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www.commprac.com
ISSN 1462 2815

EXPLORING THE ANTIOXIDANT POTENTIALS OF *SYZYGIUM CUMINI* (L.) SKEELS: A COMPARATIVE STUDY OF BARK AND LEAF EXTRACTS

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DOI: 10.5281/zenodo.12650189

Abstract

Syzygium cumini is a perennial tree native to tropical regions and belongs to the Myrtaceae family of angiosperms. However, the research on antioxidants in plants is progressing, still, there is a need to explore the phytoactive and biological potential of each plant-based product, even under a single capricious condition. The current investigation extracted the phytochemicals from the bark and leaf of *Syzygium cumini* (L.) Skeels use two types of solvent systems and further explore their total phenolic and antioxidant potentials. The dried samples were extracted using a solvent system such as aqueous ethanol (AE) and aqueous acetone (AA) by the Soxhlet apparatus. The total percentage yield, carbohydrates, amino acids, and phenolics is higher in the AE *S. cumini* leaf extract than in the bark extracts. The phytoconstituents exhibited potential antioxidant efficiency in the 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and Ferric-reducing antioxidant power in a dose-dependent mode. Significant lipid peroxidation inhibition efficiency of the extracts was observed in the linoleic acid emulsion assay. The results indicated that *S. cumini* extracts were tested, and have significant promise as organic sources of Phytoactives with antioxidant activity, which could be used as an antioxidant supplement for various applications. However, additional comprehensive research is required on laboratory and animal models to clarify the phytoconstituents of plants and their modes of action.

Keywords: Antioxidant, Malabar Plum, Phenolics, Phytoactive, Reducing Power, *Syzygium Cumini.*

INTRODUCTION

Typically, fruits and vegetables are high in compounds that possess diverse biological actions and, offer protection against various diseases. Vitamins, fiber, minerals, polyphenols, and, flavonoids were found to be the major antioxidants in plants [1]. Hence, nowadays consumers are attracted to functional foods. Research on the exploitation of plants and plant-based products for the development of antioxidant agents is still in progress. *Syzygium cumini* (L.) Skeels are perennial trees native to tropical regions and are classified under the family Myrtaceae, which consists of

flowering plants. It has common names such as Jambolan or Jamun, Java plum, and Malabar plum. The tree is indigenous to the subcontinent of India, and adjacent regions of Southeast Asia, including Sri Lanka, the Andaman Islands, and Myanmar. The tree has a lifespan of more than a century and a maximum height of 30 m (98 ft). As a fast-growing plant, it is widely considered for its edible fruit, timber, and ornamental value [2].

S. *cumini* attributed Indian traditional medicine practices to its medicinal properties. The fruit pulp possesses a pleasant taste, but the seeds have a bitter, acidic, and invigorating flavour. The pulp is rich in numerous minerals and nutrients, including sodium, calcium, potassium, iron, phosphorus, and zinc, as well as vitamins such as carotenoids, niacin, ascorbic acid, and thiamine [3]. The plant bark possesses digestion enhancement, diuretic effects, fever reduction, antiparasitic properties, stomachic effects, constipation relief, and antibacterial activity. The fruit and seed are used to address diverse health ailments, such as pharyngitis, diabetes splenopathy, ringworm infection, and urethrorrhea. In traditional practices, the leaf is expansively used to treat diabetes, constipation, dermopathy, and to inhibit blood discharges in the feces, stomachalgia, gastropathy, leucorrhea, and strangury [4]. The plant contains phytocompounds such as triterpenoids, ellagic acid, acetyl oleanolic acid, kaempferol, isoquercitin, quercetin, and myricetin in altered concentrations. *S*. *cumini* fruits and leaves have been widely studied for their biological activity in various aspects [5]. However, there are limited data available regarding the relative antioxidant effectiveness of *S. cumini* bark and leaf extracts from a specific variety of *S. cumini* in a single study using a particular solvent system, such as aqueous ethanol 50% v/v and aqueous acetone 50% v/v.

Manifold factors, including agriculture practices (such as geographical regions, cultivar, climate, season, water availability, and other environmental factors) and formulation practices (such as plant type, parts used, solvent system, and extraction techniques), influence the phytochemical content and associated biological potentials of plants [6]. Exploring the chemical constituents and their action of each plant-based product with a single variable condition is also a key element to be considered in any study of nutraceuticals for the benefit of mankind. Hence, this research aimed to determine the antioxidant capacity of aqueous ethanol 50% v/v and aqueous acetone 50% v/v solvent extracts of *S*. *cumini* bark and leaf.

MATERIALS AND METHODS

Materials:

Chemicals such as ethanol, acetone, reagents of 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's Phenol, sodium carbonate, tannic acid, linoleic acid, potassium ferricyanide, ferric chloride, ammonium thiocyanate, and trichloroacetic acid were purchased from Merck, India, and S.D. Fine Chem, India. All chemicals of analytical grade were used in this study.

Collection, processing, and extraction of *S. cumini* bark and leaf:

Fresh *S. cumini* bark and leaves were collected from Kanjamalai Forest, Salem, Tamil Nadu, India. The samples were segregated, rinsed with tap water, and then with distilled water again to flush out any soil or undesirable residues. The samples are dried out by exposure to air in a shaded area at ambient temperature for 7-8 days, and then crushed into a fine powder for subsequent procedures. The powdered samples

were extracted using an aqueous ethanol 50 % v/v and aqueous acetone 50% v/v solvent system in the Soxhlet apparatus. Using a vacuum evaporator, the collected solvents are evaporated at 50^{o} C. The proportion of dry matter in the plant sample was measured to quantify the extracted product. For additional analysis, the dried materials are dissolved in the appropriate solvent system. The samples were designated as follows: *S. cumini* leaf aqueous ethanol (SCLAE), *S. cumini* leaf aqueous acetone (SCLAA), *S. cumini* bark aqueous ethanol (SCBAE), and *S. cumini* bark aqueous acetone (SCBAA).

Assessment of the total soluble carbohydrate (TSC) and the total free amino acid (TFAA):

The total carbohydrates in the extracts were tested using the Anthrone method [7]. The known concentration of the individual extract was increased by adding distilled water (1 mL). Glucose standards were prepared at standard concentrations. In each tube, 4 mL of the anthrone reagent was added, sheltered by glass marbles, and further stored in a hot water bath for 8–10 min, then made cool. The reaction mixture (observed at green product) absorbency was measured at 630 nm using a spectrophotometer (Scanning Mini spec SL, 177, Elico).

The ninhydrin method was used to quantify the overall concentration of free amino acids in the extracts. Aliquots of extracts and standard (Leucine) solutions were added with ninhydrin reagent (1 mL) and homogenized. After being heated in a water bath for 20 min, the reaction tubes were brought to room temperature. Five milliliters of solutions were added to the reaction tube, and the absorbance at 570 nm was calculated.

Evaluation of the total phenolic content:

The Folin-Ciocalteu Phenol Reagent technique was used to determine the extracts' total phenolic content, as previously indicated by Rizvi et al., [8], using tannic acid as a standard. 490 µL of distilled water was added to 10 µL of the extract (10 mg/2 mL), and 250 µL of Folin-Ciocalteu phenol reagent (1:1) 1N. The reaction mixture was allowed to rest for 3 min and added with 20% Na_2CO_3 (w/v) solution (1.25 mL). After thoroughly mixing the components, incubated at room temperature for 40 min in the dark. At a wavelength of 725 nm, the spectrophotometer measured the reaction mixture's absorbance.

Antioxidant properties:

DPPH free radical scavenging activity:

The extracts' effectiveness as antioxidants was evaluated on the basis of their ability to donate hydrogen or scavenge DPPH radicals. 0.5 mL of extracts at various concentrations were combined with 0.3 mL of DPPH solution (0.5 mM) ranging from 20-100 µg/mL) using 3 mL of 100% ethanol. For one hour, the reaction mixture was vigorously stirred and maintained in the dark. Using a reference blank, the absorbance was measured at a wavelength of 517 nm. The typical positive control was ascorbic acid [9]. By using the following formula to determine the sample's percentage of free radical inhibition, the effectiveness of radical scavenging was measured:

DPPH radical scavenging activity (%) = (Control OD–Sample OD/Control OD)100.

Reducing antioxidant power assay:

The effectiveness of the extracts' reducing antioxidant power was examined by employing a potassium ferricyanide-ferric chloride method [9]. with tannic acid as a standard. In summary, a range of concentrations (20-100 µg/ 1 mL) of extract were combined with 0.2 M phosphate buffer (2.5 mL) at pH 6.6, along with potassium ferricyanide (0.1%) as 2.5 mL. For 20 min, the reaction mixture was incubated at 50 °C, and then supplemented with trichloroacetic acid as 2.5 mL (10%). An equal volume of purified water and 0.5 mL of ferric chloride (0.1%) is added to the supernatant as 2.5 mL is produced from the reacted solution following centrifugation (at 8000 g for 10 min). To measure the absorbance, a spectrophotometer was used at a wavelength of 700 nm.

Test for lipid peroxidation:

The linoleic acid emulsion technique was used to evaluate the extracts' capacity to stop lipid oxidation, using α-tocopherol as a standard. 0.5 mg of 0.25% w/v of extracts diluted in absolute ethanol (0.5 mL) was mixed with 0.5 mL of 2.5% linoleic acid in 99.5% absolute ethanol. An additional 1 mL of 0.05 M phosphate buffer (pH 7.0) and 0.5 mL of distilled water were introduced into the reaction tube. The reaction mixture contained in the screw-capped tube is placed inside an oven set at a temperature of 40 °C, while ensuring that it is kept away from any sources of light. At 12 h intervals, a portion (0.1 mL) of the reaction mixture was extracted and combined with 9.7 mL of ethanol (75%) and 0.1 mL of ammonium thiocyanate (30%). Following a 3 min incubation period, ferrous chloride (0.1 mL) (2×10^{-2} M) in hydrochloric acid (3.5%) was introduced. At a wavelength of 500 nm, absorbance was measured up until the control sample reached its highest value.

Statistical analyses:

The tests were conducted in triplicates, and the repeat analysis's standard deviation (±) was presented with the mean values.

RESULTS AND DISCUSSION

Total recovery yield, carbohydrate content, and amino acid content

Due to the intricate phytoconstituents and pharmacological properties of plants, it is necessary to develop an *in vitro* bioassay guide that, can be efficiently used to monitor the biological behaviors that indicate significant attributes for evaluating the bioactive qualities. The recovery yield, total dissolved carbohydrates, and total free amino acids of SCLAE, SCLAA, SCBAE, and SCBAA are shown in **Figure 1**. The results reveal that SCLAE and SCLAA have high recovery of 22.4 % and 20.2 %, respectively, whereas it is found as 3.1% and 2.5% for SCBAE and SCBAA respectively. The total carbohydrates (mg/100 g) were measured as 6.33, 4.24, 1.72, and 1.07 for SCLAE, SCLAA, SCBAE, and SCBAA, respectively. It's been clear that the total soluble carbohydrates are found to be higher in SCLAE and lower in SCBAA. The total free amino acids (mg/ 100g) for SCLAE, SCLAA, SCBAE and SCBAA are recorded as 5.9, 3.9, 2.60, and 2.26, respectively.

Extraction is an essential method used to recover and separate bioactive components from plant sources. The literature acknowledges that the solvent plays a crucial role in the extraction process [10]. In the current study, ethanol (50 %) and acetone (50%) solvents were used to extract the constituents from SCL and SCB. In general, the

current results indicated that the recovery percentage of extracts, TSC, and TFAA is found to be higher in the order SCLAE > SCLAA >SCBAE > SCBAA, which is primarily due to the nature of solvent that has the high recovery of phytochemicals and secondary the nature of the material, where the leaves have more constituents than bark [11]. The physicochemical and nutritional value of varieties of *S. cumini* and the literature reports that its various components include fruit, pulp, and seeds [12]. It's been evident that the occurrence and quantity of bioactive compounds and their antioxidant capacity of plants might vary due to their genetic diversification as well as cultivation factors [6].

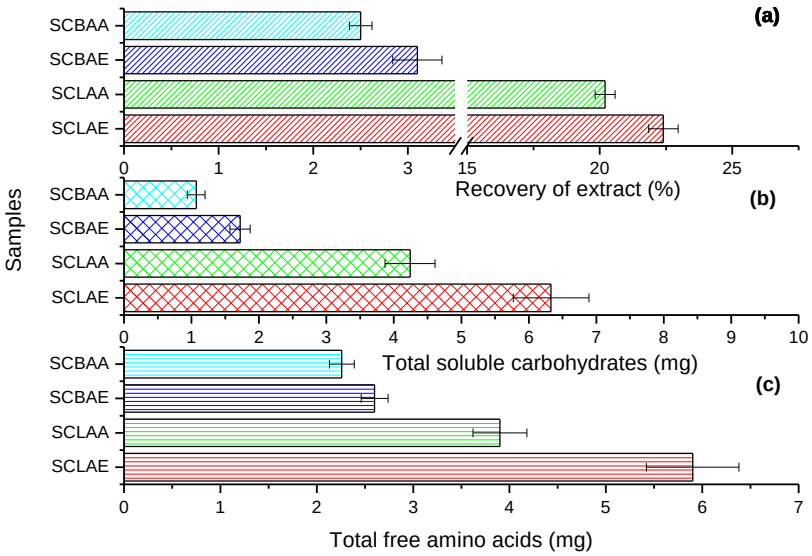

Figure 1: Recovery percentages of the extracts (a), total soluble carbohydrate (TSC), and total free amino acid (TFAA) (c) of selected solvent extracts from *S. cumini*'s leaf and bark (g/100 g)

Total phenolic content of the extracts:

Phenols are important constituents in plants, because of their hydroxyl groups that possess radical scavenging efficiency. The phenolic substance may be attributed to the antioxidative mechanism, and it is advised that polyphenols possess inhibitory qualities in the development of cancer and genetic mutations in humanoids. The antioxidant properties of plant extracts are mostly explained with a strong link to their total phenolic contents [5]. The samples were analyzed to determine the total phenolic contents in percentage as 44.9, 38.06, 9.64, and 8.87% for SCLAE, SCLAA, SCBAE, and SCBAA, respectively (**Figure 2**). The results demonstrate that high phenolic content is found in the order SCLAE > SCLAA > SCBAE > SCBAA.

Previous studies have reported variations in the phenolic content of various solvent systems, types of *S. cumini,* and its parts [13-14]. Sukmasari et al. [15] reported a variation in the phenolic content of Leaves of *S. cumini* gathered from Wonosobo, Central Java, Indonesia. In his study, the phenolic contents were measured as 3.98 ± 1.22, 24.27 ± 8.29, 5.82± 2.33, and 6.07 ± 0.59 mg for the solvent petroleum ether, toluene, ethyl acetate, and acetone. This finding is different from that of Kaur et al. [16], in which *S. cumini* leaves were collected from Punjab, India. This study reported

the total phenol content in mg as 36.05 ± 1.47 for water and 20.86 ± 1.15 for ethyl acetate. These differences are expected because of differences in plant origin, plant sources, solvent system, and extraction processes [17].

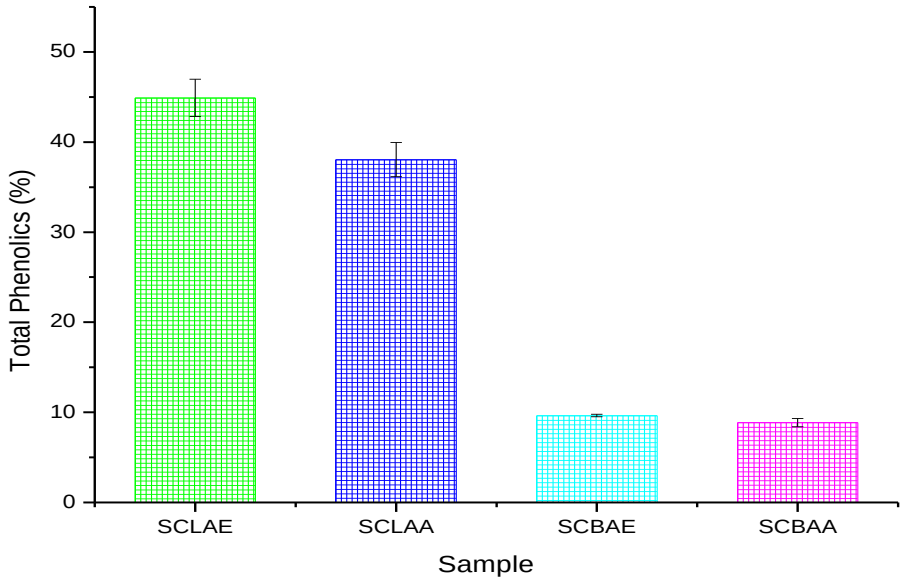

Figure 2: Total phenolic content of *S. cumini* leaf and bark extracts

Antioxidant potential measurement:

Fruits and vegetables have been widely recognized for their antioxidant properties, which include several modes of action such as radical scavenging activity, reducing and chelating ability of metal ions, breakdown of peroxides, and inhibition of chain initiation. The antioxidant effectiveness of SCL and SCB was evaluated in this study using DPPH scavenging and antioxidant reducing power tests.

DPPH scavenging activity of SCL and SCB extracts:

The free radical scavenging activity of SCLAE, SCLAA, SCBAE, and SCBAA exhibited a positive correlation with increasing dosage, as shown in **Figure 3.** The results indicate that SCLAE has more scavenging efficacy than other extracts. The leaf extracts of both solvent systems have almost similar results at a lower dose, whereas vast differences are observed at their higher doses [18]. Whereas, the SCBAA has very low activity in both low and higher doses in comparison with other extracts. The IC50 values are expressed as µg of ascorbic acid equal to antioxidant activity in each gram of the dried sample and are calculated as 61.23, 70.35, 90.17, and 101.38 for the samples SCLAE, SCLAA, SCBAE, and SCBAA, respectively. The order of the scavenging potential is observed as SCLAE > SCLAA > SCBAE > SCBAA, which correlates with the total phenolic content.

The stability of the DPPH radical allows for its bleaching when the test compound donates a hydrogen atom, resulting in the formation of stable diamagnetic molecules. This bleaching process is detected at 515 nm and serves as an indicator of the free radical scavenging capabilities of the target compounds. The DPPH radical is typically used in research because it does not exhibit the side effects of reactions such as metal chelation and enzyme inhibition [19]. Therefore, in this investigation, DPPH

scavenging activity was used as a factor to assess the antioxidant effectiveness of the extracts. The results indicate that the sample extracts have a unique scavenging effect on DPPH radicals, which differs from that of ascorbic acid. The observed increase in activity in this study is consistent with the findings reported in the literature on *S. cumini*, and the extent of the increase depends on the dose [20].

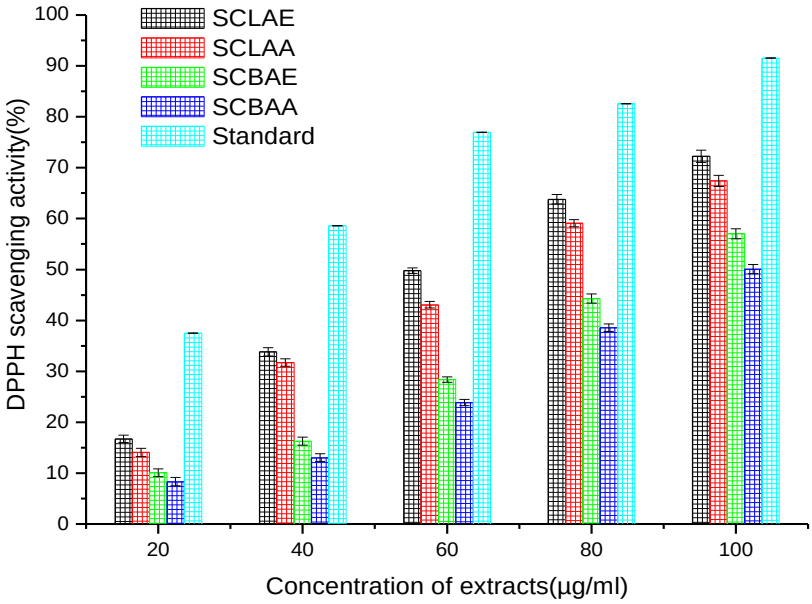

Figure 3: DPPH scavenging potential of *S. cumini* leaf and bark extracts

Reducing activity of SCL and SCB extracts:

The extracts' reducing ability is directly correlated to the increase in absorbance, which corresponds to the concentration of the reaction mixtures. This relationship is illustrated in **Figure 4**. The findings demonstrated an increase in the ability to reduce substances because of the enhanced development of the complex. SCLAE exhibited a high reducing capacity over other extracts. The activity of SCLAE and SCLAA was comparatively more prominent than the standard (tannic acid), whereas SCBAE and SCBAA activity was observed lesser than the standard. The results show that increasing the concentration of all extracts led to a higher level of inhibition, displaying the samples' efficacy in a concentration-dependent manner. These findings are consistent with those of the existing literature [18].

The capacity of test compounds can be assessed to reduce by directly converting Fe^{3+} to Fe^{2+}. A highly concentrated Perl's Prussian blue complex was produced when free Fe^{3+} was added to the reduced reaction products, which exhibited significant absorbance at 700 nm. This test is commonly used as a pointer for the electron-donating process and is a vital tool for analyzing the antioxidant efficacy of plant extracts [19]. Several investigations have confirmed that the reducing power activity of certain plant extracts, and antioxidant efficacy are directly correlated [21]. In our results, high reducing activity was observed in the order of SCLAE >SCLAE>SCLAA>SCBAE>SCBAA, which is in direct correlation with the DPPH scavenging assay. Recent findings indicate that the extract can provide electrons to reactive free radicals [22]. Consequently, this process transforms them into more

stable and non-reactive materials, effectively stopping the continuous chain reaction of free radicals [23].

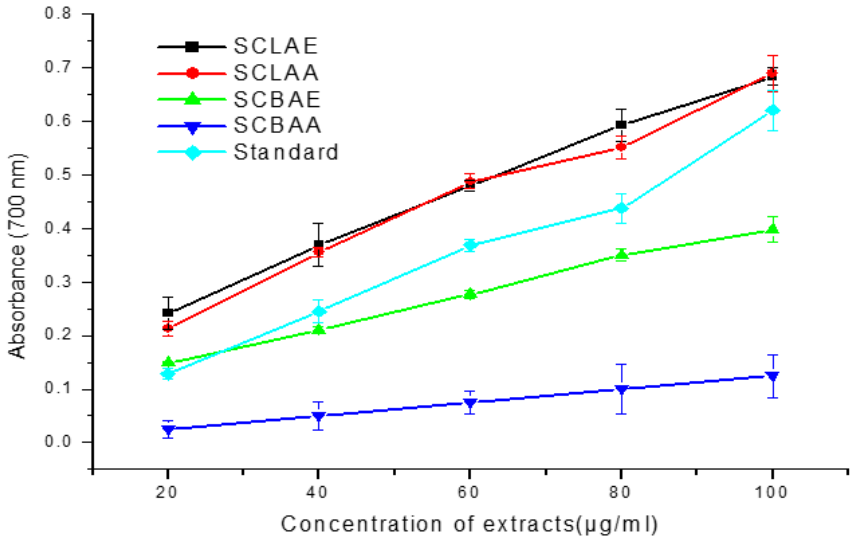

Figure 4: Reducing the power efficiency of *S. cumini* leaf and bark extracts

Lipid peroxidation activity of SCL and SCB extracts:

Figure 5 shows the peroxidation potentials of the 0.5 mg extracts in the linoleic acid emulsion system. When compared to the reference standard (α-tocopherol), the extracts showed a significant inhibitory capacity. The peroxidation inhibition percentage was recorded as 26.58, 25.07, 24.84, 24.01, and 33.86 for SCLAE, SCLAA, SCBAE, SCBAA, and standard, respectively. In comparison with extracts, the lipid peroxidation efficacy is as follows: SCLAE>SCLAA>SCBAE>SCBAA, which are in agreement with the outcome of reducing power and DPPH assays. The peroxidation assay operates by producing peroxyl radicals through the oxidation of linoleic acid, which occurs because of their thermal and catalytic degradation. The level of scavenging indicates, the antioxidant compounds present in the sample. Peroxide radicals that are not scavenged by this process are oxidized from ferrous to ferric, forming an ammonium thiocyanate complex [24].

The complexity of the extract composition represents the extracts' prevention of linoleic acid oxidation, specifically the differences between the aqueous and hydrophobic nature. In addition, a possible interaction between the emulsion and the extract exists, namely at the interfaces between oil and water, and between lipid and air [25]. The extracts' significant antioxidant potential is connected with DPPH, reducing power, and lipid peroxidation assay. The antioxidant effectiveness of the current study aligns with the findings of earlier research by Ruan et al. [5]. The substantial concentration of phenolic compounds in the extracts may cause the antioxidant activity shown in this investigation [12]. The presence of several phenolic components in *S. cumini* extracts may be responsible for their antioxidant ability, including rutin, quercetin, gallic acid, catechins, narigin, caffeic acid, delphinidin, and chlorogenic acid [26].

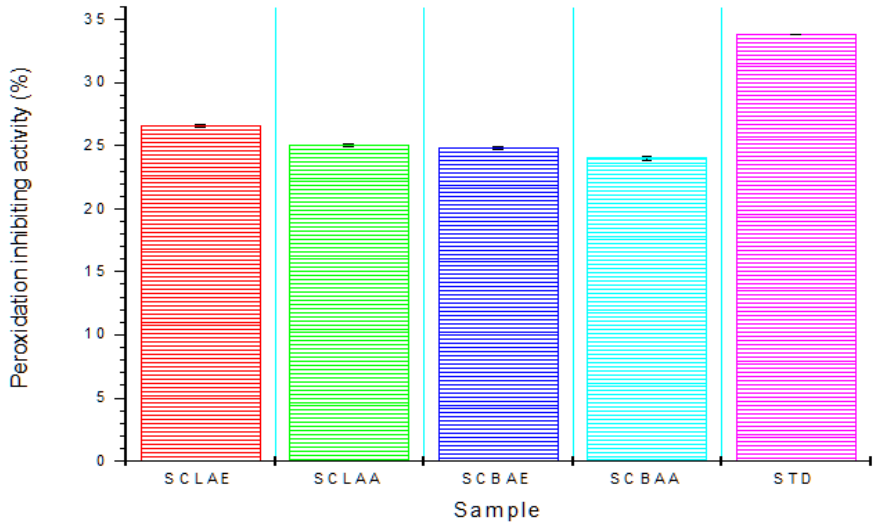

Figure 5: Peroxidation inhibiting activity of *S. cumini* leaf and bark extracts

CONCLUSION

Using two different solvent systems, the phenolic content and antioxidant activity of extracts from the leaves and bark of *S. cumini* were studied. The results indicate that the tested extracts demonstrate antioxidant properties, as evidenced by the DPPH, reducing power, and peroxidation assays. The extract contents the total phenol and the antioxidant efficacy of the extracts were to be observed as high in the order of SCLAE>SCLAA>SCBAE>SCBAA. Results for antioxidants and total phenolics showed a strong association. By calculating the percentage of dry matter in the plant sample, the extracted product's quantity was established, which might be due to the fact that the antioxidant potentials of the extract are linked with their phenol contents. The experimental work confirms that the leaf of *S. cumini* has more potential antioxidant contents than the bark of *S. cumini.* In addition, the aqueous-ethanol solvent system has more extraction efficiency than the aqueous acetone solvent system. The present work recommends further investigation involving these extracts and *S. cumini* phytoconstituents as a valuable source of alternative antioxidants for use in nutraceutical and pharmaceutical applications.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Funding

The authors have no relevant financial or non-financial interests to disclose.

Authors' contributions

J. Manjunathan: Conceptualization, Data curation, Formal analysis; Investigation, Methodology, Validation, Supervision, Visualization, Writing - original draft, and Writing - review & editing.

P K Ananthakumar: Conceptualization, Data curation, Formal analysis; Investigation, Methodology, Validation, Supervision, Visualization, Writing - original draft, and Writing - review & editing.

S. Shyamala Gowri: Conceptualization, Data curation, Formal analysis; Investigation, Methodology, Validation, Supervision, Visualization, Writing - original draft, and Writing - review & editing.

M. K. S. Pavithra: Conceptualization, Data curation, Formal analysis; Investigation, Methodology, Validation, Supervision, Visualization, Writing - original draft, and Writing - review & editing.

Sowmya Hari: Conceptualization, Data curation, Formal analysis; Investigation, Methodology, Validation, Supervision, Visualization, Writing - original draft, and Writing - review & editing.

Mukesh Kumar Dharmalingam Jothinathan: Conceptualization, Data curation, Formal analysis; Investigation, Methodology, Validation, Supervision, Visualization, Writing - original draft, and Writing - review & editing.

Archana Behera: Resources, Validation, Visualization, Writing – review & editing.

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