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Pharmacognostic and pharmacological evaluation of *Syzygium caryophyllatum* L. and *Syzygium cumini* L.

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Abstract

India is one of the nation blessed with a rich heritage of traditional medical systems and rich biodiversity to complement the herbal needs of the treatment administered by these traditional medical systems. The recognized Indian Systems of Medicine are Ayurveda, Siddha and Unani, which use herbs and minerals in the formulations. The antioxidant compounds are mostly produced in plants in the form of secondary metabolites. Phytochemicals can be literally referred to as 'plant-chemicals'. They are the non-nutritive chemical components of plants that possess numerous health benefits and disease prevention properties. Leaf chlorophyll concentration is an important parameter that is regularly measured as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. Chlorophyll is an antioxidant compounds which are present and stored in the chloroplast of green leaf plants and mainly it is present in the green area of leaves, stems, flowers and roots. The aim of this study was to observe antioxidant activity from the leaves of *S. caryophyllatum* and *S. cumini* and also to evaluate the qualitative and quantitative phytochemical analysis. The present study emphasizes the pharmacognostical study of the plant, chief chemical constituents, present in the plant *S. caryophyllatum* and *S. cumini*, and their pharmacological properties. The selected plants (*S. caryophyllatum* and *S. cumini*) have been used as a source for the development of medicines and neutraceuticals. Both plant samples contained bioactive chemicals that have pharmacological or toxicological effects. Both showed the presence of secondary metabolites such as steroids, glycosides, phenolics, tannins, anthocyanins, flavonoids, and alkaloids. Plant extracts antioxidant activity may be attributed to their phenolic and flavonoid content. Because of their phytoconstituents, antioxidants, and antibacterial properties, plants offer enormous potential for use in the pharmaceutical business and medicine.

Keywords: Antioxidant; Larvicidal Activity; Medicinal Plants; Phytochemicals

1. Introduction

Plants are a reservoir of chemical compounds that can combat many human diseases. Natural products have traditionally been utilized as a valuable source of novel medicinal agents. Many plant-based compounds are used as lead molecules to generate synthetic molecular analogs (Annadurai *et al.*, 2012). Natural products and their derivatives have historically been exploited as a valuable source of novel therapeutic agents (Koehn and Carter, 2005). Further, a large proportion of plant-based compounds are used as lead molecules in drug discovery to produce synthetic molecular analogs, implying that phytochemicals play a critical role in diversity-oriented synthesis (DOS) of natural product-like pharma-compounds (Marcaurelle and Johannese, 2008). Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga *et al.*, 2005; Mann, 1978). These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy,

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veterinary, agriculture, scientific research and countless other areas (Vasu *et al.,* 2009). A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro (Cowan, 1999). Antioxidants are substances that may protect cells from the damage caused by unstable molecules (free radicals) (Sies, 1997). Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of cancer, cardiovascular diseases, cataracts, immune system decline, and brain dysfunction (Mark, 1998).

The genus Syzygium consists of 1200 species, and these plants are native to the tropics, mainly tropical America and Australia (Annadurai *et al*., 2012). Plants of the Syzygium family are considered rich in volatile oils. These plant species have different biological properties such as antifungal, antibacterial, antiviral, anticarcinogenic, antioxidant, anaesthetic, and repellent activity. The Syzygium species having appreciable medicinal properties have drawn the attention of the researchers in recent times. Many pharmacological studies have been carried out in different Syzygium species. Syzygium is a genus of flowering plants comprising of about 1200 species, having a native range in tropical Africa, subtropical to tropical Asia, Australia, New Caledonia, New Zealand, Pacific islands (Elliot and Jones, 2010). 80 species are reported from China (Jie and Craven, 2007) and more than 75 species from India (Anand *et al*., 1999). Syzygium species exhibit antidiabetic (Kumar *et* al., 2008), antifungal (Park *et al*., 2014; Kiruthiga *et al*., 2011), anti-inflammatory (Chaudhuri *et al*., 1999), antibacterial (Kiruthiga *et al.,* 2011), antioxidant, hyper lipidemic (Modi *et al*., 1999) and growth inhibitory effects against oral pathogens (Cai and Wu.,1990). The Syzygium species are also found to possess antihyperglycemic activity, cytotoxic (Aisha *et al*., 2011) anti-angiogenic (Rekha,2010) and anti-nociceptive activity (Avila *et al.,* 2007). Further, the organoleptic analysis, functional properties, phytochemical constituents quantification of primary and secondary metabolites, chlorophyll content, mineral detection, antioxidant activity and larvicidal activity of aqueous, ethanol and methanolic extract of the selected plants were evaluated.

2. Material and methods

2.1. Sample collection and identification

The selected plant samples *Syzygium caryophyllatum* L. were collected from Kundarapadavu, Paivalige village in Kasaragod and *Syzygium cumini* L. were collected from Morgans Gate, Mangalore. The plant materials were authenticated by the help of taxonomist of Department of Biosciences, Mangalore University, Mangalagangothri, Karnataka, India.

Figure 1 *Syzygium cumini L.*

Figure 2 *Syzygium caryophyllatum L.*

Syzygium caryophyllatum, an endangered evergreen tree, belongs to the Myrtaceae family. This tree is native to Sri Lanka and India; in India, it is only found in the Western Ghats forests of Kerala, Karnataka, and Tamilnadu. The tree's namesake is known in Hindi as Jangli Jamun, in Karnataka as Kunta nerale, and in Malayalam as Kattunjara or Jnarapazham. Due to the threat posed by habitat loss and biotic pressure, this tree is listed on the IUCN Red List Endangered Class.

Syzygium cumini is an evergreen tropical tree belonging to the family Myrtaceae and native to Bangladesh, India, Nepal, Pakistan, Sri Lanka, Philippines and Indonesia. *Syzygium cumini* is also referred as *Syzygium jambolanum* and *Eugenia cumini*. Common names are Jambul, Black Plum, Java Plum, Indian Blackberry, Jamblang, Jamun etc.

2.2. Organoleptic evaluation and Functional properties

The organoleptic characters of the sample were evaluated. Organoleptic evaluation refers to evaluation of the formulation by colour, odour, taste and texture etc. Physical appearance, weight was determined. Functional properties were evaluated by studying its dispersibility, swelling power, water and oil absorption capacity, emulsion and foaming property and bulk density.

2.3. Method for preparation of extracts

To the dark-colored flasks, powdered plant material (10 g) was added and mixed with 200 ml of solvents with different polarities (water, ethanol, methanol) respectively. The flask containing the sample was placed in shaker for 8 hours and stored at room temperature for 24 hours. After 24 hours, Whatman No. 1 filter paper was used for the infusions and residue was reextracted with equal volume of solvents. The collected extract was preserved for the estimation of various parameters. After 48 hours, the process was repeated. Combined supernatant was pooled, put into evaporating dishes and evaporated to dryness at room temperature. 5 ml of distilled water was added to the residue and dissolved for storage at 4-8 °C in a refrigerator for further analysis (Thirumalaisamy *et al*., 2009).

2.4. Preliminary phytochemical screening

Chemical tests were carried out using the aqueous, alcoholic and methanolic extraction for selected medicinal plant powder, using standard procedure to identify the constituents as described by Harborn, (1973).

2.5. Quantification of primary metabolites

2.5.1. Determination of Total Carbohydrate

Weighed 100 mg of the sample into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl and cooled to room temperature. Neutralized it with solid sodium carbonate until the effervescence ceases made up the volume to 100 ml and centrifuged, collected the supernatant and take 0.5 and 1.0 ml for analysis. Prepared the standards by taking 0.2-1.0 ml of the working standards. 1.0 ml of water serves as a blank made up the volume to 1.0 ml in all the tubes with distilled water, and then added 4.0 ml of Anthrone reagent, heated for eight minutes in a boiling water bath, cooled rapidly and read the green to dark green colour at 630 nm. A standard graph was drawn by taking the concentration of glucose on X axis and spectrophotometer reading on Y axis. From the graph the concentration of glucose in the sample was calculated 100mg of the sample.

2.5.2. Estimation of Protein

500 mg of sample was grinded well with a pestle and mortar in 5-10 ml of the buffer. Centrifuged and the supernatant was used for protein estimation. 0.2 ml to 1ml of the working standard was pipette out into a series of test tubes. 0.1 ml and 0.2 ml of the sample extract was pipette out into other two test tubes. The volume was made upto 1ml in all the test tubes by using distilled water. A tube with 1 ml of water serves as the blank. 5 ml of alkaline copper solution was added to all the test tubes, mixed well and allowed to stand for 10 min. Then 0.5 ml of Folin-ciocalteau reagent was added, mixed well and incubated at room temperature in the dark for 30 min. Blue colour is developed and read at 660 nm. The amount of protein content in the sample was found out using the standard graph and expressed in mg/g or 100g sample.

2.5.3. Determination of total fat content

0.5 g of the powdered sample was mixed with 5ml of chloroform and 5ml of methanol solvent. The mixed solution with the sample was kept overnight on a mechanical shaker. The solution was filtered by using Whatman's filter paper and the filtrate was collected in the pre-weighed crucible. The collected filtrate was then kept in the oven for drying and the dry weight was taken.

2.5.4. Estimation of Ascorbic acid

1g of powdered sample extract was homogenized in 10 ml of 4% TCA and centrifuged at 2000 rpm for 10 min. The supernatant was treated with a pinch of activated charcoal, shaken well and kept it for 10 min. Centrifugation was repeated twice to remove the charcoal residues. The volume of the clear supernatants obtained was noted. 0.5 ml and 1 ml of the supernatant was taken for the assay and the volume was made up to 2.0 ml with 4% TCA. Series of working standard ascorbic acid solution (0.2 to 1.0 ml) was made up to 2.0 ml with 4% TCA and to this 0.5 ml of Dinitrophenylhydrazine (DNPH) reagent and two drops of 10% thiourea solution to all the test tubes. The osazones formed after incubation at 37 ºC for 3 hrs, were dissolved in 2.5 ml of 85% H_2SO_4 in ice cold, with no appreciable rise in

temperature. To the blank alone, DNPH reagent and thiourea solutions were added after the addition of 85% H_2SO_4. After incubation for 30 min. at room temperature, the solutions were read at 540 nm and the levels of ascorbic acid in the samples were determined using the standard graph and expressed as mg of ascorbate /g extract.

2.6. Determination of Bioactive compounds

2.6.1. Flavonoids determination

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

2.6.2. Determination of Total Phenolic Content

The concentration of phenolics in plant extracts was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml). 1 ml of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25 ml. A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV)/Visible spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract.

2.6.3. Determination of Tannin Content

The tannin content was estimated by the method of Price and Butler, 1977 with slight modifications. 20 µl of the sample was aliquoted into a test tube containing 980 µl of distilled water. To this, 500 µl of 1% $K_3Fe(CN)_6$ and 100 µl of 1% ferric chloride ($FeCl_3$) were added and made up to 3 ml with distilled water. After 10 min, the reaction mixture was measured using a UV spectrophotometer at 720 nm. The tannin content was expressed as µg of tannic acid equivalents/mg of extract.

2.6.4. Alkaloid determination

5g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.7. Mineral content

Mineral content in sample was detected using EDS system [Carl Zuiess-Fesem attached with oxford instruments EDAX systems].

2.8. Evaluation of *In vitro* Antioxidant Activity

2.8.1. Total Antioxidant Capacity

Methanol extract is added into a series of test tube containing methanol and mixed with 2 ml of Phosphomolybdenum reagent solution. Then the tubes have been kept in water bath for 90 min at 95°C. The resultant mixture was cooled to room temperature and the absorbance was read at 695 nm against blank. The experiment has been conducted in triplicates and values are expressed as equivalents of Ascorbic acid (mg) /g of the sample.

2.8.2. Reducing Power Assay

Different concentrations of methanol extracts were prepared in methanol solvent and assorted with 2.5ml of 0.2 M phosphate buffer and 2.5ml of freshly prepared 1% potassium ferricyannide ($K_3Fe(CN)_6$). This mixture was incubated for 20 minutes at 50°C. 2.5 ml of 10% trichloro acetic acid (TCA) was added and centrifuged at 3000 rpm for 10 minutes. 2.5ml of the clear extract was assorted with 2.5ml of methanol and 0.5ml of 0.1% ferric chloride ($FeCl_3$) and the absorbance was measured at 700 nm. The experiment has been conducted in triplicates and the reducing power was expressed as (mg) equivalents of Ascorbic acid /g of extract.

2.8.3. 1, 1Diphenyl-2-picryl hydrazyl (DPPH) Radical Scavenging Activity

Various concentrations (20–100 µg/ml) of a plant extract (2 ml) were taken in a number of vials containing 3 ml of 0.1 mM methanolic solution of DPPH. The test tubes were shaken gently and kept aside for 30 min at room temperature in the dark. An optical density of the sample was measured at 517 nm against blank. Ascorbic acid was used as the standard control. All the tests were performed in triplicates.

$$\text{Percentage inhibition (\%)} = ((\text{O.D of Control} - \text{O. D of Sample}))/\ (\text{O.D of Control}) \times 100$$

2.9. Total Carotenoids and Chlorophylls

Fresh leaf samples were washed thoroughly in a running tap water, rinsed with distilled water and blotted to dry in room temperature. 0.5 g of fresh plant leaf sample were taken and homogenized in tissue homogenizer with 10 ml of different solvents. Homogenized sample mixture was centrifuged at 10,000 rpm for 15min. at 40 ˚C. 0.5 ml of supernatant was mixed with 4.5 ml each of respective solvents (80% acetone, 100% acetone and ethanol). The solution mixture was analysed for Chlorophyll-a, Chlorophyll-b and carotenoid content in spectrophotometer (Sytronic). The equation used for the quantification of Chlorophyll-a, Chlorophyll-b, and carotenoids by different extractants are given in the table 1 (Porra *et al*, 1989; Lichtenthaler and Wellburn, 1983).

Table 1 Equations to determine the concentrations (µg/ml) of chlorophyll a (Chl-a), chlorophyll b (Chl-b) and total carotenoids (Cx+c) by different extractants in spectrophotometer

Extractants	Equations/Formula
80% Acetone	Chl-a=12.21A663.2 – 2.81A646.8 Chl-b=20.13A646.8 – 5.03A663.2 C x+c=(1000A470 – 3.27Ca –104Cb)/229
100% Acetone	Chl-a = 1 1.75A662-2.35A645 Chl-b = 18.61A645-3.96A662 Cx+c = (1000A470-2.27Ca-81.4Cb)/227
95% Ethanol	Chl-a=13.36A664 – 5.19 A649 Chl-b=27.43A649 – 8.12 A664 C x+c=(1000A470 –2.13Ca- – 97.63Cb)/209

A = Absorbance, Chl-a = Chlorophyll a, Chl-b = Chlorophyll b, C x+c =Carotenoids

2.9.1. Larvicidal Activity

Powdered plant material was soaked sequentially in solvent (96%), each for 48 hrs. The extracts were filtered and solvents removed using rotary evaporator at a temperature of 40 ˚C. The extracts were further dried in a freeze dryer to remove any residual water and then stored in a freezer at -20 ˚C until the day of use. Solutions of the extracts were made in DMSO, at varying concentrations, and incubated in duplicate vials with the mosquito larvae in a total volume of 10 ml. Ten brine mosquito larvae were placed in each of the duplicate vials. Others were placed in a mixture of DMSO (30 µl) and water to serve as a negative control. Cyclophosphamide, an anticancer drug was used as a positive control. After 24 hrs the mosquito larvae were examined the average number of survived larvae was determined. The mean percentage mortality was calculated.

2.10. Statistical Analysis

The experimental results were expressed as mean ± Standard Error Means (SEM) of triplicates. Analysis of data was carried out by applying one-way analysis of variance (ANOVA) using SPSS. P-value lesser than 0.05 ($p < 0.05$) was considered as statistically significant.

3. Results and discussion

3.1. Organoleptic analysis

The organoleptic test is useful to know the general characteristics of the plant as it would be further analysed and used for drug synthesis. *S. caryophyllatum* tastes bitter, has unpleasant smell and are amorphous in appearance whereas *S. cumini* also tastes bitter, but has pleasant odour and are soft in appearance.

3.2. Functional properties

Dispersibility was found to be high (6.623±0.040 %) in *S. caryophyllatum* than the *S. cumini.* Swelling power was high in *S. caryophyllatum* than *S. cumini.* Water absorption capacity (5.49±0.036 ml/g) and oil absorption capacity (3.103±0.105 ml/g) is high in *S. cumini* leaf sample. Emulsion property and emulsion stability was high (57.176±0.068 % and 4.213±0.005 %) in *S. caryophyllatum* leaf sample. Foam property was high (3.966±0.020 %) in *S. caryophyllatum* whereas foam stability was high (7.413±0.080 %) in *S. cumini*. Bulk density was high in *S. cumini* leaf sample (0.506±0.057 %). Dispersibility, swelling power, oil and water absorption capacity, foam property, foam stability, and emulsification property were all demonstrated by the plant's samples. All of these characteristics lead to the conclusion that both plant samples can be employed as a drug in the development of medicines.

Table 2 Functional properties of *S. caryophyllatum* and *S. cumini*

Plant samples	***S. caryophyllatum***	***S. cumini***
	LEAF	**LEAF**
Dispersibility (%)	6.623±0.040	6.353±0.050
Swelling power(g/g)	2.760±0.000	1.975±0.000
Water absorption capacity (ml/g)	5.1±0.1	5.49±0.036
Oil absorption capacity (ml/g)	3.063±0.055	3.103±0.105
Emulsion property (%)	57.176±0.068	40.11±0.115
Emulsion stability (%)	4.213±0.005	2.22±0.01
Foam property (%)	3.966±0.020	3.846±0.011
Foam stability (%)	5.613±0.015	7.413±0.080
Bulk density(g/ml)	0.363±0.000	0.506±0.057

The experimental results were expressed as mean ± standard error means (SEM) of triplicates, $P<0.05$ considered as significant.

3.3. Preliminary Phytochemical Screening

Table 3 Qualitative phytochemical screening of *S. caryophyllatum* and *S. cumini*

Phytochemicals	***S. caryophyllatum***			***S. cumini***		
	Ethanol	**Methanol**	**Aqueous**	**Ethanol**	**Methanol**	**Aqueous**
	Leaves	**Leaves**	**Leaves**	**Leaves**	**Leaves**	**Leaves**
Carbohydrate	+	+	+	+	+	+
Protein	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Glycoside	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	-
Saponins	-	-	+	-	-	+
Phenols	-	-	+	-	-	+

Flavonoids	-	-	+	-	-	+
Tannins	+	+	+	+	+	+

(+) indicates the presence and (-) indicates the absence of the phytochemicals

Phytochemical in plants have been extensively studied since the past decade as these phytochemicals have an impact on pharmaceutical, nutraceutical, and functional food industries. Many of these secondary plant metabolites are explored from initial identification as advanced clinical research before officially being used as pharmaceutical and nutraceutical ingredients. The phytochemical analysis shown in Table 3.

3.4. Quantification of primary metabolites

The quantitative estimation of the primary and secondary metabolites may be useful in the analysis of compounds that would be essential for the growth and development of the plant cell and also the nutritive and therapeutic properties present in the sample. The results of quantification of primary metabolites of *S. caryophyllatum* and *S. cumini* are shown in Table 4.

Table 4 Quantification of primary metabolites of *S. caryophyllatum* and *S. cumini*

Parameters	***S. caryophyllatum***	***S. cumini***
	LEAF	**LEAF**
Carbohydrate (mg/g)		
Aqueous	603.52±0.015	107.12±0.01
Ethanol	942.08±0.568	682.83±0.026
Methanol	928.9±0.01	893.75±0.015
Protein (mg/g)		
Aqueous	129.58±0.01	191.51±0.01
Ethanol	308.67±0.005	230.35±0.01
Methanol	214.28±0.01	832.61±0.001
Ascorbic acid (mg/g)		
Aqueous	314.65±0.05	321.93±0.066
Ethanol	120.59±0.079	653.24±0.01
Methanol	847.12±0.001	292.47±0.002
Fat (mg/g)	7.95±0.030	6.86±0.0057

The experimental results were expressed as mean ± standard error mean (SEM) of triplicates, $P<0.05$ considered as significant

The primary metabolite carbohydrates were high in ethanol extract (942.08±0.568 mg/g) of *S. caryophyllatum* and methanol extract (893.75±0.015 mg/g) in *S. cumini*. Protein was high in ethanol extract (308.67±0.005 mg/g) of *S. caryophyllatum* and methanol extract (832.61±0.001 mg/g) in *S. cumini*. Ascorbic acid was high in methanol extract (847.12±0.001 mg/g) of *S. caryophyllatum* and ethanol extract (653.24±0.01 mg/g) in *S. cumini.* Carbohydrates act as a source of energy essential to carrying out normal functions such as growth, movement and metabolism.

Proteins are the primary components of living things. Proteins was very high in *S. cumini* leaves (832.61±0.001 mg/g). Proteins from plants are an important source in food and feed. Ascorbic acid (vitamin C) is an abundant component of plants. It reaches a concentration of over 20 mM in chloroplasts and occurs in all cell compartments, including the cell wall. It has proposed functions in photosynthesis as an enzyme cofactor (including synthesis of ethylene, gibberellins and anthocyanins) and in control of cell growth (Nicholas and Glen, 2000).

3.5. Quantitative Analysis of Secondary Metabolites

Tannin was found to be most abundant one in ethanol extracts (1261.85±0.001 mg/g and 1304.52±0.01 mg/g). Phenolics is found to be high in ethanol extract (1304.52±0.01 mg/g) of *S. caryophyllatum* and aqueous extract (417.40±0.001 mg/g) *of S. cumini*.

Table 5 Quantification of secondary metabolites of *S. caryophyllatum* and *S. cumini*

Parameters	***S. caryophyllatum***	***S. cumini***
	LEAF	**LEAF**
Phenolic(mg/g)		
Aqueous	89.92±0.0001	417.40±0.001
Ethanol	138.81±0.0005	183.153±0.001
Methanol	125.65±0.001	160.36±0.01
Tannin (mg/g)		
Aqueous	321.87±0.01	995.7±0.1
Ethanol	1261.85±0.001	1304.52±0.01
Methanol	638.51±0.001	1298.81±0.015
Flavonoid (mg/g)	0.0677±0.0002	0.0967±0.0001
Alkaloids (mg/g)	0.1245±0.0001	0.0977±0.0001

The experimental results were expressed as mean ± standard error means (SEM) of triplicates, P<0.05 considered as significant.

Secondary metabolites are known to have pharmacological activity, one of which is antidiabetic. Alkaloids belong to the group of plant secondary metabolites that contains a basic nitrogen atom in the compound. In addition to the nitrogen atom alkaloids may also contain sulphur and oxygen atoms (Kabera *et al.,* 2014; Chauhan *et al.,* 2017). Most of the alkaloids produced by plants are toxic and are generally produced as a defense molecule against other organisms. Alkaloids are basic in nature and are mostly derived from the plant sources. Alkaloids are known to have several beneficial properties such as anti-inflammatory, anti-psychotic, anti-plasmodic activity and some alkaloid may also act as anti-neoplastic agent (Debnath *et al.,* 2018). Flavonoids are antioxidants that play an essential role in the prevention and treatment of diabetes mellitus. There are two distinct types of tannins. Condensed tannins which are large polymers of flavonoids and hydrolysable tannins which are polymers composed of a monosaccharide core (most often glucose) with several catechin derivatives attached. The two types of tannins have most properties in common, but hydrolysable tannins are less stable and have greater potential to cause toxicity. The water solubility is restricted and decrease in general with the size of the tannin molecule. Tannins indiscriminately bind to proteins and larger tannins are used as astringents in cases of diarrhea, skin bleedings and transudates. Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colours. Phenolics are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, etc.) and beverages (tea, coffee, beer, wine, etc.), and partially responsible for the overall organoleptic properties of plant foods.

3.6. Mineral content

The mineral content present in both the leaf samples of *S. caryophyllatum* and *S. cumini* include oxygen, magnesium, iron, potassium, calcium, copper, chlorine and silicon. The weight of mineral content present in each leaves sample are given in the below Table 6. Plants are complex organisms whose mineral needs are determined by a number of molecular, cellular and whole-plant events. Biochemically, plant nutrition deals with a complex of biosynthetic events by which organic plant substance is produced from inorganic materials in the environment. These elements include C, H, O, N, P, K, S, Ca, Fe, Mg, Si, Cl etc. These elements play an important role in development of plant. It may be directly involved in structure of a cell material or may be helping in metabolic process in combination with cellular enzymes. In absence of these essential element the plant gets deficiency symptoms and shows morphological abnormalities.

Table 6 Mineral composition of *S. caryophyllatum* and *S. cumini*

Parameters	Weight %	
	S. caryophyllatum	***S. cumini***
	LEAF	**LEAF**
O	12.94	14.26
Mg	0.51	0.20
Fe	0.30	0.35
K	0.48	0.42
Ca	0.55	5.35
C	4.88	0.43
Cl	0.25	0.63
Si	0.19	7.09

3.7. Evaluation of In vitro Antioxidant Activity

The reducing power concentration was high in methanolic extract (124.562±0.0015 mg/g) of *S. cumini* leaf samples when compared to other plant samples which is shown in the Table 7.

Table 7 Screening of antioxidant assay of *S. caryophyllatum* and *S. cumini*

Antioxidant Activity	***S. caryophyllatum***	***S. cumini***
	Leaf	**LEAF**
Total antioxidant activity (mg/g)		
Aqueous	76.1607±0.006	80.22±0.0015
Methanol	130.433±0.0152	81.1907±0.004
Ethanol	159.956±0.043	80.996±0.001
Reducing power (mg/g)		
Aqueous	71.768±0.117	116.47±0.02
Methanol	69.690±0.054	124.562±0.0015
Ethanol	78.126±0.093	165.55±0.01
DPPH (%)		
Aqueous (0.5ml)	89.41±0.060	60.366±0.230
Aqueous (1.0 ml)	87.436±0.011	27.743±0.063
Methanol (0.5ml)	84.49±0.060	50.796±0032
Methanol (1.0 ml)	81.035±0.003	16.826±0.048
Ethanol (0.5ml)	71.43±0.015	58.05±0.017
Ethanol (1.0 ml)	32.22±0.005	4.128±0.0005

The experimental results were expressed as mean ± standard error means (SEM) of triplicates, $P<0.05$ considered as significant.

Reducing power is associated with antioxidant activity and serve as a significant reflection of the antioxidant activity. Total antioxidant activity concentration was high ethanolic extract (159.956±0.043 mg/g) of *S. caryophyllatum* samples compared to other samples. Total antioxidant capacity exerted by the extract is concentration dependent. DPPH assay was high in all the extract of *S*. *caryophyllatum* sample compared to other samples. DPPH is a stable, synthetic radical

that does not disintegrate in water, methanol or ethanol. An antioxidant may be defined as 'any substance that when present at low concentrations, compared with those of the oxidizable substrate, significantly delays or inhibits oxidation of that substrate'.

3.8. Total Carotenoids and Chlorophylls

The chlorophyll pigment occupies a unique role in the economy of green plants. Quantity of chlorophyll is an indicator of photosynthetic capacity of a plant and this quantity is influenced dramatically by biotic and abiotic stresses. In 80% acetone extractant solvent *S. caryophyllatum* have high Chl a, Chl b and carotenoid (4.191±0.000, 5.425±0.000 and5.425±0.000) contents. In 100% acetone extractant solvent of *S. cumini* leaves have high Chl a, Chl b and carotenoids (1.539±0.000, 19.09±0.000 and 0.134±0.001). In this study, chlorophyll concentration extracted by 100% acetone was higher than that of extracted by either 80% acetone or 95% ethanol.

Table 8 Estimation of chlorophyll pigment in of *S. caryophyllatum* and *S. cumini*

Extractant Solvent	***Syzygium caryophyllatum* L.**			***Syzygium cumini* L.**		
	Chl a	**Chl b**	C_{X+C}	**Chl a**	**Chl b**	C_{X+C}
80% acetone	4.191±0.000	5.425±0.000	0.245±0.000	2.928±0.006	3.382±0.001	0.007±0.000
100% Acetone	1.188±0.001	14.92±0.001	0.488±0.000	1.539±0.000	19.09±0.000	0.134±0.001
95% Ethanol	3.505±0.000	4.59±0.000	0.245±0.001	6.22±0.000	11.19±0.000	1.62±0.000

Chl a- chlorophyll a, Chl b- chlorophyll b, Cx+c-carotenoid; The experimental results were expressed as mean ± standard error means (SEM) of triplicates,P<0.05 considered as significant.

3.9. Larvicidal activity

Mosquitos are vectors of various diseases such as dengue, chikungunya, encephalitis, malaria, and filariasis; most countries use chemicals to kill larvae, but chemicals are harmful to the atmosphere and many species of mosquitoes have developed DDT resistance to other pyrethroid insecticides. Hence the best option is conventional larvicidal plants.

Table 9 Larvicidal activity of mosquito larvae against *S. cumini*

Time	Water					Ethanol					Methanol				
	20	**40**	**60**	**80**	**100**	**20**	**40**	**60**	**80**	**100**	**20**	**40**	**60**	**80**	**100**
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	1	-	-	-	-	2	2
12	-	-	-	-	-	-	-	-	1	1	-	-	-	2	1
14	-	-	-	-	-	-	-	-	1	3	-	-	-	3	1
16	-	-	-	-	-	-	-	1	1	1	-	3	2	1	1
18	-	-	-	-	-	1	1	1	1	1	-	2	1	1	3
20	-	-	-	-	-	1	1	1	1	1	1	3	3	1	1
24	-	-	-	-	-	1	1	1	1	1	2	1	2		1
48	-	-	-	-	-	1	1	2	1	1	1	1	2		
72	-	-	-	-	-	1	1	1	1	1	1				

Table 10 Larvicidal activity against *S. caryophyllatum*

Time	**Water**					**Ethanol**					**Methanol**				
	20	40	60	80	100	20	40	60	80	100	20	40	60	80	100
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	1	2	1	2	-	-	-	-	-	-
6	-	-	-	-	-	1	1	1	1	-	-	3	2	1	1
8	-	-	-	-	-	2	1	1	1	2	1	1	1	2	3
10	-	-	-	-	-	1	1	3	1	3	1	1	3	3	2
12	-	-	-	-	-	1	2	2	3	3	3	1	2	1	2
14	-	-	-	-	-	3	1	1	2	2	1	2	1	1	2
16	-	-	-	-	-	1	2	1			1	1	1	2	
18	-	-	-	-	-						3	1			
20	-	-	-	-	-										
22	-	-	-	-	-										
24	-	-	-	-	-										
48	-	-	-	-	-										
72	-	-	-	-	-										

Total number of larvae per tube – 10: (-) – all alive: numbers – death of larvae

Table 11 Larvicidal activity of mosquito larvae against *S. caryophyllatum* and *S. cumini*

Mosquito larvae *Aedes aegypti*				
Plant samples	**Mortality (%)**			
	Concentration	**Aqueous**	**Ethanol**	**Methanol**
S. caryophyllatum Leaf	20	0	100	100
	40	0	100	100
	60	0	100	100
	80	0	100	100
	100	0	100	100
	Control	0	0	0
S. cumini Leaf	20	0	50	50
	40	0	50	100
	60	0	70	100
	80	0	90	100
	100	0	0	100
	Control	0	0	0

The evaluation of the mortality rate was performed at every 2 hours till 72 hours after the beginning of the experiment, verifying the number of dead larvae. Cyclophosphamide, an anticancer drug which was used as a positive control showed death of all mosquito larvae. The results show that the larvicidal activity of particular mosquito larvae rises as plant extract concentration increases. At all doses in methanol and ethanol extract, the medicinal plant extract of *S. caryophyllatum* demonstrated a significant death percentage against larvae. *S. cumini's* methanol extract shown an increasing death rate with concentration. Both leaf samples in methanol extract demonstrated substantial mosquito larval mortality rates.

4. Conclusion

Syzygium plants are used to treat a wide range of diseases. Many Syzygium species are well-known for their traditional usage in a variety of ailments. In some species, the medicinally helpful portion is the leaves, whereas in others, it may be the root, fruit, seed, or bark. Plant products from some Syzygium species, such as fruits, are consumed as food., whereas buds are used as spices for their flavourful characteristics. Few species are used as flavoring agents for their attractive glossy foliage, while other species look ornamented. Various plant components of the tree are renowned for ethnomedicinal applications, and the fruits of the *S. cumini* tree in particular are widely known for medicinal usage. Although the *S. cumini* fruit is a fantastic nutraceutical due to its medicinal characteristics, additional scientific evidence is needed before using these products to cure ailments. Bark and leaf extracts of *S. caryophyllatum* plant are well known for its antibacterial and antioxidant efficacy. Despite the fact that many reports on medicinal uses are known, exact usage and knowledge about this are restricted. As a result, scientific validation of its qualities and principles is urgently required. In this study, organoleptic, solubility, functional property, phytochemical analysis, quantification of primary and secondary metabolites, mineral content, and antioxidant property of *S. caryophyllatum* and *S. cumini* aqueous and alcoholic extract of leaf was evaluated. The results revealed that aqueous, methanol and ethanol extracts possess very high activity against antioxidant activity and gave good results to all the test. The *S. cumini* plant contains numerous essential chemicals which bestow the greatest of the properties of the plant. There aren't many studies on the pharmacological actions of plants phytochemical ingredients. This study leads us to the conclusion that both plant samples, *S. cumini* and *S. caryophyllatum* leaves, are high in phytochemicals. This is also a possible ingredient for the formulation of nutraceutical products for medical and veterinary uses.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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