Evaluation of Sri Lankan Wild Fruits based on Free Radical Scavenging Activity, Polyphenolic Content and Cytotoxic Activity

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Abstract: *The study of free radical chemistry has been of recent interest in the scientific community and represents an emerging paradigm in nutraceuticals and disease management. The objective is to incorporate phytochemicals into nutraceutical preparations as an alternative to natural antioxidants, which are being phased out due to possible health hazards and toxicity. This study examined the free radical structure, phenolic content, and cytotoxic nature of different wild fruits (Syzygium caryophyllatum, Careya arborea, and Mangifera zeylanica) in Sri Lanka. Hexane (Hex) ethyl acetate (EA) and aqueous (AQ) fractions were fractionated from crude methanolic extracts (CR) of fruits and assessed for antioxidant activity by 1-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) tests. The results revealed that EA and AQ fractions of Careya arborea fruit showed the highest values for DPPH radical scavenging activity, and CR and EA showed the highest ferric reducing power significantly compared to other solvent fractions. The total phenolic capacity of the evaluated fruit species ranged from 22.8 to 285.3 mg GAE/g dry weight. The present study revealed a strong correlation between free radical scavenging activity and total phenol activity, representing an R^2 = 0.9989 value. Moreover, neither plant extracts nor fractions were toxic to a normal Vero cell line. Thus, it was concluded that Syzygium caryophyllatum, Careya arborea, and Mangifera zeylanica species are positive free radical resources*.

Keywords: *free radicals, antioxidants, cytotoxicity*

1. Introduction

Free radicals are the specific oxygen-containing chemical groups that have few unpaired electrons in the external shell. In general, they are highly reactive and exceedingly unstable (Tawaha et al., 2007). since they act as oxidants or reductants by donating electrons or receiving electrons. Hydroxyl radical, hydrogen peroxide, superoxide anion radical, hypochlorite, oxygen singlet,

In particular, nitric oxide radicals and peroxynitrite radicals are particular free radicals with oxygen molecules responsible for the disease (Lobo et al., 2010). Numerous studies suggest that free radicals have a remarkable role in the development of several diseases such as cardiovascular diseases, carcinogenesis, arthritis, aging, ischemia, Alzheimer's and Parkinson's disease, etc. (Bagchi et al., 2000). Antioxidants are special substances that interact with free radicals in a way that can prevent them from damaging biological components. A diverse variety of synthetic and biological antioxidants have been shown to have therapeutic impacts on human

health owing to their encountering different free radicals (Radicals, 2018). Due to the examination of the high toxicity and other harmful benefits of synthetic antioxidants, including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), etc., there is a current tendency to study natural plant-based antioxidants as an alternative natural remedy for various diseases.

Sri Lanka is enriched with a variety of wild fruits, especially in comparison with other countries. Approximately 120 species of wild fruits have been noted, with most of them being distributed among the dry and intermediate zones. These trees are great sources of income apart from the fruit since most of the parts like the flowers, bark, leaves, roots, and seeds are used as an alternative medicine to treat various diseases. They have been used in folklore or Ayurveda traditional medicines for a long time ago (Weerasekara, Withanachchi, and Ginigaddara, 2018). Fruits are mostly targeted and have been considered while possessing a rich number of polyphenols and flavonoids. In addition, high quality and quantity of other secondary metabolites are believed to be rendering different pharmacological properties against non-communicable diseases (NCDs). Although the underutilized wild fruit species are known to be having high unrecognized nutritional value and medicinal properties, having a demand to utilize them because fruits and fruit plant parts have not been assessed.

The main intention of this study was designed to screen underutilized wild fruit species in Sri Lanka with a view to assessing their total phenolic activity, free radical scavenging capacity, and toxicity level by static investigation. For the study, Syzygium caryophyllatum, Careya arborea, and Mangifera zeylanica fruits were selected as testing species based on their revealed properties.

Syzygium caryophyllatum (L.) Alston (Heen Dan), of the family Myrtaceous, endemic to Sri Lanka and India, acquires advantageous remedial properties such as anti-cancer, anti-oxidation, anti-inflammatory, etc. (Shilpa, Krishna Kumar and Dc, 2015). And Careya arborea Roxb (Kahata), of the family Lecythidaceae, is native to India. Afghanistan and Sri Lanka. It is a famous herbal remedy, widely used for skin diseases, ulcers, cough, genito-uterine diseases, etc. (D and G, 2019).

Mangifera zeylanica (Etamba), known as “Sri Lankan Mango,” is an endemic, rare wild fruit in Sri Lanka, in the family of Anacardiaceous that has been designated as' Vulnerable 'in the IUCN Red Listed Species (Weerarathne, Samarajeewa, and Nilanthi, 2005). Mangiferin is derived from the bark and has the promising potential to fight against cardiac and cancerous diseases. There are several studies that examined the nutritional, pharmacological, physiochemical, and antimicrobial characteristics of this selected wild fruit’s various parts (leaves, bark, roots, fruit, etc.), whereas the antioxidant, polyphenolic content, and cytotoxicity profile of this S. caryoplyllatum, C. arborea, and M. zeylanica specie’s edible parts have not been revealed completely. Hence, the present study evaluates the crude, hexane, ethyl acetate, and aqueous fractions free radical potency and toxicity of the above-mentioned plant species.

2. Methodology and Experimental Design

A. Plant materials

The edible part of the selected wild fruit plants (S. caryophyllatum, C. arborea and M. zeylanica) were collected from different geographical areas in Sri Lanka and were cleaned, freeze-dried and ground to find powder by laboratory mill. Each material was given identification number and stored in – 20 °C freezer prior to the analysis.

B. Preparation of plant extracts and fractions

The 40g of selected Sri Lankan selected underutilized wild fruits namely S.

caryophyllatum L; Family-Myrtaceae fruit, C. arborea; Family-Lecythidaceae and M. zeylanica Lyophilized samples were extracted in to 100 % methanol by using sonication three times each for 90 minutes and filtered through Whatman No. 1 filter paper. Using the rotary evaporator, the collected extracts were evaporated to get dry material and known amount of sample was dissolved in DMSO for further analysis. Then, the extracted samples were partitioned into nonpolar and polar solvents with increasing polarity, hexane, and ethyl acetate, respectively. This solvent–solvent partitioning procedure was allowed to generate hexane (Hex) fraction, ethyl acetate (EA) fraction, and aqueous (AQ) fraction which was labelled and stored in −20°C prior to analysis.

C. DPPH Radical Scavenging Activity Assay

1,1-diphenyl-2-picryl- hydrazyl (DPPH) stable radical used to determine the radical scavenging activity. According to this procedure, purple coloured 1,1-diphenyl-2-picrylhydrazyl converted to a yellow-coloured diphenyl picrylhydrazine. Based on the method (Loo, Jain and Darah, 2007; Qader et al., 2011) with slight modification, stock solution (1 mg/1 mL) of the selected fruits fractions and Gallic acid as antioxidant standard was prepared and then diluted to get five different concentrations. A quantity of each plant extract (5 µL) and standards were mixed with DPPH (195 µL). The incubation period for the assay 30 min at 37 °C for 30 min. The absorbance value was measured spectrophotometric ally by a UV at 517 nm.

D. Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay was performed according to the method of and P. Ranasinghe (Wimalasiri et al., 2016), (Ranasinghe et al., 2012), (Pathiranage et al., 2020). The assay procedure was according to the reduction of Fe3+ to Fe2+ by the electron donation ability of antioxidant compound which later form the intense blue coloured complex (Fe2+-tripyridyltriazine). The freshly prepared FRAP reagent was mixed with acetate buffer (300 Mm, pH 3.6) solution of TPTZ in HCL and FeCl3 (20 mM) in 10:1:1 ratio and incubated at 37°C. The reaction volume of 200 µl containing FRAP reagent and sample was incubated at room temperature and absorbance was measured at 600 nm. As the reference standard; Vitamin E analogue trolox were examined and the results presented as mg of trolox equivalent per gram of extract (mg TE/g of extract).

E. Estimation of Total Polyphenol Content

The byusingfolin-ciocalteu method including slight alterations used to evaluate total polyphenol content (TPC) in this study (Lamuela-ravents, 1999; Pathiranage et al., 2020) that was used to 96 well microplates other than 6 well plates. The FC reagent should be prepared freshly. 110 µl of FC reagent was added with 20 µl of sample. Then, the pre-plate reading was measured and after that treated with with 10 % sodium carbonate and incubated at room temperature for 30 minutes and absorbance was measured at 765 nm. Gallic acid was used as reference standard and results presented as milligram gallic acid per equivalent per gram of sample (mg GAE/g of sample).

F. Cytotoxicity Assay

The cytotoxic activity of crude, hexane, Ethyl acetate and aqueous fractions of three selected Sri Lankan wild fruits were carried out, using Methyl Tetrazolium -MTT colorimetric assay (Qader et al., 2011; Das and Devi, 2015; Aslantürk, 2018).

The Vero (isolated from kidney epithelial cells extracted from an African green monkey) monolayer cells were seeded in a 96-well plate using DMEM supplemented with 10% FBS, the cell amount per ml was calculated as to 1.0×10^5 cells. Then, started 24hr incubation period under 37 °C under 5% CO_2 in an atmosphere before the addition of plant extract. Each diluted

extracts, ranging from 50 µg/ml to 200 µg/ml, were treated to the 96-well plate in triplicate and incubated under the similar environmental conditions. After 72 h, the treated samples in the wells were discarded and 50 µl of MTT dissolved in PBS was added to each

Table 1. Antioxidant properties and total phenolic content (TPC)of Crude, Hexane, Ethyl acetate and Aqueous fractions of three selected Sri Lankan wild fruits.

Plants	**Fractions**	**DPPH%**	**FRAP (mg TE/g)**	**TPC(mg GAE/g)**
Syzygium caryophyllatum	CR	45.71±0.04	8.95±2.12	12.51±0.09
	HEX	18.21±0.01	47.5±0.04	5.43±0.53
	EA	56.78±2.87	51.62±0.01	2.57±1.21
	AQ	34.37±0.31	18.43±1.14	7.48±0.03
Careya arborea	CR	92.25±0.98	347.34±2.30	231.26± 3.16
	HEX	79.54±6.25	117.58±0.07	61.3±5.31
	EA	95.51±0.63	261.84±0.00	126.41± 0.02
	AQ	115.74±4.15	165.27±1.02	118.72±1.42
Mangifera zeylanica	CR	63.21±0.53	97.52±2.53	76.42±0.01
	HEX	24.17±1.90	64.38±0.52	15.81±0.23
	EA	59.01±4.55	125.72±1.03	35.67±0.46
	AQ	38.5±0.87	100.42±0.05	61.38±0.03
Gallic acid		92.05±0.15	_	_

Each value represents mean ± SD. treated well. Then the plates were kept in orbital shaker for incubate again for 3 h. The absorbance was measured using a microplate reader at a wavelength of 540 nm.

G. Evaluation of DPPH Scavenging Activities

DPPH free radical scavenging activity of Crude, Hexane, Ethyl acetate and aqueous fractions of three selected fruits were examined to evaluate their free radical activity. The results are presented in Table

The DPPH free radical scavenging activity assessed with the control sample and three different fruit fractions are presented as a percentage of inhibition. As showed in Table 1. AQ fraction of *C. arborea* fruit species exhibited highest DPPH radical scavenging percentage as 115.74±4.15. Respectively, EA and CR fractions of *C. arborea* showed significantly high amount of DPPH % inhibition values following 95.51±0.63 and 92.25±0.98values.EA and AQ fractions of *S. caryophyllatum* and *M. zeylanica* reported similar % inhibition values.

Significantly positive relationship among the TPC and DPPH % scavenging activity of tested Sri Lankan fruit's crude, hexane, ethyl acetate and aqueous fractions showed in Figure 1. Based on the present date, the crude fraction of every tested species have showed strong

antioxidant potential, R^2 = 0. 997.And hexane, ethyl acetate and aqueous fractions following R^2=0.9924, R^2=0.945 and R^2=0.805 respectively responsible for inducing antioxidant capacity. This is supported by Pathiranage and et al. (Pathiranage *et al.*, 2020), who demonstrated that the TPC verses DPPH positive corelationship of S*. caryophyllatum* has showed R^2 =0.9921.

H. Ferric Reducing Antioxidant Power (FRAP)

The Ferric reducing capacity of the evaluated Sri Lankan wild fruit plants is also showed in Table 1. There is revealed that considerable variances in the reducing capabilities of the three plant species. The FRAP values of the fractions ranged from 8.95±2.12 mgTE/g of the extract to 347.34±2.30 mgTE/g of extract. The FRAP values 347.34±2.30 and 261.84±0.00 mgTE/g of C. arborea presented the higher reducing power for both crude and ethyl acetate fractions, respectively. Where the Hexane fractions of all tested wild fruit samples showed the lowest ferric reduction capacity. Since these results exhibited that the crude extract and ethyl acetate fractions have higher reducing activity, which highlight the significant corelationship among the polar molecules and reducing power. It is also revealed that there is a strong correlation between TPC and FRAP, the relationships between each fraction showed in Figure 2. According to the tested linearity curves, the crude fraction exhibited a higher corellationship among TPC and FRAP activity, R^2 =0.9989. The DPPH and FRAP radical scavenging activity of C. arborea all fractions (CR>AQ>EA>HEX) reported highest antioxidant activity, Since the positive amount of phenolic and other responsible compounds.

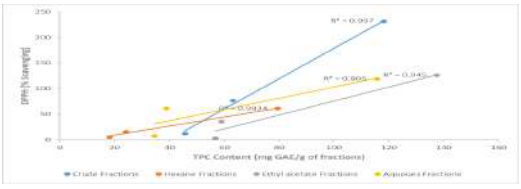

Figure 1. Correlation between selected fruits CR, HEX, EA and AQ fractions TPC content verses DPPH (% scavenging)

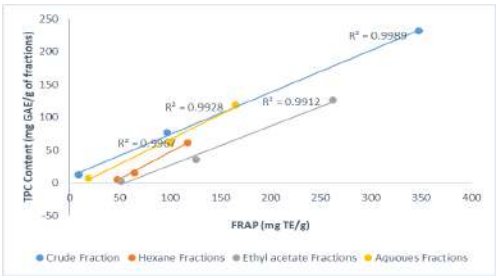

Figure 2. Correlation between selected fruits CR, HEX, EA and AQ fractions TPC content verses FRAP reducing power

I. Total Phenolic Content (TPC)

Total polyphenol content was measured as milligram Gallic acid equivalent per gram of sample (mg GAE/g). Total polyphenol values varied from 2.57±1.21 mg GAE/g of sample to 12.51±0.09 mg GAE/g of sample, from 61.3± 5.31 mg GAE/g of sample to 231.26± 3.16 mg GAE/g of sample and from 15.81±0.23 mg GAE/g of sample to 76.42±0.01 mg GAE/g of sample for, *S. caryophyllatum*, *C. arborea* and *M. zeylanica* extracts/fractions respectively. Table 1 shows the Polyphenolic activity of each plant. *C.arborea* was found to have the highest TPC values 231.26±3.16 mg GAE/g and 126.41± 0.02 mg GAE/g in both crude and ethyl acetate fractions, respectively. Based on (D and G, 2019) data, *C. arborea* leaves ethanolic and ethyl acetate extracts exhibited 33.03±1.39 and 26.36±2.40 mg GAE/g TPC content. Therefore, it has clearly showed that *C. arborea* fruit contain considerably high amount of TPC content over leaves. As showed from Table 1 that the TPC for most of the crude and aqueous fractions were

recorded as higher than hexane and ethyl acetate fractions. This is similar to the findings of Annadurai and co-workers and Pathiranage and co-workers (Annadurai *et al.*, 2012; Pathiranage *et al.*, 2020) that recorded higher TPCs activities in crude and aqueous fractions compared to hexane and ethyl acetate fractions.

J. Cell Cytotoxicity

The results of cytotoxic activity of selected Sri Lankan three wild fruits species were showed in Figure 3,4,5 and 6 and were exhibited % cell viability versus three different dosages (50 µg/ml ,100 µg/ml and 200 µg/ml) with control sample. As can be observed, none of the sample fractions showed below 70% cell viability against normal Vero cells. Therefore, Different fruits fractions presented varies amount of % cell viability. Crude fractions of *S. caryophyllatum, C. arborea* and *M. zeylanica* species reported 78.46 to 94.99 % cell viability dose depending manner. In every fraction highest % cell viability were noted in lowest concentration (50µg/ml).

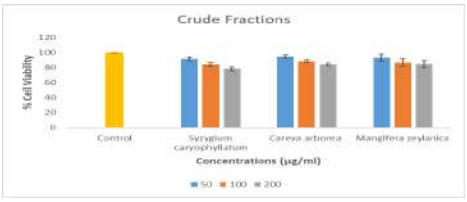

Figure 3. Cytotoxity effect of Crude Fractions for three selected wild fruits (*S. caryophyllatum, C. arborea and M. zeylanica)*

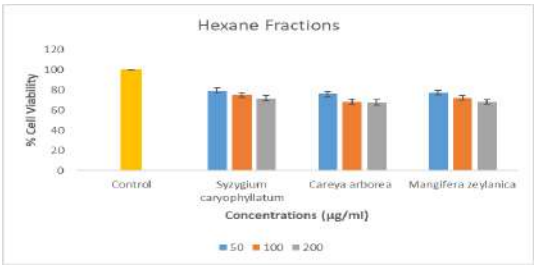

Figure 4. Cytotoxity effect of Hexane Fractions for three selected wild fruits (*S. caryophyllatum, C. arborea and M. zeylanica*)

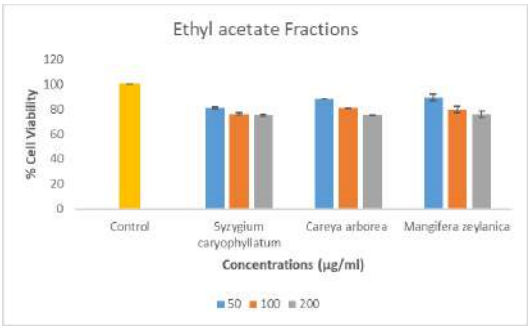

Figure 5. Cytotoxity effect of Ethyl Acetate Fractions for three selected wild fruits *(S. caryophyllatum, C. arborea and M. zeylanica*)

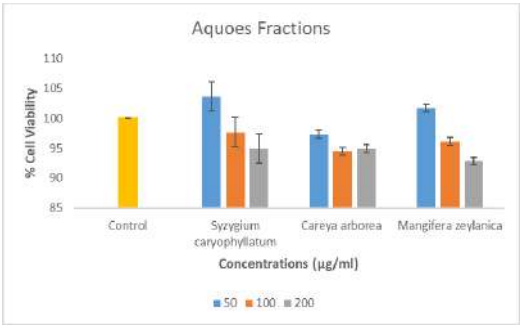

Figure 6. Cytotoxity effect of Aqueous Fractionsfor three selected wild fruits (*S. caryophyllatum, C. arborea and M. zeylanica*)

4. Conclusions

Based on the examined data and the previously revealed findings, we can conclude that the EA and AQ fractions of C. arborea Roxb. have the highest antioxidant and total phenolic activity among S. caryophyllatum and M. zeylanica species. The strong relationship between DPPH vs. TPC and FRAP vs. TPC further confirmed the prominent antioxidant activity of the tested three wild fruit fractions. The findings also revealed that there is no toxicity of selected fruit fractions against the Vero cell line. Since they exhibited high antioxidant and high cell

viability, S. caryophyllatum, C. arborea, and M. zeylanica can be used as a natural free radical agent with enriched high phenolic activity in future analysis and nutraceutical products. Therefore, further study is required to evaluate the particular active component responsible for this significant antioxidant activity.

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Abbreviations and Symbols

CR = Crude
HEX = Hexane
EA = Ethyl acetate
AQ = Aqueous

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