

## Diversity of antioxidant properties among Madan (*Syzygium cumini*) trees in Belihuloya Region, Sri Lanka: Potential for improvement for community use

Prasajith Kapila Dissanayake, P.W.S.N. Dharmasena, G.E.M. Wimalasiri

*Department of Export Agriculture, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka*

**Abstract:** *Syzygium cumini* (L.) Skeels, (Madan, Jambolan) is widely distributed in tropical and subtropical regions with high diversity and considered as underutilized fruit crop in all over the world. Though *S. cumini* is underutilized fruit crop it shows high potential of uses as multipurpose tree such as food crop (Fruit), medicinal plant, timber crop, tree for forestry etc. *S. cumini* traditionally uses as medicinal plant as treatment for various disease conditions. To improve the plant for promising uses it requires intensive study of diversity for different characters. In Sri Lanka it naturally growing in wide range of areas from North to South but pay remarkably low attention for use it as fruit crops or as medicinal plant. In Belihuloya region it grows naturally without having much intention to use as fruit crop. As the tree adapted to the condition in the region, improved varieties would have high potential to use by the community in the region. The present study is to investigate the diversity of antioxidant properties among *S. cumini* tree population in Belihuloya region. Phytochemical investigation was carried out on the crude ethanol and methanol extracts of the pericarp and seed of *S. cumini* fruit. The results showed that the methanol extracts were more active than ethanol extracts. The Folin-Ciocalteu method was used to find the Total Phenolic Content (TPC). Highest total phenolic content was recorded in methanol seed extracts and varies from  $98.73 \pm 2.64$  to  $18.970 \pm 0.11$  mg Gallic acid equivalent/ g of fruit among trees. The lowest TPC was recorded by ethanol pericarp extracts which is vary from  $5.77 \pm 0.04$  to  $0.99 \pm 0.004$  mg GAE/ g of fruit. Total Flavonoid Content (TFC) was detected using Aluminium chloride. Highest total flavonoid content was recorded in methanol seed extracts which was varied from  $2.38 \pm 0.02$  to  $0.64 \pm 0.05$  mg Quercetin equivalent/ g of fruit while lowest resulted by ethanol pericarp extracts range from  $1.52 \pm 0.03$  to  $0.25 \pm 0.01$  QE/g of fruit. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay was done to find out the antioxidant activity. The highest  $IC_{50}$  resulted by seed extracts which is range from  $1.63 \pm 0.02$  to  $0.60 \pm 0.01$  trolox/ g of extract. *S. cumini* trees exhibit wide range of antioxidant variability in their natural population in the region and this results will be source for consider the breeding for improve promising *S. cumini* varieties to the region.

**Keywords:** *Syzygium cumini*, phytochemical, phenolic, flavonoid, variability,  $IC_{50}$ , population.

### Introduction

*Syzygium cumini* (L.), which belongs to the family Myrtaceae, is Considered as underutilized fruit crop in Sri Lanka and commonly known as Madan, Indian blackberry, Java plum [1]. It grows well in both the tropical and sub-tropical regions like India, Bangladesh, Nepal, Pakistan, Sri Lanka, Philippines and Indonesia [2].

It has diverse uses such as food crop, medicinal plant, timber plant, and as landscaping plant. Medicinal properties have been explored by different research studies such as use as effective medicine against diabetics, heart and liver diseases in ayurvedic medicine [3]. It also showed anticancer and anti-hyperlipidemic activities [4], hypoglycemic, antibacterial [5], anti-HIV activity [6], antidiarrheal effects [7] and antioxidant activity. It's proven that these effects may due to the polyphenols, carotenoids, flavonoid, tannins, vitamin C and E present in fruits which have antioxidant and free radical scavenging activities [8].

Antioxidants are important in eliminating the effect of free radicals which cause oxidative damage to bioactive molecules like carbohydrates, proteins, lipids and DNA in foods and other living systems [9]. Free radicals are responsible for accelerating aging, cancer, cardiovascular diseases, Neuro-degenerative diseases and inflammations [10].

*S. cumini* in Sri Lanka, the one with small oblong shaped deep purple fruit with mildly sour and astringent flavored sweet juicy flesh. But it's not popular among consumers as common fruit due to lack of information on its nutritional composition, less available in the market and scare of research studies on antioxidant properties. Sri Lanka pays less attention for this multipurpose tree which is widely distributed throughout the country with enormous variability in respect to morphology and physico-chemical characters of fruits. There for lack of improved commercial cultivar available in Sri Lanka. However, it is highly importance fruit due to its nutritional and health benefits [3,4,5]. Traditionally, Madan is propagated by seeds, hence there is considerable variation among the trees, due to its cross pollination in nature [11]. Thus, a great deal of variation is observed in trees and fruits within Madan populations. Khan *et al* [12] Showed high genetic variation among *S.cumini* populations in different regions of India.

Study based on chemical characters along with phytochemical screening and fruit quality are very useful for crop improvement, varietal selection for high yielding clones with higher medicinal and nutraceutical attributes in order to enhance the food security. There is still lack of promotion, less planting regions and low economic values of *S. cumini* in Sri Lanka. There is a strong need to collect the existing variability of Madan within the country by exploring variation of fruit quality and antioxidant properties of the *S. cumini*. High diversity of genetic variation [12] and diversity in fruit quality, morphology emphasis potential of *S. cumini* improvement for better varieties or cultivar for community uses.

Present study was undertaken to study the variability of total phenolic content, total flavonoid content and fruit quality among *S. cumini* available in Belihuloya region of Sri Lanka seeking potentials for improvement for community use in the region.

## **Materials and methods**

### *Experimental location plants*

The study was carried out at the laboratory of Faculty of Agricultural sciences, Sabaragamuwa University of Sri Lanka. Healthy and disease free fruits were collected from nine plants of *S. cumini* within the Belihuloya region, during the season of May-August. They were named as Sc-1, Sc-2, Sc-3, Sc-5, Sc-7, Sc-9, Sc-10, Sc-11, and Sc-22 respectively. Fruit samples were air dried, sealed, labeled and stored in -80°C until analysis.

### *Preparation for analysis*

Seed and pericarp of stored ripen *S. cumini* fruits were separated. Five gram (5g) of air dried pericarp and 5g of seed were taken separately and were finely ground homogenously by using cooled mortar and pestle. Finely ground seed and pericarp were put into 50ml conical flasks separately and add 50ml of methanol/ethanol (analytical reagent (99.9%)).

Conical flasks were covered with Aluminium foil and were kept in a digital Heated Ultrasonic Bath (Soner 206H, German) at about 35-40°C for about 30 minutes. Then extracts were filtered using Buchner funnel and Whatman No. 1 filter papers. The residue particles on the filter paper after filtering, were extracted for twice using the above mentioned procedure. It means a sample is extracted three times by total 150 ml of absolute solvent.

The extraction was transferred to 500 ml round bottom flask and concentrated to dryness under reduced pressure at 32°C using a Rotavapor® (J.P.SELECTA RS 3000V, Spain) to obtain respective crude methanol and ethanol extracts. Dried crude extract was transferred to Eppendorf tubes (1.5 ml) and weight was measured and recorded using analytical balance. They were Labeled and stored in -80 °C until use.

*In vitro* Antioxidant assays

**Total Polyphenolic Content (TPC) assay**

The Folin-Ciocalteu method [13] was adopted for UV-spectrophotometer to determine total phenolic content in extracted *S. cumini* seed and pericarp of ripen fruits.

10% Sodium Carbonate solution and 10 times diluted 2X Folin Ciocalteu Reagent (FCR) was prepared for the TPC assay.

The sample series of 5,2,1,0.1 mg/ml solutions were prepared in 2 ml eppendorf tubes from the previously prepared stock solutions by diluting with distilled water. The reaction mixture was consists with 125 µl of extracts, 1 ml of Folin-Ciocalteu reagent, 625 µl of distilled water and 750 µl of 10% sodium carbonate solution. Absorbance were measured at 765 nm in spectrometer (GENESYS 10S UV-Vis, USA) after 30 minutes of incubation at room temperature (25±°C). Five different concentrations of gallic acid (0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml) were used to draw standard calibration curve. TPC of *S. cumini* ethanol and methanol extracts were expressed as mg gallic acid equivalents per gram of fruit.

**Total Flavonoid Content (TFC) assay**

Total flavonoid content of seed and pericarp of ripen fruits of *S. cumini* was determined by Aluminium chloride method [14] using UV spectrophotometer.

The sample series of 5,2,1,0.1 mg/ml solutions were prepared in 2 ml eppendorf tubes from the previously prepared stock solutions by diluting with distilled water. The reaction mixture was consists with 20µl of sample, 80µl of methanol, 100 µl of 2% Aluminium chloride in methanol and absorbance was recorded at 415 nm after ten minutes of incubation at room temperature (25±°C). Five different concentrations quercetin (1, 0.5, 0.25, 0.125, 0.0625 mg/ml) were used to construct the calibration curve. TFC of *S. cumini* ethanol and methanol extracts were expressed as mg quercetin equivalents per gram of dried fruit.

**ABTS free radical scavenging assay**

Free radical scavenging activity of *S. cumini* was determined using 2,2-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS<sup>+</sup>) radicals. Five different concentrations (0.5, 0.25, 0.125, 0.0625, 0.03125 mg/ml) of each *S. cumini* samples were prepared in 50 mM Phosphate Buffer Saline (PBS pH 7.4).

ABTS<sup>+</sup> radical was generated according to the method described by [15] incubating, equal quantities of 10 mM ABTS and 2.5Mm Potassium persulphate solution at 37 °C temperature for 16 hours. Then fresh ABTS<sup>+</sup> solution was diluted seven times with 50 mM Phosphate Buffer Saline (PBS pH 7.4) just before use. In the assay 50µl from sample was mixed with 110 µl of 50 mM PBS in cuvette and 40 µl of ABTS<sup>+</sup> solution was added in dark and incubated 25± °C for 10 minutes. Absorbance was recorded at 734 nm. 0.5, 0.25, 0.125, 0.0625, 0.03125 mg/ml of 6-hydroxy-2-5-7-8-tetramethylchroman-carboxylic acid (Trolox) was used to construct the standard curve.

*Data analysis*

Data of each experiment were calculated by using Microsoft Excel 2016 and statistically analyzed using GLM procedure of followed by comparison of means using Duncan’s Multiple Range Test (DMRT). All the results presented in tables and graphs are mean of samples with a standard error of the mean.

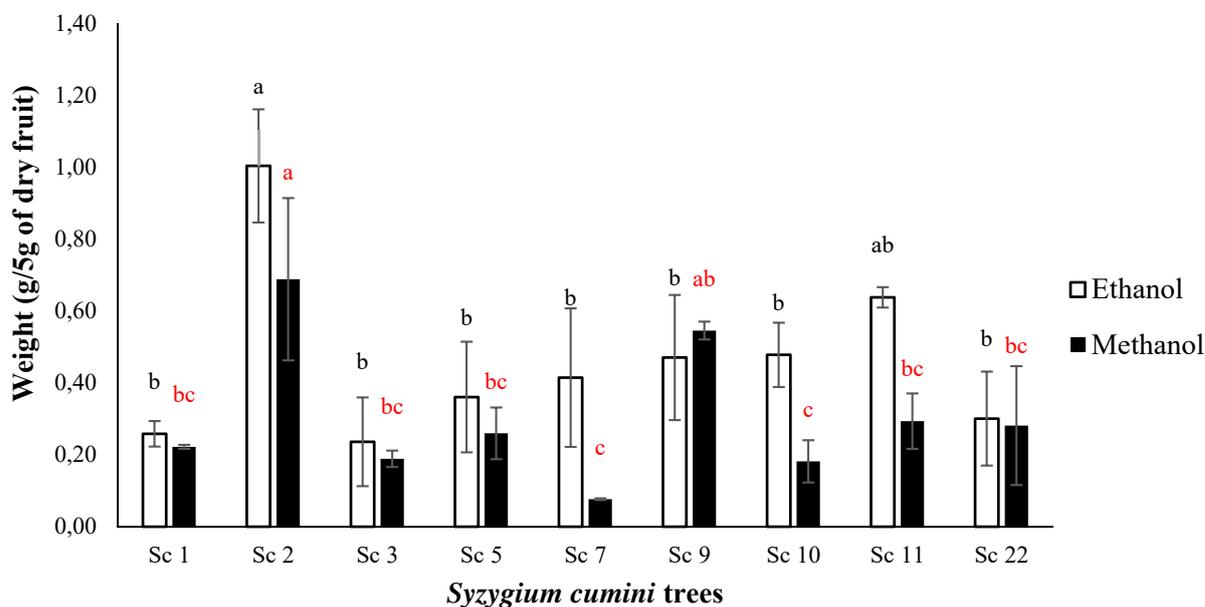
**Results and discussion**

*Variation of amount of crude extracts*

Amount of crude ethanol extracts were significantly different (p<0.05) among used *S. cumini* trees. The highest crude yield was resulted in Sc2 ethanol extract (1.00±0.15 g/ 5 g of dry fruit) and followed by Sc11, while lowest crude yield resulted in Sc3 ethanol extracts (0.24±0.12 g/ 5 g of dry fruit), but it was not significantly different among Sc1, Sc5, Sc7, Sc9, Sc22 and Sc10 (Figure 1).

Crude extracts of methanol was shown significant different (p<0.05) among the *S. cumini* trees. The highest crude yield was resulted in Sc2 methanol extract (0.69±0.23 g/ 5 g of dry fruit)

while lowest crude yield resulted in Sc7 methanol extracts ( $0.08 \pm 0.002$  g/ 5 g of dry fruit), but it was not significantly different from crude extract yield of Sc11, Sc22, Sc5, Sc1, Sc3 and Sc7 (Figure 1).



**Fig. 1.** Dry weight of ethanol and methanol extracts of ripen fruits of *S. cumini* trees

The yield of extract depends on the solvent with varying polarity, pH, temperature, extraction time and composition of the sample. Under the same extraction time and temperature, solvent and composition of sample are known as the most important parameters [16].

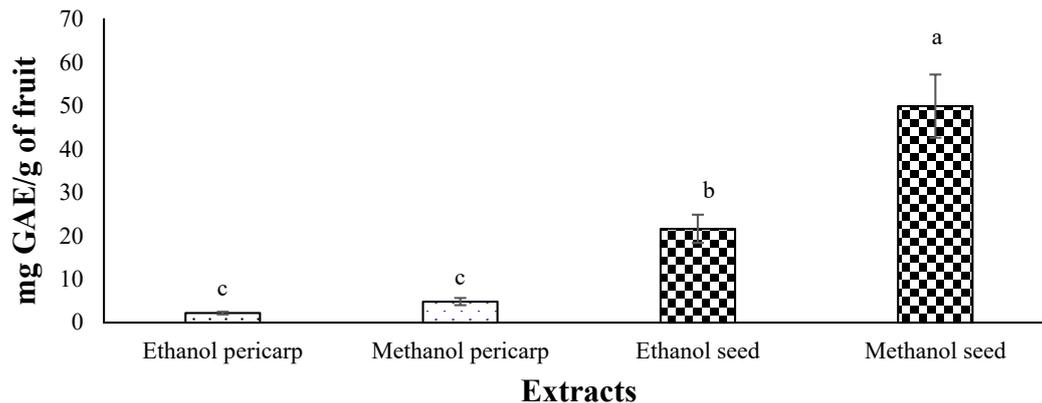
Polar solvents are frequently used for recovering polyphenols from plant materials. Ethanol has been known as a good solvent for polyphenol extraction. Methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols. Do *et al.* [16] shows that 100% methanol gives highest crude yield compared to 100% ethanol, due to increasing polarity of the solvent contribute to higher yields. In contrast Chan[17] proved that ethanol extract had the highest yield followed by methanol for *Gracilaria changii*.

Present study showed that extraction yield of ethanol was higher than methanol extracts in some of *S. cumini* trees. The difference between the results of this study and those of other studies may be attributed to several factors such as difference in plant matrix, different solvent used in extraction resulted in differences in compositions in extracts, the method and conditions of extraction [16]. Also compounds other than phenolics may have been extracted such as sugar, amino acid, glycoside compounds [18]. In this study it is obvious that the amount of crude dry sample extracted from seed or pericarp highly varied among different trees. This could be a reason of variation in chemical contents of fruit among trees [18]. Though, there was high variation of amount of crude extract among trees, in average, the best solvent to have more crude extract was absolute ethanol (100%) compared to (100%) methanol.

#### *Total Phenolic Content (TPC) of Syzygium cumini fruits*

Total Polyphenolic Content (TPC) of sample extracts were estimated as Gallic Acid Equivalents (GAE) using Gallic acid standard curve. The TPC value of each fruit extracts were estimated as mg GAE/g of fruit.

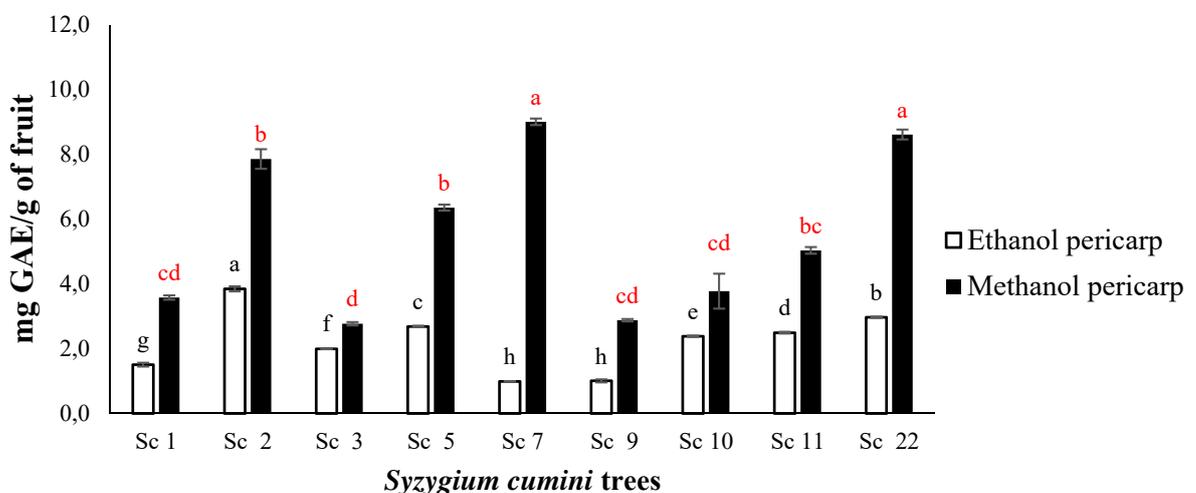
Average TPC of seed and pericarp crude extracts of *S. cumini* were significantly difference among ( $p < 0.05$ ) each ethanol and methanol extracts. The highest TPC resulted in methanol seed extracts ( $49.92 \pm 7.30$  GAE/g of fruit) and lowest resulted in ethanol pericarp extracts ( $2.20 \pm 0.31$  GAE/g of fruit) (Figure 2).



**Fig. 2.** Total phenolic content mg Gallic Acid Equilant/g of *S. cumini* pericarp and seed

The TPC was significantly different among ( $p < 0.05$ ) each ethanol extracts of *S. cumini* pericarps. The highest TPC value was resulted in Sc2 extract ( $5.77 \pm 0.04$  GAE/g of fruit) while lowest was resulted in Sc7 extract ( $0.99 \pm 0.004$  GAE/g of fruit) (Figure 3).

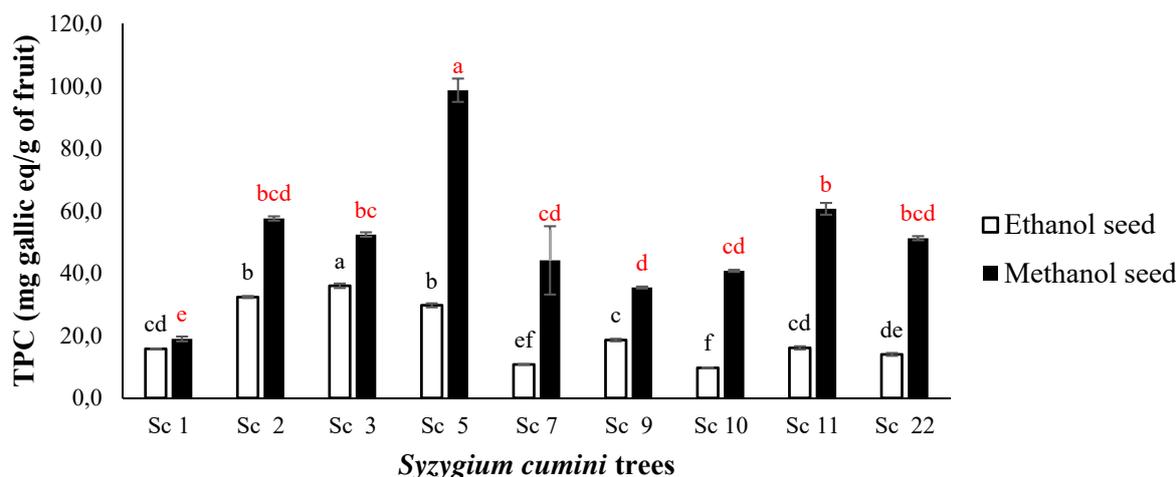
Crude pericarp extracts which was extracted by methanol shown significant difference ( $p < 0.05$ ) of TPC among each *S. cumini* trees. The results from mean comparison among them revealed that the highest TPC resulted in Sc7 extract ( $9.00 \pm 0.07$  GAE/g of fruit), and then the Sc22 extract while the lowest TPC was resulted in Sc3 extract ( $2.77 \pm 0.04$  GAE/g of fruit) and it was not significantly different from Sc10, Sc1 and Sc9 (Figure 3).



**Fig. 3.** Variation of Total Phenolic content (mg GAE/g) in fruit pericarp extracts of *S. cumini* trees using Ethanol and Methanol as extracting solvents

The TPC was significantly different among ( $p < 0.05$ ) each ethanol seed extracts of *S. cumini* trees. The highest TPC was resulted in Sc3 extract ( $36.02 \pm 0.403$  GAE/g of fruit) while the lowest TPC resulted in Sc10 ethanol seed extract ( $9.69 \pm 0.029$  GAE/g of fruit) (Figure 4).

The TPC of seed extracts which were extracted by using methanol was significantly different ( $p < 0.05$ ) among each *S. cumini* trees. The results from mean comparison among them revealed that the highest TPC resulted in Sc5 ( $98.73 \pm 2.64$  GAE/g of fruit) while the lowest TPC resulted by Sc1 ( $18.97 \pm 0.11$  GAE/g of fruit) (Figure 4).



**Fig. 4.** Variation of Total Phenolic content (mg GAE/g) in fruit seed extracts of *S. cumini* trees using Ethanol and Methanol as extracting solvents

The solvent effect is an essential parameter for the chemical behavior of antioxidant compounds. The choice of extracting solvents with different polarities can have a significant effect on the performance of Hydrogen Atom Transfer and Singlet Electron Transfer based antioxidant reactions due to the different solubility of the antioxidant compounds in different solvents, the yield of polyphenols and type of phenolic group extracted [19].

Phenolic compounds are vital plant constituents and are major factors responsible for biological activities such as antioxidant, antimicrobial, antiviral and anticancer activities [20]. Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids [21]. Phenolic compounds are organic acids composed of one or more hydroxyl groups linked to a single or multiple aromatic rings. The phenolic compounds are plant secondary metabolites can directly scavenge molecular species of active oxygen. According to the structure of phenol compound electron-donating activity is different and antioxidant ability is allowing them to act as reducing agents, Hydrogen donors, and singlet and triplet oxygen quenchers [22].

Koffi *et al.* [23] found that methanol was more effective in at a large amount of phenolic contents from walnut fruits when compared to ethanol. Iloki-Assanga [24], proved that, methanol be the most effective solvent for isolation of phenolic compounds from samples of *Bucida buceras* and *Phoradendron californicum*. Mohamed *et al.* [25], found that methanol extract possesses significant activity in releasing most secondary metabolites from leaves of *S. cumini*.

Even though the highest crud yield resulted by Ethanol extracts, the highest TPC resulted by methanol extracts. Because of TPC value of the Folin-Ciocalteu method [13] deteriorate from a number of interfering substances such as sugars, aromatic amines, sulphur dioxide, ascorbic acid, organic acids and  $Fe^{2+}$  in the ethanol extracts. Additional nonphenolic organic substances such as ascorbic acid, Cysteine, Fructose, histamine, methylamine, proteins, sucrose and some inorganic substances such as hydrazine, iron sulphate, manganese sulfate, potassium nitrate etc. may also react with the Folin- Ciocalteu reagent to give elevated apparent phenolic concentrations in methanol extracts [20].

Also the possible complex formation of some phenolic compounds in the extract that are soluble in methanol. These phenolic compounds may possess more phenol groups or have higher molecular weights than in the ethanol extracts. Based on the present results of TPC, the best extracting solvent was methanol.

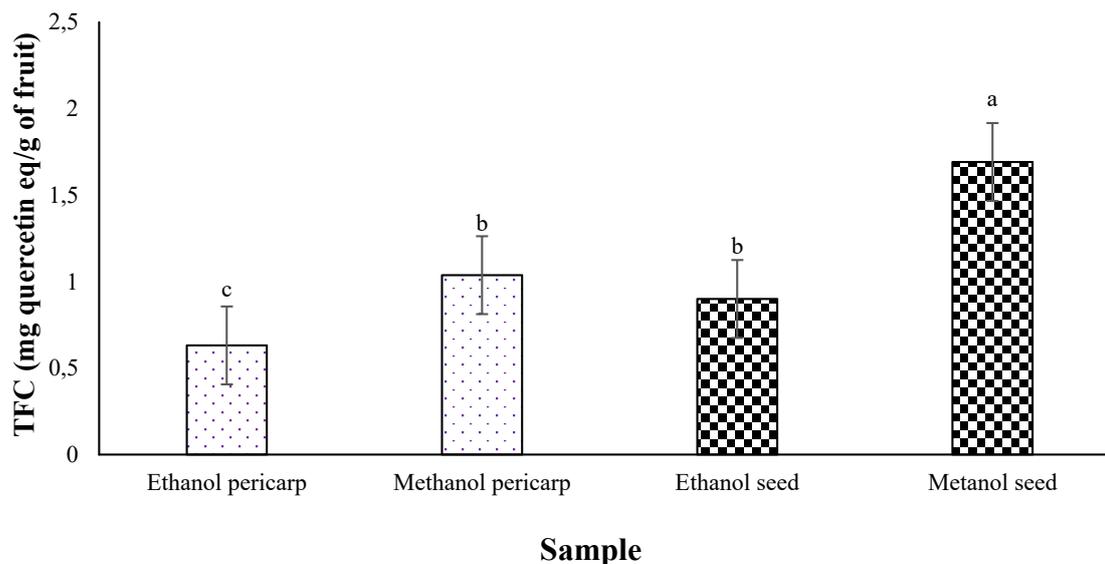
In this study it is obvious that the amount of polyphenolic content extracted from seed or pericarp highly varied among different trees of *S. cumini*. This could be a reason of variation in

chemical contents of fruit among trees [18]. This high variation in chemical nature gives promising potential of improving cultivars better community use in the area concern.

*Total Flavonoid Content (TFC) of Syzygium cumini fruits*

Total Flavonoid Content of fruit pericarps extracts were estimated as Quercetin Equivalents (QE) using Quercetin standard curve. TFC value was calculated as QE/g of extract.

Average TFC of ethanol and methanol crude extracts were significantly different among ( $p < 0.05$ ) seed and pericarp of all *S. cumini* trees. The results from mean comparison among them revealed that the highest TFC resulted in methanol seed extracts ( $1.69 \pm 0.11$  GAE/g of seed) and lowest resulted in ethanol pericarp extracts ( $0.63 \pm 0.07$  GAE/g of pericarp) (Figure 5).



**Fig. 5.** Total flavonoid content of *S. cumini* pericarp and seed extracts

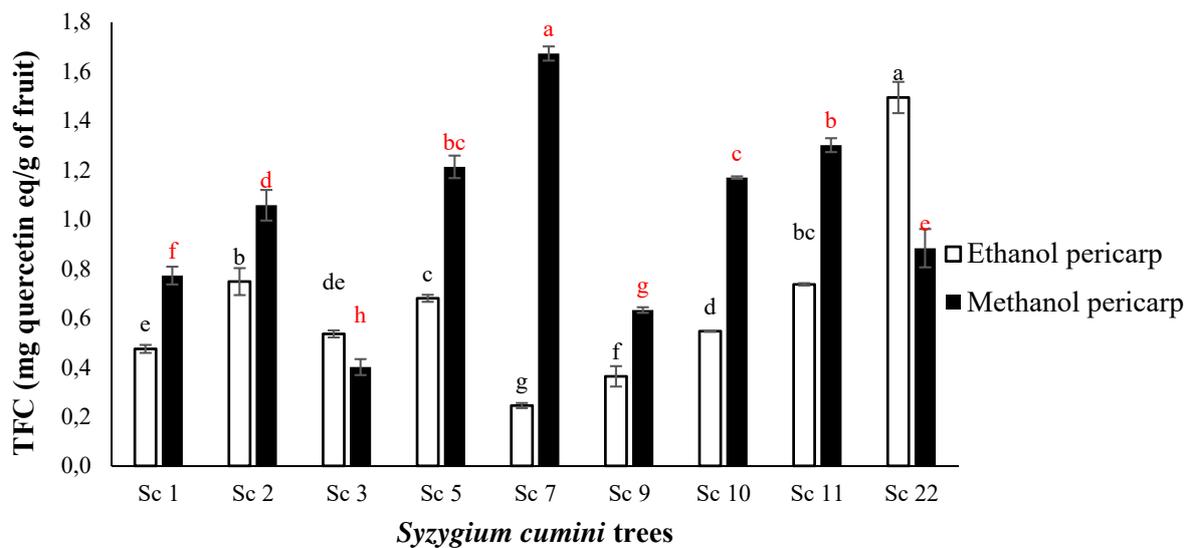
The TFC was significantly different among ( $p < 0.05$ ) each ethanol pericarp extracts of *S. cumini* trees. The highest TFC resulted in Sc22 ethanol extracts ( $1.52 \pm 0.03$  QE/g of fruit) while lowest resulted in Sc7 ( $0.25 \pm 0.01$  QE/g of fruit) (Figure 6). The TFC was significantly different among ( $p < 0.05$ ) each methanol pericarp extracts of *S. cumini* trees. The results from mean comparison among them revealed that the highest TFC resulted in Sc7 methanol pericarp extract ( $1.67 \pm 0.01$  QE/g of fruit) while the lowest TFC resulted in Sc3 extract ( $0.40 \pm 0.01$  QE/g of fruit).

The TFC of pericarp extracts were also significantly different among ( $p < 0.05$ ) each ethanol and methanol extracts of *S. cumini* trees. The highest TFC resulted in methanol pericarp extracts compared to ethanol extracts but it was deviated in Sc3 and Sc22 *S. cumini* trees (Figure 6).

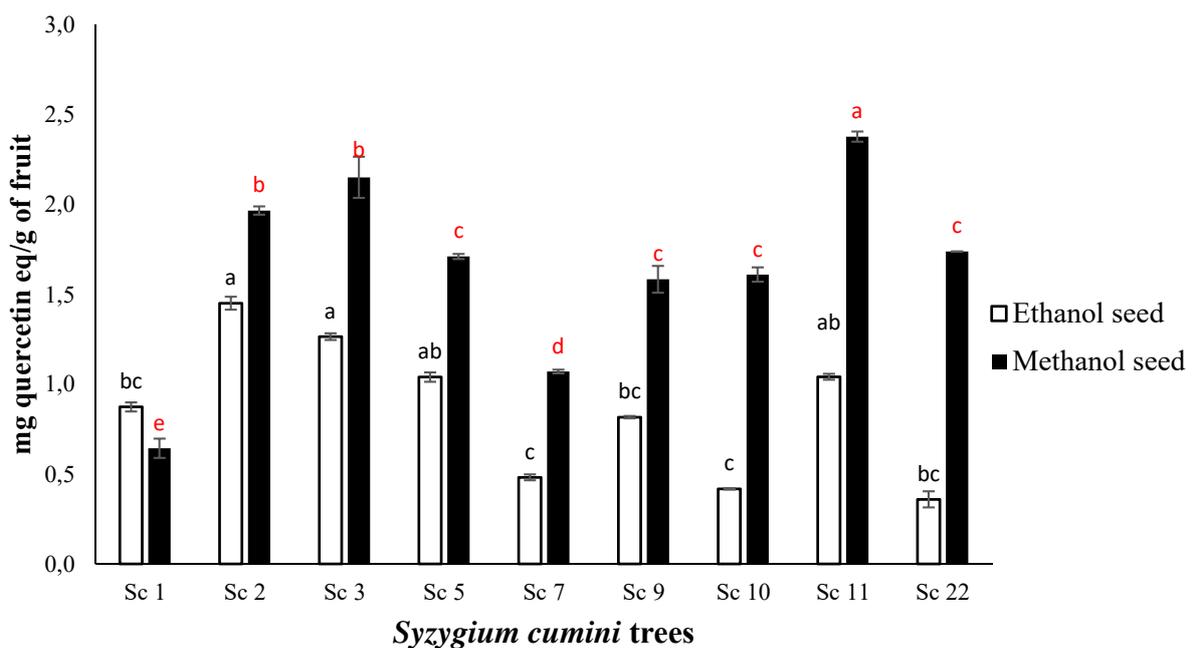
The TFC was significantly different among ( $p < 0.05$ ) each ethanol seed extracts of *S. cumini* trees. The results from mean comparison among them revealed that the highest TFC resulted in Sc2 ethanol seed extract ( $1.45 \pm 0.03$  QE/g of fruit), but it is not significantly different from Sc3, Sc11 and Sc5. The lowest TFC resulted in Sc10 ethanol seed extract ( $0.42 \pm 0.01$  QE/g of fruit) (Figure 7).

The TFC was significantly different among ( $p < 0.05$ ) each methanol seed extracts of *S. cumini* trees. The results from mean comparison among them revealed that the highest TFC resulted in Sc11 methanol seed extract ( $2.38 \pm 0.02$  QE/g of fruit) while the lowest TFC resulted in Sc1 ethanol seed extract ( $0.64 \pm 0.05$  QE/g of fruit) (Figure 7).

The TFC of seed extracts were significantly different among ( $p < 0.05$ ) each ethanol and methanol extracts of *S. cumini* trees. The highest TFC resulted in methanol seed extracts compared to ethanol extracts but it was deviated in Sc1 *S. cumini* tree (Figure 7).



**Fig. 6.** Variation of Total flavonoid content of *S. cumini* pericarp extracts and solvent used to extract Total flavonoid content (mg quercetin Eq/g) in fruit pericarp extracts of *S. cumini* trees using Ethanol and Methanol as extracting solvents



**Fig. 7.** Variation of Total flavonoid content of *S. cumini* seed extracts and solvent used to extract Total flavonoid content (mg quercetin Eq/g) in fruit pericarp extracts of *S. cumini* trees using Ethanol and Methanol as extracting solvents

The antioxidant ability of flavonoids depends on the molecular structure and position of hydroxyl groups [26]. Both flavonoids and flavonols possess antioxidant activity as a result of a native scavenging or chelating attribute [27].

Result of this assays proved to be the most effective solvent for isolation of flavonoid compounds from *S. cumini* trees is methanol. The phenomena were similar to total phenolic assay because flavonoid was major phenolic compounds in plants with concentration around 80% [28,14].

Phytochemical compounds in methanol extract were potential to donating hydrogen atom so that these compounds could form complex compounds with aluminium ion at total flavonoid assay.

In this study it is obvious that the amount of flavonoid content extracted from seed or pericarp highly varied among different trees of *S. cumini*. This could be a reason of variation in chemical contents of fruit among trees [14].

*ABTS*<sup>+</sup> radical scavenging activity as Inhibitory concentration 50%

A *BTS*<sup>+</sup> radical scavenging activity of standard (trolox) was used to calculate  $IC_{50}$  values.

Average  $IC_{50}$  of all trees was significantly difference among ( $p < 0.05$ ) each methanol extracted pericarp and seed of *S. cumini* trees. The highest  $IC_{50}$  resulted in seed extracts ( $30.55 \pm 4.42$  mg trolox/ g of extract) and lowest  $IC_{50}$  resulted in pericarp extracts ( $0.98 \pm 0.08$  mg trolox/ g of extract) (Figure 8).

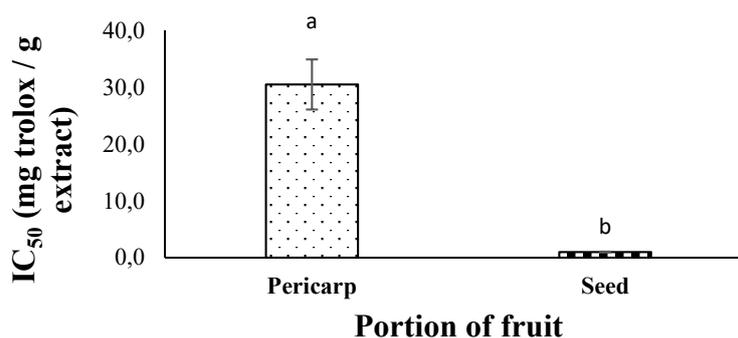


Fig. 8.  $IC_{50}$  of *S. cumini* pericarp and seed extracts

The  $IC_{50}$  was significantly different among ( $p < 0.05$ ) each pericarp extracts of *S. cumini* trees. The results from mean comparison among them revealed that the highest  $IC_{50}$  resulted in Sc10 pericarp extract ( $80.12 \pm$  mg trolox/ g of extract) while the lowest  $IC_{50}$  resulted in Sc22 pericarp extract ( $9.88 \pm$  mg trolox/ g of extract) (Figure 9).

The  $IC_{50}$  was significantly different among ( $p < 0.05$ ) each methanol seed extracts of *S. cumini* trees. The results from mean comparison among them revealed that the highest  $IC_{50}$  resulted in Sc7 methanol seed extract ( $1.63 \pm 0.02$  trolox/ g of extract) while the lowest  $IC_{50}$  resulted in Sc5 extract ( $0.60 \pm 0.01$  trolox/ g of extract) but it is not significantly different from Sc11, Sc1 and Sc9 (Figure 9).

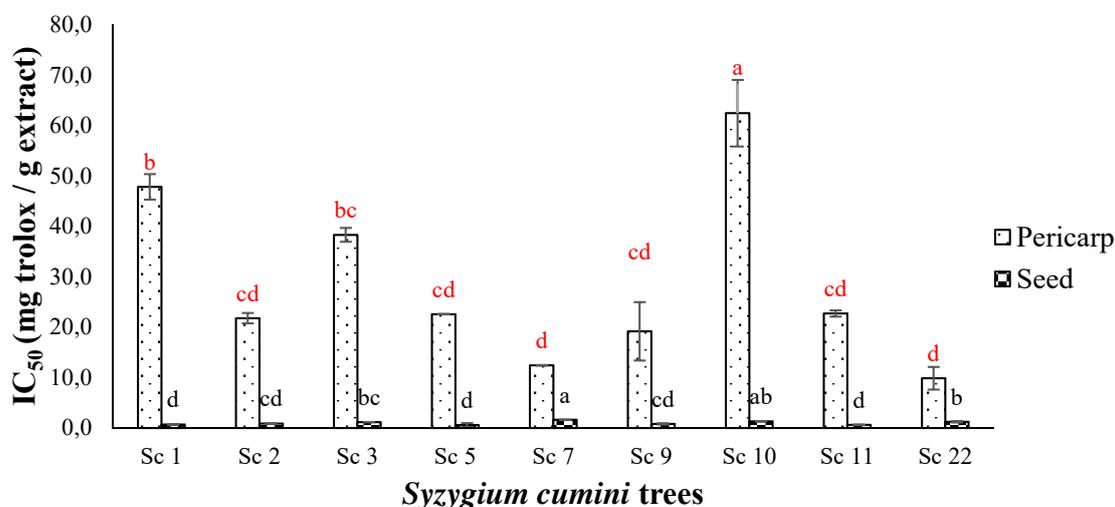


Fig. 9. Variation of  $IC_{50}$  values which represent *ABTS*<sup>+</sup> Radical Scavenging Activity of *S. cumini* seed extracts and using Ethanol and Methanol as extracting solvents

The IC<sub>50</sub> of a compound is inversely related to its antioxidant capacity, as it expresses the amount of antioxidant required to decrease the ABTS concentration by 50%. A lower IC<sub>50</sub> indicates a higher antioxidant activity of a compound. Phenolics were the main antioxidant components, and their total contents were directly proportional to their antioxidant activity [29]. Pearson correlation resulted negative correlation between TPC and IC<sub>50</sub>. In present study methanol seed extracts resulted the highest IC<sub>50</sub>. In between the seed and pericarp extracts of *S. cumini* resulted high variability.

*S. cumini* seed extracts resulted higher TPC, TFC and IC<sub>50</sub> compare to pericarp extracts. This may be attribute the content of more phenolic compounds in seed extracts such as alkaloid, jambolin, glycoside jambolin, gallic acid, etc. than pericarp extracts [30]. Antolovich *et al.* [31], determined that seed has assumed increasing phenols, often a source of unique phenols or compounds in much higher concentration than in the flesh. Previous studies reported that *S. cumini* seed extracts given the higher TPC, TFC and antioxidant activity compare to pericarp [32,33]. It indicates wide variation in the TPC, TFC and IC<sub>50</sub> according to the portion of fruit used.

In this study, high variation was observed within *S. cumini* trees, which is collected from Belihuloya region in Sri Lanka. According to recent reports, polyphenolic content which responsible for antioxidant capacity in plant foods varies greatly even among cultivars of the same species [34]. The presence of polyphenolic factors largely influenced by genetic factors, environmental conditions, variety processing and storage conditions [35].

It also indicates wide variation in the Crude extract yield, TPC, TFC and IC<sub>50</sub> according to the *S. cumini* trees. It may be due to rich diversity of phenolic compounds in different trees. Traditionally, *S. cumini* is propagated by seeds, hence there is considerable variation among the trees, due to its cross pollination in nature [11]. Thus, a great deal of antioxidant variation is observed in trees and fruits within *S. cumini* population.

### Conclusion

The best solvent for the extraction of antioxidant compounds is methanol. *S. cumini* seed contains the highest antioxidant compounds compare to pericarp.

It is evident from that *S. cumini* trees possess diverse antioxidant actions among the population as determined by ABTS radical scavenging assay. Phenolic and flavonoid compounds are diversified among the *S. cumini* trees which imply the potential of improving better cultivar for the community in the region with best antioxidant properties.

### Bibliography

1. Sivasubramaniam, K. and Selvarani, K. (2012). Viability and vigor of jamun (*Syzygium cumini*) seeds. *Brazilian Journal of Botany*, 35, 397-400.
2. Chaudhary, B. and Mukhopadhyay, K. (2012). *Syzygium cumini* (L.) Skeels: A potential source of nutraceuticals. *Int J Pharm Biol Sci*, 2, 46-53.
3. Kumar, A., Ilavarasan, R., Deecaraman, M., Aravindan, P., Padmanabhan, N., & Krishan, M.R.V. (2013). Anti-diabetic activity of *Syzygium cumini* and its isolated compound against streptozotocin-induced diabetic rats. *Journal of Medicinal Plants Research*, 2, 246-249.
4. Afify, A.E.M.M., Fayed, S.A., Shalaby, E.A., & El-Shemy, H.A. (2011). *Syzygium cumini* (pomposia) active principles exhibit potent anticancer and antioxidant activities. *African Journal of Pharmacy and Pharmacology*, 5, 948-956.
5. Bhuiyan, M.A., Mia, M.Y, & Rashid, M.A. (1996). Antibacterial principals of the seed of *Eugenia jambolana*, *Banga J. Botany*, 25, 239-24.
6. Kusumoto, I.T., Nakabayashi, T., Kida, H., Miyashiro, H., Hattori, M., Namba, T., & Shimotohno, K. (1995). Screening of various plant extracts used in ayurvedic medicine for inhibitory effects on human immunodeficiency virus type 1 (HIV-1) protease. *Phytotherapy Research*, 9, 180-184.
7. Indira, G., & Mohan, R.J. (1993). National institute of Nutrition Indian council of Medical Research, Hyderabad, pp: 34-37.

8. Velioglu, Y.S., Mazza, G., Gao, L., & Oomah, B.D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of agricultural and food chemistry*, 46, 4113-4117.
9. Wiseman, H., & Halliwell, B. (1996). Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochemical Journal*, 313, 17.
10. Stadtman, E.R. (1992). Protein oxidation and aging. *Science*, 257, 1220-1224.
11. Purseglove, J.W. (1981). *Tropical Crops – Dicotyledons*. The English Language Book Society and Longmann, 28.
12. Khan, S., Vaishali and Sharma, V. (2010). Genetic differentiation and diversity analysis of medicinal tree *Syzygium cumini* (Myrtaceae) from ecologically different regions of India. *Physiol. Mol. Biol. Plants*, 16, 149-157.
13. Singleton, V.L., Orthofer, R., & Lamuela-Raventós, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in enzymology*, 299, 152-178.
14. Siddhuraju, P. & Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of agricultural and food chemistry*, 51, 2144-2155.
15. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, 26, 1231-1237.
16. Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S., & Ju, Y.H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Linnophila aromatica*. *Journal of food and drug analysis*, 22, 296-302.
17. Chan, P.T. (2014). Antioxidant activities and polyphenolics of various solvent extracts of red seaweed, *Gracilaria changii*. *Journal of Applied Phycology*, 26.
18. Widyawati, P.S., Budianta, T.D.W., Kusuma, F.A., & Wijaya, E.L. (2014). Difference of solvent polarity to phytochemical content and antioxidant activity of plu-chea indicia less leaves extracts. *International Journal of Pharmacognosy and Phytochemical Research*, 6, 850-855.
19. Wong, J.Y., & Chye, F.Y. (2009) Antioxidant Properties of Selected Tropical Wild Edible Mushrooms. *Journal of Food Composition and Analysis*, 22, 269-277.
20. Kucuk, M., Kolayh, S., Karaoglu, S., Ulusoy, E., Baltaci, C., & Candan, F. (2007). Biological activities & chemical composition of three honeys of different types from Anatolia. *Food Chemistry*, 100: 526-534
21. Wojdyło, A., Oszmiański, J., & Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food chemistry*, 105, 940-949.
22. Pietta, P.G., (2000). Flavonoids as antioxidants. *Journal of natural products*, 63, 1035-1042.
23. Koffi, E., Sea, T., Dodehe, Y., & Soro, S. (2010). Effect of solvent type on extraction of polyphenols from twentythree Ivorian plants. *Journal of Animal and Plant Sciences (JAPS)*, 5, 550-558.
24. Iloki-Assanga, S.B., Lewis-Luján, L.M., Lara-Espinoza, C.L., Gil-Salido, A.A., Fernandez-Angulo, D., Rubio-Pino, J.L., & Haines, D.D. (2015). Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. *BMC research notes*, 8, 396.
25. Mohamed, A.A., Ali, S.I., & El-Baz, F.K. (2013). Antioxidant and antibacterial activities of crude extracts and essential oils of *Syzygium cumini* leaves. *Plos one*, 8, e60269.
26. Samanta, A., Das, G. and Das, S.K. (2011). Roles of flavonoids in plants. *carbon*, 100(6).
27. Pourmorad, F., Hosseinimehr, S.J., & Shahabimajd, N. (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African journal of biotechnology*, 5(11).

28. Aberoumand, A., & Deokule, S.S. (2008). Comparison of phenolic compounds of some edible plants of Iran and India. *Pakistan Journal of Nutrition*, 7, 582-585.
29. Liu, S.C., Lin, J.T., Wang, C.K., Chen, H.Y., & Yang, D.J. (2009). Antioxidant properties of various solvent extracts from lychee (*Litchi chinensis* Sonn.) flowers. *Food Chemistry*, 114, 577-581.
30. Ayyanar, M., & Subash-Babu, P. (2012). *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asian Pacific journal of tropical biomedicine*, 2, 240-246.
31. Antolovich, M., Prenzler, P., Robards, K., & Ryan, D. (2000). Sample preparation in the determination of phenolic compounds in fruits. *Analyst*, 125, 989-1009.
32. Margaret, E., Shailaja, A.M. and Venugopal Rao, V. (2015). Evaluation of Antioxidant Activity in Different Parts of *Syzygium cumini* (Linn.). *International Journal of Current Microbiology and Applied Sciences*, 4(9), pp. 372-379.
33. Saha, R.K., Zaman, N.M. and Roy, P. (2013). Comparative evaluation of the medicinal activities of methanolic extract of seeds, fruit pulps and fresh juice of *Syzygium cumini* in vitro. *Journal of Coastal Life Medicine*, 1(4), pp. 300-308.
34. Urquiaga I, and Leijhton F. (2000). Plant polyphenol antioxidant and oxidative stress. *Biological Research*, 33, 1-14.
35. Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*, 56, 317-33