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Analysis of phenolic compounds including tannins, gallotannins and flavanols of *Uapaca kirkiana* fruit

M. Muchuweti *, A.R. Ndhlala, A. Kasiamhuru

Department of Biochemistry, University of Zimbabwe, P.O. Box MP 167, Mount Pleasant, Harare, Zimbabwe

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Abstract

Tannins and phenolic acids were extracted from *Uapaca kirkiana* unripe pulp, seed coat, peel and embryo, as well as from ripe pulp, seed coat, embryo and peel portions of the fruit. Extraction was done using 50% methanol. More tannins were detected in the embryos of both ripe and raw fruit. Both ripe and raw seed coat contained the least amounts of tannins. The flavanoids and gallotannins also followed the same trend, with most of them in the embryos of both, the ripe and raw, fruit portions whilst the least were found in the seed coat. Tannins were, however lost during sun-drying compared to oven-drying, even though the differences were not very large. Also, raw fruits showed high concentrations of tannins compared to the ripe fruits. Hydroxybenzoic acid was found in the peel and pulp but none was detected in the seed coat or in the embryo. Similar phenolic acids were detected in the seed coat, except for caffeic acid and protocatechuic acid, detected in the embryo by both the UV florescence and Folin spray.

Keywords: *Uapaca kirkiana*; Tannins; Plant; Fruit

1. Introduction

The *Uapaca kirkiana* tree, known as *Muzhanje* in Shona, wild loquat in English and *Umhobhohobho* in *Ndebele*, is an evergreen tree, some 5–6 m high, with rounded crown and rough grey bark. The fruit is a fleshy round belly, up to forty millimeters across, with a tough reddish brown skin surrounding yellow brown pulp in which several ridged seeds are embedded. The fruits are among the most popular in Zimbabwe. The ripe fruits are sold at roadsides and at country markets. The pulp is honey – sweet with a slight flavour of oranges (Tredgold, 1986).

The *U. kirkiana* fruits improve the nutritional status, especially in times of food emergency, and have been used for a long time to supplement dietary needs. The fruits contain vital nutrients and essential vitamins, which are important, especially for growing children, who often suffer from malnutrition and related diseases (Saka & Msonthi, 1994). In northern Zambia, a hungry season precedes the first rains in November when the busiest time of field preparation and planting normally begins. The fresh fruits of *U. kirkiana* contribute significantly to diet during this period, whilst the sale of fruit generates much needed cash for farm inputs and other household requirements (Packham, 1993). In Malawi, *U. kirkiana* fruits with 27.4% dry matter were found to contain: 86.5% total carbohydrate, 8.4% fibre, 1.1% fat and 1.8% crude protein (Saka, Msonthi, & Maghembe, 1994). Ascorbic acid content is 16.8 mg per 100 g fresh weight (Saka, 1995).

The fleshy pulp of the *U. kirkiana* fruit is eaten fresh or processed into a variety of products, including juices, squashes, wines, sweet beer, porridge, jams and cakes (Ngulube, 1995). The National Breweries of Zambia are brewing *musuku* wine from *U. kirkiana*. Systematic collection of the fruit is organised by local people and

* Corresponding author. Tel.: +263 4 308047; fax: +263 4 308046.
E-mail address: muchuweti@medic.uz.a.zw (M. Muchuweti).

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brought to the roadside. Local people in the Southern Province of Zambia are informed by the brewery when the collection trucks will arrive for collection (Packham, 1993).

The aim of the work was to extract and determine the distribution of tannins and phenolic acids in both raw and ripe peel, pulp, seed coat and embryo of the *U. kirkiana* fruits, and also to compare the amounts of the tannins and phenolics recovered after sun-drying and oven-drying. The peel and pulp can be eaten, fresh, while, together with the seed coat and the embryo, they can be dried into a cake which can be stored and eaten later. This study was carried out using the *U. kirkiana* fruit because it has an astringent taste due to the presence of tannins and, also, the fruit is one of the common wild fruits widely consumed in Zimbabwe.

Tannins are naturally-occurring uncrystallisable colloidal substances with pronounced astringent properties. Their main characteristic is that they bind and precipite gelatin from solution and form insoluble compounds with gelatin-yielding tissue which is the property which enables them to convert raw hide and skin into leather, consolidating the dermal network of hide into firmer and drier structures of improved thermal stability, durability and water resistance. Because of their protein-binding properties, tannins are of considerable importance in food processing (Millic & Stojanovic, 1972; Reed, 1995). Tannins have been reported to exert other physiological effects; e.g., they can reduce blood pressure, accelerate blood clotting, decrease the serum lipid level, modulate immunoresponses and produce liver necrosis. The dosage and kind of tannins are critical for these effects. Tannins in wine have potent antioxidancy against low-density lipoprotein (LDL) of which the oxidized form is a precursor of coronary heart disease (Teissedre, Frankel, Waterhouse, Peleg, & German, 1996). Plant phenols can be considered a nuisance because they can complex proteins by hydrogen bonding. Phenolic monomers have been implicated in inhibition of digestion. Ferulic acid and *p*-coumaric acid are toxic to cellulolytic bacteria (Ayaz, Kucukislamoglu, & Reunaen, 2000; Cherney, Anliker, Albrecht, & Wood, 1989; Häkkinen, 2000).

2. Materials and methods

2.1. Samples

Ripe fruits were bought from a local market place in Harare (*Mbare* Market) and the unripe samples were collected from Glendale, about 10 km from Harare.

The peel, pulp, seed coat and seed embryo of the fruits were completely separated and two methods of drying were used, sun drying and oven drying. For sun-drying, the samples were exposed to sunlight until they were completely dry. They were then stored in dark cupboards. For oven-drying, the samples were dried at 52° until they completely dried as reported by Makkar (1999). The dried samples were ground into very fine particles using a mortar and pestle. They were then placed in sample bottles in dark cupboards.

2.2. Chemical standards and reagents

The chemical standards were of the highest purity grade. Tannic acid, gallic acid, catechin, vanillic acid, caffeic acid, *p*-coumaric acid, protocatechuic acid, ferulic acid, *p*-hydroxy-benzoic acid and *p*-hydroxybenzaldehyde were all obtained from Sigma–Aldrich Chemie (Steinheim, Germany). Folin–Ciocalteau and rhodamine were also obtained from Sigma–Aldrich Chemie (Steinheim, Germany). Methanol, Na_2HPO_4, butanol, glacial acetic acid, ethyl acetate and sodium sulphate were obtained locally.

2.3. Extraction of polyphenols

Ground fruit material (2 g) was placed in an Eppendof plastic tube of 50 ml capacity on ice. Methanol–water, 1:1 v/v (10 ml), was added and the tubes were suspended on a shaker for a minute. The tubes were then centrifuged for 10 min at 3000*g* using a bench centrifuge. The supernatants were collected, filtered and stored in a deep freezer. The pigments were removed using diethyl ether in 1% acetic acid (Makkar, 1999).

2.4. Extraction of phenolic acids

The sample (5 g) of *U. kirkiana* fruit pulp, peel, seed coat and embryo material were extracted twice each with ethyl acetate (10 ml). The ethyl acetate was dried over sodium sulphate and then the sample was concentrated by rotary evaporation at 30 °C, a method modified from the method by Ayaz et al. (2000). The concentrates were transferred into small sample bottles and stored in a freezer for analysis.

2.5. Quantification of the polyphenolics

In methanol solution, the total phenolics were quantified using the Folin–Ciocalteau method, gallotannins by the rhodamine assay, and flavanols by the vanillin assay. All the methods were adopted from Makkar (1999).

2.6. TLC of phenolic acids

For identification of the phenolic acids, standard phenolic acids (1%, 10 μl) and samples (10 μl) were applied on of Whatman K5 plate impregnated with 0.2 M Na_2HPO_4. After developing the plates in butanol:glacial acetic acid:distilled water (11:3:6 v/v), the plates were

viewed under UV fluorescent light before spraying with 1 N Folin–Ciocalteau reagent (Mueller-Harvey, 2001). After 20 min, the spots were visible and the plates were scanned using a high resolution Hp scanjet 7400 scanner.

3. Results and discussion

Concentrations of total tannins varied amongst the fruit portions, sun-dried, oven-dried, as well as the ripe and unripe fruits, of *U. kirkiana*. More tannins were detected in the embryo portion than in the peel, pulp and the seed coat, as represented in Fig. 1, having 0.046 mg/g of dry mass of sun-dried fruit and 0.057 mg/g dry mass of oven-dried fruit sample. The seed coat had the lowest concentrations of tannins, as low as 0.0133 mg/g of dry mass of sun-dried fruit and 0.046 mg/g of dry mass of oven-dried fruit, as represented in Fig. 1 as well. Morrison, Asiedu, Stuchbury, and Powell (1995) determined the content of tannins in cowpea seed coats to be 0.56 mg/g after extraction with methanol while Chavan, Shahidi, and Naczk (2001) reported the content in grass pea to be 2.1 mg/g, their finding rather higher than that of Morrison et al. (1995). Nevertheless both of the results are comparable to our results.

Tannins were lost during sun-drying compared to oven-drying with the embryo having a difference of 0.057 mg/g tannins in the dry mass of fruit sample, also represented in Fig. 1. Fig. 2 represents the same trend in amounts of tannins in the unripe fruit portions. The embryo had the highest amounts of tannins, containing as much as 0.161 mg/g in the dry mass of sun-dried fruit and 0.171 mg/g in the dry mass of oven-dried fruit. The peel had the second highest concentration, followed by the pulp and finally the seed coat. Fig. 2 also shows that tannins are lost during fruit ripening with the embryo having a difference of 0.115 mg/g of the dry mass of the sun-dried fruit and 0.118 mg/g of the dry mass of oven-dried. Ayaz et al. (2000) determined the total tannin concentration in strawberry fruit to be 0.12 mg/g, a value comparable to the amounts of tannins in the unripe fruit portions, especially the embryo and peels.

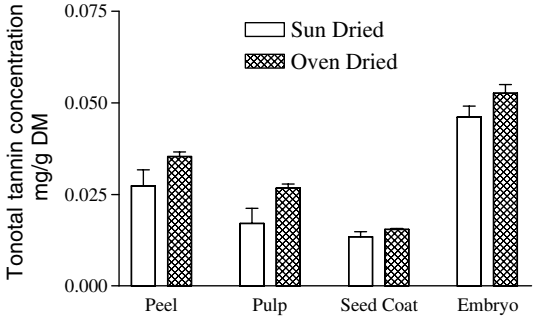

Fig. 1. Total tannin concentration in different portions of 1 g of ripe sun dried and oven dried *U. kirkiana* extracted with cold 50% methanol.

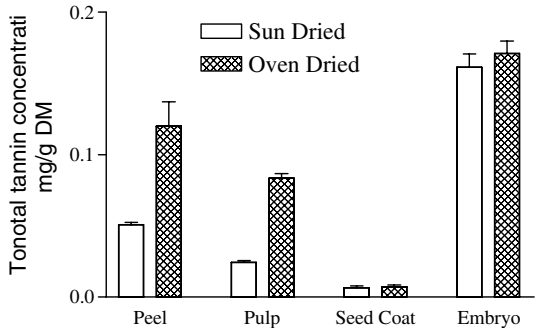

Fig. 2. Total tannin concentration in different portions of 1 g of unripe sun dried and oven dried *U. kirkiana* extracted with cold 50% methanol.

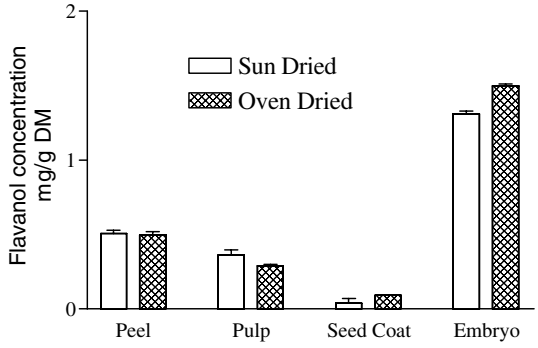

Fig. 3. Flavanol concentration in different portions of 1 g of ripe sun dried and oven dried *U. kirkiana* extracted with cold 50% methanol.

Most of the flavanoids were also found to be dominant in the embryo, followed by the peel, pulp and lastly the seed coat, a trend similar to that obtained for total tannins. In Fig. 3, the embryo had concentration of flavanoids as high as 0.0130 mg/g in the dry mass of sun-dried fruit and 0.0149 mg/g in dry mass of oven-dried fruit. The peel had 0.005 mg/g of the dry mass of sun dried fruit and 0.00496 mg/g of the dry mass of oven-dried fruit. Flavanoids were almost the same in concentrations, in the sun-dried and oven-dried samples with the sun-dried peel having more than the oven-dried sample, represented in Fig. 3. However, more flavanoids were detected in the unripe fruit portions, as was the case with total tannins (Fig. 4). There were also almost equal amounts of flavanoids in sun-dried samples as oven-dried samples as shown in Fig. 4.

The gallotannins (Figs. 5 and 6) also followed a similar trend, with most of the gallotannins in the embryo, then the peel, pulp and lastly the seed coat. There were also very small variations of gallotannins in the oven-dried compared to sun-dried samples. There were also small variations in the gallotannins in the ripe and

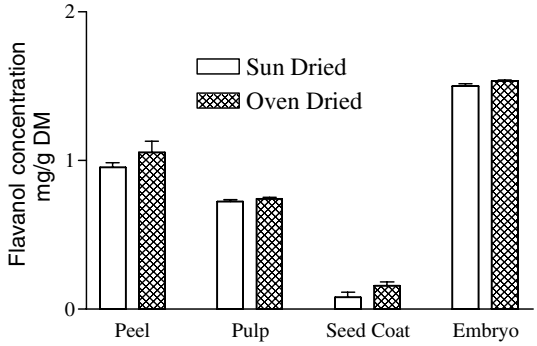

Fig. 4. Flavanol concentration in different portions of 1 g of unripe sun dried and oven dried *U. kirkiana* extracted with cold 50% methanol.

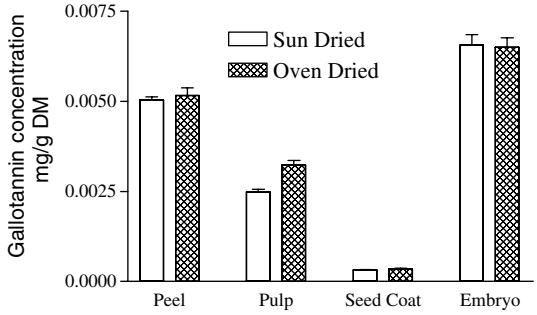

Fig. 5. Gallotannin concentration in different portions of 1 g of ripe sun dried and oven dried *U. kirkiana* extracted with cold 50% methanol.

unripe fruit samples, with the unripe embryo having 0.0076 mg/g of dry mass of sun-dried fruit and the ripe sun-dried fruit sample having 0.0075 mg/g dry as mass represented in Fig. 6. The ripe fruit had 0.0067 mg/g of dry mass of sun-dried sample and the oven-dried sample had 0.006 mg/g of dry mass, as represented in Fig. 5.

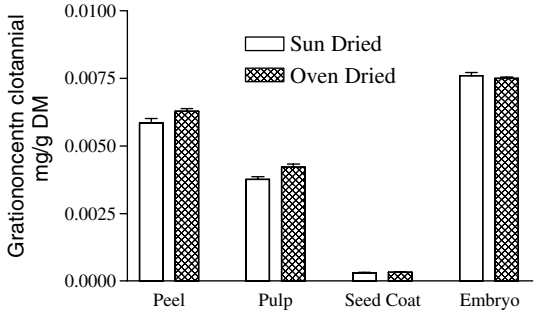

Fig. 6. Gallotannin concentration in different portions of 1 g of unripe sun dried and oven dried *U. kirkiana* extracted with cold 50% methanol.

As seen by UV fluorescence, the embryo of *U. kirkiana* fruit contains caffeic acid, with an Rf value of 0.821 cm as seen in Table 1. Table 1 also shows the lack of caffeic acid in other portions of the *U. kirkiana* fruit, namely the peel, pulp and the seed coat, since there was fluorescence detected.

The study identified *p*-hydroxybenzoic acid in the peel and pulp of the *U. kirkiana* fruit, as shown in Table 1. The *p*-hydroxybenzoic acid is absent in the seed coat as well as the embryo. However, the other six phenolic acids were not detected in the peel on the pulp. The seed coat contains ferulic acid, *p*-coumaric acid and vanillic acid. The phenolic acids detected in embryo were caffeic acid, ferulic, *p*-coumaric acid, vanillic acid and protocatechuic acid, as shown in Table 1.

The phenolic acids in the peel and pulp were similar (*p*-hydroxybenzoic acid). No *p*-hydroxybenzoic acid was detected in the seed coat or in the embryo. The phenolic acids in the seed coat were similar except for caffeic acid and protocateic acid, detected in the embryo by both the UV fluorescence and Folin–Ciocalteau reagent spray.

Table 1
Spots detected from the TLC plates of standards and *U. kirkiana* samples by UV fluorescence and after spraying with the Folin reagent

Sample	UV Fluorescence and Rf value (cm)	Rf value (cm) after spraying with Folin reagent	Phenolic acids detected by UV and Folin reagent
Peel	None	0.933	*p*-Hydroxybenzoic acid
Pulp	None	0.933	*p*-Hydroxybenzoic acid
Seed Coat	None	0.911	Ferulic acid, *p*-coumaric acid, vanillic acid
Embryo	Detected-0.821	0.821, 0.873 and 0.918	Caffeic acid, Ferulic acid, *p*-coumaric acid, vanillic acid, protocatechnic acid
Vanillic acid	None	0.911	
Caffeic acid	Detected-0.821	0.821	
p-Coumaric acid	None	0.918	
Protocatechuic acid	None	0.881	
Ferulic acid	None	0.911	
p-Hydroxybenzaldehyde	None	0.866	
p-Hydroxybenzoic acid	None	0.933	

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