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Original Article

EVALUATION OF PHYTOCHEMICALS AND FREE RADICAL SCAVENGING BEHAVIOR IN DIFFERENT PARTS OF *SYZYGIUM CUMINI*

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ABSTRACT

Objective: The present study was aimed at free radical scavenging behaviour in leaves, bark and seeds of *Syzygium cumini* (L.)

Methods: The ability to scavenge free radicals of the different extracts of *syzygium cumini* was determined by using 1,1-diphenyl2-picrylhydrazyl (DPPH), reducing power, hydroxyl radical scavenging assay, superoxide radical scavenging, Total flavonoid assay and Total phenolic assay. Another aspect of the study was to evaluate the phytochemicals of the different parts of the plant, keeping in view its pharmacological potential. The quantitative determination of compounds viz., phenolics and flavonoids supposed to be antioxidants was made and overall antioxidant activity was measured using standard methods.

Results: The results demonstrate that the total phenolic and flavonoid content of *syzygium cumini* seed is greater than the content found in leaves and bark extracts. A linear correlation between total phenolic content and antioxidant activity has been reported. The results suggested that the phenolic compounds contribute effectively to the antioxidant activity. The highest antioxidant property of bark is noteworthy as compared to leaves and seed in the present study.

Conclusion: The study suggests that *syzygium cumini* have a better source of natural antioxidants, which might be helpful in preventing the progress of oxidative stress.

Keywords: Syzygium cumini, Phytochemicals, Free radicals, Oxidative stress, Antioxidant activity

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INTRODUCTION

The plant produces many phytochemicals to protect itself and recent research demonstrates that these chemicals can protect humans against diseases [1]. Phytochemicals possess free radical scavenging ability. Free radicals are molecules containing one or more unpaired electrons in an atomic or molecular orbital. There is increasing evidence that abnormal production of free radicals leads to increased oxidative stress that underpins the pathogenesis of several important diseases, including cardiovascular diseases and neurological diseases [2]. Our metabolism protects the body from damage caused by oxidative stress with the help of vital substances called antioxidant [3]. It was also investigated by several researchers that oxidative damage plays a role in the development of acute and chronic age-related degenerative diseases and that dietary antioxidants oppose this and lower risk of disease [4, 5]. And thus it becomes necessary to extract these antioxidants from the medicinal plants. For several years, many researchers have been searching for powerful but non-toxic antioxidants from edible parts of plants because of their potent antioxidant activities, lack of side effects, and economic viability [6].

Jamun is an edible fruit from tropical regions belong to family Myrtaceae. Botanically, it is known as *Syzygium cumini*. The ripe fruits are used for health drinks, making preserves, squashes, jellies and wine.

In association to its dietary use, all parts of the tree and, importantly the seeds are used to treat a range of ailments, the most important being diabetes mellitus. Research studies accomplished in last twenty years has explored that *Syzygium cumini* have an outstanding complex of naturally present antioxidant compound [7]. There are a large amount of phytonutrients present in jamun fruit and seed that have been reported for their antiallergic, anti-inflammatory, antidiabetic, anticancer, free radical scavenging (ROS) and antioxidant properties. The bark of the plant is astringent, sweet, refrigerant, carminative, diuretic, digestive, antihelminthic, febrifuge, constipating, stomachic and antibacterial [8].

The present study was aimed at evaluation of free radical scavenging ability in the aqueous and ethanolic extract of leaves and bark of *Syzygium cumini*. Another aspect of the study was to evaluate the phytochemical potential in terms of phenolic and flavonoid content of the different parts of the plant.

MATERIALS AND METHODS

Sample collection and preparation

The plant *Syzygium cumini* was collected from the Mansarovar area of Jaipur, Rajasthan and was taxonomically verified from the specimen number RUBL 211552 of the herbarium, Department of Botany, University of Rajasthan, Jaipur, Rajasthan. Fresh and healthy plant parts viz. Leaves, bark, fruit pulp and seed are processed in to dry form through washing, draining, drying followed by grinding [9]. The powder material of plant parts was then extracted using solvents in the ration of 1:20 using soxhlet apparatus. After each extraction, the solvent was recovered using distillation assembly, and the extract was concentrated under reduced pressure. The final yield of extract was calculated and stored in air tied container for the experiment. Both the extracts (Aqueous and Ethanolic) were tested at the concentration of 100µg/ml (Low dose), 150µg/ml (Medium dose), and 200µg/ml (High dose).

Chemicals

All chemicals used including the solvents were of analytical grade. Quercetin, Nitro-blue tetrazolium, Folin–Ciocalteu reagent, trichloroacetic acid, aluminium chloride and ascorbic acid etc. were arranged by Dr. B. Lal Institute of Biotechnology (Rajasthan, India). All other chemicals and reagents used were of the highest commercially available purity.

Preliminary phytochemical screening

The aqueous and ethanolic extracts of various parts of *Syzygium cumini* plant was subjected to different chemical tests for the detection of different phytoconstituents [10-12].

Test for tannins

1 ml of the sample was taken in a test tube and then 1 ml of 0.008 M Potassium ferricyanide was added. 1 ml of 0.02 M Ferric chloride containing 0.1 N HCl was added and observed for blue-black coloration.

Test for phlobatannins

When a crude extract of each plant sample was boiled with 2 % aqueous HCl. The deposition of a red precipitate was taken as evidence for the presence of phlorotannins.

Test for saponins

The crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

Test for flavonoids

5 ml of dilute ammonia solution were added to a portion of the crude extract followed by addition of concentrated H2SO4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Test for alkaloids

The crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown coloured precipitate indicates the presence of alkaloids.

Test for quinines

Dilute NaOH was added to the 1 ml of crude extract. Blue green or red colouration indicates the presence of quinones.

Test for coumarin

10 % NaOH was added to the extract and chloroform was added for observation of yellow color, which shows the presence of Coumarin.

Test for terpenoids

5 ml of extract was mixed with 2 ml of chloroform and 3 ml of concentrated H2S04 was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides

5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated H2SO4. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

Estimation of total phenol and flavonoid content

Total flavonoids (TF) content

Aluminium chloride method was used for flavonoids determination [13]. Mix 0.5 ml plant part extracts separately with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M sodium potassium tartrate and 2.8 ml of distilled water. Allow it to stand for 30 min room temperature. Absorbance measured at 415 nm. The flavonoid content is expressed in terms of Quercetin equivalent (mgg-1 of the extracted compound).

Total phenolic (TP) content

Total phenolic content determined by standard Folin-Ciocalteu reagent method [14], using tannic acid as a standard phenolic compound in the range 100 µg/ml to 1000 µg/ml. 250 µl of extract was mixed with 1 ml of distilled water followed by the addition of 250 µl of Folin-Ciocalteu reagent. Mixed well and left to stand for 5 min at room temperature. 2.5 ml of 7 % sodium carbonate aqueous solution were added and the final volume was made up to 6 ml with distilled water. The absorbance was measured at 760 nm. The Phenolic content is expressed in terms of tannic acid equivalent (mgg-1 of the extracted compound).

Determination of antioxidant capacity

DPPH free radical scavenging activity

This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the non radical form DPPH-H [15] 1.5 ml of DPPH (0.1 mmol in methanol) and 1.5 ml extract incubated for 10 min in dark. Measured absorbance at 517 nm. Quercetin standard used as a positive control and methanol used as a blank. The percentage of inhibition DPPH scavenging activity was calculated using the following formula:

$$Scavenging effect(\%) = 1 - (sample_{OD}/control_{OD}) \times 100$$

Super oxide free radical scavenging assay

This assay was based on the reduction of nitro blue tetrazolium [16]. 150 µl varying concentrations of extracts were mixed with 100 µl of riboflavin, 200 µl of EDTA, 200 µl of methanol and 100 µl of nitro-blue tetrazolium. The above reaction mix was diluted up to 3 ml with phosphate buffer. Absorbance was measured at 590 nm after 5 min using phosphate buffer as a blank Quercetin was taken as positive control. Percentage scavenging of super oxide free radical was calculated as per following formula-

$$Scavenging effect(\%) = 1 - (sample_{OD}/control_{OD}) \times 100$$

Hydroxyl radical scavenging (HO) assay

The reaction mixture (1.0 ml) consist of 100 µl of 2-deoxy-Dribose (28 mmol in 20 mmol KH2PO4-KOH buffer, pH 7.4), 500 µl of the extract of different concentration, 200 µl EDTA (1.04 mmol) and 200 µl FeCl3 (1.0 mmol), 100 µl of H2O2 (1.0 mmol) and 100 µl ascorbic acid (1.0 mmol) which is incubated at 37 °C for 1 h. 1.0 ml of thiobarbituric acid (1%) and 1.0 ml of trichloroacetic acid (2.8%) were added and incubated at 100 °C for 20 min. After cooling, absorbance is measured at 532 nm [17]. KH2PO4 buffer used as a blank. Quercetin can be used as a positive control. Percentage scavenging of hydroxyl radical was calculated as per following formula-

$$Scavenging effect(\%) = 1 - (sample_{OD}/control_{OD}) \times 100$$

Reducing power ability

Reducing the power of the extracts was determined by using potassium ferricyanide-ferric chloride system [18]. Briefly, 1 ml of extract at different concentration was added with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), mixed with 2.5 ml of potassium ferricyanide (0.1%) and the mixture was incubated at 50 °C for 20 min. 2.5 ml of trichloroacetic acid (10%) was added to the reaction mixture and centrifuged at 8000g for 10 min. 2.5 ml of supernatant was mixed with equal volume of distilled water. 0.5 ml of 0.1% ferric chloride was added. Absorbance was measured at 700 nm. Quercetin can be used as a reference control.

Different phytochemicals viz. Tannin, Saponin, Flavonoid, Coumarin and Cardiac glycosides have been found to possess a wide range of activities which may help in the protection against chronic diseases. For example, phytochemicals such as saponin, tannins, terpenoids, and alkaloids have anti-inflammatory effects. Tannins have amazing astringent properties. They are known to hasten the healing of wounds and inflamed mucous membranes. Flavonoids are also present as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity [19, 20]. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, antiallergenic, antispasmodic, anti-hyperglycemic, anti-inflammatory and immunomodulatory properties [21, 22]. In addition; terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties as well [23]. Numerous studies have confirmed that saponins possess the unique property of precipitating and coagulating red blood cells [24]. Plants containing carbohydrates, glycosides and coumarins are known to exert a beneficial action on the immune system by increasing body strength and hence are valuable as dietary supplements. Coumarins can be suggested to be beneficial for hyper proliferative skin diseases on the basis of their antimicrobial and anti-inflammatory effects [25].

RESULTS

Table 1: **Phytochemical screening of leaves, seed and bark of syzygium cumini in aqueous and Ethanolic extract**

S. No.	Test	Aqueous			Ethanolic		
		Leaves	Seed	Bark	Leaves	Seed	Bark
1	Tannin	+	+	+	+	+	+
2	Phylobatannin	-	-	-	-		-
3	Saponin	+	+	+	+	+	+
4	Flavonoid	+	+	+	+	+	+
5	Alkaloid	-	-	-	+	-	+
6	Quinone	-	-	-	-	-	-
7	Coumarin	-	+	+	+	-	+
8	Terpenoid	+	-	+	-	-	+
9	Cardiac glycosides	+	+	+	+	+	+

Quantification of total phenolic content and flavonoid content of leaves, seed and bark of *Syzygium cumini* in aqueous and ethanolic extract

Table 2: Total flavonoid content of *syzygium cumini* extract-Quercetin equivalent/mg of extract

Extract	Leaves	Bark	Seed
Aqueous	32 µg	15 µg	17µg
Ethanolic	23 µg	25 µg	12µg

Table 3: Total phenolic content of *syzygium cumini*-tannic acid equivalent/mg of extract

Extract	Leaves	Bark	Seed
Aqueous	77 µg	78 µg	57µg
Ethanolic	62 µg	71µg	76µg

The total phenolic content in the aqueous and ethanolic extract of *syzygium cumini* leaf was 77 µg/mg and 62 µg/mg, the seed was 57µg/mg and 76µg/mg and the bark was 78 µg/mg and 71 µg/mg respectively. While the flavonoid content in leaf aqueous and ethanolic32 µg/mg and 23 µg/mg, the seed was 17 µg/mg and 12 µg/mg and bark was 15µg/mg and 25 µg/mg. These results demonstrated that the total phenolic content of *syzygium cumini* bark (aq.) was greater than the content found in seed and leaves extracts. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable.

The results strongly suggest that the phenolics are important components of this plant and some of the pharmacological effects could be attributed to the presence of this invaluable component

Analysis of free radical scavenging behaviour of leaves, seed and bark of *Syzygium cumini* in aqueous and ethanolic extract

Table 4: % Inhibition–DPPH radical scavenging assay of *Syzygium cumini* extract

Doses concentration	Leaves		Bark		Seed		Positive control	
	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic
100 µg/ml	69.23	6	21.65	33.6	14.43	80.59	51.36	86.9
150µg/ml	82.6	9	23.1	61.3	26.15	82.24	60.118	87.5
200µg/ml	87.13	25	61.2	86.03	40.82	84.61	62.248	87.9

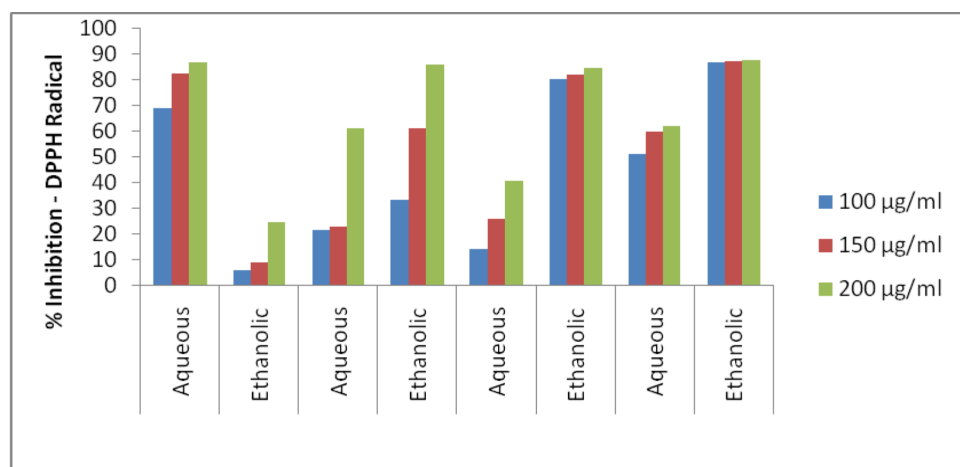

Fig. 1: % Inhibition DPPH radical

This method is based on scavenging of the DPPH radical from the antioxidants, which produces a decrease in absorbance at 517 nm. *syzygium cumini l*eaves aqueous showed highest DPPH scavenging activity with 87.13% inhibition in the dose concentration of 200µg/ml followed by *syzygium cumini* bark ethanolic extract with 86.03%. (fig. 1)

Table 5: % Inhibition–Hydroxyl radical scavenging assay of *Syzygium cumini* extract

Doses concentration	Leaves		Bark		Seed		Positive control	
	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic
100 µg/ml	22.25	78.041	19.88	81.6	37.6	71.8	18.1	89.3
150µg/ml	33.93	79.821	39.46	84.569	42.4	67.9	30.26	90.5
200µg/ml	52.07	81.6	46.88	88.13	47.1	59.05	35.01	92.2

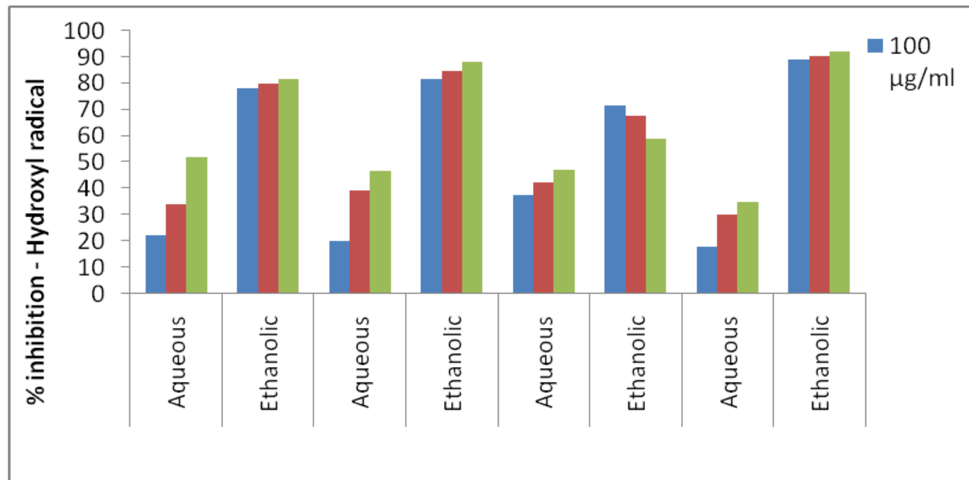

Fig. 2: % Inhibition hydroxyl radical

The ability of the ethanolic extract of *Syzygium cumini* to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and seems to be a good scavenger of active oxygen species. *Syzygium cumini bark* ethanolic showed highest hydroxyl scavenging activity with 88.13% inhibition in 200µg/ml concentration followed by *Syzygium cumini* bark ethanolic extract with 84.56% and minimum activity showed by bark aqueous with 19.88% (fig. 2).

Table 6: % Inhibition–Superoxide radical scavenging assay of *Syzygium cumini* extract

Doses concentration	Leaves		Bark		Seed		Positive control	
	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic
100 µg/ml	34.41	88.311	46.75	88.96	72.07	66.22	67.79	19.04
150µg/ml	35.71	91.558	51.29	91.55	73.37	74.67	72.88	24.02
200µg/ml	41.55	94.805	73.37	96.1	77.27	74.67	79.66	33.1

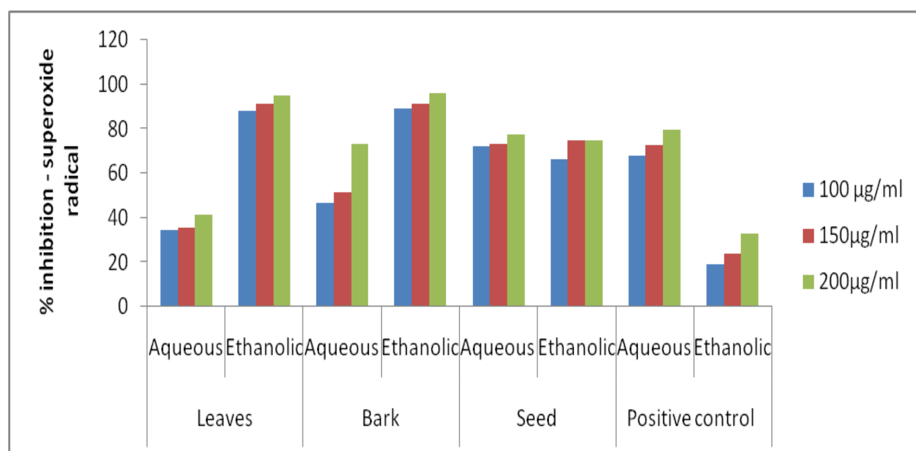

Fig. 3: % Inhibition superoxide radical

Table 7: Reducing power activity of *Syzygium cumini* extract

Doses concentration	Leaves		Bark		Seed		Positive control	
	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic
100 µg/ml	0.07	0.161	0.09	0.182	0.056	0.332	1.17	1.22
150µg/ml	0.1	0.217	0.11	0.23	0.128	0.565	1.45	1.88
200µg/ml	0.15	0.269	0.18	0.32	0.139	0.642	2.37	2.16

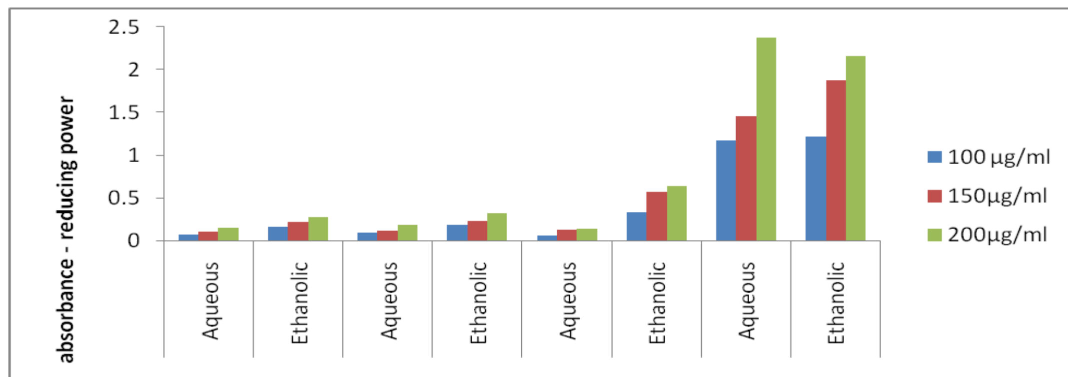

Fig. 4: Absorbance of reducing power

The superoxide radical reduced NBT to blue-coloured formazan that can be measured at 560 nm. *syzygium cumini l*eaves ethanolic showed highest Superoxide scavenging activity with 94.805% inhibition in 200µg/ml concentration followed by *syzygium cumini* bark ethanolic extract with 91.55% (fig. 3).

An increase in the absorbance indicates an increase in the antioxidant activity. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloroacetic acid, and ferric chloride, which is measured at 700 nm. *syzygium cumini seed* ethanolic showed highest reducing power absorbance with 0.64 in 200µg/ml concentration followed by *syzygium cumini* minimum absorbance showed by leaves aqueous 0.07 (fig. 4).

DISCUSSION

The present study investigated the antioxidant activity of the of *Syzygium cumini* at different concentration. The ability of scavenging free radicals of the different extracts of *syzygium cumini* was determined by using 1,1-diphenyl2-picrylhydrazyl (DPPH), reducing power, hydroxyl radical scavenging assay, superoxide radical scavenging, total flavonoid assay and total phenolic assay.

Our Research studies showed that *Syzygium cumini* has an outstanding complex of naturally present antioxidant compounds. There are a large amount of phytonutrients such as flavonoids and phenolic acids present in different parts of jamun.

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract. In the present study, In its radical form, DPPH has disappears on reduction by an antioxidant compound or a radical species to become a stable diamagnetic molecule resulting in the colour changes from purple to yellow, which could be taken as an indication of the hydrogen donating ability of the tested sample [26,27]. Although syzygium cumini leaves aqueous possess good DPPH scavenging activity, it was evident that the extract could serve as free radical inhibitors or scavengers.

Hydroxyl radicals are most reactive species, initiating the peroxidation of the cell membrane. The lipid radical, thus generate would initiate a chain reaction in the presence of oxygen, giving rise to lipid peroxide, which breaks down to aldehydes such as malondialdehyde. The highly reactive hydroxyl radical can cause oxidative damage to DNA, lipid and protein [28]. In this study, the extracts exhibited concentration dependent scavenging activity against hydroxyl radical generated. *Syzygium cumini* was found to scavenge hydroxyl radical significantly and in dose dependent manner and may protect the DNA, protein and lipid from damage.

A reducing power is an indicative of reducing agent having the availability of atoms which can donate an electron and react with free radicals and then convert them into more stable metabolites and terminate the radical chain reaction [29]. Accordingly, *Syzygium cumini* might contain a sizable amount of reductants which may react with the free radicals to stabilize and terminate from free radical chain reaction. The reducing potential of the bark, leaves and seed extracts measured for the concentration at 100 mg/ml, 150 mg/ml and 200 mg/ml, showed a general increase in activity when concentration increased.

Superoxide anion plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA [30]. It was reported that the superoxide anion scavenging activity could be due to the action of a free hydroxyl group of phenolic compounds were generated by the hypoxanthine-xanthine oxidase and the NBT system. Our data demonstrated that the leaves ethanolic showed highest Superoxide scavenging activity with 94.80% inhibition in 200µg/ml concentration

The antioxidant activities of the plant extracts are often explained by their total phenolics and flavonoid contents. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [31]. Interest in phenolics is increasing in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [32]. There is a wide range of total phenol content in the extracts of the plant under study. The results demonstrated that the total phenolic content of *Syzygium cumini* bark (aq.) was greater than the content found in seed and leaves extracts.

CONCLUSION

Currently, there has been an increased interest globally to identify antioxidant compounds from plant sources which are pharmacologically potent and have low or no side effects for use in protective medicine and the food industry. Plants produces a large amount of antioxidants to prevent the oxidative stress, they represent a potential source of new compounds with antioxidant activity. Increasing knowledge in antioxidant phytoconstituents and include them in daily uses and diet can give sufficient support to the human body to fight those diseases. Phytoconstituents and herbal medicine are also important to manage pathological conditions of those diseases caused by free radicals. Explore the antioxidant principles from natural resources; Identification and isolation of

those phytoconstituents are simultaneously presenting enormous scope for their better therapeutic application for treatment of human disease. Therefore it is time for us, to explore and identify our traditional therapeutic knowledge and plant sources and interpret it according to the recent advancements to fight against oxidative stress, in order to give it a deserving place. The results demonstrate that the total phenolic and flavonoid content of *Syzygium cumini* seed is greater than the content found in leaves and bark extracts. A linear correlation between total phenolic content and antioxidant activity has been reported. The highest antioxidant property of *Syzygium cumini* is noteworthy in the present study. Thus, the study suggests that *Syzygium cumini* has a better source of natural antioxidants, which might be helpful in preventing the progress of oxidative stress.

CONFLICT OF INTERESTS

Declared none

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