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ORIGINAL PAPER

Evaluation of the Total Antioxidant Capacity, Polyphenol Contents and Starch Hydrolase Inhibitory Activity of Ten Edible Plants in an *In vitro* Model of Digestion

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Abstract The total phenolics contents, total antioxidant capacity (TAC) and starch hydrolase inhibitory activity of the aqueous extracts of 10 edible plants and the stability of these parameters after the gastric and duodenal digestion in an *in vitro* model was investigated. The TAC was evaluated using the oxygen radical absorbance capacity (ORAC) assay, ferric reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH•) and 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical scavenging assays. Characterization and quantification of five polyphenol compounds which were previously identified to be present in all the selected plants were carried out. None of the extracts showed a decrease in the total phenolics content or the ORAC and FRAP values following digestion. None of the quantified phenolic compounds had decreased during any of the digestion phases – an observation which was deemed as beneficial in terms of therapeutic properties. Overall, the parameters analyzed were relatively stable throughout the digestive process in all the extracts.

Keywords DPPH · FRAP · Gallic acid · ORAC · Polyphenols

Abbreviations

ABTS	2, 2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EA	Ellagic acid
FRAP	Ferric reducing antioxidant power
GA	Gallic acid
GC	Gallocatechin
HAT	Hydrogen atom transfer
HPLC	High-performance liquid chromatography
ORAC	Oxygen radical absorbance capacity
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SEM	Standard error mean
SET	Single electron transfer
TAC	Total antioxidant capacity
VA	Vanillic acid

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Introduction

Polyphenol consumption has been associated with several health benefits such as reducing the incidence of diabetes and its related complications, cancer and cardiovascular diseases [1]. It is believed that this effect is achieved by cumulative biological exposure to antioxidants which are able to quench the proliferation of radical oxygen (ROS) and reactive nitrogen (RNS) species which are implicated in the pathology of these diseases [2]. Several chemical and biochemical assays have been utilized for the quantification of total antioxidant capacity (TAC) of plant products which involve either single electron transfer (SET) or hydrogen atom transfer (HAT) reaction kinetics [3]. In addition a number of studies also present a measure of the total polyphenol content of food products in order to draw comparisons with other similar products, and to provide more detailed information about this sub-group of antioxidants comprising flavanoids, lignins and tannins [4, 5]. However, a major obstacle in evaluating the role of individual food components in modifying disease risk is the

scarcity of information on factors that influence their bioavailability and bioaccessibility [6].

Several *in vitro* methods have been used to determine the bioaccessibility and bioavailability of individual antioxidant compounds in order to isolate those which remain stable and active throughout the digestion and absorption processes [4, 7]. However, no study to date has been able to show a protective effect of an acute dose of any individual compound [7]. Focus has therefore returned to classical research work which have succeeded in the reduction in disease rates over time when higher amounts of fruit and vegetables were consumed [8]. Measurement of TAC before and after *in vitro* digestion offers a method of ranking food products (especially those of plant origin) in order of their therapeutic capabilities [3, 9]. In addition to TAC, incorporation of starch hydrolase inhibitors into the diet have also been clinically identified as an effective remedy of chronic disease prevention [9]. These inhibitors retard the absorption of glucose through inhibition of the starch-hydrolyzing enzymes such as α-amylase and α-glucosidase which are present in the small intestinal brush border. Thus, the aim of this research was to quantify polyphenol contents, TAC and starch hydrolase inhibitory activity of the aqueous extracts of 10 commonly consumed edible plants and assess the stability of these parameters after the gastric and duodenal digestion phases of an *in vitro* model. The TAC was evaluated using the Oxygen Radical Absorbance Capacity (ORAC), Ferric Reducing Antioxidant Power (FRAP) and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH•) and 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical scavenging assays. High-throughput measurements of the inhibition of α-amylase and α-glucosidase was also carried out. Quantification of five polyphenol compounds, namely gallic acid (GA), vanillic acid (VA), gallocatechin (GC), catechin and ellagic acid (EA), which have been previously identified to be present in all of the 10 edible plants was carried out as well using High-Performance Liquid Chromatography (HPLC). This study is novel, since previous publications detailing the antioxidant capacity and the starch hydrolase inhibitory activities of these selected edible plants in particular and their stability in *in vitro* digestion models are less in number.

Materials and Methods

All reagents, chemicals and HPLC standards used for this study were purchased from Sigma Chemicals (St. Louis, MO, USA).

Preparation of Edible Plants Powders and *In vitro* Digestion Procedure

The following edible plants were chosen for the study based on their therapeutic properties and previous studies on antioxidant and starch hydrolase inhibitory properties as documented in the authoritative literature [2, 10, 11]: *Acacia arabica*, *Aegle marmelos* flower, *Aegle marmelos* root bark, *Aerva lanata*, *Asteracantha longifolia*, *Cassia auriculata*, *Hemidesmus indicus*, *Hordeum vulgare*, *Phyllanthus emblica*, *Tinospora cordifolia*. Dried powders of the plants were obtained from the Ayurvedic Medicinal Hall in Kandy, Sri Lanka. The methodology by Wu *et al.*, was followed for the preparation of the herbal extracts using acetone/water/acetic acid (70:29.5:0.5) [12]. The *in vitro* digestion model was adapted from Ryan, O'Connell, O'Sullivan Aherne & O'Brien [7], where detailed methodological information are available in the Electronic supplementary material (ESM). Digested samples were analyzed within 2 weeks.

Total Phenolics Content and Antioxidant Activity Assays

The total phenolics contents were determined according to Wijeratne, Abou-Zaid & Shahidi [13]. The values were expressed as μg gallic acid equivalents per gram fresh weight (μg GAE/g) of sample. The ORAC assay was carried out according to the method by Prior *et al.*, using a Thermo Scientific Multiskan FC Microplate Reader [14]. The DPPH• method was conducted according to Brand-Williams *et al.*, [15]. The FRAP assay was carried out as described by Benzie & Strain [16]. In addition, the antioxidant activities of the samples were analyzed by investigating their ability to scavenge the ABTS•+free radical using the method by Ozgen *et al.* [17].

Assays of α-Amylase and α-Glucosidase Inhibitory Activities

The α-amylase inhibitory activity of the extracts was carried out according to Liu, Song, Wang & Huang [18], while the α-glucosidase inhibitory activity was carried out according to Koh, Wong, Kasapis & Huang [19]. Data were expressed as IC_{50} (mg/mL).

High Performance Liquid Chromatography Determination of the Anthocyanins and Phenolic Compounds

The anthocyanin contents were measured according to Brown & Shipley [20]. Details of the methodology are available in the ESM. A Shimadzu (Kyoto, Japan) HPLC system equipped with a diode array detector (SPDM10Avp) and a phenomenex Luna C-18(2) column (4.6 mm i.d. × 25 cm, 5 μm) was used for the quantification of GA, VA, GC, catechin and EA. A gradient profile using two solvents—where Solvent A: 8 % aqueous formic acid and solvent B: acetonitrile/methanol (10:90, v/v), at a flow rate of 0.9 mL/min was applied following the method by Wijeratne, Abou-Zaid & Shahidi [13].

Statistical Analysis

All results are presented as mean±standard error mean (SEM). For comparisons, data was analyzed by ANOVA and Tukey's multiple comparison test (SPSS, version 17). A probability of 5 % or less was accepted as statistically significant.

Results and Discussion

The total phenolics content and results from the antioxidant assays are shown in Fig. 1. All plant extracts displayed a significant presence of phenolic compounds ranging from 235 to 390 μg GAE/g prior to digestion. All extracts with the exception of *A. marmelos* flower and *A. marmelos* root bark displayed a statistically significant increase ($P<0.05$) in the total phenolics content after both phases of digestion. None of the plant extracts showed a decrease in the total phenolics content at any of the phases of digestion. The ORAC values had the better correlation with the total phenolics content ($R^2=0.988$) as compared with FRAP ($R^2=0.887$), DPPH ($R^2=0.812$) and ABTS ($R^2=0.698$). This may have been due to the phenolic compounds having the ability to be better scavengers of peroxyl radicals, which are generated during the assay. The ORAC values of the extracts varied between 795 and 1875 μmol TE/g. With the exception of *H. indicus* and *P. emblica*, all remaining extracts showed a statistically significant increase ($P<0.05$) in the ORAC values following the duodenal phase. As shown in Fig. 1c, the FRAP values showed a statistically significant increase ($P<0.05$) from the gastric phase itself, which was further statistically significantly increased ($P<0.05$) during the duodenal phase. As for the DPPH radical scavenging activities, there was no statistically significant difference observed when comparing the values before and after the digestion. On the other hand, *C. auriculata* and *H. vulgare* showed a statistically significant difference ($P<0.05$) for the ABTS•+radical scavenging activity assay, where the ABTS•+radical scavenging activity value of *H. vulgare* decreased following duodenal digestion.

The anthocyanin contents in all the herbs were observed to be negligible as compared with the total amount of phenolics present. The values are shown in Table 1 of the ESM. Changes to GA, VA, GC, catechin and EA are shown in Fig. 2. Representative HPLC diagrams of the phenolic compounds as present in *A. marmelos* flower and *C. auriculata* are shown in Fig. 1 of the ESM. The GA contents had statistically significantly increased ($P<0.05$) in both digestion phases only in *P. embelica*, *T. cordifolia*, *A. arabica*, *A. longifolia*, *A. lanata* and *H. vulgare*. No changes in the GA contents were observed in *A. marmelos* root bark and *C. auriculata*. The VA, EA, GC and catechin contents had undergone statistically significant

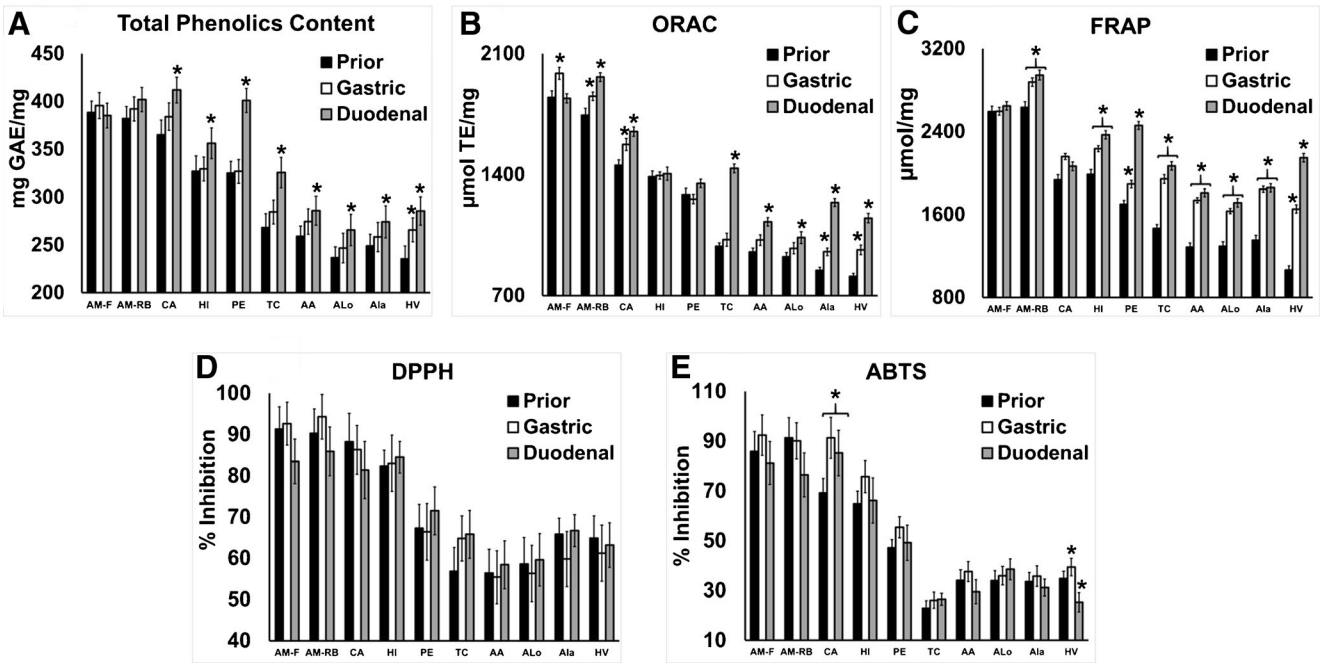

Fig. 1 **a** Total phenolics content **b** ORAC **c** FRAP **d** DPPH and **e** ABTS radical scavenging activities of the edible plant extracts prior to digestion and following the gastric and duodenal phases. Abbreviations: AA—*A. Arabica*, AM-F—*A. marmelos* flower; AM-RB—*A. marmelos* root bark, ALa—*A. lanata*; ALo—*A. longifolia,* HI—*Hemidesmus indicus*, HV—*Hordeum vulgare*, PE—*Phyllanthus emblica*, TC—*Tinospora cordifolia*. * $P<0.05$, denotes statistically significant difference as compared with prior to *in vitro* digestion. Values represent mean±SEM of 3≤independent experiments

Table 1 α-Amylase and α-glucosidase inhibitory activities of the edible plant extracts prior and following digestion in the gastric and duodenal phases

Sample Code	α-Amylase Inhibitory Activity			α-Glucosidase Inhibitory Activity		
	Prior (IC_{50}, μg/mL)	Gastric (IC_{50}, μg/mL)	Duodenal (IC_{50}, μg/mL)	Prior (IC_{50}, μg/mL)	Gastric (IC_{50}, μg/mL)	Duodenal (IC_{50}, μg/mL)
A. marmelos (flower)	56.9±5.5	66.5±2.5 *	71.5±6.1 *	65.9±2.1	72.5±1.9 *	73.9±2.1 *
A. marmelos (root bark)	66.5±3.2	74.5±2.1 *	75.9±4.0 *	95.8±5.2	105.2±3.2 *	108.6±4.8 *
C. auriculata	89.6±2.8	98.4±3.2 *	125.9±6.9 *	120.2±6.9	134.9±6.8 *	147.6±8.1 *
H. indicus	128.9±6.5	139.5±4.7 *	159.4±8.2 *	168.4±5.9	174.6±6.2	179.4±6.5
P. emblica	157.9±9.2	187.6±8.4 *	198.6±8.6 *	169.4±7.4	175.2±5.9	179.5±6.6
T. cordifolia	184.5±7.3	194.7±8.2	195.2±9.4	187.2±6.5	188.4±6.7	189.4±6.8
A. arabica	208.4±10.4	258.4±11.2 *	269.7±11.2 *	259.4±8.7	284.3±9.1 *	287.6±9.2 *
A. longifolia	241.6±8.5	247.9±10.0	257.6±9.5	274.5±9.5	285.1±8.5	289.6±9.1
A. lanata	268.4±9.5	274.6±9.5	279.3±9.2	276.5±8.9	284.3±9.4	289.6±11.1
H. vulgare	295.6±8.5	301.5±11.6	326.9±11.6	298.6±10.4	308.4±10.4	338.4±10.5

* $P<0.05$, denotes statistically significant difference as compared with prior to *in vitro*

increases ($P<0.05$) during both digestion phases. Overall, none of the quantified phenolic compounds had decreased during any of the phases.

The α-amylase and α-glucosidase inhibitory activities of the plant extracts prior to digestion as well as afterwards are shown in Table 1. The mean IC_{50} values of the α-amylase and α-glucosidase inhibitory activities had increased following the gastric and duodenal digestion phases, indicating the reduced ability to inhibit the enzymes. Nevertheless, statistically significant increases ($P<0.05$) in the IC_{50} values of the α-amylase inhibitory activities were observed only in *A. marmelos* flower, *A. marmelos* root bark, *C. auriculata*, *H. indicus*, *P. emblica* and *A. arabica* following both phases of digestion. Statistically significant increases ($P<0.05$) in the IC_{50} values of the α-glucosidase inhibitory activities were observed in *A. marmelos* flower, *A. marmelos* root bark and *C. auriculata* following both phases of digestion. Despite the overall decrease in the IC_{50} values, they could still be

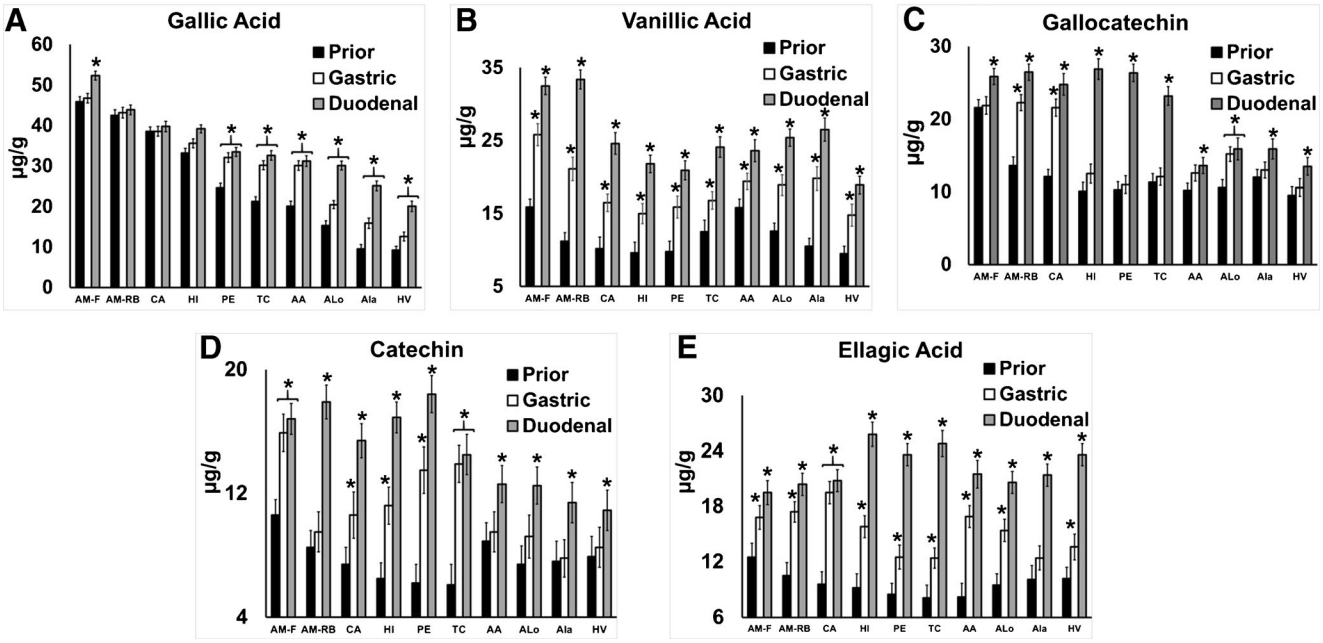

Fig. 2 Changes to the **a** Gallic Acid **b** Gallocatechin **c** Catechin **d** Ellagic Acid **e** Vanillic Acid quantities of the edible plant extracts prior to digestion and following the gastric and duodenal phases. Abbreviations: AA—*A. Arabica*, AM-F—*A. marmelos* flower; AM-RB—*A. marmelos* root bark, ALa—*A. lanata*; ALo—*A. longifolia,* HI—*Hemidesmus indicus*, HV—*Hordeum vulgare*, PE—*Phyllanthus emblica*, TC—*Tinospora cordifolia*. * $P<0.05$, denotes statistically significant difference as compared with prior to *in vitro* digestion. Values represent mean±SEM of 3≤independent experiments

considered as high, indicating the ability of all the plant extracts to sustain the α-amylase and α-glucosidase inhibitory characteristics, although the statistically significant differences in both enzymes are of more importance to be monitored during the gastric phase of digestion.

Bioaccessibility is defined as the amount of a food constituent present in the gut as a consequence of the release of this constituent from the solid food matrix, which is subsequently able to pass through the intestinal barrier [21]. Only polyphenols released from the food matrix by the action of digestive enzymes (small intestine) and bacterial microflora (large intestine) are bioaccessible in the gut and therefore, potentially bioavailable. Thus, the amount of bioaccessible food polyphenols may differ quantitatively and qualitatively from polyphenols included in food databases [22]. Overall, the results of this study showed that antioxidant capacity and the starch hydrolase inhibitory activities are relatively stable throughout the digestive process in all plant extracts analyzed. This was encouraging given that previous studies reported a reduction in various polyphenols following similar two phase digestions of a variety of fruit juices [23, 24], although in these studies, it may have been due to a structural transformation in the polyphenols which render them undetectable by HPLC analyses.

A description of the edible plants which were used in this study could be deemed as essential. *A. arabica* has been commonly used in folklore medicine throughout the world and is well known for its high content of tannins which are known to be extracted for medicinal uses [11]. *A. marmelos* is a commonly consumed tonic purported to contain various therapeutic properties [25]. The flowers and root barks of this plant has been commonly used in traditional medicinal systems, similar to *A. arabica*. *A. lanata* has proved to be a therapeutic agent for various diseases, where the bioactive components have been identified as flavonoids, glycosides, carbohydrates, alkaloids, tannins, saponoins, terpenoids, phenols and phytosterols [11]. Clinical trials of *A. longifolia* have indicated the plant extract to be highly effective against diabetes, which ascertains its role as an anti-diabetic herbs in many traditional medicinal recipes [26]. A study on *C. auriculata*—a common plant in Asia which has been widely used in traditional medicine as a remedy for diabetes, was reported to possess anti-hyperlipidaemic effects in addition to anti-diabetic properties [27]. *H. indicus* comes from a family of shrubs known to contain high amounts of polyphenols [11]. *H. vulgare* is a highly adaptable cereal grain produced in climates ranging from sub-Arctic to subtropical and is commonly consumed as a cooling beverage, regardless of its medicinal properties and reported antioxidant properties [2]. *P. emblica* contains a multitude of medicinal properties owing to the high amounts of catechins and epicatechins in its extracts [28]. It is a highly versatile herb which is incorporated into many traditional medicinal recipes throughout the world for the treatment of numerous non-communicable diseases. *T. cordifolia* is known to contain many quinones, flavonoids, tannins and coumarins resulting in its usage for many disease treatments [11].

Conclusions

This study was able to prove that the 10 selected edible plants were a significant source of total phenolics, antioxidant activity and starch hydrolase inhibitory activities. Additionally, all of the plant extracts were either stable or enhanced in terms of TAC following *in vitro* digestion, although there was a large variation in the responses of individual extracts to this process. This research highlights the need for further investigation in several areas. First, it could be considered as important to factor in the contribution of structurally transformed molecules and other antioxidant metabolites to the TAC and associated therapeutic properties. Next, it is important that future studies aim to provide biologically relevant information on antioxidants by providing data concerning the bioaccessibility and bioavailability of antioxidants in a human system. Finally, in support of evidence provided by previous studies, this research work highlights the importance of using multiple methods of analysis for the measurement of TAC in the absence of any single accepted assay.

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Conflict of Interest As the corresponding author, I declare that there are no conflicts of interest involved during the preparation of the manuscript and that the work reported herein is original and has not been accepted for publication elsewhere. This article also does not contain any studies with human or animal subjects.

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