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FULL PAPER

Comparison of phytochemicals, antioxidant and anti-cholinesterase activity of unripe and ripe fruit of *Sonneratia caseolaris*

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Abstract

Sonneratia caseolaris, also known as mangrove apple produces the edible fruit which can be found mainly on tidal mud in mangroves area. Since this fruit is considered as underutilized fruit as it is not fully discovered for its potential health benefits and not fully commercialized, hence this study aimed to determine the phytochemicals contents (i.e. total flavonoid, phenolic, anthocyanins and carotenoids contents), antioxidant and anti-cholinesterase activity of different parts (flesh and stem cap) of the unripe and ripe fruit extracts. Phytochemicals, antioxidant and anti-cholinesterase activities were determined using standard methods of spectrophotometric analysis. The flesh part of unripe *S. caseolaris* displayed the highest total phenolic, flavonoid and carotenoid contents as compared to the other parts in 80% methanol extract with the values of 67.67±0.10 mg (GAE/g), 37.06±0.30 mg (RE/g) and 5.41±0.10 mg (BC/100 g), respectively. The flesh part of unripe *S. caseolaris* in 80% methanol extract exhibited the best antioxidant properties in three different assays (i.e. DPPH, FRAP and ABTS) with the values of 98.32±0.28%, 67.72±0.74 mmol/g and 91.24±1.23 mg/g, respectively. The acetylcholinesterase inhibition also was found to be higher in the flesh part of unripe 80% methanol *S. caseolaris* extract with 47.18±0.68% at the concentration of 250 µg/mL. Therefore, utilization of this fruit as natural antioxidant and acetylcholinesterase inhibition sources may develop new pharmaceutical and nutraceutical products.

1. Introduction

Fruits from tropical and subtropical climates have long historical evidence for their medicinal and nutraceutical properties (Loganayaki and Manian, 2010). Naturally occurring phytochemicals such as phenols, flavones and lignan in fruits are reported to have health-promoting properties (Lin *et al.,* 2008). Mangrove plants not only offer the ecological function to the environment but also have medicinal properties (Bunyapraphatsara *et al.,* 2003). *Sonneratia caseolaris* is one of the mangrove fruits found in the state of Sabah, Malaysia which remains underutilized. *S. caseolaris* or locally known as 'Pidada' among Bruneian in Sabah, and 'Berembang' or 'Perepat laut' among Malay community in Peninsular Malaysia (Tangah, 2006; Rahim and Bakar, 2018). Because of the apple-like shape of the fruit, *S. caseolaris* is also known as 'Mangrove Apple' (Rahim and Bakar, 2018). Traditionally, the extract of this plant has been used as an astringent and antiseptics to stop haemorrhage (Ghani, 1998). Besides that, the juice of unripe fruit can be used to treat coughs (Peter and Sivasothi, 1999). The pounded leaves of *S. caseolaris* can also be used for hematuria and smallpox (Perry and Metzger, 1980).

Alzheimer's disease (AD) is a progressive neuro-degenerative disorder associated with memory impairment and cognitive deficit which is the most common cause of dementia. It is characterized by low levels of acetylcholine in the brain of AD patients due to the presence of large amounts of the enzymes; acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) which hydrolyse the ester bond in the acetylcholine molecule, leading to loss of stimulatory activity (Orhan *et al.,* 2004; Murray *et al.,* 2013). Thus, inhibition of AChE activity is a gold standard for inhibiting AD. As reviewed by Dos Santos *et al.* (2018), the most prevalent plant families studied for anti-AChE activity were Amaryllidaceae, Lycopodiaceae, and Polygonaceae. As reported by Rawa *et al.* (2019) for

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Malaysian medicinal plants, five from the 10 plant species from Fabaceae family showed active against cholinesterase inhibition. Other previous studies also reported the anti-AChE potential of Malaysian plants (Noridayu *et al.,* 2011; Nour *et al.,* 2014).

In this study, the fruit of *S. caseolaris* is being selected due to its long history in traditional medicine which remains underutilized as it is not fully commercialized, and the lacks of scientific data on its potential health benefits. Hence, the aim of this study is to determine the phytochemicals contents, antioxidant and anti-cholinesterase activities of different parts (flesh and stem cap) of the unripe and ripe fruit in 80% methanol and aqueous extracts.

2. Materials and methods

2.1 Fruit collection

The *S. caseolaris* fruit was collected between October and December 2011 in Kg. Weston, Sabah. The herbarium specimens after collection were authenticated by Mr Johnny Gisil and deposited in BORNEENSIS, Universiti Malaysia Sabah, Malaysia with voucher specimen number of BORH 1037.

2.2 Sample preparation

After collection, the fruits were washed with tap water, weighed, chopped into small pieces and freeze-dried (Thermo Fisher Scientific, USA). The dried samples were ground into powder form and then sieved (estimated size from 590 to 250 micron) to get uniform size. It was then stored in freezer at -20°C till use.

2.3 Extraction

Two hundred mg of sample were extracted 2 hrs with 2 mL of 80% methanol or distilled water at 25°C using orbital shaker set at 200 rpm which was then centrifuged at 1000 x *g* for 15 mins and the supernatant was decanted into vials. The supernatant was used for the determination of total phenolic, flavonoid, anthocyanin and carotenoid contents and antioxidant assessment (Velioglu *et al.*, 1998). For acetylcholinesterase inhibition assay, 6 g of the lyophilized sample was extracted with 100 mL of 80% methanol or 100 mL of distilled water for 2 hrs at 25°C using orbital shaker set at 200 rpm (Wettasinghe *et al.*, 2002).

2.4 Phytochemical content

2.4.1. Total phenolic content

Total phenolic content was determined using a Folin-Ciocalteu method as described by Velioglu *et al.* (1998). Gallic acid (concentration range 0 to 100 µg/mL) was used as a standard and the calibration curve was plotted to calculate the total phenolic content in the sample. The results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g).

2.4.2 Total flavonoid content

Total flavonoid content was determined using a colorimetric method according to Dewanto *et al.* (2002) with slight modification with no distilled water added at the last step of the experiment. Rutin (concentration range 0 to 100 µg/mL) was used as a standard and the calibration curve was plotted to calculate the total flavonoid content in the sample. The results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/g).

2.4.3 Total anthocyanin content

The total anthocyanin content (TAC) was determined using pH-differential method demonstrated by Giusti and Wrolstad (2001). The results were expressed as mg c-3-gE/100 g dried sample which were determined using this formula:

Total anthocyanin content = A x MW x DF x 1000/(ε x C)

Where A = (A515 – A700) pH 1.0 – (A515 – A700) pH4.5; MW (molecular weight) = 449.2 $g.mol^{-1}$ for cyanidin-3-glucoside; DF = the dilution factor of the samples, ε is the molar absorptivity of cyanidin-3-glucoside = 26900; C is the concentration of the buffer in mg/mL.

2.4.4 Total carotenoid content

The total carotenoid content was determined according to the method by Hess (1990). β-carotene was used as a standard and the findings were expressed as mg of β-carotene in 100 g of dried sample (mg BC/100 g dried sample).

2.5 Antioxidants

2.5.1 DPPH free-radical scavenging assay

The 1,1-diphenyl-2-pycrylhydrazyl (DPPH) was used as a free radical model in the assay done using a method demonstrated by Magalhaes *et al.* (2006). The free-radical scavenging activity was calculated using the equation below:

Scavenging effect (%) = [1- {absorbance of sample/ absorbance of control}] x 100

The resulting percentages were plotted and the final percentage of the scavenging activity was at 100 µg/mL concentration

2.5.2 FRAP (Ferric Reducing/Antioxidant Power) assay

The FRAP assay was performed according to the method previously described by Benzie and Strain (1996). A standard curve was plotted and the result was expressed as the concentration of antioxidant having a ferric reducing ability in 1 g of sample (μM/g).

2.5.3 ABTS decolourization assay

The ABTS free-radical-scavenging activity was determined by ABTS radical cation decolourization assay as described by Re *et al.* (1999). A standard curve was plotted to obtain the final result expressed as mg ascorbic acid equivalent antioxidant capacity in 1 g of sample (mg AEAC/g).

2.6 Anti-cholinesterase inhibition assay

The anti-cholinesterase inhibition assay was carried out according to a method demonstrated by Atta-ur-Rahman and Thomsen (2001), and the percentage inhibition was calculated using the formula below:

% inhibition = [control absorbance (without extract) – tested extract absorbance/control absorbance] x 100.

2.7 Statistical analysis

All tests in this study were carried out in triplicates and in three independent experiments. Using Prism 5 statistical software, the results were presented as mean ± standard deviation. The results were statistically analysed by one-way analysis of variance (ANOVA) and Duncan post-hoc test with $p \leq 0.05$ set as the level of statistical significance. To determine the relationship between phytochemicals and bioactivity potential of the samples, Pearson's correlation analysis was conducted.

3. Results and discussion

3.1 Phytochemical content

3.1.1 Total phenolic content

The oxidation of phenolates complex molybdenum-tungsten blue can be determined using Folin-ciocalteu reagent and measured spectrophotometrically at 725 nm (Singleton and Rossi, 1965). The flesh of unripe *S. caseolaris* displayed the highest total phenolic content in this study with the value of 67.67±0.10 mg GAE/g for 80% methanol extract (Table 1) followed by stem cap of unripe fruit (27.89±0.38 mg GAE/g), flesh of ripe fruit (22.40±0.16 mg GAE/g) and stem cap of ripe fruit (14.34±0.80 mg GAE/g). The same trend was also found in the aqueous extract in the order of flesh of unripe fruit (27.67±0.82 mg GAE/g) > stem cap of unripe fruit (19.57±0.18 mg GAE/g) > flesh of ripe fruit (17.16 0.29 mg GAE/g) and stem cap of ripe fruit (13.57±0.22 mg GAE/g) (Table 2). Significant different of total phenolic content in edible (flesh) and non-edible (stem-cap) of *S. caseolaris* ($p<0.05$) was observed. The result is in agreement with the previous study by Prasad *et al.* (2013) and Lim *et al.* (2006) where the unripe fruits of *Nypha fruticans* Wurmb and *Psidium guajava* displayed higher phenolic content as compared to the ripe fruits. This condition might be as a result of degradation of some of the phenolic phytochemicals during the ripening process of *S. caseolaris* fruit. The degradation of phenolic compounds during fruit ripening are related to their biosynthesis pathways, which are mainly governed by enzyme expression and various genetic as well as environmental factors (Belwal *et al.,* 2019).

Table 1. Total phenolic, total flavonoid, total anthocyanin and total carotenoid contents in 80% methanol extract of *S. caseolaris*

Samples	Total phenolics[1]	Total flavonoids[2]	Total anthocyanins[3]	Total carotenoid[4]
Unripe Flesh	67.67±0.10^{a}	37.06±0.30^{a}	ND	5.41±0.10^{a}
Unripe stem cap	27.89±0.38^{b}	22.89±1.44^{c}	ND	3.17±0.20^{c}
Ripe flesh	22.40±0.16^{c}	15.00±0.32^{d}	ND	4.12±0.07^{b}
Ripe stem cap	14.34±0.80^{f}	10.71±0.23^{e}	ND	2.56±0.11^{c}

The results were presented as mean ± SD (n = 3), the values with different superscript letters within the column indicate significant difference at $p < 0.05$. ND = Not detected, [1]Total phenolic content was expressed as mg GAE/g, [2]Total flavonoid content was expressed mg RE/g, [3]Total anthocyanin content was expressed as mg C-3-GE/100 g dried sample, [4]Total carotenoid content was expressed as mg BC/100 g dried sample

Table 2. Total phenolic, total flavonoid, total anthocyanin and total carotenoid contents in aqueous extract of *S. caseolaris*

Samples	Total phenolics[1]	Total flavonoids[2]	Total anthocyanins[3]	Total carotenoid[4]
Unripe Flesh	27.67±0.82^{b}	26.68±0.01^{b}	ND	4.49±0.14^{b}
Unripe stem cap	19.57±0.18^{d}	14.19±0.19^{d}	ND	2.63±0.05^{c}
Ripe flesh	17.16±0.29^{e}	11.55±0.29^{e}	ND	3.25±0.72^{c}
Ripe stem cap	13.57±0.22^{f}	10.67±0.14^{e}	ND	2.31±0.14^{c,d}

The results were presented as mean ± SD (n = 3), the values with different superscript letters within the column indicate significant difference at $p < 0.05$. ND = Not detected, [1]Total phenolic content was expressed as mg GAE/g, [2]Total flavonoid content was expressed mg RE/g, [3]Total anthocyanin content was expressed as mg C-3-GE/100 g dried sample, [4]Total carotenoid content was expressed as mg BC/100 g dried sample

3.1.2 Total flavonoid content

In the present study, the unripe flesh of *S. caseolaris* displayed the highest total flavonoid content for both 80% methanol and aqueous extracts with 37.06±0.30 mg RE/g and 26.68±0.01 mg RE/g, respectively (Tables 1 and 2). The total flavonoid content in different parts of *S. caseolaris* (80% methanol) in descending order are as follows: flesh of unripe fruit (37.06±0.30 mg RE/g) > stem of unripe fruit (22.89±1.44 mg RE/g) > flesh of ripe fruit (15.00±0.32 mg RE/g) and stem cap of ripe fruit (10.71±0.23 mg RE/g) (Table 1). In addition, total flavonoid content for aqueous extract in different parts of *S. caseolaris* were in the order of flesh of unripe fruit (26.68±0.01 mg RE/g) > stem cap of unripe fruit (14.19±0.19 mg RE/g) > flesh of ripe fruit (11.55±0.29 mg RE/g) and stem cap of ripe fruit (10.67±0.14 mg RE/ g) (Table 2). The previous study reported the presence of two flavonoids namely luteolin and luteolin 7-O-β-glucoside in *S. caseolaris* fruit which contribute to the antioxidant activity of the fruit (Sadhu *et al.,* 2006). Hence, this also might explain the presence of flavonoid compounds in the present study. In fact, the mixture of methanol and water was believed to facilitate the extraction of more phytochemical compounds due to more polar medium present (Adil *et al.,* 2017).

3.1.3 Total anthocyanin content

In the present study, the total anthocyanin content was determined at two different pH values (1.0 and 4.5) by evaluating the change in absorbance value (Sondheimer and Kertesz, 1948). The results of this study showed that anthocyanin was not detected in all parts of the fruit for both 80% methanol and aqueous extracts.

3.1.4 Total carotenoid content

In this study, the presence of the carotenoid was found in unripe and ripe of both flesh and stem cap of *S. caseolaris*. For 80% methanol extract, the flesh (unripe) contained highest carotenoid with 5.41±0.10 mg BC/100 g, followed by stem cap (unripe), flesh (ripe) and stem cap (ripe) with 3.17±0.20, 4.12±0.07 and 2.56±0.11 mg BC/ 100 g, respectively (Table 1). For aqueous extract, the flesh (unripe) showed highest carotenoid content with 4.49±0.14 mg BC/100 g, followed by flesh (ripe), stem cap (unripe) and stem cap (ripe) with 3.25±0.72, 2.63±0.05 and 2.31±0.14 mg BC/ 100 g, respectively (Table 2). Carotenoids consist of 40 carbon skeleton of isopropene units where they have provitamins and antioxidants role (Liu, 2004). Carotenoids usually contribute to the bright colour of the plants such as orange, red and yellow colour.

3.2 Antioxidant content

3.2.1. Scavenging activity on 2,2-diphenyl-2-picrylhydrazyl radical (DPPH)

In this assay, the antioxidant activity is determined using spectrophotometer at 518 nm where the changes in the purple colour of 2,2-diphenyl-1-picrylhydrazyl (DPPH.) to yellow hydrazine indicates the successful of scavenging process by antioxidant in the samples. It was shown that for both 80% methanol and aqueous extracts, unripe flesh of *S. caseolaris* exhibited higher antioxidant activity as compared to the other parts as shown in Tables 3 and 4. For 80% methanol extract, the flesh of unripe fruit displayed the highest percentage DPPH free radical scavenging with 98.32±0.28%, followed by stem cap (unripe), flesh (ripe), and stem cap (ripe) with 95.79±0.65%, 87.43±0.30%, and 80.34±0.02% at concentration of 100 µg/mL, respectively (Table 3). Meanwhile for aqueous extract, the same trend was observed as the scavenging effect was highest in flesh of unripe fruit (81.34±1.26%), followed by stem cap of unripe fruit (78.29±0.98%), flesh of ripe fruit (62.30±3.71%) and stem cap of ripe fruit (56.56±0.34%) (Table 4). The unripe parts of the fruit showed higher antioxidant activity compared to ripe parts of the fruit where this result was in line with the previous study on unripe and ripe of *Carica papaya* (Maisarah *et al.,* 2013).

3.2.2 Ferric reducing antioxidant power (FRAP) assay

In this assay, the Fe^{3+} complex of tripyrilhydrltriazine (TPTZ) are reduced to bluish colour of Fe^{2+} complex in acidic environment (Benzie and Strain, 1996). The results showed that the reducing ability of *S. caseolaris* was higher in flesh part of unripe fruit than in other parts for both 80% methanol and aqueous extracts as presented in Tables 3 and 4. For 80% methanol extract, the reducing ability of the tested extracts was found highest in flesh of unripe fruit (67.72±0.74 Mm Fe^{2+}/litre), followed by stem cap of unripe fruit (51.66±1.30 Mm Fe^{2+}/litre), flesh of ripe fruit (32.52±0.54 Mm Fe^{2+}/litre) and stem cap of ripe fruit (28.52±0.46 Mm Fe^{2+}/litre) at concentration of 1000 µg/ml (Table 3). Meanwhile for aqueous extract, the flesh of unripe fruit also showed the highest reducing ability with 56.00±0.10 Mm Fe^{2+}/litre, followed by stem cap of unripe fruit (45.81±0.22 Mm Fe^{2+}/litre), flesh of ripe fruit (16.91±0.44 Mm Fe^{2+}/litre) and stem cap of ripe fruit (13.24±0.22 Mm Fe^{2+}/litre) (Table 4). The properties of phenolic compounds as electron donors might contribute to the reducing ability of the sample tested. The results were supported by the previous study by Prasad *et al.* (2013) on *Nypha fruticans* Wurmb. fruit where the antioxidant activity was higher in unripe fruit as compared to ripe fruit.

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Table 3. Antioxidant activity of the extracts of different parts of 80% methanol extract of *S. caseolaris* evaluated by three different assays

Samples	DPPH assay (%)[1]	FRAP assay[2]	ABTS assay[3]
Unripe Flesh	98.32±0.28[a]	67.72±0.74[a]	91.24±1.23[a]
Unripe stem cap	95.79±0.65[a]	51.66±1.30[c]	68.14±4.44[c]
Ripe flesh	87.43±0.88[b]	32.52±0.54[e]	56.38±0.36[d]
Ripe stem cap	80.34±0.30[c]	28.52±0.46[f]	40.76±1.04[e]

The results were presented as mean ± SD (n = 3), the values with different superscript letters within the column indicate significant difference at $p < 0.05$. [1]DPPH free radical scavenging activity was expressed as % of scavenging at 100 mg/mL, [2]FRAP was expressed as mM ferric reduction to ferrous in 1 g of dry sample, [3]ABTS free radical scavenging activity was expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC) in 1 g of dry sample

Table 4. Antioxidant activity of the extracts of different parts of aqueous extract of S. caseolaris evaluated by three different assays

Samples	DPPH assay (%)[1]	FRAP assay[2]	ABTS assay[3]
Unripe Flesh	81.34±1.26[c]	56.00±0.10[b]	81.52±0.16[b]
Unripe stem cap	78.29±0.98[c]	45.81±0.22[d]	62.81±3.88[c,d]
Ripe flesh	62.30±3.71[d]	16.91±0.44[g]	42.05±0.33[e]
Ripe stem cap	56.56±0.34[e]	13.24±0.22[h]	35.81±2.03[e]

The results were presented as mean ± SD (n = 3), the values with different superscript letters within the column indicate significant difference at $p < 0.05$. [1]DPPH free radical scavenging activity was expressed as % of scavenging at 100 mg/mL, [2]FRAP was expressed as mM ferric reduction to ferrous in 1 g of dry sample, [3]ABTS free radical scavenging activity was expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC) in 1 g of dry sample

3.2.3 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging assay

The disappearance of radical chromagen compounds in a sample is caused by the antioxidant activity present in the samples (Arnao, 2000). For 80% methanol extract, the results showed that the flesh of unripe *S. caseolaris* displayed the highest antioxidant capacity with 91.24±1.23 mg AEAC/g, followed by stem cap (unripe), flesh (ripe) and stem cap (ripe) with 68.14±1.44, 56.38±0.36 and 40.76±1.04 mg AEAC/g, respectively (Table 3). For aqueous extract, the flesh of unripe *S. caseolaris* also exhibited highest antioxidant activity with 81.52±0.16 mg AEAC/g (Table 4), followed by stem cap (unripe), flesh (ripe) and stem cap (ripe) with 62.81±3.88, 42.05±0.33 and 35.81±2.03 mg AEAC/g, respectively. This study was in an agreement with the previous study where the unripe cactus berry displayed better antioxidant activity compared to the ripe cactus berry (Herrera-Hernández *et al.,* 2001).

3.3. Anti-cholinesterase activity

The enzyme activity is measured based on the yellow colour produced from thiocholine when it reacts with dithiobisnitrobenzoate ion. In this study, all parts of *S. caseolaris* exhibited anti-cholinesterase activity with the concentration ranged from 50 to 250 µg/mL. Based on the results obtained, the dose-dependent trend was observed as the anti-cholinesterase activity of the sample increased when the concentration of the samples increased in both 80% methanol and aqueous extracts. For 80% methanol extract, the ability of the samples to inhibit acetylcholinesterase at concentration 250 µg/mL was in the descending order of flesh of unripe fruit (47.18±0.68%), followed by stem cap of unripe fruit (43.30±2.85%), flesh of ripe fruit (38.45±1.97%) and stem cap of ripe fruit (36.23±1.64%). For aqueous extract, the flesh of unripe *S. caseolaris* (7.80±0.94%) exhibited the highest acetylcholinesterase inhibition, followed by the stem cap of unripe fruit (5.60±0.35%), flesh of ripe fruit (4.74±0.16%) and stem cap of ripe fruit (2.52±0.51%). All samples extracted with 80% methanol exhibited moderate anti-cholinesterase potential (30-50%) while all the samples extracted with aqueous extract show inactive or low anti-cholinesterase potential (<30% inhibition) (Vinutha *et al.,* 2007). Hence, this study showed that the types of extraction had a significant effect on the anti-cholinesterase study.

3.4 Relation between phytochemicals, antioxidants and anticholinesterase

A positive correlation between phytochemicals and antioxidants (Hassan *et al.,* 2013; Prasad *et al.,* 2013; Bakar *et al*., 2015) as well as antioxidants and anti-cholinesterase activity in the fruits and vegetables (Rahman *et al.,* 2012; Hassan and Bakar, 2013) were observed in many studies from the previous years. In this study, there was a strong positive correlation between the antioxidant activity (i.e. DPPH, FRAP and ABTS) with the total phenolic, flavonoid and carotenoid contents in all samples tested (Tables 5 and 6). Besides, ABTS was strongly positive correlated with the total phenolic ($r = 0.825$), flavonoid ($r = 0.944$) and carotenoid ($r = 0.822$) contents. The same trend was observed between DPPH

Table 5. Correlation analysis between phytochemicals contents, antioxidant and anti-cholinesterase inhibition activity in 80% methanol extract of *S. caseolaris*

	Total phenolics	Total flavonoids	Total anthocyanins	Total carotenoid	DPPH assay	FRAP assay	ABTS assay	Anti-cholinesterase inhibition activity
Total phenolics	1	*	*	*	0.48	0.894	0.857	0.808
Total flavonoids	*	1	*	*	0.55	0.937	0.875	0.853
Total anthocyanins	*	*	1	*	-0.155	-0.153	-0.17	-0.443
Total caratenoids	*	*	*	1	-0.797	-0.348	-0.229	-0.44
DPPH assay	0.48	0.55	-0.155	-0.797	1	*	*	0.537
FRAP assay	0.894	0.937	-0.153	-0.348	*	1	*	0.87
ABTS assay	0.857	0.875	-0.17	-0.229	*	*	1	0.841
Anti-cholinesterase inhibition activity	0.808	0.853	-0.443	-0.44	0.537	0.87	0.841	1

Table 6. Correlation analysis between phytochemicals contents, antioxidant and anti-cholinesterase inhibition activity in aqueous extract of *S. caseolaris*

	Total phenolics	Total flavonoids	Total anthocyanins	Total carotenoid	DPPH assay	FRAP assay	ABTS assay	Anti-cholinesterase inhibition activity
Total phenolics	1	*	*	*	0.849	0.932	0.901	0.376
Total flavonoids	*	1	*	*	0.871	0.938	0.905	0.359
Total anthocyanins	*	*	1	*	-0.053	-0.143	-0.123	-0.028
Total caratenoids	*	*	*	1	-0.143	-0.395	-0.279	-0.343
DPPH assay	0.849	0.871	-0.053	-0.143	1	*	*	0.413
FRAP assay	0.932	0.938	-0.143	-0.395	*	1	*	0.421
ABTS assay	0.901	0.905	-0.123	-0.279	*	*	1	0.303
Anti-cholinesterase inhibition activity	0.808	0.853	-0.443	-0.44	0.413	0.421	0.303	1

and flavonoid content with positive strong correlation (r = 0.723). The moderate positive correlation between anti -cholinesterase and antioxidant activity such as FRAP and ABTS were also found in this study with the values of (r = 0.476) and (r = 0.385), respectively.

4. Conclusion

In conclusion, unripe flesh of *S. caseolaris* displayed the highest total phenolic content, total flavonoid content, antioxidant activity and acetylcholinesterase inhibition properties as compared to the other parts which suggested that it can be used in cooking. Intensive efforts devote to the strategy of discovering natural antioxidants which also possess anti-Alzheimer's potential is greatly recommended. Hence, *S. caseolaris* is suggested as having promising phytochemicals properties with natural antioxidants and further alleviating Alzheimer's disease.

Conflict of Interest

The authors declared no conflict of interest.

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