Comparative Phyto and Physicochemical Parameters of the Therapeutic Plant *Syzygium cumini* (L.) Skeels in Jaffna District

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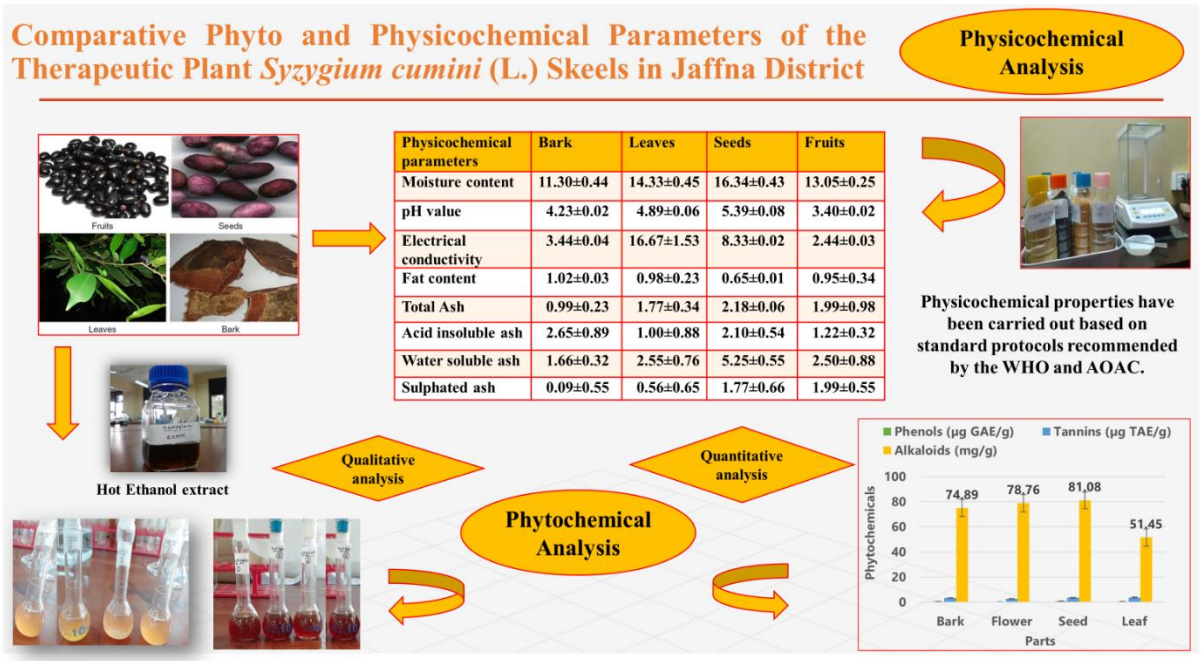

Abstract

Syzygium cumini (L.) Skeels (Myrtaceae) is an evergreen tree, grown widely in Sri Lanka. It is regularly recognized as jambolan, black plum, and jamun. It is an extensively used therapeutic herbal in the treatment of innumerable illnesses, particularly in Diabetes mellitus in traditional medicine. The goal of the research was to compare the phytochemical and physicochemical properties between the different parts of jambolana tree which is naturally found in Sri Lanka. Proximate estimation of physicochemical properties of dried matured different parts as bark, leaves, fruits, and seeds of the *Syzygium cumini* have been done in the current study rendering to the standard protocols recommended by the World Health Organization (WHO) and Association of Analytical Chemist (AOAC). This study includes electrical conductivity value, moisture content, fat content, and different ash contents analysis. It was found that the leaves had the highest electrical conductivity (16.67±1.53) and the bark had the lowest electrical conductivity (2.44±0.03). The moisture content of the bark was lower (11.30±0.44) in comparison to that of the seeds (16.34±0.43). Furthermore, the bark had the highest fat content (1.02±0.03) and acid

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insoluble ash (2.65±0.89), when compared to the seeds (0.65±0.01) and leaves (1.00±0.88) respectively. The seeds had the highest pH (5.39±0.08); total ash (2.18±0.06) and water-soluble ash (5.25±0.55), while the fruits had the highest sulphated ash (1.99±0.55). Qualitative inspection revealed that hot ethanolic seed extracts contained high levels of flavonoids, tannins, phenols, glycosides, alkaloids, terpenoids and quinones, in addition to other parts, which also had high levels of alkaloid content (81.06 mg/g). The current study will contribute useful data in the precise documentation and validation of various parts of *S. cumini* and may aid in removing contaminants in the preparation of nutraceuticals or medicine.

Keywords: *Medicinal Plant, Parts, Parameters, Physicochemical, Phytochemical, Syzygium cumini*

1. Introduction

Syzygium cumini (*S. cumini) (L.)* Skeels (Myrtaceae) recognized as jambolana is regularly utilized as a therapeutic agent of several illnesses, including in the control of blood sugar; it is one of the best-known frequently cultivated species (Ayyanar and Subash-Babu, 2012). It is recognized as an underutilized fruit crop in Sri Lanka (Prasajith et al., 2018) and it is usually known as jamun, Indian blackberry, Malabar plum, black or purple plum and etc. (Warrier et al., 1996). All parts of jambolana possess therapeutic potential and it has been utilized in alternative medication for several disorders for an extended period (Ayyanar and Subash-Babu, 2012).

Controlling and treating diabetes has consistently been made easier by the isolation of constituents and extracts from various natural resources, particularly plants (Sanmugarajah & Rajkumar, 2022). This plant has been regarded as an antidiabetic plant since it became commercially available several decades ago (Komal et al., 2018). Over the previous four decades, the antidiabetic activity of this plant has been reported in literature. The immense quantity of literature and scientific studies found in the database revealed that the extracts of jambolana possess significant pharmacological activity, especially anti-diabetic and antioxidant activities (Ramirez & Roa, 2003; Helmstädter, 2008; Bopp et al., 2009; Ayyanar & Subash-Babu, 2012; Baliga et al., 2013; Srivastava & Chandra, 2013; Chagas, et al., 2015; Baldissera et al., 2016; Krishnasamy et al., 2016; Bitencourt et al., 2017; Rekha, 2021; Qamar et al., 2022; Uddin et al., 2022). The usage of *Syzygium cumini* (*S. cumini*) was presented in allopathy in the mid-nineteenth period, when the initial reports on the study of its antidiabetic possessions were published (Chagas et al, 2015).

In the treatment of many diseases, the traditional medicine system in Sri Lanka has displayed much promise with fewer side effects (Sanmugarajah et al., 2022) than modern synthetic drugs (Rajkumar et al., 2022[a]). The human liver can be protected by phytochemicals (Rajkumar et al., 2022[b]). Although numerous phytochemical and physicochemical studies for leaves, bark, seeds, fruits of *S. cumini* have been performed separately worldwide (Bigoniya et al., 2012; Srilakshmi, 2014; Swapnil et al., 2015; Ghosh et al., 2016; Alston & Dighe, 2016; Cartaxo-Furtado et al., 2017), the present study focused on the comparative evaluation of the phytochemical and physicochemical properties between the selected parts such as leaves, bark, seeds and fruits of *S. cumini* which is native in Jaffna District, Sri Lanka because the constituents of medicinal plants are different in various regions due to the influence of the environmental and climatic conditions. The pharmacological effects of these constituents can vary depending on their chemical structures and compositions. This comparative study will motivate future

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scientific studies aimed at extracting the most important chemical composition from suitable parts of *S. cumini.*

2. Methodology

2.1. Plant Materials

Matured bark, leaves, fruits and seeds of *S. cumini* were collected from September to October 2021 from the Jaffna District, Sri Lanka and systematically authenticated by the Department of Botany, Faculty of Science, University of Jaffna.

2.2. Preparation of Samples and Extracts

Each plant part was washed with tap water and allowed to air-dry thoroughly under shade for 3 weeks. Then, dried material was pulverized and the powder was kept in a labelled air-tight container until further use. Each powdered plant part was extracted with ethanol using the hot extraction technique and each extract was concentrated using a rotary evaporator (Buchi) before further analysis.

2.3. Proximate estimation of physicochemical properties

Three samples of each powdered part of *S. cumini* were exposed for assessment such as moisture content, ash values, fat content, pH in 1% and 10% solution, and electronic conductivity values were performed based on the standard procedures approved by the World Health Organization and Association of Analytical Chemist (WHO, 1998; AOAC, 2016).

2.3.1. Determination of moisture content

An amount of 1.0 g of *S. cumini* powder was added separately, to a moisture disc that has been properly weighed (Electronic measurement scale – Mettler Toledo) and dried for 5 hours at 105 °C in an oven (Memmert) and cooled for 30 minutes in a desiccator. Thereafter it was immediately weighed to estimate the moisture content. The amount of in mg/g of air-dried material was used to calculate the loss of weight (WHO, 1998).

$$\text{Percentage of moisture content} = \frac{\text{Loss of moisture}}{\text{Weight of sample}} \text{x} 100$$

2.3.2. pH value determination

At room temperature, the pH of various preparations (water-soluble portions) in 1% w/v (1 g: 100 ml) and 10% w/v (10 g: 100 ml) containing the powder from each plant part of *S. cumini* were measured with standard simple glass electrode pH meter (Neeraj & Bhupinder, 2011).

2.3.3. Determination of electrical conductivity

Using an electrical conductivity meter, the powder samples of each material were tested for electrical conductivity in 1% and 10% aqueous solutions based on Association of Official Analytical Chemistry method 981.121 (AOAC, 2016).

2.3.4. Determination of ash contents

2.3.4.1. Total ash

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An accurately weighed crucible was pre-ignited at 350 °C for an hour. Sample was placed in the crucible in an even layer, and it was heated gradually to 550 °C for five hours in a muffle furnace (Nabertherm) until it turned white in colour. After that, the sample was placed in a desiccator to cool down to room temperature and weighed. Its weight was recorded in mg/g of air-dried material (WHO, 1998).

2.3.4.2. Acid-insoluble ash

The crucible with the total ash was filled with 25 ml of HCl (70 g/l) TS, fully covered with a lid, and slightly boiled for 5 minutes. Hot water (5 ml) was used to rinse the lid and added to the crucible. The insoluble materials were filtered using an ashless Whatman filter paper without ash, and the filtrate was then thoroughly washed with hot water until it was neutral. The filter paper comprising the insoluble materials were added to the crucible, and ignited slowly raising the temperature in a muffle furnace to 550 °C for three hours, keeping the weight constant. After 30 minutes of cooling in a suitable desiccator, weight was recorded of the residue. The acid insoluble ash content was calculated as mg/g of air-dried material (WHO, 1998).

2.3.4.3. Water-soluble ash

The crucible with the total ash was filled with 25 ml of water, covered with a lid, and heated gently for 5 minutes. The insoluble materials were collected using an ashless filter paper, washed in hot water and ignited in a crucible for 15 minutes in a muffle furnace at 450 °C. After 30 minutes of cooling in a suitable desiccator, weigh of the residue was recorded. The water-soluble ash content was calculated as mg/g of air-dried material (WHO, 1998).

2.3.4.4. Sulfated ash

A crucible made of silica was ignited at 650 °C for 30 minutes, cooled in a desiccator, and then precisely weighed. A crucible that had already been lit was filled with one gram of the plant powder, which was gently ignited at first until the substance was completely white. The sample was cooled and moistened with 1 ml of sulfuric acid (1760 g/l) TS, then gently heated until it was completely charred. Once the deposit has cooled, it was moistened with a small amount (typically 1 ml) of sulfuric acid (1760 g/l) TS, heated gently until white fumes were no longer released, and ignited at 800 °C + 25 °C until the residue was completely consumed (WHO, 2012).

Calculation method for total ash, acid insoluble, water soluble and sulfated ashes is according to the following equation.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of original sample}} \text{x} 100$$

2.3.5. Crude fat content

To make sure the weight of the flask is stable, a flask and its lid were left in an incubator overnight at 105 °C. A weighed sample was placed in an extraction protector and transferred to a Soxhlet apparator. The flask was filled with 250 ml of petroleum ether, which was heated for about 14 hours at a rate of 150 drops per minute. Utilizing a vacuum condenser, solvent was evaporated. The flask was incubated at 80–90 °C until the solvent had completely evaporated and the flask had dried completely. The flasks were then moved to the desiccator for cooling, and the residue weight was noted (WHO, 1998).

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$$\text{Fat content (\%)} = \frac{\text{Final weight}}{\text{Initial weight of sample}} \text{x}100$$

2.4. Preliminary Phytochemical Screening

To identify the occurrence of the following secondary metabolites, the hot ethanol extracts of each part of *S. cumini* powder were first screened for phytochemical compounds using standard laboratory techniques (Kokate et al., 1995; Farnsworth, 1996; Harborne, 1998; Gupta et al., 2008, Prashant et al., 2011; Tiwari et al., 2011; Saxena et al., 2012; Kamal, 2014; Bijekar, and Gayatri 2015; Gul et al., 2017; Rajkumar et al., 2021 & 2022[a]).

2.4.1. Identifying phenolic compounds

2 ml (1%) of each extract received two to three droplets of a 1% ferric chloride solution. With ferric ions, phenolic compounds produce a deep violet colour (Tiwari et al., 2011).

2.4.2. Identifying Tannins (Ferric chloride test)

Testing for ferric chloride involved boiling and filtering a small amount of the extract with distilled water. Two drops of ferric chloride were mixed to the filtrate, and it was determined that the presence of a blue-black or greenish-black precipitate in the presence of ferric chloride was proof that tannins were present (Harborne, 1998).

2.4.3. Identifying Flavonoids (Shinoda test)

The extract was dissolved in methanol (50%, 1-2 ml) by heating. Three pieces of magnesium chips and a few droplets of concentrated hydrochloric acid were poured to an alcoholic solution of each extract. Flavonoids are present when an orange, pink, red, or purple color is present (Bijekar, and Gayatri 2015).

2.4.4. Identification of Coumarins

With 1% KOH in pure ethanol, coumarins produce a yellow color. Three to four drops of 1% KOH in pure ethanol were added to 1 ml of portions of 1% solutions of each in test tubes (Farnsworth, 1996).

2.4.5. Finding Anthraquinones

A sample in potassium hydroxide solution was prepared to examine the anthraquinone derivatives; anthraquinones have a blood-red color (Gupta et al., 2008).

2.4.6. Identification of Quinones

The sodium hydroxide is added to the test sample. Quinones are present when blue, green, or red color formation occurs (Kokate et al., 1995).

2.4.7. Identification of Alkaloids

Mayer's Test: 1-2 drops of 1M hydrochloric acid and 4-5 drops of Mayer's regent (Potassium Mercuric Iodide) were used to acidify and treat one ml portions of each extract, respectively. Alkaloids can be detected by the appearance of a precipitate that is yellow or white in color or turbidity.

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Dragendroff's Test: Extracts were individually dissolved in diluted hydrochloric acid and filtered. Dragendroff's reagent (potassium bismuth iodide solution) was applied to the filtrates. Alkaloids are present when red precipitate forms (Kokate et al., 1995).

2.4.8. Identification of Glycosides (Keller-Kiliani Test)

Each 2 ml of filtered sample was mixed with 0.5 ml glacial acetic acid, 3 drops of 1% aqueous $FeCl_3$ solution, and 0.5 ml of conc H_2SO_4. The formation of brown ring between the layers indicated the presence of glycosides (Gul et al., 2017).

2.4.9. Identification of Amino acids (Xanthoproteic Test)

A few drops of concentrated nitric acid were applied to the extracts. Formation of yellow colour indicates the occurrence of amino acids (Tiwari et al., 2011).

2.4.10. Determination Saponins (Foam Test)

Shaken with 2 ml of water and 0.5 g of extract. It is a sign that saponins are present if the foam created lasts for ten minutes (Kamal, 2014).

2.4.11. Detection of reducing sugar (Fehling's test)

Fehling's A and B solutions were added and mixed in a test tube with 1 ml of each. This was heated for ten minutes over a boiling water bath with two milliliters of added plant extract. Precipitate that is brick red or orange in color forms when reducing sugars are present (Saxena et al., 2012).

2.4.12. Detection of Steroids (Libermann Burchards test)

Extract was dissolved in equal volumes of anhydrous acetic acid and chloroform ($CHCl_3$) and cooled to 0 °C. The mixture was transferred to a dry test tube and concentrated sulfuric acid (H_2SO_4) was added to the bottom of the tube. Formation of a reddish brown or violet- brown ring at the interface of the two liquids indicated steroids (Saxena et al., 2012).

2.4.13. *Detection of Terpenoids (Chloroform test)*

Each 2 mL of filtered sample was added with 0.5 mL chloroform with 0.5 mL of acetic anhydride and a few drops of concentrated sulfuric acid. The formation of reddish-brown precipitate leads the occurrence of terpenoids (Kamal, 2014).

2.5. Quantitative analysis of phytochemicals

2.5.1. Total Phenolic Content (Folin-Ciocalteu colorimetric method)

About 20 µl of each filtered sample was added to the test tube by using a micropipette. 1.58 µl water and 100 µl folin reagent were added to each of above test tubes. They were mixed well by using a magnetic stirrer, and they were allowed to stand for 8 minutes after stirring. 300 µl sodium carbonate solution was added to each stirred solution. They were heated in a water bath at 40 °C for 30 minutes and permitted to cool. They were again stirred well and the absorption of each sample was measured by spectrophotometer at 765 nm wavelength. A curve chart for each solution was prepared using absorbance and concentration (Singleton et al., 1999). Total phenol content was expressed as µg GAE/g (Rajkumar et al., 2021 & 2022[a]).

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2.5.2. Total Tannin Content (Folin-Ciocalteu colorimetric method)

Each of the filtered samples (0.5 ml) was added into 3.75 ml of distilled water and 0.25 ml of Folin reagent, 0.5 ml of 35% of sodium carbonate. The absorbance of each sample was measured at 725 nm using a spectrophotometer. The blank was prepared by using the above reagents with distilled water instead of the sample. A curve chart for each solution was prepared by using absorbance and concentration (Kavitha Chandran & Indira, 2016). Total tannin content was expressed as µg TAE/g (Rajkumar et al., 2021 & 2022[a]).

2.5.3. Total Alkaloid Content

About 5 g of the three samples of each plant material were loaded into a 250 ml beaker. An amount of 200 ml of 20% acetic acid was added and enclosed to stand for 4 hours. They were filtered, and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to each extract until the precipitous was completed. The whole solution was permitted to settle down, and the precipitate was collected by filtration through the accurately weighed filter paper. The filtrate is the alkaloid that was dried out in the oven for 4 hours and weighed. Total alkaloid content was measured as mg per g of air-dried material (Edeoga et al., 2005; Aliyu et al., 2008; Rajkumar et al., 2021 & 2022[a]).

2.6 Statistical data analysis

Data were statistically analysed by Analysis of Variance (ANOVA) using a SAS statistical package (version 9.1.3) and mean values along with standard deviation were recorded. Statistical analysis was done for three replicates of each part of selected plant.

3. Results and Discussion

3.1. Physicochemical parameters

The average physicochemical parameters of each part of *S. cumini* course powder are tabulated as Table 1. From the findings, it was found that the leaves had the highest electrical conductivity (16.67±1.53) and the bark had the lowest electrical conductivity (2.44±0.03). The moisture content of the bark was the lowest (11.30±0.44) when compared to the seeds (16.34±0.43). Furthermore, the bark had the highest fat content (1.02±0.03) and acid insoluble ash (2.65±0.89), when compared to the seeds (0.65±0.01) and leaves (1.00±0.88), respectively. The seeds had the highest pH (5.39±0.08), total ash (2.18±0.06), and water-soluble ash (5.25±0.55), whereas fruits had the least pH (3.40±0.02); seeds had the least fat content (0.65±0.01) and leaves had the lowest acid insoluble ash (1.00±0.88). The amount of sulphate ash in fruits (1.99±0.55) was higher than in bark (0.09±0.55).

A reduced amount of moisture content of preparations can avert microbial, fungiform or yeast growth while being stored (Pandey et al., 2012). The quality, authenticity, and purity of crude drugs are determined by ash values, which are significant measurable standards (Paramjyothi and Syed, 2010). The extractive values are helpful in determining the chemical components that are existing in the crude drug and also help in determining which elements are soluble in a certain solvent (Shwetajain et al., 2011).

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Table 1: Physicochemical parameters of each part of *S. cumini powder*

Physicochemical parameters	Samples			
	Bark	**Leaves**	**Seeds**	**Fruits**
Moisture content	11.30±0.44	14.33±0.45	16.34±0.43	13.05±0.25
pH value	4.23±0.02	4.89±0.06	5.39±0.08	3.40±0.02
Electrical conductivity	3.44±0.04	16.67±1.53	8.33±0.02	2.44±0.03
Fat content	1.02±0.03	0.98±0.23	0.65±0.01	0.95±0.34
Total Ash	0.99±0.23	1.77±0.34	2.18±0.06	1.99±0.98
Acid insoluble ash	2.65±0.89	1.00±0.88	2.10±0.54	1.22±0.32
Water soluble ash	1.66±0.32	2.55±0.76	5.25±0.55	2.50±0.88
Sulphated ash	0.09±0.55	0.56±0.65	1.77±0.66	1.99±0.55

Based on the results, the seeds have high moisture content than other parts. Ash values were also different in each part. It may be due to variations in the preparation procedure, and/or drying method of parts or geographical variations in the environment from which plant materials were collected, or standardization method used. which may be due to high content of a mixture of impurities like carbonates, phosphates, oxalates, silicates and silica.

1.2. Phytochemical parameters

1.2.1. Qualitative *analysis of phytochemicals*

Based on Table 2, the hot ethanolic seed extracts contained high levels of flavonoids, tannins, phenols, glycosides, alkaloids, terpenoids, quinones and amino acids, in addition to other parts of *S. cumini*.

Table 2: Qualitative analysis of phytochemicals of different parts of *S. cumini*

Phytochemicals	Seed	Fruit	Leaf	Bark
Flavonoids	++++	++	+	-
Tannins	++++	++++	++++	++++
Phenols	++++	++++	++++	+++
Glycosides	++++	+++	-	+++
Terpenoids	++++	-	-	++++
Alkaloids	++++	+++	++	+++
Anthraquinones	-	-	-	++
Saponins	+	++++	-	++++
Quinones	++++	+	++	+
Coumarins	+	++	++++	-
Amino Acids	++++	+	-	-
Reducing Sugar	+	+	-	++++
Steroid	-	-	-	++++

(++++) abundant, (+) present, (-) absent

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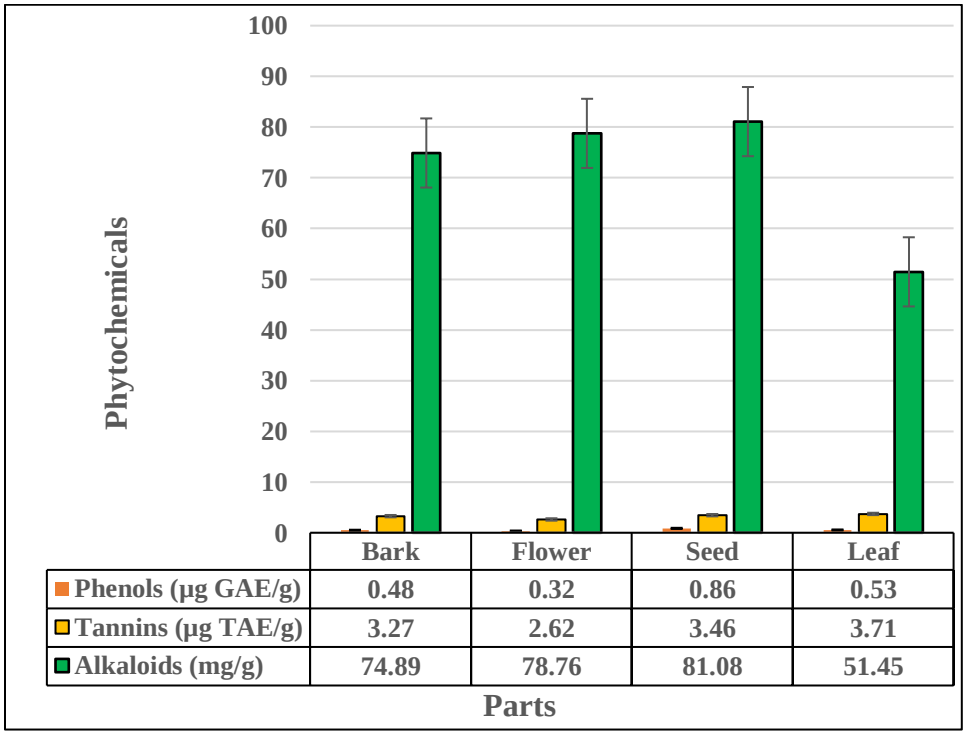
Tannins are represented in all selected parts of *S. cumini* in high levels of concentration. Majority of these phytochemicals are commonly known as hypoglycemic agents and have therapeutic properties, such as anti-diabetic action (Mujeeb et al., 2014). The seeds of *S. cumini* can be used as an astringent and diuretic, and they also have hypoglycemic, antipyretic, anti-inflammatory, psychopharmacological, hypo-lipidemic, and antioxidant activities (Sharma et al., 2019).

The current results of *S. cumini* could be comparable to those of other phytochemical studies conducted in different countries related to its different parts (Murti et al., 2012; Kamal, 2014; Prabakaran & Shanmugavel 2017; Aziz & Banerjee, 2018).

1.2.2. Quantitative analysis of phytochemicals

According to Figure 1, the comparison of the total phytochemical contents of the different parts of *S. cumini* showed that the alkaloid content was highly represent in their concentration in all selected parts of *S. cumini* rather than other phytochemicals.

As shown in Figure 1, the alkaloid contents were found to be 81.08% in seeds extract of *S. cumini* when compared with the other parts. Alkaloids are organic molecules made up of nitrogen which occur naturally (Kurek, 2019). The primary function of alkaloids in the body is to manage diabetes and prevent starch from turning into sugar in the blood (Gaikwad et al., 2014). Based on the published research articles, researchers studied that the *S. cumini*'s alkaloid contains different chemical constitution (Omar et al., 2012) and seeds are more alkaloid-rich than other parts of plants (Sing et al., 2015).



	Bark	Flower	Seed	Leaf
Phenols (µg GAE/g)	0.48	0.32	0.86	0.53
Tannins (µg TAE/g)	3.27	2.62	3.46	3.71
Alkaloids (mg/g)	74.89	78.76	81.08	51.45

Figure 1: Quantitative analysis of phytochemicals of different parts of *S. cumini*

Different parts of *S. cumini* contain numerous phytochemicals such as alkaloids, carbohydrates, tannins, flavonoids, glycosides, etc. (Bandiola et al., 2017). The ethanol extracts from Jamun stem bark,

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leaf, seed, and fruit pulp revealed the presence of alkaloids, anthraquinone glycosides, flavonoids, tannins, saponins, phenols, cardiac glycosides, terpenoids, phytosterols, steroids and amino acids (Chaudhary and Mukhopadhyay, 2012). At the same time, another study stated that the leaf extract did not contain terpenoids and phytosterols (Jagetia et al., 2005). In Jaffna District, a comparative quantitative analysis revealed that *S. cumini* seeds possess higher levels of phenolics, alkaloids, and tannins content than other seed extracts such as *Brassica alba*, *Trigonella foenum-graecum*, and *Nigella sativa* (Rajkumar et al., 2021). Also, Rajkumar et al., found that the seed extract of *S. cumini* that was collected in Jaffna District had a great anti-microbial activity against *E. faecalis*, *S. aureus* and *E. coli* and which were comparable to the positive control streptomycin (Rajkumar et al., 2023).

A number of research studies also found that the *S. cumini* seed had hypoglycemic effect as well as glucose tolerance in alloxan or streptozotocin induced diabetes rats (Nair and Santhakumari, 1986; Pandey and Khan, 2002; Prince et al., 2003; Singh and Gupta, 2007; Kumar et al., 2008; Ahmad et al., 2017; Ulla et al., 2017; Arafa et al., 2020).

4. Conclusion

The present study revealed that the seeds had the highest pH (5.39±0.08); total ash (2.18±0.06) and water-soluble ash (5.25±0.55), while hot ethanolic seed extracts also contain main phytochemicals and higher alkaloid content than other parts. The current study will contribute useful data in the precise documentation and validation of various parts of *S. cumini* and may aid in avoiding its contamination in the preparation of nutraceuticals or medicine. Furthermore, to confirm its therapeutic potential, future scientific studies could focus on extracting the major chemical composition from *S. cumini*.

5. Acknowledgement

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