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Evaluation of Antioxidant and Antimicrobial Activity of *Syzygium cumin* Leaves

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A B S T R A C T

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. 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 District Fatehgarh Sahib (Punjab), India for the determination of antioxidant activity. The four strains of bacteria like *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and two fungi such as *Aspergillus niger* and *Trichoderma viru* were used to measure the antimicrobial and 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 and antioxidant activity and antimicrobial activity. A high content 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.

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 (Tanaka *et al*., 2006).

Herbal medicines are the important part of traditional therapeutic system. Medicinal plants could be source of noble antimicrobial age, which may effective against pathogen strain. In recent years, exploration and use p1 derived products have been increased rapidly. Medicinal properties of plants need to

evaluating scientifically, for the treatment of infectious disease produced by pathogens.

The medicinal values of *Syzygium cumini* has been recognized in different traditional medication system, for treatment of various diseases of human being. The fruits and seeds *Syzygium cumini* have been studied mainly for its anti diabetic properties. The folkloric use of *Syzygium cumini* to treat infectious diseases stimulated the investigation 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 bio chemical procedures and antimicrobial activity in *Syzygium cumini* leaves extracts.

Materials and Methods

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

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

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, Chunni Kalan District, 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 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 grinded into fine powder.

Aqueous extract: Powdered leaves sample was soaked 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 flash was filled with acetate. Soxhlet assembly was assembled and placed on the heating mental. The assembly was run at 75°C for 24 h. After 24 h, crude extract was collected in round bottom flask. These crude extract was transferred to the beaker. Extract was evaporated in the water bath at 70ºC. Extract was collected in the form of powder.

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 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 grinded into fine powder.

Aqueous extract

Powdered leaves sample was soaked 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 soxhiet assembly. Ethyl acetate was poured into the assembly. Half of round bottom flash was filled with acetate. Soxhlet assembly was assembled and placed on the heating mental. The assembly was run at 75°C for 24 h. After 24 h, crude extract was collected in round bottom flask. These crude extract was transferred to the beaker. Extract was evaporated in the water bath at 700C. 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), as modified by Lister and Wilson (2001). 1 ml of each sample, 2.5 ml of 10% dilution of Foiin-Ciocalteu reagent and 2 ml of Na2CO3 (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 mm 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 (Official method of analysis, 1999). 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. 5m1 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 (29) (Zhang *et al*., 2000). 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 NaS_2O_3 until yellow color disappeared. Percentage of scavenging of hydrogen peroxide was calculated as:

% Inhibition = (VO - Vi)/ VO x 100

Here VO was volume of NaS_2O_3 solution used to titrate the control sample in the presence of hydrogen peroxide (without extract), Vi was the volume of NaS_2O_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 Thiocynate Method (FTC). The standard method was given by (Nakatani *et al*., 1987). Each extract (1mg/m1) was mixed with reaction mixture in a screw cap 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.imL 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.

Inhibition of lipid peroxidation (%) = [1- (A sample)/ (A control)] x 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 viride*). 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 final suspension was standardized spectrophotometrically to an absorbance of 0.600 at 450 rim.

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 tg/ml), ethyl acetate extract (100 tg/ml) conc. and aqueous extract (100 [tg/ml)) was poured in rest of four wells of respective extract plate. Standard antibiotic tetracycline (30 tg/ml) was poured in respective Petri plates except of *P. aeruginosa* and fungus. In case of *P. aeruginosa*, erythromycin (30 tg/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 ± 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 anthroguinones, flavanoids, aromatic acid and tannin have been shown to scavenging the reactive oxygen species (ROS) and also playing important role in preventing lipid peroxidation (Odukaya *et al.*, 2004). It is well documented in literature that there is direct relationship between antioxidant activity and phenolic content of plant extracts (Kaur *et al*., 2002 and Ivanova *et al*., 2005). 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 does not cause cellular damage (Riemersma *et al*., 2000).

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 from 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, peroxyl 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 (Larson, 1988).

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 Tepe *et al*., (2005).

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.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_2O_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
Staphylococcus aureus	4.25 ± 0.5	7.25 ± 0.5	2.25 ± 0.5	12.5 ± 0.57
Salmonella typhi	5.5 ± 0.57	5.75 ± 0.5	3.75 ± 0.5	11 + 0.81
E.coli	0	0	0	9.25 ± 0.95
Pseudomonas aeruginosa	0	0	0	9.75±0.5
Aspergillus niger	3.25 ± 0.5	3.75 ± 0.5	2.5 ± 0.57	9.75 ± 0.5
Trichoderma viridae	0	0	0	9.5 ± 0.57

Values are mean ± SD of three different observations.

Table 4 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 viride*. These result 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 possess antimicrobial activities against a number of microorganisms. In Conclusion, all the above observations suggested that *Syzygium cumini* leaves have antioxidant and Antimicrobial activity.

References

Ivanova, D., Gerova, D., Chervenkov, T. 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants, *J Ethnopharmacol.*, 95: 145-150.

Kaur, C., Kapoor, H.C. 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. *Int. J. Food. Sci. Technol.*, 37: 153-161.

Larson, R.A. 1988. The antioxidant of higher plants. *Phytochem.*, 27: 969- 978.

Lister, E., Wilson, P. 2001. Measurement of total phenolics and ABTS assay for antioxidant activity (personal communication); Crop Research Institute, Lincoln, New Zealand.

Nakatani N., Kikuzaki, H. 1987. A New Antioxidative Glucoside Isolated from Oregano (Origanum vulgare L.); *Agric. Biol. Chem.*, 51: 2727-2732.

Odukaya, A., Olukemi lIon, Olukimi, Sofidiya, M., Oluwatoyin Aniunoh, Austin Lawal, B., Mansurat, Tade, I., Olufunmilola. 2005. Antioxidant activity of Nigerian dietary species; *Elect. J. Environ. Agric. Food Chem.*, 4(6): 1086-1093.

Official methods of analysis. 1999. 10th Edition, 5th Reversion, AOAC International, Gaithersburg, MD, method 939.13 and 966.18.

Riemersma, R.A., Carruthers, K.F., R.A. Elton, R.A., K.A. Fox, K.A. 2000. Vitamin-C and the risk of acute myocardial infarction; *Am. J. Clin. Nutr.*, 179(71): 1181-1186.

Spanos, G.A., Wrolstad, R.E. 1990. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice; *J. Agri. Food Chem.*, 38: 1565-1571.

Tanaka, J.C.A., De Silva, C.C., De Oliveira, A.J.B., Nakamura, C., Dias Filho, B.P. 2006. Antibacterial activity of iodole alkaloids from Aspidoperma ramifloram; *Br. J. Med. Biol. Res.*, 39(3): 387-391.

Tepe, B., Daferera, D., Sokmen, A., Sokmen, M., Polissiou, M. 2005. Antimicrobiol and antioxidant activities of the essential oil and various extracts of Salvia tomentosa Miller (Lamiacese); *J. Food chem.*, 90: 333-340.

Zhang X.Y. 2000. Principles of Chemical Analysis; Beijing: China Science Press: 275-2.

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