Antioxidant Potential of Selected Underutilized Fruit Species Grown in Sri Lanka

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Abstract— *The aim of this study was to assess the potential of aqueous extracts of three underutilized fruit species namely Dialium guineense, Solanum nigrum and Carissa carandas grown in Sri Lanka. Aqueous extracts of fruits were obtained by extracting accurately measured and finely ground fresh fruit samples with distilled water for 90 min followed by centrifuging at 4500rpm. The supernatants were collected separately and the extraction was repeated twice with residues. The collected supernatants were combined and lyophilized. Total phenolic contents (TPC) were determined using a spectrophotometric technique, based on the Folin-Ciocalteau reagent, calculated as gallic acid equivalents GAE/g fresh weight. Antioxidant potential was determined using DPPH˙ and ABTS˙+ scavenging assays. The DPPH radical scavenging activity of the extracts was expressed as IC_{50} values that denote the concentration of the sample required to scavenge 50% of radicals. The radical scavenging activity (RSA) was calculated as percentage of ABTS˙+ discoloration over six minutes. The TPC varied among species from 1.16mg ± 0.049 GAE/g of fresh fruit of D. guineense to 3.12 mg ± 0.43 GAE/ g of fresh fruit of S. nigrum. The highest antioxidant potential was possessed S. nigrum as it showed about 66% ABTS˙+discoloration over six minutes and 12.23mg ± 0.11 of fresh fruit needed for IC_{50}. Therefore these results suggested that wild fruit species could be exploited as an ingredient in developing a potential antioxidant supplement.*

Keywords— *Total Phenolic Content, Antioxidant Potential, D.guineense, S. nigrum, C. carandas*

I. INTRODUCTION

Phenolic compounds such as phenolic acid, flavanoids, stilbenes, lignans and tannins are commonly found in both edible and non-edible plants and they have been reported to have multiple biological effects, including antioxidant activity (Marja, *et al*., 1999). Antioxidants neutralize the effect of free radicals through different ways and may prevent the body from various diseases. Synthetic antioxidants like butylated hydroxy anisole (BHA) butylated hydroxy toluene (BHT), tertiary butylated hydroxy quinone and gallic acid esters have been suspected to be carcinogenic (Rajan e*t al.,* 2011). Hence, strong limitations have been placed on their use and there is a trend to replace them with naturally occurring antioxidants.

A number of underutilized fruits are adequately rich in antioxidants and phytochemicals besides necessary nutritional components such as vitamins, minerals, and dietary fibre. Hence, more emphasis is being given to some of these fruit crops due to their high nutraceutical values. They are found in wild or in the home gardens, which vary from 100 m^2 to 1000 m^2 in extent and are commonly found in many rural areas of Sri Lanka (Rajapaksha, 2007) such as *Tamarindus indica, Flacouria indica, Elaeocarpus serratus, Syzygium cumini,* ect. The villagers often collect the produce of them and sell them in village fairs or supply them to the collectors. Therefore these fruit crops are being life-servers for thousands of resource poor people in regions where food and nutrition security are significant problem. Some of these fruits are processed by small entrepreneurs into jam, jelly and ready to serve drinks and sold in groceries and supermarkets.

Solanum nigrum L. grows as a common weed in cultivated lands all parts of the country. Fruits of *S. nigrum* have been used in traditional medicine to treat fever, diarrhoea, and heart and eye diseases. Many scientific evidences indicate that *S. nigrum* possesses beneficial activities such as antioxidant and hepatoprotective activities. *Carissa carandas* Lill. grown in home gardens of Sri Lanka as an ornamental tree. The unripe fruit used in pickles whereas ripened ones in jams and other preserves and also used in traditional folk medicine to treat malaria, rabies, epilepsy and skin diseases. There is a remarkable advance in pharmacology of *C. carandas* where it possesses antibacterial and free radical scavenging activities. *Dialium guineense* Willd grows wild in the dry zone of the country producing large number of fruits during the season. The molluscicidal activity of the fruits and leaves of *D. guineense* have been reported (Odukoya *et al.,* 1996). The antioxidant potential of these fruits is still not fully explored. Therefore this study was carried out to evaluate the antioxidant activity and total phenolic content of selected three underutilized fruit species grown in Sri Lanka in order to find new potential sources of natural antioxidants.

II. MATERIALS AND METHODS

A. Collection and Preparation of Samples

S. nigrum, C. carandas and *D. guineense* fruits were collected from Regional Agriculture Research and Development Centre (RARDC), Bandarawela, Agriculture Research station, Maduruketiya and natural habitat in Moneragala, respectively. All fruits were transported to the laboratory in RARDC, Bandarawela under cold conditions and cleaned with clean water and then distilled water, allowed to drain. Aqueous extracts of fruits were obtained by extracting accurately measured and finely ground fresh fruit samples with distilled water for 90 min followed by centrifuging at 4500 rpm. The supernatants were collected separately and the extraction was repeated twice with residues. The collected supernatants were combined and lyophilized, stored below 0 °C until further analysis.

B. Chemical Regents

Folin-Ciocalteu's phenol reagent, gallic acid, sodium carbonate, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-Azobis (2-amidinopropane) dihydrochloride (AAPH), 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) were purchased from Analytical Instruments Limited, Sri Lanka.

C. Quantification of Total Phenolic Content

Total phenolic content (TPC) was determined colorimetrically using Folin-Ciocalteu's method as described by Yu *et al.* (2002) with slight modifications. To 20 µl of each sample in three replicates, 100 µl of 2 N Folin-Ciocalteu regent and 1.58 ml of distilled water were added, shaken vigorously, incubated at room temperature for 8 minutes, 300 µl of 0.7 M $NaCO_3$ was added and incubated at room temperature for 30 minutes. The absorbance of all samples were measured at 765 nm using a Helios Omega – UV – VIS spectrophotometer. Results were expressed as milligrams of gallic acid equivalent per gram of fresh weight (mg GAE/g FW) by computing with the standard calibration curve constructed for different concentrations of gallic acids.

D. Determination of Antioxidant Potential

1) DPPH radical scavenging activity: Antioxidant potential was determined by using DPPH radical scavenging assay according to the method described by Lan *et al.* (2007) with some modifications. Six different concentrations of each sample were prepared ranging from 10 mg/ml to 0.5 mg/ml and 0.2 ml of each was added to 1.8 ml of 0.1 mM methanolic DPPH solution. After leaving for an hour in the dark at room temperature, the absorbance was read at 517 nm using Helios Omega – UV – VIS spectrophotometer. The control was prepared by adding 0.2 ml of methanol into 1.8 ml of DPPH radical.

The percentage of radical scavenging activity (RSA) was calculated as follows;

$$RSA\% = \{1- (A_{sample}/A_{control})\} * 100$$

Where A_{sample} = Absorbance of sample at 517 nm
$A_{control}$ = Absorbance of blank at 517 nm

The results were expressed as IC_{50} values that denote the concentration of the sample required (mg of fresh weight/ml) to scavenge 50% of DPPH radicals.

2) ABTS radical scavenging capacity: The ABTS assay was performed according to the previously reported method by Zhou and Yu (2004) with some modifications. To obtain working solution, 2.5 mM ABTS and 2 mM AAPH in PBS buffer solution (pH 7.4) were mixed in 1 : 1 ratio and incubated at 60 ^{0}C in a shaking water bath to obtain bluish green colour with the absorbance between 0.3 and 0.5 at 734 nm. To 40 µl of each sample, 1.96 ml of working solution was added and absorbance was measured over six minutes at one minute interval at 734 nm using Helios Omega – UV – VIS spectrophotometer. The RSA was calculated as percentage inhibition of $ABTS^+$ radical.

E. Statistical Analysis

Data were reported as mean ± SD for triplicate determinations. Analysis of variance and least significant difference tests were conducted to identify differences among means using SAS 9.1.3 statistical software. Statistical significance was declared at p = 0.05.

III. RESULTS AND DISCUSSION

Total Phenolic Content (TPC)

Total phenolic content (TPC) was determined by Folin-Ciocalteu's method, based on the reduction of metal oxides by phenolic acid resulting in a blue solution that has an absorption maximum at 765 nm (Farrawati *et al.,* 2012). The TPC was calculated using regression equation of calibration curve (Y = 0.0149X; R^2 = 0.9673). Phenolics are the largest group of phytochemicals and have been reported to account for most of the antioxidant activity of plants (Ogu *et al.* 2013). The TPC varied among species from 1.16 ± 0.049 mg GAE/g FW of *D. guineense* to 3.12 ± 0.43 mg GAE/ g FW of *S. nigrum* which shown the highest (Figure 1).

All three fruit species contained high TPC compared to most commonly available fruits. Mango recognized as "king of the fruits" reported to have TPC of 1.39 - 0.32 mg GAE/g FW (Liu *et al* 2013). Luximon-Ramma *et al.* (2003) illustrated that the TPC of pineapple, mango, papaya and litchi are 47.9, 56.0, 57.6, and 28.8 mg/100g, respectively. TPC of methanolic extracts of Fruit, fruit peel, and fruit pulp of *C. carandas* were 14.8 ± 0.3, 10.5 ± 0.4 and 13.4 ± 0.5 mg GAE/g respectively (Dhan *et al.,* 2011). TPC of *D. guineense* reported 5.79 ± 6.013 mg GAE/g of fruit (Aline *et al.,* 2008). Methonolic extract of S. nigrum fruits reported TPC of 5.73 mg GAE/g (Veeru *et al.,* 2009). It was found that the yield in total phenols depends on the method and the choice of solvent (Goli *et al.,* 2005). The phenolic compositions of plant tissues are reported to be varying considerably with seasonal, genetic, and agronomic factors (Hilton and Palmer-Jones, 1973). In addition, a large variability at different stages of maturation and growing conditions such as temperature and rainfall is known to affect the TPC (Wang and Zheng, 2001).

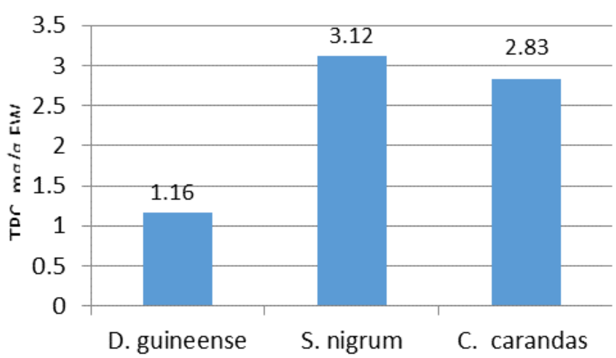

Figure 1. Total Phenolic Content (TPC) of three fruit species

Antioxidant Potential

Results of DPPH radical scavenging activity assay were expressed as IC_{50} values that denote the concentration of the sample required to scavenge 50% of $DPPH^{+}$ radicals. The weight of fresh fruit needed for IC_{50} values of *S. nigrum, C. carandas* and *D. guineense,* were 12.99^{a} ± 0.12 mg, 14.02^{b} ± 0.03mg and 14.99^{b} ± 0.11mg, respectively (Table 1).

Table 1. DPPH and ABTS radical scavenging activity of Fruits extracts

Fruit Species	IC_{50} Value mg of fresh fruit	ABTS inhibition percentage
D. guineense	14.99^{b} ± 0.11	23.27^{c} ± 1.0
S. nigrum	12.99^{a} ± 0.12	66.14^{a} ± 0.71
C. carandas	14.02^{a} ± 0.03	50.26^{b} ± 0.61

Figure 2 shows that inhibition of $ABTS^{\cdot+}$ by aqueous extracts of three species over six minutes. Among the three species, *S. nigrum* displayed the highest inhibition percentage (66.14 % ± 0.71) over six minutes in ABTS+ radical scavenging assay followed by *C. carandas* (50.26 % ± 0.61) and *D. guineense* (23.27 % ± 1.0) at a concentration of 4 mg/ ml. In both assays *S. nigrum* showed the highest antioxidant capacity and free radical scavenging activity. *S. nigrum* elaborates a wide range of pharmacological properties such as antioxidant, anticancer, hepatoprotective, neuroprotective and antiulcerogenic properties (Arulmozhi *et al.,* 2012). According to the study carried out by Karmakar *et al.* (2010) concluded that fruit extracts of *S. nigrum* contains reducing sugar, tannins, saponins, gums, steroids, alkaloids, and glycosides which have potential role in its' cytotoxic and antioxidant activity.

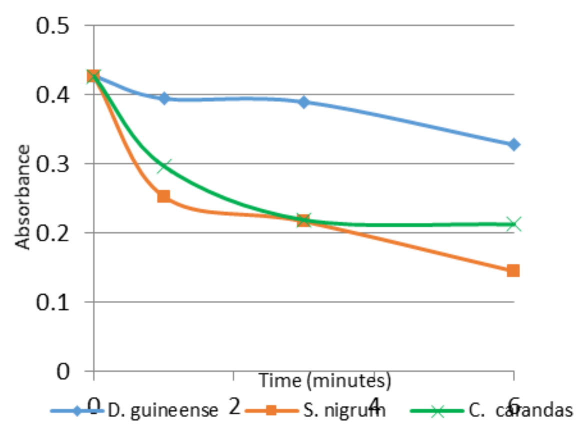

Figure 2. Inhibition of $ABTS^{+}$ over six minutes

IV. CONCLUSION

The results reveal that water extracts of *S. nigrum, D. guineense* and *C. carandas* show strong antioxidant activity. This study indicates that these underutilized fruits may bring about health benefits. In addition, the easy availability of these plants makes them promising sources of natural antioxidants and other bioactive compounds in food and pharmaceutical industries. However, further research is needed to identify individual components forming antioxidative system and develop their applications for food and pharmaceutical industries.

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