

Research article

Evaluation of *Semecarpus kurzii* Engler from Bay Islands for oxidative DNA damage protective activity and *in vitro* antioxidant potentialSonali Das¹, Raghavendra L.S. Hallur¹, Asit B. Mandal²¹Centre for Biotechnology, Pravara Institute of Medical Sciences-DU, Loni (Bk), 413736, Taluk Rahata, District Ahmednagar, Maharashtra, India²Division of Crop Improvement, Central Research Institute for Jute & Allied Fibres (ICAR), Barrackpore, Kolkata, 700 120, West Bengal, India

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ABSTRACT

Introduction and Aim: Harnessing traditional knowledge of medicinal plants is important for the betterment of mankind. We must safeguard traditional knowledge from misuse by miscreants living in modern societies and knowledge must be available in the public domain for use in drug designing in the healthcare system. Since the beginning of recorded history, Indians have used plants as a source of medicine, and their cultural past is incredibly rich. This study was conducted to evaluate the oxidative stress-induced DNA damage prevention and antioxidant potential of *Semecarpus kurzii* found in the Bay Islands.

Materials and Methods: The scavenging of superoxide, hydroxyl radicals, nitric oxide (NO), 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) radicals, ferric reducing power (FRAP), and lipid peroxidation inhibition activity were tested to determine antioxidant activity. DNA damage inhibition test was used to evaluate the protection against oxidative DNA damage.

Results: The *S. kurzii* extract showed dose-dependent scavenging of DPPH, superoxide anion, nitric oxide, hydroxyl radical and reducing power evaluated by comparing with standard antioxidants (ascorbic acid, α -tocopherol and BHT). However, the nitric oxide scavenging ($IC_{50} = 186.47 \mu\text{g/mL}$) and superoxide scavenging ($IC_{50} = 678.32 \mu\text{g/mL}$) was more than that of DPPH ($IC_{50} = 28.03 \mu\text{g/mL}$) and Hydroxyl radical ($IC_{50} = 89.10 \mu\text{g/mL}$) scavenging capacity. In addition, *S. kurzii* extract and ascorbic acid showed lipid peroxidation inhibition activity. The extract also exhibited DNA protection which was confirmed by the DNA damage inhibition assay.

Conclusion: Our results corroborated that, the methanolic extract of *S. kurzii* bark has substantial antioxidant activity and DNA damage inhibition. The potential antioxidant and oxidative DNA damage preventive activity could be due to the existence of polyphenolic compounds in the *S. kurzii* bark.

Keywords: Bay Islands; antioxidant; DNA damage assay; ethnomedicine; *Semecarpus kurzii*.

INTRODUCTION

Andaman and Nicobar Islands is a group of islands popularly known as Bay Islands with 2500 angiosperm species distributed across the island territory among which 45 species are traditionally used as medicine by tribals and aboriginals (Great Andamanese, Onges, Shompens and Nicobarese) (1). About 223 species of medicinal plants are endemic to these islands, which are found to be more effective for diseases like the common cold to some life-threatening diseases (2, 3). Several ethnobotanical studies have been carried out on the Great Andamanese, Onges, and Jarawas tribes but detailed information is not available on the folklore medicines of these *Negrito* tribes (4,5).

Plants are potent biochemical reactors that have proved to be a good source of phytochemicals with potent medicinal properties since time immemorial. All plant parts, including the leaves, bark, roots, flowers, fruits, and seeds, contain natural antioxidants that guard the human body against many diseases (6, 7). The combined actions of secondary

metabolites in a specific plant that is distinct in a taxonomic sense determine the synergistic effects of medicinal plants, which are unique to plant species or groupings (8). For the prevention and treatment of complicated illnesses including cancer, Alzheimer's disease, atherosclerosis, stroke, and diabetes, antioxidant-based drugs and formulations have been created in the last three decades (9,10). By forming covalent bonds with enzymes, superoxide anions (O_2^-), nitric oxide (NO), and hydroxyl radicals ($OH^\cdot$) render them inactive (11). The oxidative stress is caused due to the increased production of toxic oxygen derivatives. Plants and other organisms can synthesize a variety of antioxidant molecules thereby evolving a wide range of mechanisms to avert different disease conditions. In the modern food industry, synthetic antioxidants are commonly used, and it has been suggested that their indirect inclusion in the human diet may have cancer-causing consequences (12). In addition to their ability to scavenge free radicals, natural antioxidants are preferred over synthetic antioxidants because they are safer and healthier. This has led to an increased

interest in identifying and characterising these natural compounds.

Semecarpus kurzii Engler (Family: Anacardiaceae), commonly known as *Bara Bhilawa* by local people, is widely distributed throughout the tropical rain forest of Andamans, especially in the Koriaghat area of South Andaman. Local tribes use the leaves of this tree as elephant fodder, while fruits eaten by Imperial pigeons are reported to be antihelminthic and used in treating wounds. The leaves are also used for treating malarial fever and as an anthelmintic agent. The resin of this tree is used for curing skin allergic infections. Interestingly it was reported that eruptions and blisters developed upon touching the tree or by just passing nearby (13) in some people having very sensitive skin. Medicinal plant species with prophylactic and therapeutic properties are abundant in these remote islands, which are cut off from mainland India by a distance of about 1200 km. Even today, rural folks and tribals in India largely depend upon the surrounding medicinal plants and forests for their daily needs.

There is a lurking threat to the medicinal plant population in India due to ever-increasing demand, ruthless over-exploitation by the manufacturing sector and destructive and unsustainable harvesting practices. The medicinal plants in the Bay Islands are no exception, facing multifaceted problems ranging from the immediate threat of extinction to the impending danger of biopiracy and related IPR issues. The population density of humans in these islands has already exceeded its carrying capacity. Furthermore, the increased human intrusion into the natural habitats, together with destructive traditional harvesting and pilfering by the tourists have taken a toll leading to the extinction of many valuable species including medicinal plants and such losses “are forever” and beyond resurrection. Besides, the traditional knowledge gleaned over centuries by the total Baidya/Hakims, etc. is fast draining away due to the overall development of the islands. At this crucial juncture, it is essential to revisit the medicinal plants of Bays Islands through a detailed survey to catalog the medicinal plants used by the people residing in this union territory over many years. The purpose of the current study was to evaluate *S. kurzii*, an Andaman and Nicobar Islands traditional ethnomedicine, for its *in vitro* antioxidant activity while taking all of the aforementioned factors into consideration.

MATERIALS AND METHODS

Plant material collection

The bark of *Semecarpus kurzii* was obtained from several tropical rainforest areas in the Andaman Islands. The *S. kurzii* was identified by the Botanical Survey of India, Andaman and Nicobar Circle, Port Blair, Andaman and Nicobar Islands.

Extraction procedure

The powdered *S. kurzii* bark (50 g) was extracted for 72 h with methanol in a Soxhlet extractor. Whatman filter paper No. 1 was used to filter the extract. A vacuum rotary evaporator (40–45°C) was used to evaporate the filtrate. For further research, a yield of 7.8 0.2% (w/w) was collected and stored in a desiccator (14).

Total phenol content (TPC)

The TPC in the methanolic extract of *S. kurzii* bark was detected using Folin-Ciocalteu's reagent (15). In a nutshell, the reaction mixture was combined with 1 ml of Folin-Ciocalteu's reagent and 1 mg of extract that had been dissolved in methanol. 3 ml of Na₂CO₃ (2%) was added to the reaction mixture after three minutes. The reaction mixture was continuously shaken for two hours at room temperature before the absorbance at 760 nm was spectrophotometrically determined. The outcomes were shown as the equivalent of pyrocatechol (µg) using a typical pyrocatechol graph.

Total flavonoid content (TFC)

The TFC of the methanolic *S. kurzii* bark extract was calculated using aluminium chloride (16). 20 µg/mL of *S. kurzii* bark extract was combined with 1.5 ml of methanol, followed by the addition of 5% sodium nitrate (75 µL), 10% aluminium chloride (150 µL), 1 M sodium hydroxide (0.5 ml), and double-distilled water (275 µL). The reaction mixture was allowed to sit at room temperature (RT) for 30 minutes. At 510 nm, the absorbance of quercetin was measured using it as a reference. Using the quercetin standard curve, the result was expressed as the amount of quercetin in mg/g of the extract.

Antioxidant activity of *S. kurzii* bark

The ability of the methanol extract of *S. kurzii* bark to neutralise free radicals was investigated using different *in vitro* assay models, including the phosphomolybdate assay, DPPH assay, reductive ability, nitric oxide assay, hydroxyl radical assay, superoxide radical assay, and inhibition of lipid peroxidation (17-20).

Phosphomolybdate assay

To evaluate the overall antioxidant potential of the *S. kurzii* bark methanolic extract, phosphomolybdenum test (21) was performed. The methanolic extract of *S. kurzii* bark (0.1 mL) was agitated with 1 mL of reagent solution containing 0.6 M sulfuric acid, 28 M sodium phosphate, and 4 M ammonium molybdate. The tubes were incubated in a 95 °C water bath for 90 minutes before being cooled to room temperature. The absorbance was read spectrophotometrically at 695 nm. Ascorbic acid equivalents (µg/mL of AAE) were used as a standard unit of measurement to represent the antioxidant capacity of the methanolic

extract of *S. kurzii* bark.

DPPH assay

The DPPH radical scavenging ability of the methanolic extract of *S. kurzii* bark was assessed, as was previously described by Cotellet et al. (22). A reaction mixture of 3.0 ml was incubated for 30 min at 37 °C and contained 2.8 mL of different *S. kurzii* bark extract strengths in methanol and 0.2 mL of DPPH (100 M in methanol). The absorbance was measured at 517 nm. The % inhibition was calculated using the following calculation:

$$\text{DPPH scavenging activity (\%)} = \frac{(A_c - A_s)}{A_c} \times 100$$

where A_c and A_s represent the sample's and the control's respective absorbances.

Reductive ability

Using the method outlined by Maji et al., (18), the reductive ability of the methanolic extract of *S. kurzii* bark was assessed. The various doses of *S. kurzii* bark extract were combined with 2.5 mL of phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The reaction mixture was heated to 50 °C and incubated for 20 min. 2.5 mL of 10% trichloroacetic acid was added to the reaction mixture, and it was then centrifuged at 3000 rpm for 10 min. Water (2.5 mL), 0.1% FeCl_3 , and 0.5 mL of supernatant were added to the 2.5 mL of supernatant. The reaction mixture was spectrophotometrically measured at 700 nm. A strong reductive potential of the extract is shown by the reaction mixture's increased absorbance. The % inhibition was calculated using the following calculation:

$$\text{Reductive ability (\%)} = \frac{(A_c - A_s)}{A_c} \times 100$$

where A_c and A_s represent the sample's and the control's respective absorbances.

Nitric oxide assay

There was NO radical scavenging activity tested on the *S. kurzii* bark methanolic extract (18). In a nutshell, several amounts of extract were combined with 1 mL of 10 mM sodium nitroprusside in a phosphate buffer (pH 7.4). The reaction mixture was heated to 25 °C and kept there for 150 minutes. The extract combination was then added to an equivalent amount of Griess' reagent, which comprised naphthalene diamine dihydrochloride (0.1%), sulphanilamide (1%), and o-phosphoric acid (2%). At 546 nm, absorbance was detected. The % inhibition was calculated using the following calculation:

$$\text{Nitric Oxide Scavenging activity (\%)} = \frac{(A_c - A_s)}{A_c} \times 100$$

where A_c and A_s represent the sample's and the control's respective absorbances.

Hydroxyl radical scavenging activity

By examining the antagonistic relationship between deoxyribose and *S. kurzii* bark for the hydroxyl radicals produced by Fenton's reaction, Maji et al., (18) approach was used to assess the ability of the *S. kurzii* bark extract to scavenge hydroxyl radicals. In a nutshell, 1.0 ml of a reaction mixture including 500 μL of various extract solutions and 100 μL of 28 mM 2-Deoxy-D-ribose, 1.04 mM EDTA, 0.2 mM FeCl_3 , and 1.0 mM ascorbic acid were incubated for one hour at 37 °C. Thiobarbituric acid reactive (TBARS) measurements were used to colorimetrically assess the harm that free radicals caused to deoxyribose. The % inhibition was calculated using the following calculation:

$$\text{Hydroxyl radical scavenging activity (\%)} = \frac{(A_c - A_s)}{A_c} \times 100$$

where A_c and A_s represent the sample's and the control's respective absorbances.

Superoxide radical scavenging assay

The procedure indicated by Nishimiki et al., (20) was followed in order to test the methanolic extract of the bark of *S. kurzii* for its ability to scavenge superoxide anions. In a phosphate buffer with a pH of 7.4, 100 mM, all the solutions needed for this experiment were made. A mixture of the extracts at various concentrations was made using 1.0 mL each of 156 μM NBT and 468 μM NADH. 100 mM phosphate buffer (pH 7.4) was used to increase the reaction mixture's final volume to 5.0 mL. A 100 μL solution of PMS at a concentration of 60 μM was added to start the reaction, which was then left to sit at 25 °C for five minutes. It was 560 nm where the absorbance was measured. As a rule, the BHT was applied. To get the percentage inhibition, the following calculation was used:

$$\text{Superoxide radical scavenging activity (\%)} = \frac{(A_c - A_s)}{A_c} \times 100$$

where A_c and A_s represent the sample's and the control's respective absorbances.

Inhibition of lipid peroxidation

By using the TBARS technique (21) and rat liver homogenate as the source of polyunsaturated fatty acids, it was possible to demonstrate that the methanolic extract of *S. kurzii* bark inhibited lipid peroxidation. 40 mM Tris-HCl buffer (pH 7.2) was used to homogenise the liver. For ten minutes at a speed of 3000 rpm, the liver homogenate was centrifuged. KCl (0.15 M), FeSO_4 (15 mM), and ascorbic acid (6 mM) were added to the reaction mixture (4 mL), which was then incubated at 37 °C for one hour. The reaction mixture also contained 0.5 ml of clear supernatant, various plant extract concentrations, and 100 μL of each. Centrifuging at 3000 rpm for 20 min. at 4 °C after adding 10% TCA (1.0 mL) to the mixture eliminated the insoluble

proteins. Finally, the supernatant (2.0 mL) was removed, and this fraction was treated with 0.8% trichlorobutyric acid (TBA) in 1 mL. A water bath was used to heat the entire mixture to 90 °C for 20 minutes, after which it was cooled to room temperature. It took 2.0 mL of butanol to extract the TBA-MDA complex. 532 nm was used to measure the reaction mixture's absorbance. To get the percentage inhibition, the following calculation was used:

$$\text{Lipid Peroxidation inhibition activity (\%)} = \frac{(A_c - A_s)}{A_c} \times 100$$

where A_c and A_s represent the sample's and the control's respective absorbances.

DNA damage activity

The ability of *S. kurzii* bark extract at various doses to protect the pUC19 plasmid DNA from hydroxyl radicals produced by Fenton's reagent (23). Plasmid DNA (3 mL), 10 µL of Fenton's reagent (100 mM H_2O_2 , 1 mM Ascorbic acid, and 1 mM $FeCl_3$), and various doses of *S. kurzii* bark extract were added to the reaction mixture. Using double-distilled water, the mixture's final volume was prepared to 20 µL,

and it was then incubated at 37°C for 30 min. After the incubation period, 0.25 percent of bromophenol blue dye (in 50% glycerol) was added. Applied on 0.8% agarose gel were the 20 µL reaction solutions. The electrophoresis was run for 1 hour at 90V. Ethidium bromide was used for staining. The pUC19 was visualized and quantified. Fenton's reagent was usually used to oxidize organic contaminants, and, in this assay, it was used to destroy the DNA.

Statistical study

The experiments were run in triplicates, and the results are shown as mean standard deviation (SD). Using Microsoft Excel's linear regression analysis, the IC_{50} values were calculated.

RESULTS

TPC and TFC

The TPC and TFC of the methanolic *S. kurzii* bark extract are shown in Table 1. TPC in *S. kurzii* bark extract was 190.4 µg pyrocatechol equivalents. The TFC in the *S. kurzii* bark extract was found to be 65 µg quercetin equivalents (Fig. 1).

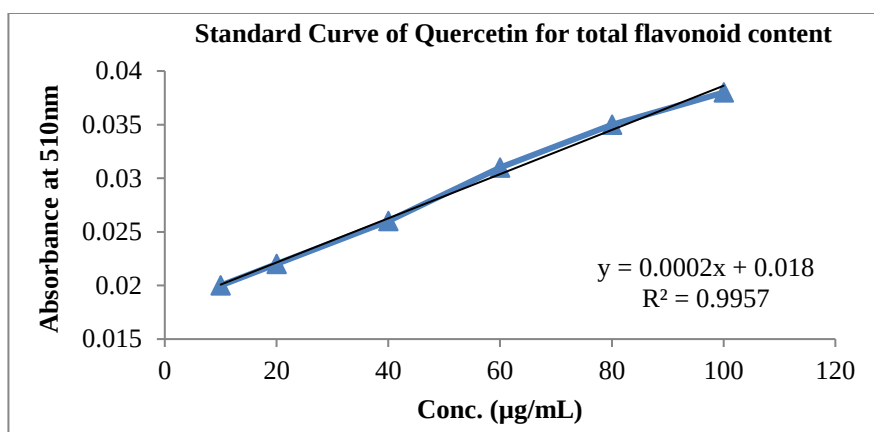


Fig. 1: Quercetin standard graph for total flavonoid content

Total antioxidant capacity

The overall antioxidant strength of the methanolic *S. kurzii* bark extract is shown in Table 1. The quantity

was found to be 201 µg ascorbic acid/g extract. Fig. 2 displayed the conventional graph for ascorbic acid.

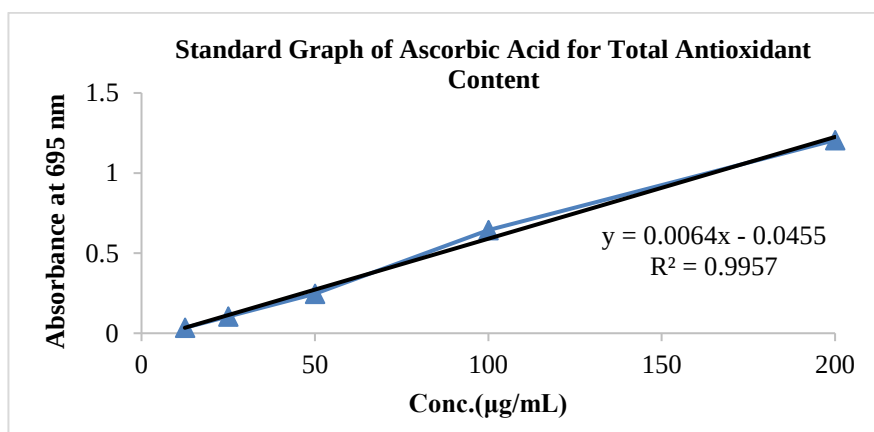


Fig. 2: Ascorbic acid standard graph for total antioxidant activity

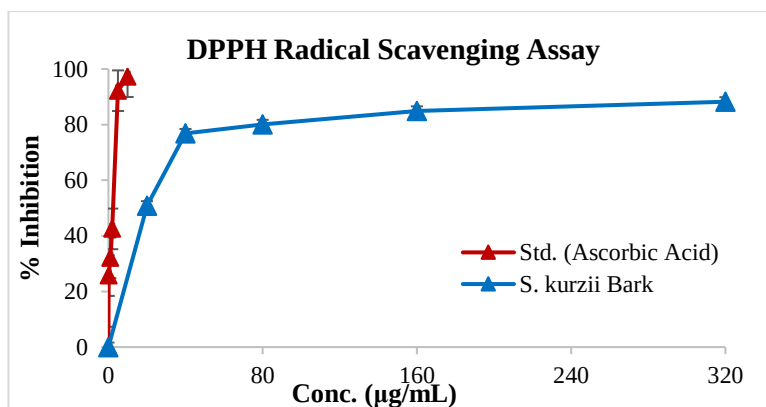
Table 1: The TPC and TFC and antioxidant contents of methanolic extracts of *S. kurzii* bark

Sample	TPC (μg Pyrocatechol/g)	TFC (μg Quercetin/g)	Total Antioxidant (μg Ascorbic Acid/g)
<i>S. kurzii</i> (bark)	190.4	65	201.0

DPPH assay

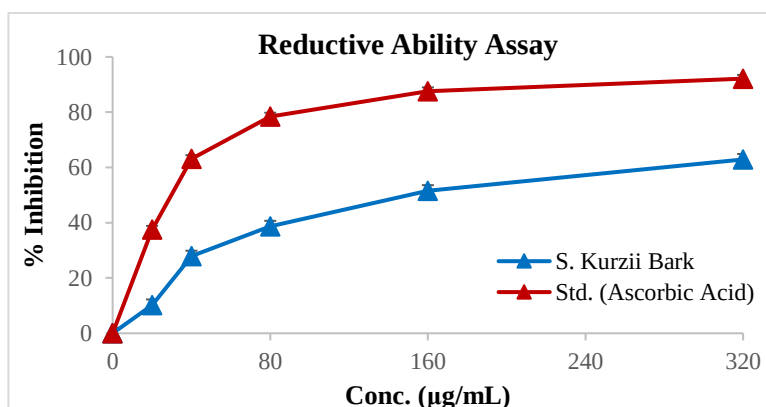
Standard vitamin C and a methanolic extract of *S. kurzii* bark (20–320 $\mu\text{g/mL}$) are both shown to have DPPH free radical scavenging properties (Fig. 3). The amount of plant extract needed to scavenge 50%

of the DPPH free radical (IC_{50} value) was discovered to be 28.03 $\mu\text{g/mL}$. The maximum inhibition (%) of DPPH was 88.23% at 320 $\mu\text{g/mL}$ concentration and was compared against vitamin C which showed 97.23% inhibition of the DPPH radical at $\mu\text{g/mL}$.

**Fig. 3:** DPPH radical scavenging activity of *S. kurzii* bark extract**Reductive ability**

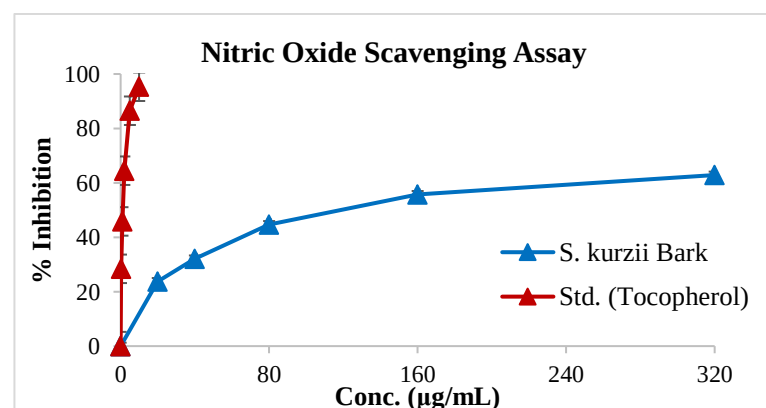
The reductive ability methanolic extract of *S. kurzii* bark (20–320 $\mu\text{g/mL}$) and the standard ascorbic acid are shown in Fig. 4. The bark extract was shown to

have noticeably good reduction power. In the existence of the plant extract Fe^{3+} was transformed to Fe^{2+} . The methanolic extract was shown to have a 62.90% inhibitory percentage at 320 $\mu\text{g/mL}$.

**Fig. 4:** Reductive ability of *S. kurzii* bark extract**Nitric oxide assay**

S. kurzii bark methanolic extract demonstrated considerable nitric oxide scavenging action, with the

greatest percentage of nitric oxide radical inhibition being 62.87% (Fig. 5). The results were compared against the standard α -tocopherol.

**Fig. 5:** Nitric oxide scavenging activity of *S. kurzii* bark extract

Hydroxyl radical scavenging activity

The methanolic extract of *S. kurzii* bark was tested for its capacity to scavenge hydroxyl radicals ($\bullet\text{OH}$) using the deoxyribose technique. An inhibition of

70.27% was discovered at 320 $\mu\text{g/mL}$ extract. The resultant figure, Fig. 6, shows the IC_{50} value to be 89.10 $\mu\text{g/mL}$.

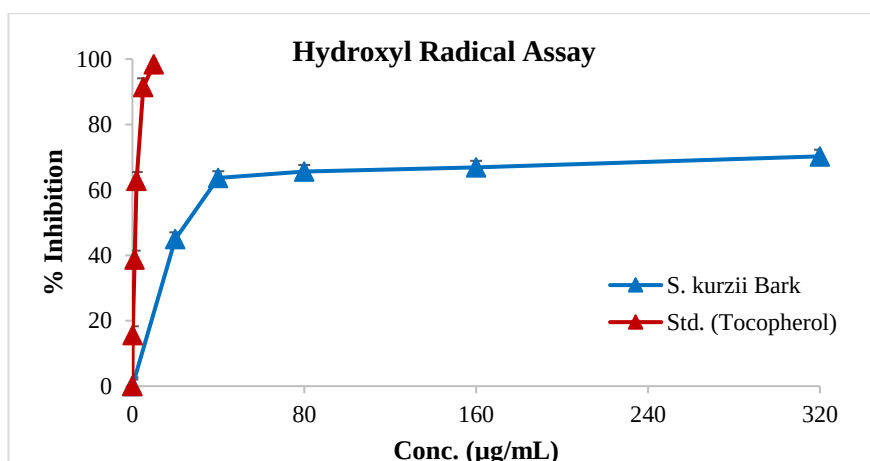


Fig. 6: Hydroxyl radical scavenging activity of *S. kurzii* bark extract

Superoxide radical scavenging assay

The decline in absorbance at 560 nm with the methanolic extract of *S. kurzii* bark and the standard compound (BHT) indicates the quenching ability of

superoxide radicals. At 20–320 $\mu\text{g/mL}$, the % inhibition of *S. kurzii* methanolic extract was 23.82%, while standard BHT was 89.26%. The IC_{50} for *S. kurzii* extract was found to be 678.32 $\mu\text{g/mL}$ (Fig. 7).

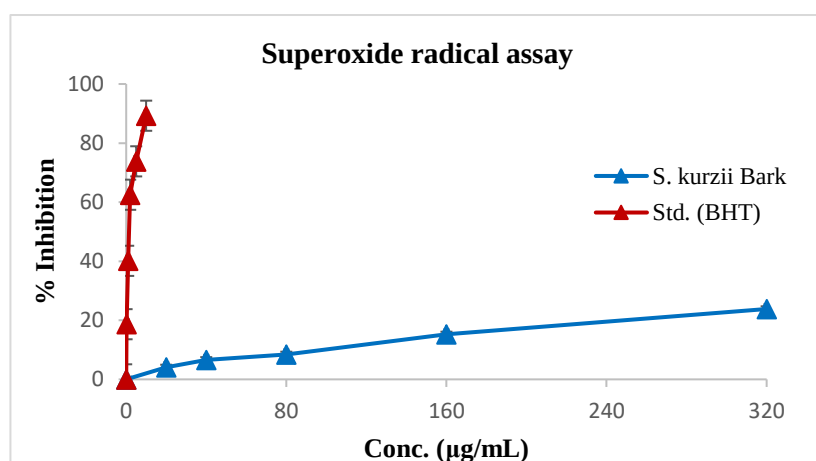


Fig. 7: Superoxide radical scavenging activity of *S. kurzii* bark extract

Inhibition of lipid peroxidation

Fig. 8 illustrates the impact of plant extract and standard ascorbic acid on the inhibition of lipid peroxidation. The production of lipid peroxides by

Fe^{2+} - ascorbate in rat liver homogenate appears to be reduced (64.28% at 320 $\mu\text{g/mL}$ of extract) by *S. kurzii* bark extract.

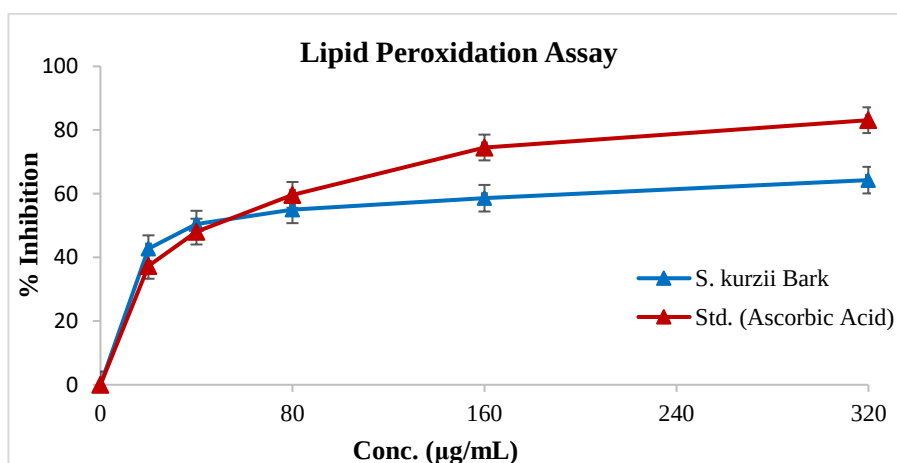


Fig. 8: Lipid peroxidation inhibitory activity of *S. kurzii* bark extract

DNA damage protection

DNA nicking caused by Fe^{3+} -dependent hydroxyl radicals is decreased by the effect of *S. kurzii* bark metabolic extract. The objective of this test is to evaluate if *S. kurzii* bark extract can protect the pUC19 plasmid DNA from hydroxyl radical-related oxidative damage. Figure 9 demonstrates how the *S. kurzii* extracts can lessen Fe^{3+} -dependent plasmid DNA nicking. Fenton's reagent was employed to dissolve the pUC DNA, and the process resulted in the creation of supercoiled (Form I- SC) and open circular form DNA (Form II- OC).

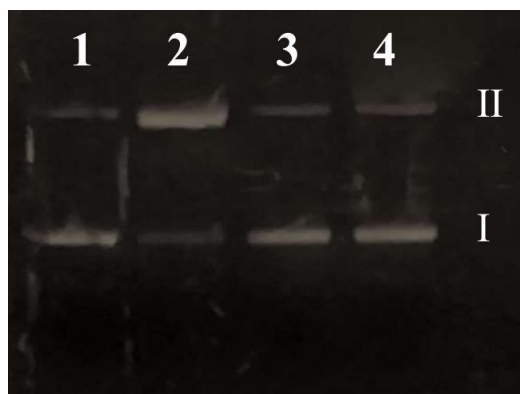


Fig. 9: Gel electrophoresis showing the cleavage of SC pUC19 DNA with methanolic extracts, incubated at 37 °C, using 0.8% agarose gel, at 90 mV for 1 h.

Legends: Lane 1: DNA control; Lane 2: DNA + Fenton's reagent; Lane 3: DNA + SKB-50 µg/mL + Fenton's reagent; Lane 4: DNA SKB- 500 µg/mL + Fenton's reagent; (Form II- OC) and supercoiled (Form I- SC).

DISCUSSION

Several earlier studies revealed that medicinal plants are very good sources of antioxidants that play a crucial part in the treatment of a number of ailments that reduce oxidative stress and therefore prevent or down-regulate degenerative diseases (24). This study examined the methanolic *S. kurzii* bark extract for its *in vitro* antioxidant properties. The methanolic extract from the bark of *S. kurzii* was shown to have antioxidant properties equivalent to those of conventional antioxidant compounds and to scavenge free radicals in a dose-dependent manner. Flavonoids have been found to exhibit high antioxidant activity through scavenging or chelating actions (24). Furthermore, phenolic compounds act as free radical terminators and represent a class of antioxidant agents. The phenol contents of the *S. kurzii* bark represented as quercetin equivalent ($y = 0.0050x + 0.0112$, $r^2 = 0.876$) were between 18.12 ± 0.15 and 75.3 ± 3.2 .

The antioxidant activity of the *S. kurzii* bark extract was evaluated using the DPPH, nitric oxide scavenging, superoxide scavenging, and lipid peroxidation comparison tests, along with standards such as ascorbic acid, α -tocopherol, and BHT. The

plant extract demonstrated dose-dependent scavenging activity in all assays and was compared against respective standards.

Because the extract (20–320 µg/mL) and standard can scavenge free radicals, there was a decrease in the amount of DPPH radicals as the concentration of the extract increased. The nuts of *Semecarpus anacardium* were the subject of a comparable investigation on antioxidant activity (25). Increased absorbance with increasing concentration was seen in the reducing power of *S. kurzii* bark extract, demonstrating the extract's superiority over vitamin C in terms of reductive abilities. Nitric oxide scavenging abilities of *S. kurzii* bark extract were equivalent to those of standard.

Superoxide radicals have been demonstrated to have a significant role in the development of several illnesses. By generating highly reactive species like the hydroxyl radical (OH^+), which causes lipid peroxidation and tissue damage, superoxide anions may interact with biological macromolecules in a number of pathological processes. According to several reports, the capacity of phenols and flavonoids from plants to scavenge superoxide anion radicals makes them efficient antioxidants.

DNA damage protection activity was not reported in this plant previously. The antioxidant compounds found in the plant provide *S. kurzii* bark extract with the potential to protect against DNA damage. However, it has been claimed that the presence of a plant extract with strong antioxidant activity (DPPH free radical scavenging activity, FRAP, and reducing power potential) also indicates the presence of a plant extract with strong DNA protection activity. The concept that phenols and flavonoids may be the main contribution to the antioxidant activity of this plant is supported by the substantial association between TPC and TFC and hydrogen peroxide radical scavenging capability.

The *K. paniculata*, *S. trifoliatius*, *A. scholaris* and *F. benjamina* plant extracts showed a protective ability against OH radicals (26) and our results are in line with these results. Naturally, the human body by producing antioxidants (catalase, superoxide dismutase, and glutathione peroxidase) will neutralize oxidative stress-induced damage and protect from many diseases (6-12). Numerous research has documented the quest for plant-based antioxidant chemicals, and as a result, herbal medications have developed in complementary and alternative folkloric medicine.

CONCLUSION

According to the findings, the *S. kurzii* bark's methanolic extract had a potent antioxidant effect by scavenging free radicals, which may be connected to the TPC and TFC. The levels of TPC and TFC show a substantial association with antioxidant activity. It

can be concluded that *S. kurzii* bark may serve as a natural antioxidant. Further phytochemical characterization is required to isolate potent natural antioxidants, and further efforts should be directed to carry out *in vivo* studies.

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CONFLICT OF INTEREST

The authors have declared that no conflicts of interest exist.

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