hyptis spicigera* Lam. which showed a high TPC ($18.5 \pm 0.2 \text{ mg GAE g DW}^{-1}$) and foods of the Amaranthaceae family (*A. hypochondriacus* L. and *Amaranthus cruentus* L.) with low TPCs of 2.6 ± 0.1 and $2.9 \pm 0.2 \text{ mg GAE g DW}^{-1}$ respectively (Table 1).

Total flavonoid content

The result in Table 1 also showed variation in the total content of flavonoids from 0.3 to 162.2 mg CE g DW^{-1} . Total flavonoid content was highest for *T. indica* L. seeds ($184.2 \pm 0.2 \text{ mg CE g DW}^{-1}$) and lowest for *A. hypochondriacus* L. ($2.6 \pm 0.1 \text{ mg CE g DW}^{-1}$). Flavonoids

Table 1. Total phenolic content, total flavonoid content and total antioxidant activity of selected traditional medicinal foods identified in Kamuli and Gulu districts.

Plant scientific name	Local names	Plant parts analyzed	TPC (mg GAE gDW ⁻¹)	TFC (mg CE gDW ⁻¹)	TAC (mg VCE gDW ⁻¹)
<i>Tamarindus indica</i> L.	Enkooge	Seeds	184.2 ± 6.4 ^a	162.2 ± 3.5 ^a	57.8 ± 0.5 ^a
<i>Ipomea eriocarpa</i> R.Br.	Padowia kuri	Leaves	91.9 ± 2.9 ^b	78.9 ± 2.7 ^b	57.1 ± 0.6 ^a
<i>Corchorus trilocularis</i> L.	Otigo lum	Leaves	76.5 ± 0.2 ^c	46.0 ± 1.4 ^c	48.7 ± 1.6 ^c
<i>Ocimum suave</i> L.	Mujaaja	Leaves	74.5 ± 3.6 ^c	50.1 ± 4.3 ^c	55.8 ± 1.0 ^{a,b}
<i>Corchorus olitorius</i> L.	Otigo Diri	Leaves	62.3 ± 0.9 ^d	36.2 ± 0.6 ^c	48.2 ± 0.8 ^c
<i>Acalypha bipartita</i> Müll.Arg.	Ayuyu	Leaves	62.0 ± 1.4 ^d	40.2 ± 0.3 ^c	49.7 ± 0.9 ^b
<i>Hibiscus acetosella</i> Welw. ex Hiern.	Gwanya	Leaves	51.7 ± 1.2 ^{d,e}	24.4 ± 0.4 ^e	36.1 ± 2.0 ^d
<i>Cassia obtusifolia</i> L.	Oyado	Leaves	48.1 ± 1.7 ^{e,f}	12.0 ± 0.2 ^{f,g}	20.0 ± 1.2 ^{e,f}
<i>Solanum aethiopicum</i> L.	Nakati	Leaves	45.7 ± 0.5 ^{e,f}	13.2 ± 0.4 ^{f,g}	19.8 ± 1.3 ^{e,f}
<i>Solanum nigrum</i> L.	Ensugga	Leaves	45.1 ± 0.5 ^{e,f}	17.5 ± 0.3 ^{e,f}	25.6 ± 2.5 ^e
<i>Vigna unguiculata</i> L.	Eggobe	Leaves	39.0 ± 1.1 ^{f,g}	6.1 ± 0.6 ^{g,h}	14.4 ± 0.9 ^{f,g}
<i>Hibiscus cannabinus</i> L.	Nyarogena	Leaves	38.4 ± 0.9 ^{f,g}	13.4 ± 0.2 ^{f,g}	22.2 ± 1.8 ^e
<i>Crotalaria ochroleuca</i> G.Don.	Lala	Leaves	38.3 ± 2.3 ^{f,g}	2.6 ± 0.3 ^{h,i}	8.8 ± 0.7 ^{g,h}
<i>Cleome gynandra</i> L.	Ejjobyo	Leaves	38.0 ± 0.8 ^{f,g}	8.7 ± 0.7 ^{g,h}	15.0 ± 0.7 ^{f,g}
<i>Hibiscus</i> sp. near <i>H. diversifolius</i> Jacq.	Malakwang	Leaves	37.7 ± 2.4 ^{f,g}	12.9 ± 1.0 ^{f,g}	19.2 ± 2.1 ^{e,f}
<i>Solanum anguivi</i> Lam.	Katunkuma	Fruits	32.7 ± 1.0 ^{f,g}	7.2 ± 0.5 ^{g,h}	11.4 ± 0.6 ^{g,h}
<i>Solanum gilo</i> L.	Entuula	Leaves	25.3 ± 0.3 ^{h,g}	2.6 ± 0.5 ^{h,i}	6.4 ± 0.5 ^{f,i}
<i>Hyptis spicigera</i> Lam	Lamola	Seeds	18.5 ± 0.2 ^h	0.4 ± 0.1 ⁱ	1.2 ± 0.6 ⁱ
<i>Amaranthus cruentus</i> L.	Dodo	Seeds	2.9 ± 0.2 ^j	0.5 ± 0.2 ^j	0.1 ± 0.0 ^j
<i>Amaranthus hypochondriacus</i> L.	Dodo	Seeds	2.6 ± 0.1 ^j	0.3 ± 0.1 ^j	0.1 ± 0.1 ^j

TPC: Total phenolic content; TFC: Total flavonoid content; and TAC: Total antioxidant activity. Data are expressed as mean ± standard error from three independent experiments (n=3). Mean values in the same column with different superscript letters are significantly different ($p < 0.05$).

are a large class of polyphenolic compounds that have been attributed to nutraceutical properties of several plants. Flavonoids are potent antioxidants and metal chelators (Tapas et al., 2008) and have long been recognized to be beneficial against many chronic diseases such as cardiovascular diseases (Kris-Etherton et al., 2002), cancer (Birt et al., 2001; Middleton et al., 2000), inflammation (Manthey et al., 2001) and neurodegenerative disorders (Lu et al., 2010; Moosmann and Behl, 2002).

Total antioxidant activity

Antioxidant activity ranging from 0.1 to 57.8 mg VCE gDW⁻¹ was recorded for the different food materials. *T. indica* L. seeds with 57.8 ± 0.5 mg VCE gDW⁻¹, *Ipomea eriocarpa* R.Br. (57.1 ± 0.6 mg VCE gDW⁻¹), *Ocimum suave* L. (55.8 ± 1.0 mg VCE gDW⁻¹), *Acalypha bipartite* Müll.Arg. (49.7 ± 0.9 mg VCE gDW⁻¹), *Corchorus trilocularis* L. (48.7 ± 1.6 mg VCE gDW⁻¹), *Corchorus olitorius* L. (48.2 ± 0.8 mg VCE gDW⁻¹) and *Hibiscus acetosella* Welw. ex Hiern. with 36.1 ± 2.0 mg VCE gDW⁻¹ exhibited the highest antioxidant activities.

The total antioxidant activity of the traditional food

plants significantly correlated to both the total phenolic content ($R^2 = 0.883$; $p < 0.05$) (Figure 2) and the total flavonoid content ($R^2 = 0.965$; $p < 0.05$) (Figure 3), suggesting that antioxidant activity may be attributed to the bioactivity of the phenolic and flavonoid compounds. Abu Bakar et al. (2009) reported a strong correlation between phenolic and flavonoid content and DPPH free radical scavenging ability.

Phytochemical content of the *Tamarindus indica* L. pulp and seed juice

Incorporation of tamarind seed powder (TSP) into tamarind pulp juice resulted into a proportionate increase in total phenolic content of the enriched juice from 24.68 ± 1.3 mg GAE 100 ml⁻¹ for the control (juice without TSP) to 33.78 ± 2.2, 39.14 ± 2.0 and 53.34 ± 0.6 mg GAE 100 ml⁻¹ for juice samples containing 0.5, 1 and 3% TSP, respectively (Table 2). The total flavonoid content (TFC) showed a similar pattern, increasing from 0.92 ± 0.0 mg CE 100 ml⁻¹ for the control to 9.62 ± 0.1, 11.46 ± 0.3 and 17.5 ± 0.1 mg CE 100 ml⁻¹ for juice samples enriched with 0.5, 1 and 3% (w/v) TSP, respectively. Similarly, total antioxidant activity increased from 8.50 ± 0.2 mg VCE ml⁻¹

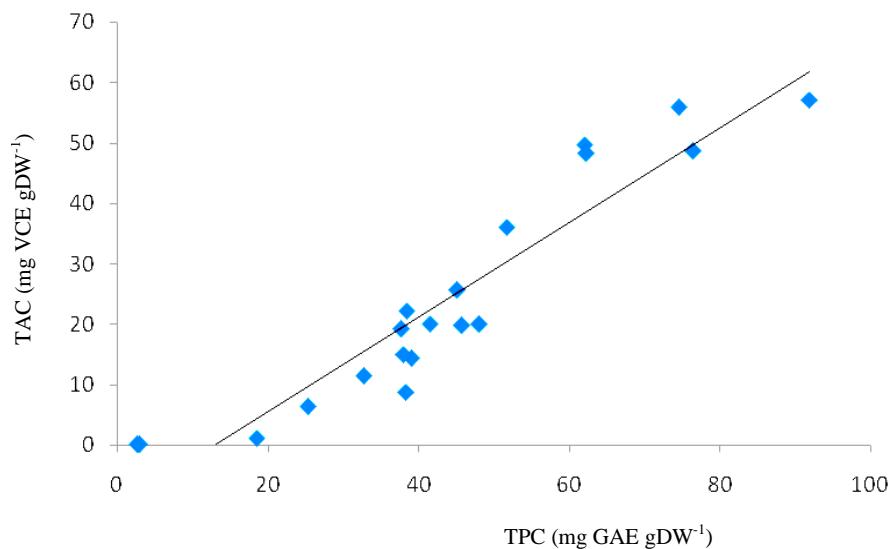


Figure 2. Relationship between antioxidant activities and the total phenolic contents in traditional food plants ($R^2 = 0.883$).

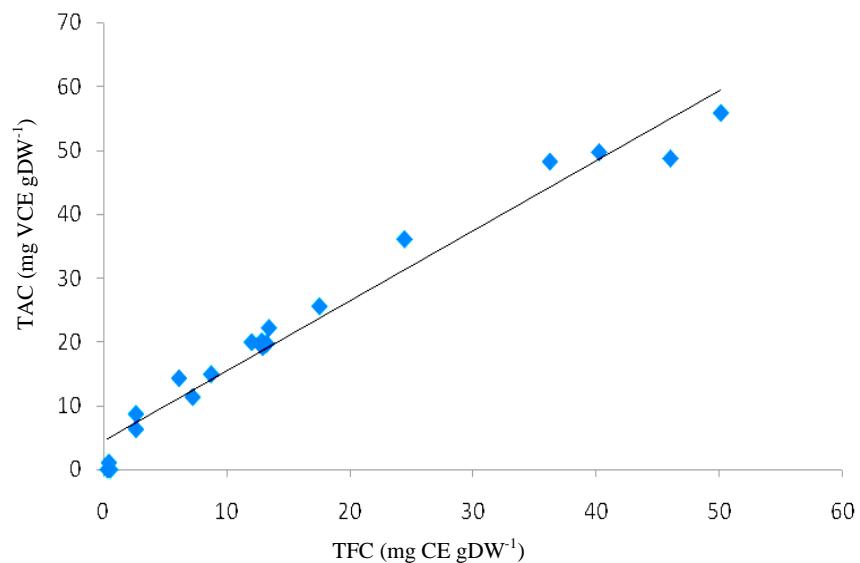


Figure 3. Relationship between antioxidant activities and the total flavonoid contents in traditional food plants ($R^2 = 0.965$).

Table 2. Total phenolic content, total flavonoid content and total antioxidant activity of tamarind pulp juices with different concentrations of tamarind seed powder.

Tamarind seed powder concentration (%)	TPC (mg GAE 100 ml ⁻¹)	TFC (mg CE 100 ml ⁻¹)	TAC (mg VCE 100 ml ⁻¹)
0	24.68 ± 1.3c	0.92 ± 0.0d	8.50 ± 0.2d
0.5	33.78 ± 2.2 ^b	9.62 ± 0.1c	12.05 ± 0.3c
1.0	39.14 ± 2.0 ^b	11.46 ± 0.3b	15.33 ± 0.7b
3.0	53.34 ± 0.6 ^a	17.50 ± 0.1a	17.22 ± 0.7a

TPC: Total phenolic content, TFC: Total flavonoid content, and TAC: Total antioxidant activity. Data are expressed as mean ± standard error from three independent experiments (n=3). Mean values in the same column with the same superscript letters are significantly different ($p < 0.05$).

Table 3. Sensory evaluation of juice prepared from tamarind pulp and seed powder.

Tamarind seed powder concentration (%)	Colour	Aroma	Taste/flavor	Mouth feel	General acceptability
0	6.70 ± 0.0 ^a	6.50 ± 0.2 ^a	6.50 ± 0.2 ^{ab}	6.90 ± 0.1 ^a	7.40 ± 0.4 ^a
0.5	6.45 ± 0.2 ^{ab}	6.80 ± 0.5 ^a	6.45 ± 0.4 ^{ab}	6.75 ± 0.1 ^a	6.65 ± 0.3 ^{ab}
1.0	6.23 ± 0.2 ^{ab}	5.34 ± 0.3 ^{ab}	6.80 ± 0.1 ^a	6.65 ± 0.2 ^a	7.01 ± 0.1 ^a
3.0	6.08 ± 0.1 ^b	4.65 ± 0.2 ^b	5.80 ± 0.1 ^b	5.30 ± 0.3 ^b	5.34 ± 0.2 ^b

Data are expressed as mean ± standard error from three independent experiments (n=3). Mean values in the same column with different superscript letters are significantly different ($p < 0.05$).

¹ for the control to 12.05 ± 0.3, 15.33 ± 0.7 and 17.22 ± 0.7 mg VCE 100 ml⁻¹ for the tamarind pulp juice sample containing 0.5, 1 and 3% (w/v) TSP, respectively. The phenolic contents of tamarind juice samples enriched with tamarind seed powder were comparatively higher than those of natural fresh juices made from apricot (23.75), pineapple (36.16), white grape (37.69), apple (45.38) and red grape juices (49.4), as reported by Mahdavi et al. (2010). The observed increments in total phenolic and flavonoid contents and in total antioxidant activity of the juice may be attributed to the high content of phenolic compounds and antioxidant activity in the *T. indica* L. seeds (Table 1). Previous studies have also reported high phenolic content and antioxidant activity in tamarind seeds (Siddhuraju, 2007). Overall, these results have demonstrated that incorporation of tamarind seed powder boosted the content of phenolic compounds and antioxidant activity of tamarind pulp juice. It further showed that the phenolic content and antioxidant activity increased proportionately with the amount of tamarind seed powder incorporated in the tamarind juice. However, the extent of incorporating tamarind seed powder in juice might be limited by the high content of tannins in tamarind seed coat and associated astringency (Pugalenthhi et al., 2004). Thus, pre-treatment methods such as autoclaving of the seeds were found to reduce the astringency associated with tamarind seed (Legesse and Emire, 2012).

Sensory evaluation of tamarind pulp and seed juice

Addition of tamarind seed powder to tamarind juice to level of 1.0% (w/v) did not result in any significant difference in the perceived sensory attributes of tamarind pulp and seed juice ($p > 0.05$) as compared to the control (juice without tamarind seed powder) (Table 3). However, incorporation of tamarind seed powder at level of 3% (w/v) in the juice resulted in reduced scores in all the sensory attributes such as colour, aroma, taste and mouth feel. The largest difference in sensory scores between the control juice and juice containing 3% tamarind seed powder was in flavor and consistency, implying that the tamarind seeds disproportionately

affected these sensory attributes. Tamarind seed kernels are known to contain some polysaccharides which when mixed with water form mucilaginous dispersions, leading to increase in viscosity of the juice. Furthermore, the presence of antinutritional factors such as tannins in tamarind seed testa renders the whole seed unsuitable for consumption (Caluwé et al., 2010). Overall, the colour, aroma, flavor and consistency of 0.5 and 1% tamarind pulp and seed powder juices were well accepted and the respective general acceptability scores for these were 6.65 ± 0.3 and 7.01 ± 0.1.

Conclusions

Biochemical analysis of traditional food plants identified to have health benefits by local communities in Gulu and Kamuli districts of Uganda showed that most exhibited high levels of total phenolic and flavonoid contents as well as high antioxidant activity. The present study therefore provides support for continued use of these investigated traditional foods for health promotion in Uganda. In particular, the seeds of *T. indica* L. and leaves of *I. eriocarpa* R.Br., *C. trilocularis* L., *O. suave* L., *C. olitorius* L., *A. bipartita* Müll.Arg. and *Hibiscus acetosella* Welw. ex Hiern. exhibited high levels of total phenolic and flavonoid contents including high antioxidant activity. The study has further demonstrated experimentally the potential use of *T. indica* L. seed powder in boosting antioxidant activity of juices as well as enhancing levels of phenolic and flavonoid compounds.

Conflict of interests

The authors did not declare any conflict of interests.

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