

A study of antibacterial, antioxidant and neuroprotective effect of stem of *Syzygium cumini*

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Abstract

Aims: The purpose of the present study was to investigate the antibacterial activity, antioxidant activity, and neuroprotective ability of aqueous and alcoholic extracts of the stem of *Syzygium cumini*. **Materials and Methods:** Antibacterial activity of the aqueous and alcoholic extracts of stem of *S. cumini* was observed against two Gram-positive bacteria (*Bacillus amyloliquefaciens* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) by agar well diffusion method. Phytochemical screening was done to reveal the presence of flavonoids in its aqueous, ethanolic, and methanolic extracts of stem of *S. cumini*. Antioxidant activity was estimated by its enzymatic and non-enzymatic biochemical assay such as superoxide dismutase, catalase, glutathione content, glutathione S-transferase, and malondialdehyde content in the aqueous and methanolic extract. Neuroprotective ability of aqueous and ethanolic extract of stem of *S. cumini* were observed on Rat pheochromocytoma (PC)-12 cell line, by giving neurotoxic shock to Rat PC-12 cells using 6-hydroxydopamine, **Statistical Analysis Used:** To estimate the accuracy of the experimental data, each experiment was performed in triplicates, and the result was expressed as mean \pm standard deviation of three replications. $P < 0.05$ was regarded as significant. **Results:** Antibacterial activity of the aqueous and alcoholic extracts of stem of *S. cumini* was observed where alcoholic extract showed maximum antibacterial activity against *B. amyloliquefaciens* and *S. aureus*. Methanolic extract showed higher level of antioxidant activity as comparative to aqueous extract. Ethanolic extract has shown maximum number of viable cells, i.e., 75% and aqueous extract showed 50% of it. **Conclusions:** This study suggests that stem can be utilized as a good source of antibacterial, antioxidant, and neuroprotective agent.

Key words: Antibacterial activity, antioxidant activity, neuroprotective, phytochemical screening

INTRODUCTION

Herbs of medicine are being used throughout the world, and these herbs are found to be original source for most of the drugs. Medicinal plants contain so many bioactive compound that acts as therapeutic agents used for the treatment of human disease. Herbal plants and their respective extracts have played a significant role as antiulcer, antipyretic, antidiabetic, and anticancer activity in different animal model.^[1] As far as the medicinal plants are concerned, they are found to be utilized in two distinct areas of health management, i.e., in traditional system of medicine and modern system of medicine. The medicinal attributes of plants are found to be present in leaves, stem, fruits, and other parts where they are used as alterative, tonic diuretics, blood purifier, and antiphlogistic. They are being used as remedy

against chronic eczema, chronic ulcer, chronic rheumatism, chronic nervous diseases, madness, cholera amenorrhoea, piles, and fistula.^[2] Natural bioactive components or natural extract has shown various therapeutic impacts such as antibacterial, antifungal, and anti-inflammatory properties. It is also been reported that each part of plants has different secondary metabolites in their particular extracts and as well as different antibacterial activity in them.^[3] Medicinal plants

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have natural antioxidant compound such as flavonoids, phenolic acid, and volatile compounds that reduce the risk of chronic diseases including cancer and heart disease. These antioxidant compounds have the ability to trap free radicals and oxygen species that may oxidize nucleic acid, proteins, lipids, or DNA.^[4] Medicinal plants or herbs have been used to treat cognitive disorders, including neurodegenerative diseases such as Alzheimer's disease and other memory-related disorders. Identification and characterization of new medicinal plants to cure neurodegenerative diseases and brain injuries resulting from stroke have become major scientific interest in recent years. There are more than 100 traditional medicines that are being used for the therapy of central nervous system (CNS) disorders. CNS cells are able to counter oxidative stress using some resources such as vitamins, bioactive molecules, lipoic acid, antioxidant enzymes, and redox-sensitive protein transcriptional factors. Phytochemical derived bioactive antioxidant compound has neuroprotective importance in slowing progression of neuronal cell loss that reduces cellular damage.^[5,6] There are many medicinal plants that have shown various antioxidant activity against the stress provided by the 6-hydroxydopamine (6-OHDA).^[7]

Syzygium cumini is commonly known as jamun, Duhat in Hindi and black plum, black plum tree, Indian blackberry, jambolan, and jambolan plum, which belongs to the family of Myrtaceae.^[8,9] These trees are found growing throughout the subcontinent, East Africa, South America, Madagascar, and United States of America. It is most widely distributed trees in India.^[10] It can grow on shallow, rocky soils and grow up to 25 m tall. *S. cumini* is also known as *Syzygium jambolanum* and *Eugenia cumini*.^[11] It has young stems grayish white with lower bark coarse and discolored. Leaves of jamun tree are simple, glossy and short pointed at tips. Flowers are from white to pinkish in branched cluster at stem tips. Fruits are ovoid, dark purplish red, and shiny with white to lavender flesh.^[12] Different parts of jamun tree were also reported for its antioxidant, anti-inflammatory, neuropsychopharmacological, antimicrobial, antibacterial, anti-HIV, antileishmanial, and antifungal activity.^[13] Leaves of jamun tree are considered to strengthen the teeth and gums, to treat leucorrhea, stomachalgia, fever, gastropathy, strangury, dermatopathy, and to inhibit food discharges in the feces.^[14,15] Crude extracts of jamun leaves have shown prominent activity against various pathogenic bacteria. Its essential oil as well as leaf methanolic extracts has also shown significant activity against both Gram-positive and Gram-negative bacteria.^[16] Methanolic extract has shown higher inhibitory effect than methylene chloride and essential oil extracts. A higher content of flavonoids was also found in methanolic extract of leaves of jamun tree, i.e., 6.22 mg/g d.w.^[4] Fruit bark of jamun tree has led to the isolation of three ellagic derivatives that are ellagic acid, ellagic acid 4-O- α -L-2'-acetyl-rhamnopyranoside, and 3-O-methylellagic acid 3'-O- α -L-rhamnopyranoside, as well as the new derivative 3-O-methylellagic acid

3'-O- β -D-glucopyranoside.^[17,18] Decoctions of jamun bark are gargled or used as mouthwash for the astringent effect on mouth ulceration.^[19] Stem bark of jamun tree has shown the astringent property of presented gallo and ellagitannins.^[10] The oral administration of fruits pulp extracts enhances insulinemia in normoglycemic and diabetic rats as well as increases the insulin secretion.^[20] Roots of jamun tree are rich in flavonoids, glycosides, and isorhamnetin-3-rutinoside.^[10,21,22] Tannin extracted from *S. cumini* fruit has shown free radical scavenging and ferric reducing power.^[23] Aqueous, ethanol and n-hexane extracts from leaves, fruits, roots-bark, and stem bark of *S. cumini* (L.) Skeels has shown antifungal activity against the disease of chickpea.^[24]

The purpose of the present study was to investigate the antibacterial activity of different extracts of stem of *S. cumini* against two Gram-positive and Gram-negative bacteria by agar well diffusion method. Phytochemical screening was done in different extracts to determine the presence of secondary metabolites in them. Antioxidant activity of aqueous and alcoholic extracts of stem of *S. cumini* was observed to determine the enzymatic and non-enzymatic antioxidant properties of extract. Neuroprotective ability, i.e., neuroprotective effect of aqueous and ethanolic extract of stem of *S. cumini* were observed on Rat pheochromocytoma (PC)-12 cell line, by giving neurotoxic shock to Rat PC-12 cells using 6-OHDA. These experiments were initialized to provide a scientific rationale for the use of stem of *S. cumini* (Jamun tree) as a traditional herbal remedy.

MATERIALS AND METHODS

Selection of Plant

The stem of *S. cumini* was collected from IARI, New Delhi and kept at Amity Institute of Biotechnology, Amity University, Uttar Pradesh. Stems were separated out from leaves, washed, dried in the shaded area at room temperature and were grounded with grinder to form a powder and used throughout the study.

Bacterial Cells

Bacterial cells of *Escherichia coli* (DH5 α), *Bacillus amyloliquefaciens*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were obtained from Helix BioGenesis Pvt. Ltd., Noida, Uttar Pradesh, and were sub cultured freshly in Luria Broth (LB) medium and used throughout the research work.

Neuronal Cells

Rat PC-12 cells were collected from National Center for Cell Science, Pune, India. They were resuspended, subcultured in fresh DMEM media and used throughout the project.

Preparation of Antibacterial Extracts

For the aqueous and alcoholic extracts, 5 g of powdered stems were dissolved in 50 ml of distilled water to prepare aqueous extracts and for alcoholic extracts in different solvents such as ethanol and methanol. Aqueous extracts were allowed to boil in water bath about 30 min at 100°C and alcoholic extracts at 65°C. The conical flasks of the extracts were covered by cotton plugs to prevent it from evaporation. Extracts were placed in shaking incubator for 24 h at 250 rpm. They were filtered with muslin cloth and again with filter paper twice. Filtered aqueous extracts were stored at 4°C, whereas alcoholic extracts were air dried by evaporation.^[25]

Antibacterial Sensitive Test (Agar Well Diffusion Method)

Antibacterial activity of prepared aqueous and alcoholic extracts of stems of *S. cumini* was determined by agar well diffusion method against different bacterial cells. LB agar media was prepared and autoclaved at 121°C for 15 min at 15 lbs. Autoclaved media was allowed to pour in sterile Petri plates up to a uniform thickness of approximately 10–15 min. 200 µl of inoculums were spread over LB agar plates using sterile spreader and after few minutes four wells were made in each plate and loaded with 100 µl of extracts and control. Plates were incubated at 37°C for 24 h. Antibacterial activity was observed by measuring its inhibition length. The experiments were done in triplicate.^[25,26]

Inhibition length = Zone of inhibition (mm) – Well diameter (mm)

Preliminary Phytochemical Screening (Qualitative Test)

Phytochemical screening of aqueous and alcoholic extracts of stem of *S. cumini* were done according to standardized protocol^[25,26] to reveal the presence of secondary metabolites such as saponin, tannin, flavonoid, terpenoid, naphthoquinone, inulin, carbohydrate, alkaloid, and phenol in the defined extracts of stem of *S. cumini*.^[25,26]

Antioxidant Activity (Enzymatic and Non-enzymatic Biochemical Assay)

Antioxidant activity of stem of *S. cumini* was determined by enzymatic and non-enzymatic biochemical assay in their aqueous and methanolic extracts. These extracts were prepared by standardized protocols.^[7] The superoxide dismutase activity (SOD), catalase activity (CAT), glutathione content (GSH), and glutathione S-transferase (GST) activity were observed in extracts of stem of *S. cumini*. SOD activity was expressed as one unit of enzyme activity, i.e., enzyme concentration required for inhibition at 560nm

absorbance of chromogen production by 50% in 1 min and was expressed as specific unit of SOD per min per mg of protein.^[27] CAT activity was expressed in enzyme activity as µ moles of H₂O₂ oxidized per min per mg protein.^[28] Lipid peroxidation, i.e. thiobarbituric acid reactive substances were determined on the principle of formation of malondialdehyde (MDA) content by breaking down of polyunsaturated fatty acids and were measured spectrophotometrically at 532 nm in its aqueous and methanolic extract. Levels of lipid peroxidation were expressed in nano mole of MDA formed per g of tissue.^[29] GSH content was estimated in their aqueous and methanolic extracts using dithiobis nitrobenzoic acid and expressed in µg per mg of protein.^[30] GST activity was assayed on the basis of GSH conjugation to 1-chloro-2,4-dinitrobenzene (CDNB-GSH) conjugate formed per min per mg of protein in the aqueous and methanolic extract of stem of *S. cumini*.^[26,31]

Quantification of Protein

Protein was quantified in the 100 µl of extract's supernatant by Lowry's method using Folin–Ciocalteu Reagent.^[7]

Neuroprotective Efficacy

Neuroprotective efficacy of extracts on Rat PC-12 cell was observed by giving neurotoxic shock to Rat PC-12 cells using 6-OHDA. After sub culturing of Rat PC-12 cells in DMEM media, 10⁶ cells/well were plated in duplicates for each sample in microtiter plates. The plates were incubated for 48 h in CO₂ incubator at 37°C. First set of wells contained only cells as positive control, i.e., only cells. Second set contained cells + 6-OHDA as negative control. Third and fourth sets of wells contained cells + 6-OHDA + aqueous and ethanolic extracts of stem of *S. cumini*, respectively. Results were obtained on the basis of percentage of viable cells.^[28]

Statistical Evaluation

To estimate the accuracy of the experimental data, each experiment was performed in triplicates and the result was expressed as mean ± standard deviation of three replications. $P < 0.05$ was regarded as significant.

RESULTS

Antibacterial Sensitive Test

The purpose of the present study was to investigate the antibacterial activity of aqueous and alcoholic extracts of stem of *S. cumini* by agar well diffusion method. Inhibition length was calculated to observe the inhibitory effect of defined extracts against two Gram-positive bacteria (*B. amyloliquefaciens* and *S. aureus*) and two Gram-negative

bacteria (*E. coli* and *P. aeruginosa*). From the above study, it was observed that maximum antibacterial activity found to be present in the ethanolic extract of stem of *S. cumini* against the *B. amyloliquefaciens* with an inhibition length of 12 ± 1 mm, where as its methanolic extract has also shown its antibacterial activity against the *B. amyloliquefaciens* with an inhibition length of 11.33 ± 0.577 mm, whereas no antibacterial activity was found to be present in the aqueous extract of stem of *S. cumini* as shown in Graph 1. Aqueous extract has not shown any antibacterial activity against any bacterial cells as given in Table 1. Whereas *S. aureus*, *E. coli* and *P. aeruginosa* were found to be inhibited by ethanolic extract of stem of *S. cumini* with an inhibition length of 8.33 ± 0.577 mm, 7.66 ± 0.577 and 8.66 ± 0.577 mm, respectively, as given in Table 2. As far as the methanolic extract of stem of *S. cumini* was concerned, *E. coli*, *S. aureus*, and *P. aeruginosa* was found to be simultaneously inhibited with an inhibition length of 9 ± 1 mm, 7.33 ± 0.577 mm, and 7.66 ± 1.527 mm, respectively, as given in Table 3. From the above study, this can be estimated that both ethanolic and methanolic extracts have shown antibacterial activity against both the Gram-positive bacteria and Gram-negative bacteria are shown in Figure 1.

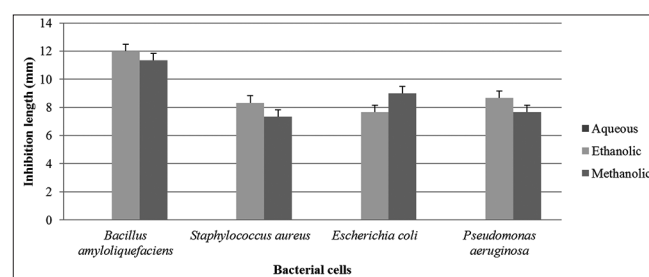
Preliminary Phytochemical Screening

Phytochemical screening was done to reveal the presence of secondary metabolites that were present in the aqueous and alcoholic extracts of the stem of *S. cumini*. The present study has shown the presence of saponins, tannins, flavonoids,

terpenoids, alkaloids, inulins, and carbohydrates, whereas naphthoquinone and phenol found to be absent in the aqueous extract. As far as the ethanolic extract was concerned, saponins, tannins, flavonoids, terpenoids, alkaloids, carbohydrates, and phenol found to be present in them, whereas naphthoquinone and inulin have not revealed any presence in the ethanolic extract of stem of *S. cumini*. The methanolic extracts of stem of *S. cumini* have revealed the presence of tannins, flavonoids, terpenoids, naphthoquinones, alkaloids, carbohydrates, and phenols, whereas saponins and inulins were found to be absent in the methanolic extract of stem of *S. cumini* are given in Table 4.

Antioxidant Activity

Antioxidant activity was observed in the aqueous and methanolic extract of the stem of *S. cumini* with respect to



Graph 1: Antibacterial activity of aqueous, ethanolic and methanolic extracts of bark of *Syzygium cumini* against *Bacillus amyloliquefaciens*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*

Table 1: Antibacterial activity of aqueous extracts of bark of *Syzygium cumini* against different bacterial cells

Bacterial cells	Well diameter (mm)	Zone of inhibition (mm)	Inhibition length (mm)
<i>Bacillus amyloliquefaciens</i>	9	Nil	Nil
<i>Staphylococcus aureus</i>	9	Nil	Nil
<i>Escherichia coli</i>	9	Nil	Nil
<i>Pseudomonas aeruginosa</i>	9	Nil	Nil

Table 2: Antibacterial activity of ethanolic extracts of bark of *Syzygium cumini* against different bacterial cells

Bacterial cells	Well diameter (mm)	Zone of inhibition (mm)	Inhibition length (mm)
<i>Bacillus amyloliquefaciens</i>	9	21 ± 1	12 ± 1
<i>Staphylococcus aureus</i>	9	17.33 ± 0.577	8.333 ± 0.577
<i>Escherichia coli</i>	9	16.66 ± 0.577	7.66 ± 0.577
<i>Pseudomonas aeruginosa</i>	9	17.66 ± 0.577	8.66 ± 0.577

Table 3: Antibacterial activity of methanolic extracts of bark of *Syzygium cumini* against different bacterial cells

Bacterial cells	Well diameter (mm)	Zone of inhibition (mm)	Inhibition length (mm)
<i>Bacillus amyloliquefaciens</i>	9	20.33 ± 0.577	11.33 ± 0.577
<i>Staphylococcus aureus</i>	9	16.33 ± 0.577	7.33 ± 0.577
<i>Escherichia coli</i>	9	18 ± 1	9 ± 1
<i>Pseudomonas aeruginosa</i>	9	16.66 ± 1.527	7.66 ± 1.527

the specific enzyme activity of flavonoids that were revealed in the phytochemical screening of aqueous and methanolic extracts of stem of *S. cumini*. The superoxide radical scavenging effects of aqueous and methanolic extract were analyzed in the terms of formazan and SOD activity which were calculated in terms of unit/mg of protein. SOD activity in the aqueous and methanolic extract of stem of *S. cumini* found to be 2.94 ± 0.094 unit/min/mg protein and 5.10 ± 0.106 unit/min/mg protein, respectively. Higher level of SOD activity in methanol extracts demonstrates the powerful superoxide anion scavenger that can used as a therapeutic against oxidative stress as shown in Graph 2. The CAT activity found to be present in both the extracts by scavenging activity of hydrogen peroxide, where methanolic extract and aqueous extract have shown 5.19 ± 0.006 μmoles of H_2O_2 consumed/min/mg protein and 2.71 ± 0.078 μmoles of H_2O_2 consumed/min/mg protein, respectively. The methanolic extract of the bark of *S. cumini* has shown maximum GST

activity, i.e., 17.47 ± 0.068 μmoles of CDNB-GSH conjugate formed/min/mg protein whereas aqueous extract has shown 7.77 ± 0.198 μmoles of CDNB-GSH conjugate formed/min/mg protein. GSH content was also found to be present in the aqueous and methanolic extracts of the stem of *S. cumini*, whereas it has shown a high level in the methanolic extract, i.e., 15.28 ± 0.079 μg of GSH oxidized/mg protein. Aqueous extract of the stem of *S. cumini* has also shown the GSH content, i.e., 3.5 ± 0.332 μg of GSH oxidized/mg protein. In this study, it was also observed that the methanolic extract revealed the highest level of GSH content and as well as GST activity then aqueous extract as shown in Graph 2. Lipid peroxidation activity was also observed in the aqueous and methanolic extract, and as per the study aqueous extract has shown a maximum level of MDA content, i.e., 7.95 ± 0.061 $\mu\text{moles/mg}$ protein sample as compared to methanol it has shown less MDA content, i.e., 1.91 ± 0.0763 $\mu\text{moles/mg}$ protein sample as shown in Graph 2.

Table 4: Phytochemical screening of aqueous, ethanolic and methanolic extract of bark of *Syzygium cumini*

Phytoconstituents	Aqueous	Ethanol	Methanol
Saponin	+	+	-
Tannin	+	+	+
Flavonoid	+	+	+
Terpenoid	+	+	+
Naphthoquinone	-	-	+
Alkaloid	+	+	+
Inulin	+	-	-
Carbohydrate	+	+	+
Phenol	-	+	+

+ indicate the presence of the constituents while - indicate the absence of constituents

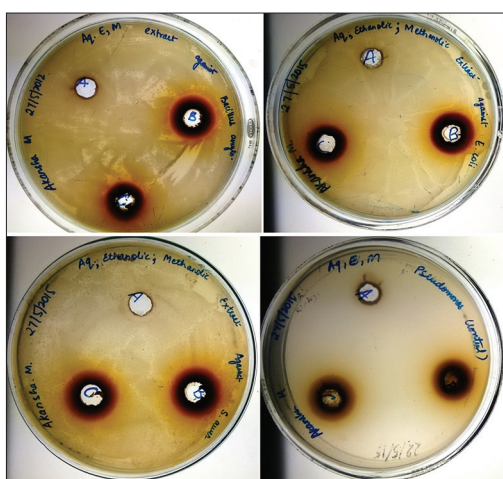


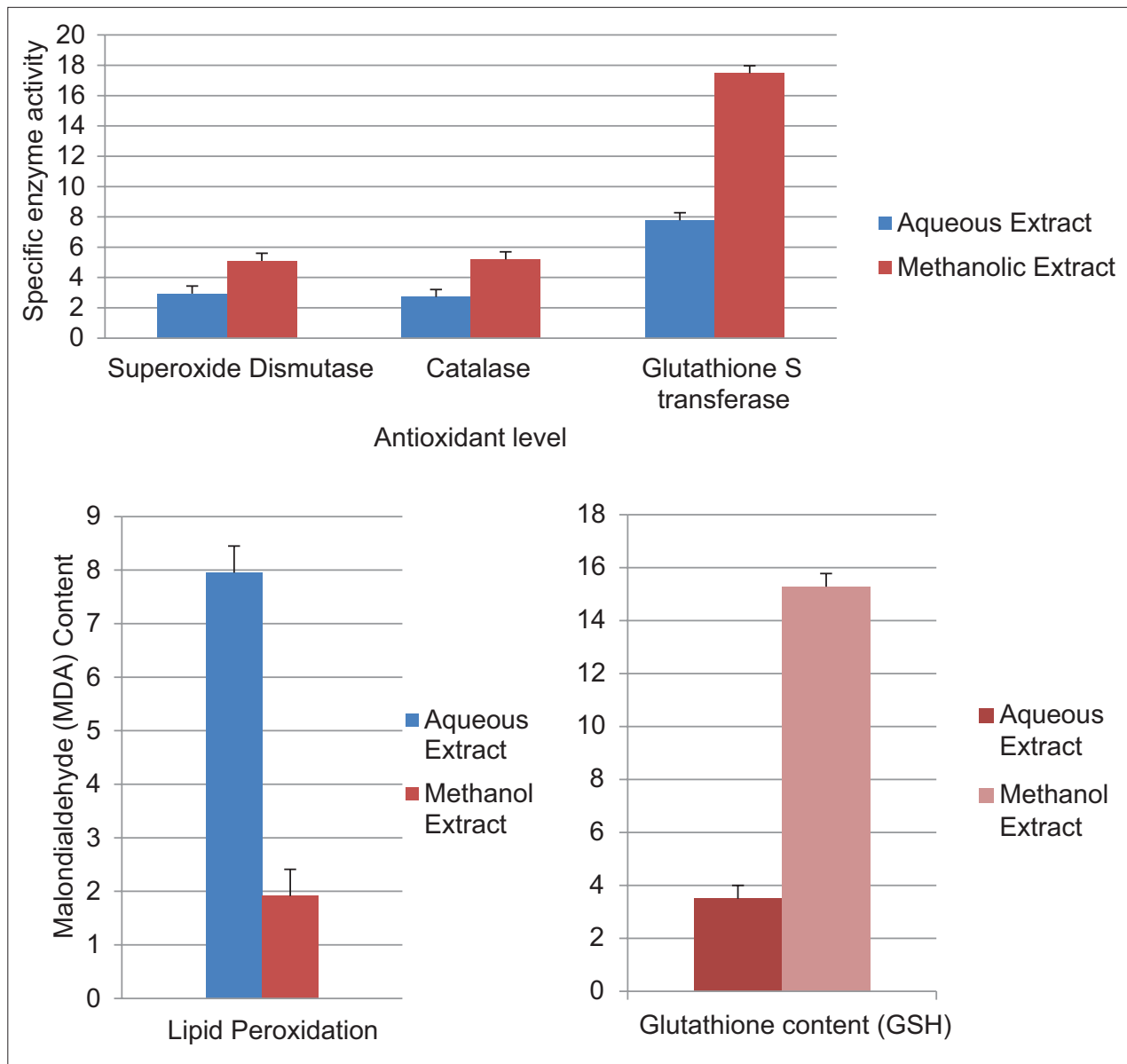
Figure 1: Antibacterial activity of (A) aqueous, (B) ethanolic and (C) methanolic extract against the *Bacillus amyloliquefaciens*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*

Neuroprotective Ability

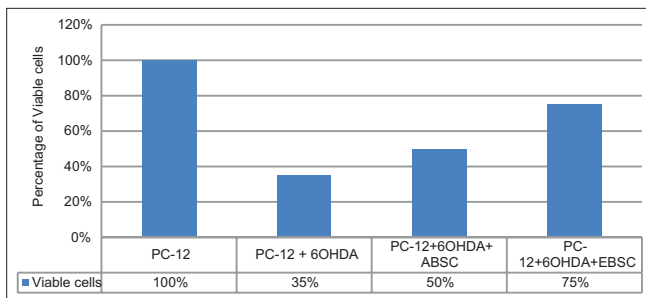
From the present study, it was observed that antioxidant secondary metabolites such as flavonoids have shown neuroprotective ability. The neuroprotective ability was observed in the aqueous and ethanolic extract of stem of *S. cumini* on Rat PC-12 cells. Ethanolic extract has shown a maximum number of viable cells, i.e., 75% and aqueous extract has shown 50% of viable cells as compared to those cells which were given as neurotoxic shock using 6-OHDA, i.e., 35% negative control as shown in Graph 3 and Figure 2.

DISCUSSION

Bioactive compound from medicinal plant has shown therapeutic impact against the various disease caused by microbes and oxidative stress. These active compounds can be act as antimicrobial agent, antioxidant agent, and many more. In ayurvedic and alternative traditional medicines, medicinal plants are used in curing general health of common people.^[26] As far as the stem of *S. cumini* was concerned, in the previous studies, eugenin and fatty acid ester found to be present as chemical constituents in its bark, as well as quercetin, kaempferol, and bergenins. The present work was an initial effort in the field of microbiology and as well as in neurobiology. The purpose of the present study was to investigate the antibacterial activity, phytochemical screening, antioxidant activity, and neuroprotective ability was observed. From the above study, it was observed that ethanolic extract of stem of *S. cumini* has shown maximum antibacterial activity against *B. amyloliquefaciens* with an inhibition length of 12 ± 1 mm. Methanolic extract of stem of *S. cumini* has also shown its antibacterial activity against *B. amyloliquefaciens*, *E. coli*, *S. aureus*, and *P. aeruginosa* with an inhibition length of 11.33 ± 0.577 mm, 9 ± 1 mm, 7.33 ± 0.577 mm, and 7.66 ± 1.527 mm, respectively. As



Graph 2: Antioxidant activity of aqueous and methanolic extracts of bark *Syzygium cumini* showing different levels of enzymatic and non-enzymatic antioxidant



Graph 3: Neuroprotective ability of aqueous and ethanolic extract of bark of *Syzygium cumini* on Rat pheochromocytoma-12 cells

compared to the previous study methanolic extract of leaves of *S. cumini* has shown an inhibitory effect against *E. coli*, *S. aureus*, and *P. aeruginosa* with a zone of inhibition, i.e., 22

± 0.9 mm, 24 ± 0.8 mm, and 19 ± 0.8 mm, respectively.^[4] As far as the aqueous extract was concerned, it has not shown any antibacterial activity against both the Gram-positive and Gram-negative bacteria. As compared to the previous studies aqueous extract of leaves of *S. cumini* has shown maximum antibacterial activity at volume of 100 μ l against *E. coli* and *S. aureus* with zone of inhibition, i.e. 18 mm individually.^[32] From the above study, it was observed that growth of microorganism found to be inhibited by ethanolic and methanolic extract of stem of *S. cumini*. Preliminary phytochemical screening was done to find the presence of secondary metabolite that was present in the stem. Flavonoids found to be present in the aqueous, ethanolic, and methanolic extracts whereas tannins, resin and phytosterols found to be reported in the previous studies.^[33] Present study has also shown the presence of tannin, terpenoid, alkaloid,

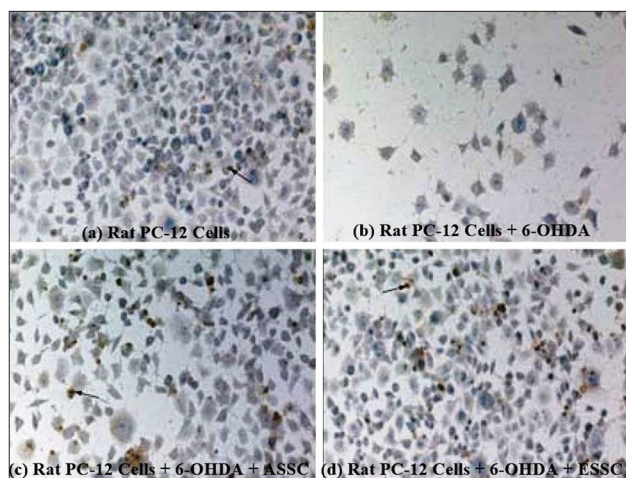


Figure 2: Neuroprotective ability of aqueous and ethanolic extract of stem of *Syzygium cumini* on Rat pheochromocytoma-12

and carbohydrate in the aqueous, ethanolic and methanolic extract of stem of *S. cumini*. Aqueous extract has also revealed the presence of saponin and inulin whereas saponin found to present in the ethanolic extract also but inulin has not shown any presence in the methanolic and ethanolic extract. As far as the phenol was concerned, this was found to be present in the ethanolic and methanolic extract.

Antioxidant activity of aqueous and methanolic extract of stem of *S. cumini* was observed by measuring the enzymatic and non-enzymatic biochemical assay. Enzymatic and non-enzymatic biochemical assay was observed to determine the antioxidant activity of present flavonoids in the aqueous and methanolic extracts. This was found from the above study that methanolic extract has shown a high level of SOD activity to scavenge the superoxide anion, i.e., 5.10 ± 0.106 unit/min/mg protein. Methanolic extract of stem of *S. cumini* also has shown a high level of CAT activity, i.e., 5.19 ± 0.006 μ moles of H_2O_2 consumed/min/mg protein. As compared to the previous study that CAT activity in liver and skeletal muscle were found to be decreased significantly in seed of *S. cumini*.^[34] GST activity was found to be present in both aqueous and methanolic extract of stem of *S. cumini* whereas compared to previous studies oral administration of aqueous extract of seed to mice treated with urethane 7,12-dimethyl benzanthracene resulted in reduced chromosomal damage, significantly inhibited hepatic lipid peroxidation, which was associated with significantly increased activity of GST, SOD, and CAT activity.^[35] GSH content in the methanolic extract has shown high level, i.e., 15.28 ± 0.079 μ g of GSH oxidized/mg protein, whereas compared to previous studies that pre-treatment of cyclophosphamide – administered rats with methanolic extract of *S. cumini* fruit resulted in increased GSH levels.^[36] MDA content was also found to be present maximum in aqueous extract, i.e., lipid peroxidation of 7.95 ± 0.061 μ moles/mg protein sample as compared to the previous studies methanolic extract of *S. cumini* fruit resulted in reduced formation of hepatic MDA.^[36] Neuroprotective

ability was also observed in the aqueous and ethanolic extract on Rat PC-12 cells that have shown 75% of viable cell in its ethanolic extract and 50% in its aqueous extract as compared to the previous studies age related neurodegenerative disease found to be prevented from the berry fruits that also improve motor and cognitive function.

CONCLUSION

From the present study, we authors would like to conclude that bioactive extracts of stem of *S. cumini* can be utilized as an active antibacterial agent against both the Gram-positive and Gram-negative bacteria. Presence of flavonoids, antioxidant activity, and neuroprotective ability in the bioactive extracts of stem suggests to use it as natural antioxidant as well as neuroprotectant that could have a great importance as a therapeutic agent. Future work requires the *in vivo* studies to use stem of *S. cumini* as a traditional herbal remedy.

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