

Full Paper

Evaluation of Extraction Yield, Total Phenolic Content, and Total Flavonoid Content of Thirteen Underutilized Fruits in North Central Province of Sri Lanka

Shashikamali W.S. Walimuni^a, Ilandarage M.N. Molagoda^b, Rajapakshagedara G.P.T. Jayasooriya^c, and K.K.A. Sanjeewa^{d,*}

Corresponding Author: asankasanjeewa@sjp.ac.lk

Received: 10 December 2024; Revised: 02 January 2025; Accepted: 05 January 2025; Published: 13 July 2025

Abstract

Sri Lanka is a biodiversity hotspot, rich with numerous fruit crops. However, most of them remain underutilized or neglected especially, in terms of industrial uses, the bioactivity of these fruit plants metabolites is largely unexplored and most of them are highly seasonal. The present study is focused on the extraction of bioactive compounds from 13 different underutilized fruits including Elaeocarpus serratus L., Aegle marmelos, Phyllanthus emblica L., Limonia acidissima, Psidium guineense, Annona reticulata L., Phyllanthus acidus, Pouteria champechiana (kunth) Baehni, Flacourtia indica Burm. f) Merr.) Syzygium cumini L. Skeels, Morus alba var. indica, Passiflora foetida L., and Ficus racemosa L., and screen their bioactivity potential to increase awareness for its usage. Fruits were dried using the heat pump drying method at 40 °C for 15-20 hours. The dried powder was extracted using the cold maceration method in 70% ethanol in a 1:20 ratio for 24 h in a mechanical shaker with stirring at 125 rpm at 30 °C, repeated thrice, and vacuum filtered. The solvent was evaporated in a rotary evaporator at 25 rpm, a pressure of 180 mbar. According to the results, the extraction yield percentage ranged from $15.73 \pm 1.67\%$ to $55.05 \pm 0.04\%$ and the highest yield was reported for *P. acidus* and the lowest yield for the F. racemosa L.. Total Polyphenolic Content (TPC) of extracts ranging from 7.94 ± 0.70 to 330.80 ± 6.26 mg Gallic Acid Equivalent/g (Dry Weight), the highest TPC showed in P. emblica L. and the lowest in P. champechiana fruits. Total Flavonoid Content (TFC) of extracts ranging from 1.88 ± 0.40 to 271.84 ± 1.91 mg Quercetin Acid Equivalent/g (Dry Weight), the highest TFC was shown in P. emblica L., and the lowest in L.acidissima fruits. These findings support the sustainable utilization of these fruits; however, further research is required to screen the specific bioactive properties of these extracts, which will enhance their industrial applicability.

Keywords: underutilized fruit, total polyphenolic content, total flavonoid content, ethanolic extraction yield, Sri Lanka

Introduction

Sri Lanka is a hub of biodiversity, rich with diverse fruit crop varieties. As a tropical island, its environmental conditions, including two major rainfall periods, high relative humidity, and consistently warm temperatures, create favorable conditions for the growth and production of fruit crops [1, 2].

^aFaculty of Graduate Studies, University of Sri Jayewardenepura, Gangodawila, Nugegoda 10250, Sri Lanka

^bCardiac Inflammation Research Group, Clinical Research Center, Lund University, Malmo 221 00, Sweden

Department of Bioprocess Technology, Faculty of Technology, Rajarata University, Mihintale 50300. Sri Lanka

dDepartment of Biosystems Technology, Faculty of Technology, University of Sri Jayewardenepura, Homagama 10206, Sri Lanka

However, despite their rich phytochemical profiles and safety, most of these fruit crops remain neglected, unrecognized, and underutilized, especially in terms of industrial uses. This less popularity is mainly due to less awareness of their safety and nutritional value [3]. Underutilized fruits represent an underexploited resource with substantial nutritional and pharmacological potential [4]. Notably, these minor fruits lack effective value-addition methods, and the bioactivity of some of their metabolites is largely unexplored. Additionally, most of these fruits are highly seasonal. Other than that, these fruits are highly perishable as they contain high water content and natural enzymatic activities, leading to rapid ripening and quick deterioration. They typically have a short postharvest shelf life at room temperature [4, 5]. For instance, *Morus alba* var. indica (mulberry fruit) and *Syzygium cumini* L. *Skeels* (java plum fruit). The fruits selected for this study are commonly found in home gardens, natural reserves, forests, and areas of limited commercial cultivation. Fruit plants are rich sources of bioactive compounds, including polyphenols, which have great potential for the development of different applications such as cosmeceuticals [6].

Nowadays, the consumption of products containing natural ingredients has increased significantly across various industries, including cosmeceuticals and nutraceuticals, as they tend to be safer, biodegradable, more environmentally friendly, and more effective compared to synthetic alternatives [7-9].

Polyphenol is a large and diverse group of abundant plant secondary metabolites. Recently, polyphenols have garnered more attention due to their potential health benefits to humans. Therefore, polyphenols have versatile applications in various industries such as pharmaceutical, nutraceutical, and functional food [10]. The consumption of underutilized fruits in Sri Lanka is significantly influenced by individual preferences, which vary based on taste, cultural practices, generational trends, the influence of modern lifestyle, knowledge of medicinal and health benefits, economic factors, accessibility, and convenience. In the rural areas of Sri Lanka, traditional practices are more prevalent and widely accepted than urban community due to their role in folk medicine and rituals [11, 12]. The fruits studied in the present research are native to tropical regions or the majority of them are indigenous to South Asia which are both edible and offer therapeutic benefits (Table 1).

Traditionally, Ceylon olive (*Elaeocarpus serratus* L.) leaves are used in anti-head lice and anti-dandruff treatments in rural areas of Sri Lanka, and the fruit is popular for curing the risk of diabetes [13, 14]. The fruits, seeds, and stem bark of Java Plum (*Syzygium cumini* L.) have been widely used in the treatment of diabetes by traditional practitioners over many centuries [15]. The decoction of the unripe fruit pulp of *Aegle marmelos* (Bael Fruit) is used as an antidiarrheal agent in an indigenous practice through generations by local communities [16]. Cluster Fig (*Ficus racemosa* L.) bark and decoction of leaves have been used to treat wound healing in Ayurveda and the indigenous systems of medicine in Sri Lanka [17]. Whereas Cluster Fig fruit is regarded as a good remedy for diabetes, diarrhea, and constipation [18]. Indian Gooseberry (*Phyllanthus emblica* L.) fruit has long been used for the treatment of numerous diseases in traditional Ayurveda including; s inflammation, diabetes, fever, cough, asthma, bronchitis, common cold, cephalalgia, ophthalmopathy, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, leprosy, anemia, hematogenesis, emaciation, hepatopathy, jaundice, diarrhea, dysentery, hemorrhages, leucorrhea, menorrhagia, cardiac disorders, skin diseases and premature greying of hair [19].

Star Gooseberry fruit is Ayurvedic for the treatment of urinary concentrations, bronchitis, biliousness, diabetes, and blood purification [20]. Furthermore, its roots are used to cure psoriasis; and root extract is used to treat headache, cough, and asthma [21]. Star Gooseberry (Phyllanthus acidus L.) leaf decoction is used as a medication for urticaria as an ethnomedicinal practices in Asian countries like India and the Philippines [22]. Besides, Custard Apple (Annona reticulata L.) plant is traditionally employed as a pain reliever, cardiac problem, fever, epilepsy, dysentery, constipation, parasite and worm infestations, bacterial infection, hemorrhage, dysuria, ulcer, and as an insecticide [23-25]. Bark serves as a powerful astringent and is used as a tonic, whereas leaves were employed for helminthiasis treatment. In folk medicine, wood apple (Limonia acidissima L.) fruit juice has been used for detoxification via blood purification and removal of toxins from the body. The leaves with milk and sugar are used as a remedy for intestinal problems and are traditionally used in snake bites and against bacterial pathogens [26, 27]. Guava (Psidium guineense) leaves have been traditionally used for hair care in Sri Lanka. Besides, it is employed for diarrhea and toothaches, and used as an antiseptic. Further, leaves and bark of P. guineense are taken to treat skin ailments, gastrointestinal problems, stomach upsets, menstrual period, rheumatism, epilepsy, cholera, sore throat, ulcers, bowel health, and used in weight loss formula [28, 29]. Flacourtia indica (Burm. f) Merr., or the governor's plum fruit plant, is widely used as a traditional medicine. For instance, dried leaves are used to treat asthma, bronchitis, and hepatic disorders. It has also been reported for the treatment of rheumatoid arthritis, fever, and respiratory infections, and fruit is used to cure enlarged spleen, appetizing, diuretic, and digestive problems [30-32]. The plant Passiflora foetida L. is commonly referred to as the "stinking passion flower" used in traditional medicine to treat diarrhea, rheumatism, inflammation, abdominal pain, ear infections, fever, and skin diseases [33]. Globally, mulberry leaves have been exploited for silkworm rearing, and other parts of the plant have not been utilized on an industrial scale. Mulberry fruit has tremendous potential for developing various industrial products, for example, the fruit can be used to develop food colorants as it contains a high content of anthocyanins [34]. Besides, the leaves are used to treat skin issues [35, 36]. Pouteria champechiana (kunth) Baehni is commonly known as 'Canistel', the parts of this plant have been used in folk medicine for centuries. The canistel bark is utilized as an antipyretic agent, to treat fevers and skin eruptions, unripe fruit is used for controlling diarrhea, and seeds are taken as a remedy for ulcers [37, 38].

Existing studies have investigated that the phytochemicals present in plant parts, including fruits, exhibit synergetic pharmacological activity to improve human health and immune function, which is directly correlated with a reduced risk of Non-Communicable Diseases (NCDs) [39, 40]. The combination of polyphenols with existing drugs and therapies could be a practicable and promising approach to achieving better results while significantly reducing their toxicity. Using a blend of various polyphenols, which possess synergistic effects, resulting in a lower required therapeutic dose and offering multitargeted action. Apart from that, these bioactive compounds could be employed as potential drug candidates to treat several diseases. Identifying phytonutrients and investigating their pharmacological and therapeutic potential is a timely need. These types of research could increase the industrial applicability and awareness among the people, leading to effective sustainable utilization of these naturally available resources.

The objective of this study is to evaluate the effects of ethanolic extract yield of fruit and leaf and to investigate the Total Polyphenolic and Total Flavonoid Content of the selected underutilized fruit plants (Table.2). We believe that the identification of novel potent candidates for pharmaceutical, nutraceutical, and functional food industry, increase the awareness among the people and establishment of suitable cultivation system for the identified plants. Other than that, it assures the development and sustainable utilization of those fruits and increases their applicability in various industries in Sri Lanka.

Table 1. Traditional/folk medicinal use of the selected underutilized fruits

Name of the	af tha				
Fruit	Plant Part/s	t Part/s Traditional or Folk Medicinal Usage/s			
Carrian Oliva	Leaves	Anti-head lice, anti-dandruff treatments	[13]		
Ceylon Olive	Fruit	Curing diabetes	[14]		
Java Plum	Fruits, Seeds, Stem Bark	Treatment of diabetes	[15]		
Bael Fruit	Unripe Fruit Pulp	Antidiarrheal agent	[16]		
Bark/ Leaves Wound healing		Wound healing	[17]		
Cluster Fig	Fruit	Remedy for diabetes, diarrhoea, and constipation	[18]		
Indian Gooseberry	Fruit	Diabetes, asthma, ulcers, anaemia, and skin diseases	[19]		
Star Coocabarre	Fruit	Treatment of urinary concentrations, bronchitis, diabetes, and blood purification	[20, 22]		
Star Gooseberry	Roots	Remedy for psoriasis, headaches, and asthma	[21]		
	Leaf Decoction	Treatment for urticaria	[22]		
Custard Apple	Whole Plant	Pain reliever, cardiac problems, fever, epilepsy, dysentery, constipation, bacterial infections, ulcers, and act as insecticide	[23-25]		
	Bark	Astringent and tonic			
_	Leaves	Treatment for helminthiasis	[25]		
	Fruit Juice	Blood purification, removal of toxins from the body	[26]		
Wood Apple	Leaves with Milk and Sugar	Milk Remedy for intestinal problems, snake bites, and bacterial			
	Leaves	Hair care, diarrhoea, toothaches, and antiseptic	[28, 29]		
Guava	Bark	Treatment of skin ailments, gastrointestinal problems, menstrual issues, and weight loss	[28, 29]		
C / M	Dried Leaves	Antidiarrheal agent Wound healing Remedy for diabetes, diarrhoea, and constipation Diabetes, asthma, ulcers, anaemia, and skin diseases Treatment of urinary concentrations, bronchitis, diabetes and blood purification Remedy for psoriasis, headaches, and asthma Treatment for urticaria Pain reliever, cardiac problems, fever, epilepsy, dysentery constipation, bacterial infections, ulcers, and act as insecticide Astringent and tonic Treatment for helminthiasis Blood purification, removal of toxins from the body Remedy for intestinal problems, snake bites, and bacterial pathogens Hair care, diarrhoea, toothaches, and antiseptic Treatment of skin ailments, gastrointestinal problems, menstrual issues, and weight loss Remedy for asthma, bronchitis, and hepatic disorders Treatment for enlarged spleen and digestive problems Treatment of diarrhoea, rheumatism, inflammation, abdominal pain, ear infections, fever, and skin diseases Treatment for skin issues Industrial food colorant	[31, 32]		
Governor's Plum	vernor's Plum	Treatment for enlarged spleen and digestive problems	[30]		
Stinking Passion Flower	Whole Plant		[33]		
N.C 11	Leaves				
Mulberry	llberry Fruit Industrial food colorant		[34, 35]		
	Bark	Antipyretic agent, treatment for fevers and skin eruptions	[41]		
Canistel	Unripe Fruit				
		<u> </u>			

Materials and Methods

This study selected a range of underutilized fruits native to the North Central Province of Sri Lanka based on their traditional use, seasonal availability, and limited scientific exploration. Representative images (Figure 1) of these fruits are provided below to aid in their identification and to highlight their morphological diversity.

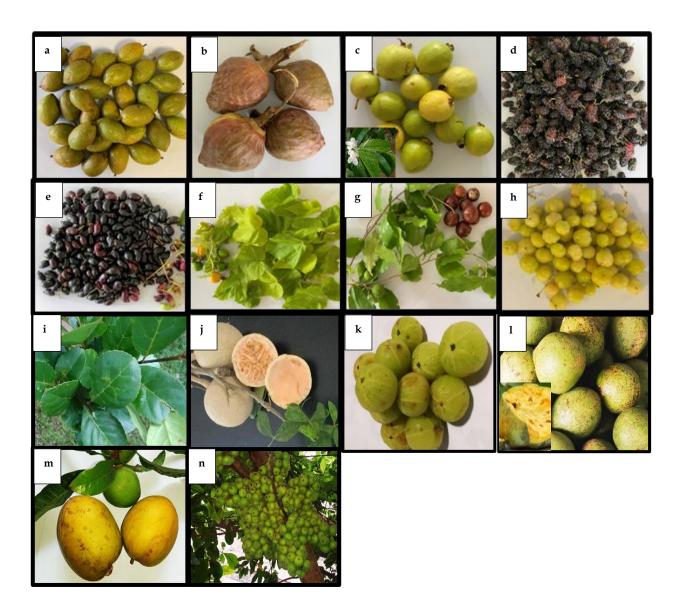


Figure 1. Selected underutilized fruits; (a,i) Elaeocarpus serratus L.fruit and leaves, (b) Annona reticulata L. fruit (c) Psidium guineense, fruit, (d) Morus alba var. indica fruit, (e) Syzygium cumini L. fruit, (f) Passiflora foetida L. fruit, (g) Flacourtia indica fruit, (h) Phyllanthus acidus L. fruit, (j) Limonia acidissima fruit, (k) Phyllanthus emblica L. fruit, (l) Aegle marmelos fruit, (m) Pouteria champechiana (Kunth) Baehni fruit, and (n) Ficus racemosa L. fruit.

Sample Collection

Different parts of underutilized ripe fruits were collected from a rural area in the North Central Province (Table 2). The fruit and leaf samples were thoroughly washed, and the seeds were removed from the fruits. The fruit pericarp and leaves were then dried using a heat pump dryer (MEIYA DRYER, WKD6070W) at 45 °C until a constant weight was obtained [43]. Plant samples were pulverized using a kitchen grinder into a fine powder.

Table 2. Selected Underutilized Fruits

Scientific name	Plant Family	Common name	Vernacular name	Plant Part/s	Collected location
Elaeocarpus serratus L.	Elaeocarpaceae	Ceylon Olive	Veralu	Pericarp/Leaves	Mekichchawa
Morus alba var. indica	Moraceae	Mulberry	Mulberry	Whole fruit	Rathmale
Annona reticulata L.	Annonaceae	Custard Apple	Weli Anoda	Pericarp	Vavniya
Phyllanthus acidus (L.) Skeels	Phyllanthaceae	Star Gooseberry	Mal nelli	Pericarp	Wijepura
Syzygium cumini L. Skeels	Myrtaceae	Java Plum	Madan	Pericarp	Nekatunuwewa
Ficus racemosa L.	Moraceae	Cluster Fig	Attikka	Whole fruit	Nekatunuwewa
Pouteria champechiana (Kunth) Baehni	Sapotaceae	Canistel/Egg Fruit	Lawulu	Pericarp	Mekichchawa
Passiflora foetida	Passifloraceae	Stinking passion flower	Padagedi	Whole fruit	Nekatunuwewa
Flacourtia indica (Burm. f) Merr.)	Salicaceae	Governor's Plum	Uguressa	Whole fruit	Kannattiya
Psidium guineense	Myrtaceae	Guava-White flesh	Jam pera	Whole fruit	Nekatunuwewa
Phyllanthus emblica L.	Phyllanthaceae	Indian Gooseberry	Beheth Nelli	Pericarp	Anuradhapura
Aegle marmelos	Rutaceae	Bael Fruit	Beli	Pericarp	Mihintale
Limonia acidissima L.	Rutaceae	Wood Apple	Diwul	Pericarp	Mihintale

Ethanolic extraction

Ethanolic extraction was selected as a suitable method for isolating bioactive compounds from the selected underutilized fruits due to its efficiency, safety, and compatibility with both polar and non-polar phytochemicals. Ethanol is a food-grade solvent that's commonly used in the extraction of phenolics and flavonoid compounds. Therefore, this method was applied to obtain crude fruit extracts for subsequent phytochemical and bioactivity analysis.

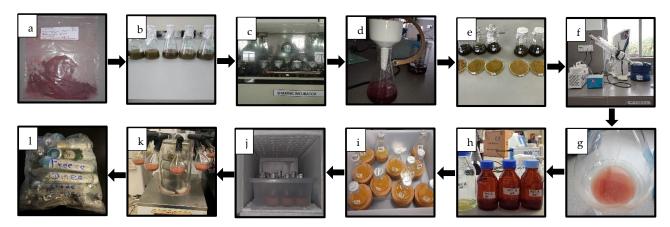


Figure 2. Steps in Ethanolic extraction of fruit samples; (a) pulverizing the fruit, (b) preparing an extraction mixture (70% ethanol + Fruit powder, 1:20 ratio), (c) shaking the mixture, (d) vacuum filtering the samples, (e) extract, (f) concentrating the extract using a rotary evaporator, (g,h) concentrated sample (i) transferring the concentrated extract to a freeze-drying flask, (j) Storing samples at – 80 °C for a day, (k) freeze-drying the samples, and (l) storing the freeze-dried crude extract at -20 °C.

As shown in Figure 2, the dried fruits were pulverized into fine powder. Five grams of the powdered fruit sample were soaked in 100 mL of 70% ethanol (1: 20) [44, 45]. Then, the mixture was extracted for 24 h in a mechanical shaker with constant stirring at a speed of 125 rpm and a controlled temperature of 30 °C. Then, the extract was vacuum-filtered, and the residue was used for another two rounds of extraction. Then, the solvent was evaporated in a rotary vacuum evaporator (BUCHI, Rotavapor® R-100, Switzerland) at 25 rpm, a pressure of 180 mbar, a water bath temperature of 45 °C, and a chiller temperature of 10 °C. Then, concentrated extract samples were freeze-dried. The freeze-dried crude extract powder was reconstituted for bioactivity assays in 10% Dimethyl Sulfoxide (DMSO) solution. The crude extract yield of each fruit was calculated using the following equation;

Yield (%) = (Weight of the crude extract / Weight of the dried fruit) ×100% [46].

Total Phenolic Content (TPC)

The total phenolic content (TPC) of the crude extracts was determined by the Folin–Ciocalteu method as previously reported with slight modifications [47]. Briefly, per each well, 110 μ L of the 1:10 (v/v) Folin-Ciocalteu reagent was added. Then, 20 μ L of the 0.5 or 1mg/mL sample was added and incubated for 5 minutes. After that 70 μ L of the 10%, w/v Na₂CO₃ was added, mixed, and incubated in the dark at room temperature for 30 minutes; absorbance was taken at 765 nm. A standard curve was plotted using gallic

acid as standard (10-100 μ g/mL), (R² = 0.9976). The total phenolic content in fruit extract was expressed as mg Gallic Acid Equivalents (GAE)/ g dry weight. All tests were performed in triplicate.

Total Flavonoid Content (TFC)

TFC was measured by the previously reported procedure of the Aluminum Chloride method [47]. Seventy μl of the 0.5 or 1mg/mL sample was mixed with 30 μl of 5% w/v NaNO₂ in a sterile microplate. The 96-well microplate was allowed to stand for 5 min, and 50 μl of 2% w/v AlCl₃.6H₂O was added. After 6 min, 50 μl of 1 M NaOH was added. Then the plate was allowed to stand at room temperature in the dark for 15 min, and absorbance was measured at 415 nm. The standard curve was plotted using quercetin as standard (3.125-200 $\mu g/mL$), (R²=0.9977). The total flavonoid content in fruit extract was expressed as mg Quercetin Equivalents (QE)/g dry weight. All tests were performed in triplicate.

Results and Discussion

Based on the results of the present study (Table 3 and Figure 3), the extraction yield varied among the fruits, ranging from 15.73 ± 1.67 % to 55.05 ± 0.04 %. The highest yield was reported for *P. acidus*, and the lowest yield for *F. racemosa* L. TPC value ranging from 7.94 ± 0.70 mg GAE/g to 330.80 ± 6.26 mg GAE/g. TFC value ranging from 1.88 ± 0.40 mg to 271.84 ± 1.91 mg QE/g. *P. emblica* exhibited the highest TPC 330.80 ± 6.26 mg GAE/g and a remarkable TFC of 271.84 ± 1.91 mg QE/g. Apart from that, *E. serratus* leaves and *A. marmelos* fruit exhibit a considerable amount of TPC and TFC (> 100 mg GAE/g or mg QE/g).

For industrial-scale production, cost-effectiveness and safety are important considerations. Ethanol is a great solvent due to its safety and efficiency for cosmeceutical and functional food applications, unlike toxic solvents such as methanol. Based on up-to-date scientific evidence, the ethanolic extract is acceptable for human consumption models [45, 48]. A study investigated that effective extraction of bioactive compounds can be achieved by using 70 % ethanol as a suitable solvent for industrial-scale bioactive compound extraction [45]. The present study further confirmed that results; maceration using 70% ethanol serves as a simple, effective, economical, and safe approach for bioactive phytonutrient extraction.

Furthermore, several studies have reported that 70% ethanol has better permeability to break down cell walls, which allows it to effectively access and release intracellular bioactive compounds. This concentration provides optimal polarity for extracting both hydrophilic and lipophilic compounds. Ethanol has great potential for the extraction of polyphenols from fruit plants [49].

Polyphenols are a major group of bioactive compounds, rich in various plants, including fruits. For example, Quercetin is a natural flavonoid present in fruits like berries. They are responsible for important pharmacological activities that are highly beneficial for human nutrition, health, and recovery from prevailing diseases. For instance, polyphenols have the ability to inhibit of tyrosinase enzyme, which could be beneficial for the development of nutraceuticals for pigmentary disorders such as melasma, freckles, and hyperpigmentation, as there is an unmet clinical need in treating these disorders. Numerous scientific

studies have demonstrated that polyphenolic compounds offer various benefits to the skin, such as eliminating factors contributing to skin aging and damage.

Table 3. Total Polyphenolic, Flavonoid Content, and Extraction yield

Name of the Fruit	TPC ± SEM (mg GAE/g Dry Weight)	TFC ± SEM (mg QE/g Dry Weight)	Extraction Yield% ± SEM
Phyllanthus emblica L.	330.80 ±6.26 ^a	271.84 ± 1.91 ^a	41.91 ± 1.75 ^{c,d}
Elaeocarpus serratus L. Leaves	142.8 ± 7.25 ^b	124.27 ± 7.48 ^b	$19.50 \pm 0.23^{\rm f}$
Aegle marmelos	103.32±1.03°	120.27 ± 1.42^{b}	54.33 ± 0.41^{a}
Elaeocarpus serratus L. Fruit	44.92 ± 0.19^{d}	25.51 ± 0.29 ^d	$16.87 \pm 0.31^{\rm f}$
Passiflora foetida	$40.37 \pm 0.87^{\rm d,e}$	42.25 ± 3.3°	36.66 ± 0.31 ^d
Annona reticulata L.	29.60±2.49e,f	26.61 ± 0.53 ^d	50.36 ± 0.65 a,b
Psidium guineense	24.26±1.67 ^{f,g}	20.35 ± 0.08 ^d	38.76 ± 0.50^{d}
Syzygium cumini L. Skeels	24.05±0.45 ^{f,g}	19.17 ± 0.45 ^d	46.33 ± 2.20 ^{b,c}
Flacourtia indica (Burm. f) Merr.)	$23.79 \pm 0.95^{f,g}$	22.38 ± 0.47 ^d	$48.80 \pm 2.80^{a,b}$
Morus alba var. indica	22.85 ± 0.68 f,g,h	19.08 ± 0.22 ^d	53.70 ± 1.07^{a}
Limonia acidissima L.	14.05±0.20g,h,i	1.88 ± 0.4°	$40.97 \pm 0.54^{c,d}$
Ficus racemosa L.	11.45±0.65g,h,i	28.3 ± 0.08d	15.73 ± 1.67 ^f
Phyllanthus acidus (L.) Skeels	9.41±0.42h,i	2.09 ± 1.44°	55.05 ± 0.04^{a}
Pouteria champechiana (Kunth) Baehni	7.94±0.70 ⁱ	16.89 ± 0.37^{d}	29.55 ± 0.35°

Means with different superscript letters in individual columns are significantly (p < 0.05) different from each other. Data are expressed as Mean \pm Standard Error Mean (SEM) (n=3). TPC: Total Polyphenolic Content, TFC: Total Flavonoid Content, GAE: Gallic Acid Equivalent, QE: Quercetin Equivalent

Flavonoids belong to a subclass of polyphenols, which are phytonutrients present in many fruit plants. These compounds mainly consist of a benzopyrone ring, which is typically attached to a phenolic or polyphenolic group at different positions [50]. Flavonoids are vastly diverse compounds that offer numerous medicinal benefits, including antioxidant, anti-inflammatory, anti-cancer, neuroprotective, and cardioprotective properties. Several studies reported that flavonoids have been used in cosmetics and skincare products, as anti-wrinkle skin agents and as natural dyes [51-54].

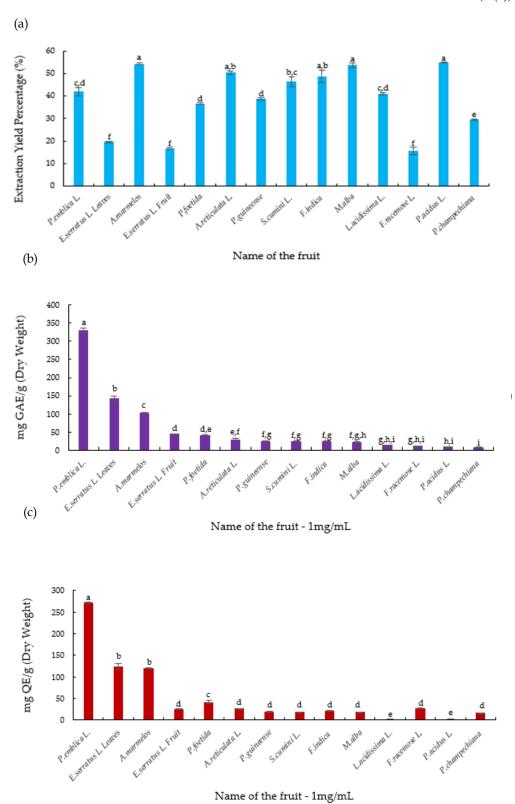


Figure 3. (a) Extraction yield percentage, (b) total polyphenolic content, and (c) total flavonoid content of the selected underutilized fruits. Values are represented as Mean ± Standard Error Mean (SEM) (n=3).

Antioxidant activity is related to polyphenols, and some study provides evidence that the use of TPC can be used to predict antioxidant activity with reasonable accuracy [55]. Polyphenols and flavonoids have radical scavenging activity, reducing Reactive Oxygen Species (ROS) [56-58]. This activity helps to prevent ROS-mediated diseases such as cancer. Utilization of fruits that are high in polyphenols and flavonoids in daily meals fulfills the nutritional requirement. It provides natural immune protection, reducing the risk of many NCDs, such as cancers, arthritis, Alzheimer's disease, diabetes mellitus, and Parkinson's disease [59]. The ethnomedicinal knowledge of the indigenous communities through generations represents a greatly valuable database for the commercial exploitation of these underutilized fruits. Further investigation of pharma-chemical and pharmacological analysis of these fruit plants is much needed to validate the safety and efficacy of the documented therapeutic potential of traditional herbal remedies.

Conclusion

The minor wild fruits studied in the present research are emerging as nutritionally rich and pharmacologically valuable wild edible fruit species with significant potential for health and therapeutic applications. The present study highlights the unexplored potential of underutilized fruits in the North Central Province of Sri Lanka as rich sources of phenolics and flavonoids. It emphasizes their significant role in ethnobotany and ethnopharmacology. The highest TPC and the highest TFC value were reported for P. emblica fruit, E.serratus leaves, and A. marmelos fruit, which also contained considerable amounts of bioactive compounds, which are showing potential for use in functional food, cosmeceutical, and pharmaceutical industry applications. Incorporating these fruits into the diet can enhance nutrition while promoting biodiversity conservation. Furthermore, ethanol serves as an effective, non-toxic solvent for bioactive compound extraction from plants for a versatile range of industrial applications due to its suitability for human consumption and cost-effectiveness. Moreover, evaluation of chemical composition, nutritional composition, toxicological studies, and further assessment of bioactive ingredients such as antioxidants, vanillin content, and proanthocyanidin content evaluation is important for specific industrial applications. For the sustainable utilization of these neglected fruits, it is essential to implement valueaddition strategies to maintain a continuous supply and demand throughout the year. Present research evidence will contribute to the future utilization and commercialization of neglected and underutilized fruits in Sri Lanka.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication.

Acknowledgment

This research was supported by the Science and Technology Human Resource Development Project, Ministry of Higher Education, Sri Lanka, funded by the Asian Development Bank (Grant No. R2/RJ4) and the Department of Bioprocess Technology, Faculty of Technology, Rajarata University of Sri Lanka, providing the necessary laboratory facilities.

Funding

This research was funded by ADB/STHRD/CRG/R2/RJ4 project.

References

- [1] E.K. Madanayake., Challenges in vegetables and fruits exports in Sri Lanka, *Journal of Economics Students' Society, University of Kelaniya, Sri Lanka.*, **2016**,11(1) 82-90.
- [2] L. Rajapaksha, D. Gunathilake, S. Pathirana, and T. Fernando, Reducing post-harvest losses in fruits and vegetables for ensuring food security—Case of Sri Lanka, *Journal of Food Process Technology*, **2021**, 9 (1), 7-16, DOI: 10.15406/mojfpt.2021.09.00255.
- [3] G. Pushpakumara, R. Silva, and T. Borelli, "Diversity of underutilized vegetables and fruit in Sri Lanka: prioritization for collection, conservation, genetic improvement, and promotion.," ed: *Alliance of Bioversity International and CIAT. Rome, Italy*, **2023**, p. 34 pages.
- [4] N. Dahanayake., Some neglected and underutilized fruit-crops in Sri Lanka, *International Journal of Scientific Research Publications*, **2015**, 5 (2),1-7.
- [5] U.M.U.R. Ariyasoma and D. L. Wathugala, Nutritional and therapeutic attributes of neglected and underutilized fruit crops in Sri Lanka and their potential applications in value addition, *International Journal of Minor Fruits, Medicinal and Aromatic Plants.*, 2023, Vol. 9 (2) 42-51, DOI: 10.53552/ijmfmap.9.2.2023.
- [6] G. D. Liyanaarachchi, J. K. R. R. Samarasekera, K. R. R. Mahanama, and K. D. P. Hemalal, Tyrosinase, elastase, hyaluronidase, inhibitory and antioxidant activity of Sri Lankan medicinal plants for novel cosmeceuticals, *Industrial Crops and Products*, **2018**, 111 597-605, https://doi.org/10.1016/j.indcrop.2017.11.019.
- [7] K.K.A. Sanjeewa, Lee, W. W. Kim, J.Jeon, and You-Jin, Exploiting biological activities of brown seaweed Ishige okamurae Yendo for potential industrial applications: A review, *Journal of applied phycology*, **2017**,29 3109-3119, https://doi.org/10.3109/09637486.2011.619965...
- [8] H.T. Hoang, J.Y. Moon, and Y. C. Lee., "Natural Antioxidants from Plant Extracts in Skincare Cosmetics: Recent Applications, Challenges and Perspectives," *Cosmetics*, vol. 8, no. 4,https://doi.org/10.3390/cosmetics8040106.
- [9] S.W.S. Walimuni, I.M.N. Molagoda, R.G.P.T. Jayasooriya, and K.K.A. Sanjeewa., Evaluation of extraction procedure for effective isolation of bioactive compounds from underutilized fruits in Sri Lanka for potential industrial applications, Conference Paper of *International Conference on Biomimicry for Sustainability-ICBS* 2024, gapHQ, Colombo 07, Sri Lanka., 23rd August 2024.
- [10] B. Mojzer, E.K. Hrncic, M.Skerget, and M. K. Z. U. Bren., Polyphenols: Extraction Methods, Antioxidative Action, Bioavailability and Anticarcinogenic Effects, *Molecules*, 2016, 21 (7), https://doi.org/10.3390/molecules21070901
- [11] R.A.U.M.U. and D.L. Wathugala., Nutritional and therapeutic attributes of neglected and underutilized fruit crops in Sri Lanka and their potential applications in value addition, *International Journal of Minor Fruits, Medicinal and Aromatic Plants.*, **2023**, 10 42-51, DOI: 10.53552/ijmfmap.9.2.2023.
- [12] S. Mihiranie, J.K. Jayasinghe, C.V.L. .Jayasinghe, and J. P. D. Wanasundara., Indigenous and traditional foods of Sri Lanka, *Journal of ethnic foods*, **2020**, 71-19, https://doi.org/10.1186/s42779-020-00075-z.
- [13] C.Y. Huang, I. H. Liu, X. Z. Huang, H. J. Chen, S. T. Chang, M. L. Chang, Y. T. Ho, and H. T. Chang, Antimelanogenesis Effects of Leaf Extract and Phytochemicals from Ceylon Olive (Elaeocarpus serratus) in Zebrafish Model, *Pharmaceutics*, **2021**, 13 (7), https://doi.org/10.3390/pharmaceutics13071059.
- [14] S.P.A.S. Senadheera, S. Ekanayake, and C. Wanigatunge., Dietary habits of type 2 diabetes patients: variety and frequency of food intake, *Journal of nutrition metabolism*, **2016**, 2016 (1), 7987395, https://doi.org/10.1155/2016/7987395.
- [15] M. Ayyanar, P.S. Babu, and S. Ignacimuthu, *Syzygium cumini* (L.) Skeels., A novel therapeutic agent for diabetes: Folk medicinal and pharmacological evidences, *Complementary Therapies in Medicine*, **2013**,21 (3),232-243,,https://doi.org/10.1016/j.ctim.2013.03.004.

- [16] S.S. Mehesare, S.P. Waghmare, M.G. Thorat, S.W. Hajare, P.R. Itankar, and S. S. Ali., Evaluation of antidiarrhoeal activity of extract of unripe fruit of *Aegle marmelos, Journal of Pharmacognosy Phytochemistry*, **2019**,8 (4),2390-2392.
- [17] N.S. Bopage, G.M.K.B. Gunaherath, K.H. Jayawardena, S.C. Wijeyaratne, A.M. Abeysekera, and S. Somaratne., Dual function of active constituents from bark of *Ficus racemosa* L. in wound healing, *BMC complementary alternative medicine*, **2018**, 18 1-13, https://doi.org/10.1186/s12906-018-2089-9.
- [18] F. Ahmed and A. Urooj., Traditional uses, medicinal properties, and phytopharmacology of *Ficus racemosa*: A review, *Pharmaceutical Biology*, **2010**, 48 (6), 672-681, https://doi.org/10.3109/13880200903241861.
- [19] R. Saini, N. Sharma, O.S. Oladeji, A.Sourirajan, K. Dev, G.Zengin, M. El-Shazly, and V. Kumar., Traditional uses, bioactive composition, pharmacology, and toxicology of *Phyllanthus emblica* fruits: A comprehensive review, *Journal of ethnopharmacology*, **2022**,282 114570, https://doi.org/10.1016/j.jep.2021.114570.
- [20] R. Brooks, A.G. Barnaby, and D. Bailey., Nutritional and Medicinal Properties of *Phyllanthus Acidus* L. (Jimbilin), *International Journal of Fruit Science*, **2020**, 20 (sup3), 1706-1710, https://doi.org/10.1080/15538362.2020.1830013.
- [21] S.-P. Tan, E. N.-Y. Tan, Q.-Y. Lim, and M. A. Nafiah, Phyllanthus acidus (L.) Skeels: A review of its traditional uses, phytochemistry, and pharmacological properties, *Journal of Ethnopharmacology*, **2020**,253 112610, https://doi.org/10.1016/j.jep.2020.112610.
- [22] Z. Siddiqui, M.I. Khan, Badruddeen, J. Akhtar, and M. Ahmad., Therapeutic Potential of Star Gooseberry (*Phyllanthus Acidus*,(Skeels)) For Treatment of Diabetes and Related Complications: A Review,**2022**,DOI: 10.35248/2155-6156.1000939.
- [23] A. Kazman, B.S.M. Hanrahan, and J. Rouse, Traditional Uses, Phytochemistry and Pharmacological Activities of Annona Genus, 2022, https://doi.org/10.3390/molecules27113462.
- [24] C. Téllez, A.V.M. Gónzalez, E. Yahia, E.M.O. Vázquez, and N. Eva, *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity, *Arabian Journal of Chemistry*, **2018**,11 (5),662-691,https://doi.org/10.1016/j.arabjc.2016.01.004.
- [25] P.G. Jamkhande and A. S. Wattamwar., *Annona reticulata* Linn. (Bullock's heart): Plant profile, phytochemistry and pharmacological properties, *Journal of Tradit Complement Medicine*, **2015**, 5 (3),144-52,https://doi.org/10.1016/j.jtcme.2015.04.001.
- [26] S.P Kerkar, S.S.S Patil, A. Dabade, A.Sonawane, and K. Sachin., *Limonia acidissima*: versatile and nutritional fruit of India, *International Journal of Fruit Science*, **2020**, 20 (sup2), S405-S413, http://dx.doi.org/10.1080/15538362.2020.1737302.
- [27] G.M.M. Parvez and R. K. Sarker., Pharmacological potential of wood apple (*Limonia acidissima*): A Review, *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **2021**,7 (2),40-47.
- [28] R.M.P. Gutiérrez, S. Mitchell, and S. V. Solis., *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology, *Journal of Ethnopharmacology*, **2008**,117 (1),1-27,https://doi.org/10.1016/j.jep.2008.01.025.
- [29] S. Naseer, S. Hussain, N. Naeem, M. Pervaiz, and M. Rahman., The phytochemistry and medicinal value of *Psidium guajava* (guava), *Journal of Clinical phytoscience*, **2018**,4 (1),1-8,https://clinphytoscience.springeropen.com/articles/10.1186/s40816-018-0093-8.
- [30] S.K. Patro, P. Behera, P.M. Kumar, D.Sasmal, R.K. Padhy, and S. K. Dash, Pharmacological Review of *Flacourtia sepiaria* (Ruxb.) *Scholars Academic Journal of Pharmacy (SAJP)* **2013**, 2(2) 89-93.
- [31] S. Tyagi, M. Singh, D. Singh, I. Yadav, S. Singh, and M. H. Mansoori., Anti-asthmatic potential of *Flacourtia indica* Merr, *African Journal of Basic Applied Sciences*, **2011**, 3 (5), 201-204.
- [32] M.K. Vijay and N. Singh, Nutritional composition, photochemistry, and Pharmacognostic activities of Flacourtia

- indica (Burm. f.) Merr.: An important wild edible fruit species of central India,2024,http://dx.doi.org/10.33545/26174693.2024.v8.i7j.1586.
- [33] M. Asadujjaman, A.U. Mishuk, M.A. Hossain, and U. K. Karmakar., Medicinal potential of *Passiflora foetida* L. plant extracts: biological and pharmacological activities, *Journal of Integrative Medicine*, **2014**,12 (2),121-126,https://doi.org/10.1016/s2095-4964(14)60017-0.
- [34] B. Jan, R. Parveen, S. Zahiruddin, M.U. Khan, S. Mohapatra, and S. Ahmad., Nutritional constituents of mulberry and their potential applications in food and pharmaceuticals: A review, *Saudi Journal of Biological Sciences*, **2021**, 28 (7), 3909-3921, https://doi.org/10.1016/j.sjbs.2021.03.056.
- [35] A.H.M.M. Rahman and A. Khanom., Taxonomic and ethno-medicinal study of species from Moraceae (Mulberry) Family in Bangladesh Flora, *Research in Plant Sciences*, **2013**,1 (3),53-57,http://dx.doi.org/10.12691/plant-1-3-1.
- [36] Q. Yuan and L. Zhao, The Mulberry (*Morus alba* L.) Fruit A Review of Characteristic Components and Health Benefits, *Journal of Agricultural and Food Chemistry*, **2017**,65 (48),10383-10394,https://doi.org/10.1021/acs.jafc.7b03614.
- [37] S.B. Aseervatham G, V. Manthra, C. Ireen, S. Thilagameena, S. Akshaya, A.C. Mary, S. Giriprashanthini, and T. Sivasudha, Free radical scavenging potential and antihaemolytic activity of methanolic extract of *Pouteria campechiana* (Kunth) Baehni. and *Tricosanthes tricuspidata* Linn, *Biocatalysis and Agricultural Biotechnology*, **2019**,18 101031, https://doi.org/10.1016/j.bcab.2019.101031.
- [38] C. Zapata, I.C. Canche, J.F. Martín, K.M. Quintal, Z.T. Romero, J.C.L. Riegos, J.C.R. Camacho, M.A.A. Argáez, and V. Ermilo, Immunomodulatory effects of the methanolic extract from *Pouteria campechiana* leaves in macrophage functions, *Journal of Food Agricultural Immunology*, **2018**,29 (1),386-399, http://dx.doi.org/10.1080/09540105.2017.1386163.
- [39] E.M.M. Yahia, M.E. Celis, and M. Svendsen., The contribution of fruit and vegetable consumption to human health, *Journal of Fruit and Vegetable Phytochemicals: Chemistry Human Health*, **2017**, 2nd Edition 1-52, https://doi.org/10.1002/9781119158042.ch1.
- [40] H.I. Abeysuriya, V.P. Bulugahapitiya, and J. L. Pulukkuttige, Total Vitamin C, Ascorbic Acid, Dehydroascorbic Acid, Antioxidant Properties, and Iron Content of Underutilized and Commonly Consumed Fruits in Sri Lanka, *International Journal of Food Science*, 2020, 2020 4783029, https://doi.org/10.1155/2020/4783029.
- [41] S.B. Aseervatham G, V. Manthra, C. Ireen, S. Thilagameena, S. Akshaya, A.C. Mary, S. Giriprashanthini, and T. Sivasudha, Free radical scavenging potential and antihaemolytic activity of methanolic extract of *Pouteria campechiana* (Kunth) Baehni. and *Tricosanthes tricuspidata* Linn, *Biocatalysis Agricultural Biotechnology*, **2019**,18 101031,https://doi.org/10.1016/j.bcab.2019.101031.
- [42] T.V.T. Do, W. Suhartini, C.U Phan, Z. Zhang, G. Goksen, and J. M. Lorenzo., Nutritional value, phytochemistry, health benefits, and potential food applications of *Pouteria campechiana* (Kunth) Baehni: A comprehensive review, *Journal of Functional Foods*, 2023, 103, https://doi.org/10.1016/j.jff.2023.105481.
- [43] S.P. Ong and C. L. Law., Drying kinetics and antioxidant phytochemicals retention of salak fruit under different drying and pretreatment conditions, *Journal of Drying Technology*, **2011**,29 (4),429-441,http://dx.doi.org/10.1080/07373937.2010.503332.
- [44] K. Šavikin, J. Živković, A. Alimpić, G. Zdunić, T. Janković, S.D. Laušević, and N. Menković, Activity guided fractionation of pomegranate extract and its antioxidant, antidiabetic and antineurodegenerative properties, *Industrial Crops and Products*, **2018**, 113 142-149, https://doi.org/10.1016/j.indcrop.2018.01.031.
- [45] B. Vongsak, P. Sithisarn, S. Mangmool, S. Thongpraditchote, Y. Wongkrajang, and W. Gritsanapan, Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method, *Industrial Crops and Products*, **2013**, 44 566-571, https://doi.org/10.1016/j.indcrop.2012.09.021.
- [46] S. Chunhakant and C. Chaicharoenpong., Antityrosinase, antioxidant, and cytotoxic activities of phytochemical constituents from *Manilkara zapota* L. Bark, *Journal of Molecules*, **2019**, 24 (15), 2798, https://doi.org/10.3390/molecules24152798.

- [47] S.D.T. Maduwanthi and R. A. U. J. Marapana., Total phenolics, flavonoids and antioxidant activity following simulated gastro-intestinal digestion and dialysis of banana (*Musa acuminata*, AAB) as affected by induced ripening agents, *Food Chemestry*, **2021**, 339 127909, https://doi.org/10.1016/j.foodchem.2020.127909.
- [48] A. Plaskova and J. Mlcek., New insights of the application of water or ethanol-water plant extract rich in active compounds in food, *Journal of Frontiers in Nutrition*, **2023**, 10 1118761, https://doi.org/10.3389/fnut.2023.1118761.
- [49] E.N. Sembiring, B. Elya, and R. Sauriasari., Phytochemical Screening, Total Flavonoid and Total Phenolic Content and Antioxidant Activity of Different Parts of *Caesalpinia bonduc* (L.) Roxb, *Pharmacognosy Journal*, **2017**, 10 (1),123-127, http://dx.doi.org/10.5530/pj.2018.1.22.
- [50] T.Y. Wang, Q. Li, and K. S. Bi., Bioactive flavonoids in medicinal plants: Structure, activity and biological fate, *Asian Journal of Pharmaceutical Sciences*, **2018**,13 (1),12-23,https://doi.org/10.1016/j.ajps.2017.08.004.
- [51] P. Chuarienthong, N. Lourith, and P. Leelapornpisid., Clinical efficacy comparison of anti-wrinkle cosmetics containing herbal flavonoids, *International journal of cosmetic science*, **2010**,32 (2),99-106,https://doi.org/10.1111/j.1468-2494.2010.00522.x.
- [52] O. Deveoğlu and R. Karadağ, A review on the flavonoids-a dye source, *International Journal of Advances in Engineering*, **2019**, 31 (3), 188-200, http://dx.doi.org/10.7240/jeps.476514.
- [53] A.S. Ribeiro, M. Estanqueiro, M.B. Oliveira, and J. M. S. Lobo., "Main Benefits and Applicability of Plant Extracts in Skin Care Products," *Cosmetics*, vol. 2, no. 2, pp. 48-65. doi: 10.3390/cosmetics2020048
- [54] D. Tungmunnithum, N. Tanaka, A. Uehara, and T. Iwashina., "Flavonoids Profile, Taxonomic Data, History of Cosmetic Uses, Anti-Oxidant and Anti-Aging Potential of *Alpinia galanga* (L.) Willd," *Cosmetics*, vol. 7, no. 4. doi: 10.3390/cosmetics7040089
- [55] Y. Kiselova, D. Ivanova, T. Chervenkov, D.Gerova, B. Galunska, and T. Yankova., Correlation between the *in vitro* antioxidant activity and polyphenol content of aqueous extracts from *Bulgarian* herbs., *An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, **2006**, 20 (11), 961-965, https://doi.org/10.1002/ptr.1985.
- [56] M. Hidalgo, C.S. Moreno, and S. d. P. Teresa., Flavonoid-flavonoid interaction and its effect on their antioxidant activity, *Journal of food Chemistry*, **2010**,121 (3),691-696,https://doi.org/10.1016/j.foodchem.2009.12.097.
- [57] Y. Lu and L. Y. Foo., Antioxidant and radical scavenging activities of polyphenols from apple pomace, *Journal of Food chemistry*, **2000**, 68 (1), 81-85, https://doi.org/10.1016/S0308-8146(99)00167-3.
- [58] A. Seyoum, K. Asres, and F.K.El-Fiky., Structure–radical scavenging activity relationships of flavonoids, *Journal of Phytochemistry*, **2006**, 67 (18), 2058-2070, https://doi.org/10.1016/j.phytochem.2006.07.002.
- [59] H. Boeing, A. Bechthold, A. Bub, S. Ellinger, D. Haller, A. Kroke, E.L. Bonnet, M.J. Müller, H. Oberritter, M. Schulze, P. Stehle, and B. Watzl., Critical review: vegetables and fruit in the prevention of chronic diseases, *Journal of European journal of nutrition*, 2012,51 637-663, https://doi.org/10.1007/s00394-012-0380-y.