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Studies on Antioxidant and Antimicrobial Potential of *Syzygium cumini* Leaves.

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ABSTRACT

Reactive oxygen species are generated in cells under normal metabolic activities are implicated in several metabolic diseases that include heart diseases, acquired immunodeficiency syndrome, diabetes mellitus, arthritis, cancer, aging, liver disorder etc. The antioxidant therapy has gained an utmost importance in the treatment of these metabolic diseases. The World Health Organization in 2006 estimated that 4 billion people use herbal medicines in some aspects of primary healthcare and there is a growing tendency to "Go Natural". In these aspects, all around the world, the medicinal properties of plants have been investigated and explored for their potential antioxidant activities to counteract metabolic disorders, which are of high economically viable, with no side effects. The present study was designed to explore the antioxidant and antimicrobial potential by measuring antioxidant activity and antimicrobial activity in *Syzygium cumini* leaves extracts. The healthy plants were collected from the fields of Chunni Kalan Distt. Fatehgarh Sahib (Punjab)-India for the determination of antioxidant activity. The four strains of bacteria like *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* & *Staphylococcus aureus* and two fungus such as *spergillks niger* & *Trichoderma viru* were used to measure the antimicrobial & antifungal activity of *Syzygium cumini*. The aqueous, ethanol and Ethyl Acetate extracts of *Syzygium cumini* leaves were prepared for the determination of total phenolic ascorbic acid, hydrogen peroxide scavenging activity & antioxidant activity and antimicrobial activity. A high contents of Phenolic Content, Hydrogen Peroxide Scavenging activity, Antioxidant Activity and Vitamin-C were observed in various leaves extracts (aqueous, alcohol and ethyl acetate) of *Syzygium cumini* were recorded. The antibacterial and antifungal activities were also observed in different extracts of by means of *Syzygium cumini*. All the above observations suggested that *Syzygium cumini* leaves have antioxidant and Antimicrobial activity.

Keywords: *Syzygium cumini* leaves, Reactive Oxygen Species, antioxidant activity, antimicrobial activity

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INTRODUCTION

Some bacterial diseases are unable to control, due to widespread presence of antibiotic resistant bacteria and extensive use of current antimicrobial agent. Plants with medicinal properties are gold mines of valuable herbal products, which are used in the treatment of various diseases [1]. Herbal medicines are the important part of traditional therapeutic system. Medicinal plants could be source of noble antimicrobial agent, which may be effective against pathogen strain. In recent years, exploration and use of plant derived products have been increased rapidly. Medicinal properties of plants need to be evaluated scientifically, for the treatment of infectious disease produced by pathogens.

The medicinal values of *Syzygium cumini* have been recognized in different traditional medication systems, for treatment of various diseases of human beings. The fruits and seeds of *Syzygium cumini* have been studied mainly for their anti-diabetic properties. The folkloric use of *Syzygium cumini* to treat infectious diseases stimulated the investigation of the antimicrobial activity of the various extracts from *Syzygium cumini* leaves against Gram positive and Gram negative bacteria, as well as against fungus. Literature reports revealed that data on the antimicrobial properties of *Syzygium cumini* leaves is scant in India. So, the present study was designed to explore the antioxidant and antimicrobial potential by measuring antioxidant activity by various biochemical procedures and antimicrobial activity in *Syzygium cumini* leaf extracts.

MATERIALS AND METHODS

All the chemicals used in the present study were of analytical grade and purchased from Himedia Laboratory Pvt. Ltd. and Titan Biotech. Ltd.

Plant Materials: The plant specimens were collected from the fields of Chunnikalan Distt. Fatehgarh Sahib (Punjab)- India. The care was taken to select healthy plants.

Microorganisms: Four strains of bacteria (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and two fungus (*Aspergillus niger* and *Trichoderma viride*) were used and obtained from the Department of Microbiology, Dolphin (PG) College of Sciences Chunnikalan, Distt. Fatehgarh Sahib (Punjab)- India.

Preparation of Leaf Extract: Leaves were washed in water containing Tween-20 solution for 15 minutes. After that leaves were washed with distilled water until lather formed due to the detergent was completely removed. 70% ethanol treatment was given to leaves for 90 seconds. Leaves were again washed and rinsed with distilled water. Surface sterilized leaves were air dried at room temperature. The dried leaves were ground into fine powder.

Aqueous Extract: Powdered leaf sample was soaked in distilled water. The mixture was stirred for 16 h at magnetic stirrer. Then the extract was filtered by Whatman filter paper. The filtrate was evaporated at 40 °C.

Ethanol Extract: Finely ground powder of leaves was soaked in ethanol. The mixture was stirred for 16 h at magnetic stirrer. The extract was filtered by Whatman filter paper followed by evaporation at 50 °C. The extract was collected in powdered form.

Ethyl Acetate Extract: Ethyl acetate extract was prepared with the help of Soxhlet assembly. Fine ground powder sample was placed in the chamber of Soxhlet assembly. Ethyl acetate was poured into the assembly. Half of round bottom flask was filled with acetate. Soxhlet assembly was assembled and placed on the heating mantle. The assembly was run at 75°C for 24 h. After 24 h, crude extract was collected in round bottom flask. This crude extract was transferred to the beaker. Extract was evaporated in the water bath at 70°C. Extract was collected in the form of powder.

Analysis of Antioxidant Activity

Estimation of Total Phenolic Contents: The amount of Total Phenolic Content in all the extracts of *Syzygium cumini* leaves was determined with Folin-Ciocalteu Reagent using the method of Spanos and Wrolstad, 1990 [2] as modified by Lister and Wilson, 2001 [3]. 1 ml of each sample, 2.5 ml of 10% dilution of Folin-Ciocalteu

reagent and 2 ml of Na_2CO_3 (7.5%, w/v) were added and the resulting mixture was incubated at 45°C for 15 mm. The absorbance of all samples was measured at 765 nm. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw)

Estimation of Ascorbic Acid (Vitamin - C): Ascorbic acid in aqueous extract was estimated by indo phenol titration method an Official method of analysis, 1999 [4]. 2 ml of distilled water, ascorbic acid and aqueous extract was added to the marked flask for the blank, ascorbic acid and aqueous extract respectively. 5ml acid stabilization solution was added to each flask. The solutions were titrated rapidly with the dye solution until a light but distinct rose pink colour persists for at least 5 sec. There was no need of indicator because it is self indicator.

Estimation of Hydrogen Peroxide Scavenging Activity: Hydrogen peroxide scavenging activity of the extract was estimated by replacement titration of Zhang et al., 2000[5]. Aliquot of 1.0 ml of 0.1 mM H_2O_2 and 1.0 ml of various concentrations of extracts were mixed, followed by 2-3 drops of 3% ammonium molybdate, 10 ml of 2 M H_2SO_4 and 7.0 ml of 1.8 M KI. The mixed solution was titrated with 5.09 mM $\text{Na}_2\text{S}_2\text{O}_3$ until yellow color disappeared. Percentage of scavenging of hydrogen peroxide was calculated as:

$$\% \text{ Inhibition} = (\text{VO} - \text{Vi}) / \text{VO} \times 100$$

Here VO was volume of $\text{Na}_2\text{S}_2\text{O}_3$ solution used to titrate the control sample in the presence of hydrogen peroxide (without extract), Vi was the volume of $\text{Na}_2\text{S}_2\text{O}_3$ solution used in the presence of the extract. Hydrogen peroxide scavenging activity of Tocopherol was taken as reference standard for comparison.

Estimation of Antioxidant Activity: -

The Antioxidant activity of each extract was tested using the Ferric Thiocyanate Method (FTC). The standard method was given by Nakatani et al., 1987 [6]. Each extract (1mg/ml) was mixed with reaction mixture in a screwcap vial. Each reaction mixture consisted of 2.88 ml, of 2.51% linoleic acid in ethanol and 9 ml, of 40 mM phosphate buffer (pH 7.0). The vial was placed in incubator at 40°C in the dark. At intervals during incubation, a 0.1ml aliquot of the mixture was diluted with 9.7 ml, of 75% ethanol, which was followed by adding 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance at 500 nm was measured.

$$\text{Inhibition of lipid peroxidation (\%)} = [1 - (\text{A sample}) / (\text{A control})] \times 100$$

The antioxidant activity of Tocopherol as reference standard was assayed for comparison.

ANTIMICROBIAL ACTIVITY: Antimicrobial activity of *Syzygium cumini* leaves extract was explored by the Agar well diffusion method.

Microorganism Used: Four strains of bacteria (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*), and two fungus (*Aspergillus niger* and *Trichoderma viridae*). These cultures were obtained from Department of Microbiology, Dolphin (PG) College of Life Sciences. The cultures were maintained on nutrient agar at 4 °C.

Preparation of inoculums: Twenty four hours old cultures of selected bacteria were mixed with physiological saline. The turbidity was corrected by adding sterile physiological saline, until a Mac Farland turbidity standard of 0.5 (106 colony forming units (CFU) per ml) was obtained. The fungal suspension was standardized spectrophotometrically to an absorbance of 0.600 at 450 nm.

Agar Well Diffusion Method: Petri plates were prepared by pouring 10 ml of Mueller Hinton Agar for bacteria and Potato Dextrose Agar for fungi and allowed to solidify. Plates were dried and 0.1 ml of standardized inoculums suspension was poured and uniformly spread. The excess inoculums was drained and the inoculums was allowed to dry for 5 mm. Five wells of size 6 mm diameters were formed. Pure solvent i.e. water, ethanol and ethyl acetate was poured in Centre well (Centre of the Petri plates) for each extract plate. Ethanol extract (100 µg/ml), ethyl acetate extract (100 µg/ml) conc. and aqueous extract (100 µg/ml) was poured in rest of four wells of respective extract plate. Standard antibiotic tetracycline (30 µg/ml) was poured in respective Petri

plates except of *P. aeruginosa* and fungus. In case of *P. aeruginosa*, erythromycin (30 ig/m1) was taken as standard where as fluconazole was taken as standard for antifungal activity. The plates were incubated in BOD incubator at 37°C for 24 h for bacteria and 28 °C for 72 hrs in case of bacteria. The inhibition zone was measured from the edge of the disc to the inner margin of the surrounding pathogens.

STATISTICAL ANALYSIS: Tests were carried out in triplicate. Results were expressed as Mean \pm Standard Deviation (SD).

RESULTS AND DISCUSSION

In the present study, we found that a high contents of phenol in different extracts of *Syzygium cumini* leaves. The contents of total phenolic contents (36.05 ± 1.47 mg GAE/ g of dry weight) found in aqueous extract of *Syzygium cumini* leaves was higher in comparison to ethanol extract (30.24 ± 1.17 mg GAE/ g of dry weight) and ethyl acetate extract (20.86 ± 1.15 mg GAE/ g of dry weight) of *Syzygium cumini* leaves (Table-1). Plants possessing polyphenols such as anthroquinones, flavanoids, aromatic acid and tannin have been shown to scavenging the reactive oxygen species (ROS) and also playing important role in preventing lipid peroxidation [7]. It is well documented in literature that there is direct relationship between antioxidant activity and phenolic content of plant extracts [8, 9]. The presence of high contents of phenol in different extracts of *Syzygium cumini* leaves suggesting the antioxidant properties to scavenging the production of ROS and lipid peroxidation.

Vitamin C/ Ascorbic acid acts as an antioxidant in biological systems and scavenges the free radicals, thereby increase the antioxidant defense in the body. Ascorbic acid acts as an antioxidant by being available for energetically favorable oxidation. Ascorbic acid reduced the ROS and itself oxidised. The oxidized form of ascorbate is relatively stable and unreactive, and do not cause cellular damage [10].

Antioxidant activity of *Syzygium cumini* leaves was also determined by measuring the levels of hydrogen Peroxide (H_2O_2) in different leaf extracts. H_2O_2 is a water oxidizing agent and can in activate few enzyme directly, by oxidation of essential thiol (-SH) groups. H_2O_2 can cross cell membrane rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+} and possibly Ca_2 to form hydroxyl radical and this may be the origin of its toxic effect. It is therefore biologically advantageous for cell to control the amount of H_2O_2 that is allowed to accumulate. Leaves extract of *Syzygium cumini* was showing H_2O_2 scavenging activity.

Free radicals have been implicated in many disease conditions, the important ones being super oxide radicals, hydroxyl radicals, peroxy radicals, and single oxygen. Herbal drugs containing free radical scavengers are gaining importance in treating such diseases. Many plants extract exhibit efficient antioxidant properties due to their phytoconstituents, including phenolics [11].

The results of present study indicating that *Syzygium cumini* have H_2O_2 scavenging activity and having antioxidant potential, which prevent the formation and scavenging of the free radicals thereby bends to the disease like cancer and ageing.

An aqueous extract, ethanol and ethyl acetate extract of *Syzygium cumini* leaves is scavenging 50 ± 6.25 %, 31.57 ± 5.26 % and 20.80 ± 2.50 % respectively in comparison to 71.92 ± 8.04 % of H_2O_2 the scavenging ability (Table-2).

The antioxidant activity was also determined by Ferric Thiocyanate method in different extracts of *Syzygium cumini* leaves. An Aqueous extract, ethanol extract and ethyl acetate extract are producing 52.30 ± 0.59 %, 47.07 ± 0.62 % and 32.85 ± 0.65 % antioxidant activity respectively, where as the reference compound tocopherol was producing 73.03 ± 0.46 % of antioxidant activity (Table-3). The plant extracts tested showed low absorbance value which indicates a high level of antioxidant activity. None of the plant extracts showed absorbance value greater than the negative controls (without plant extracts). It has been observed that the extract exhibiting strong activity with the increase in polarity (with reference to organic solvent) indicating that polyphenols may play important roles in the antioxidant potential of plants. The present findings are in agreement with the literature report [12].

Antimicrobial activity of the various leaves extract was checked by Agar Well Diffusion and Micro Dilution Broth method. Antimicrobial activity was tested by four bacteria (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and two fungus (*Aspergillus niger* and *Trichoderma viridae*).

In the Agar well diffusion method, Antimicrobial activity was determined by measuring zone of inhibition around the well. The zone of inhibition is formed due to killing of the microorganism around the well by the effect of leaves extracts. Antimicrobial activity is directly related to the zone of inhibition. Larger the zone of inhibition greater will be the antimicrobial activity

Table IV. indicates that ethanol extract was showing higher antimicrobial activity as compared to other extracts in both bacteria and fungus. It was producing 5.75 ± 0.5 mm, 7.25 10.5 mm and 3.75 10.5 mm zone of inhibition in *Staphylococcus aureus*, *Salmonella typhi*, and *Aspergillus niger* respectively. While Aqueous extract was showing moderate antimicrobial activity as compared to other extracts. It was producing 4.25 ± 0.5 mm, 5.5 ± 0.57 mm and 3.25 ± 0.5 mm zone of inhibition in *Staphylococcus aureus*, *Salmonella typhi*, and *Aspergillus niger* respectively. And ethyl acetate was showing least antimicrobial activity. It was producing 2.25 ± 0.5 mm, 3.75 ± 0.5 mm and 2.5 ± 0.57 mm zone of inhibition in *Staphylococcus aureus*, *Salmonella typhi*, and *Aspergillus niger* respectively.

Whereas none of these extract was effective against *E. coli*, *Pseudomonas aeruginosa* and *Trichoderma viridae*. These results shows that the activity of various leaves extracts of *Syzygium cumini* shows significant antibacterial and antifungal activities. These antimicrobial activities of extracts may be due to presence of different phytochemicals. Plants rich in tannin and phenolic compounds have been shown to posses antimicrobial activities against a number of microorganisms. In Conclusion, all the above observations suggested that *Syzygium cumini* leaves have antioxidant and Antimicrobial activity.

Table-1: The levels of total phenolic Content in various extracts (water, ethanol and ethyl acetate) of *Syzygium cumini* leaves

Extracts	Phenolic Content (mg GAE/ g of dry weight)	H ₂ O ₂ Scavenging activity (% inhibition)	Antioxidant Activity (% inhibition)
Aqueous	36.05± 1.47	50±6.25	52.30± 0.59
Ethanol	30.24± 1.17	31.57±5.26	47.07± 0.62
Ethyl Acetate	20.86 ± 1.15	20.80 ± 2.50	32.85 ± 0.65
Tocopherol	-	71.92± 8.04	73.03 ± 0.46

Values are mean ± SD of three different observations.

Table-2: Hydrogen Peroxide scavenging activity in various extracts of *Syzygium cumini* leaves

Extract/Standard	Hydrogen Peroxide Scavenging activity (% inhibition)
Aqueous	50±6.25
Ethanol	31.57±5.26
Ethyl Acetate	20.80 ± 2.50
Tocopherol	71.92± 8.04

Values are mean ± SD of three different observations.

Table-3: Antioxidant activity in various extracts of *Syzygium cumini* leaves by FTC Method.

Extract/Standard	Hydrogen Peroxide Scavenging activity (% inhibition)
Aqueous	52.30± 0.59
Ethanol	47.07± 0.62
Ethyl Acetate	32.85 ± 0.65
Tocopherol	73.03 ± 0.46

Values are mean ± SD of three different observations.

Table-4: Antimicrobial activity in various extracts (aqueous, ethanol and ethyl acetate) of *Syzygium cumini* leaves.

Micro-organism	Zone of Inhibition (mm)			
	Aqueous Extract	Ethanol Extract	Ethyl Acetate Extract	Standard
<i>Staphylococcus aureus</i>	4.25 ± 0.5	7.25 ± 0.5	2.25 ± 0.5	12.5 ± 0.57
<i>Salmonella typhi</i>	5.5 ± 0.57	5.75 ± 0.5	3.75 ± 0.5	11 + 0.81
<i>E.coli</i>	0	0	0	9.25 ± 0.95
<i>Pseudomonas aeruginosa</i>	0	0	0	9.75±0.5
<i>Aspergillus niger</i>	3.25 ± 0.5	3.75 ± 0.5	2.5 ± 0.57	9.75 ± 0.5
<i>Trichoderma viridae</i>	0	0	0	9.5 ± 0.57

Values are mean ± SD of three different observations.

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