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Antioxidant activity of *Annona selegalensis* - A medicinal plant collected from the Kingdom of Eswatini

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ABSTRACT

A. senegalensis (Annonaceae) is a medicinal plant and it finds widespread applications in the ethnomedicine in Africa**.** The objectives of the present study were to evaluate the antioxidant activity, determine the IC_{50} values, determine total phenolic contents (TPCs), and determine total flavonoid contents (TFCs) of methanolic crude extract and fractions obtained from this crude extract of the stem-bark of *A. senegalensis* collected in the Kingdom of Eswatini. The methanloic crude extract was obtained by a combining maceration and hot solvent extraction techniques. n-Hexane, dichloromethane, ethyl acetate, and methanol-water fractions were obtained using solvent-solvent partition technique. DPPH assay was employed to evaluate antioxidant potential. Folin-Ciocalteu and aluminium chloride colorimetric methods were employed to determine the TPCs and TFCs, respectively. At concentrations 200-3000µg/mL, the DPPH radical scavenging potential of the crude extract, the four fractions, and the positive control (ascorbic acid) was determined as 11.90±0.36-74.60±0.18%, 5.32±0.45-94.80±0.04%, and 54.81±036-89.61±0.18%, respectively. The IC_{50} values of the crude extract, the four fractions, and the positive control were determined as 945.80, 905.54, 1040.00, <200, 828.58, and <200µg/mL, respectively. Furthermore, the TPCs of the crude extract and the four fractions were determined as 144.17±7.60 and 62.55±3.84, 82.30 ±5.74, 100.30±4.50, and 157.04±1.70mg GAE/g DW, respectively. Similarly, the TFCs of the crude extract and the four fractions were determined as 130.27±7.78 and 44.09±5.14, 46.36±0.71, 115.18±5.09, and 139.54±8.69mg QE/g DW, respectively. The methanolic crude extract and its four fractions showed a moderate to significant radical scavenging activity. Notably, the ethyl acetate fraction was identified as the most potent and it showed relatively higher radical scavenging potential compared to the crude extract and the rest of the fractions. In addition, the methanolic crude extract and the four fractions possessed moderate to significant TPCs and TFCs. This study supported the traditional use of *A. senegalensis*. and further studies to develop antioxidant-based therapeutics from this plant are recommended.

Keywords: *Annona senegalensis*, Annonaceae, antioxidant activity, DPPH radical scavenging assay, IC_{50} values, total phenolic contents (TPCs), total flavonoid contents (TFCs).

1. INTRODUCTION

Annona senegalensis is a medicinal plant and it finds widespread applications in traditional medicine, especially in the African continent (Omeke et al., 2019). *A. senegalensis* belongs to the Annonaceae family of the genus Annona (Theophine et al., 2012; Koto-te-Nyiwa et al., 2017). *A. senegalensis* is known by common names such as Wild custard apple, African custard apple, and Wild soursop**.** *A. senegalensis* is also known by several other vernacular names, which include Canelle apple, Mande sounsoun, Pobilisakpeli, Dugor, Annone, Mkongonasi, Koropetaka, Badkudga, I boagilansaani, Konokono nougni, Digor, A hmanskpela, Jorqut, Uburu ocha, Gwandar daaji, Arere, Abo, Ukpokpo, Ngonowu, Uwu, and Mkonokono (Adzu et al., 2005; Barros et al., 2016; Mindiediba et al., 2024). *A. senegalensis* grows to 2-6 meters in height as a shrub, but under favorable conditions, it can grow as a small tree to a height of 11 meters (Theophine et al., 2012; Samuel et al., 2016; Koto-te-Nyiwa et al., 2017; Shadman et al., 2021; Mindiediba et al., 2024). *A. senegalensis* has simple, alternate, oblong, ovate, or elliptical-shaped leaves. The leaves begin as green to bluish green in colour and have no hairs on their upper surface but have brownish hairs on their lower surface (Samuel et al., 2016; Koto-te-Nyiwa et al., 2017; Shadman et al., 2021). The barks are either silver grey or grey-brown (Samuel et al., 2016; Koto-te-Nyiwa et al., 2017; Shadman et al., 2021). The flowers of *A. senegalensis* are approximately 2.00cm in length and 3.00cm in diameter and are arising above the leaf axils as solitary or in groups of 2-4. The fragrant (aroma) flowers are often used in culinary to flavor food (Theophine et al., 2012; Samuel et al., 2016; Koto-te-Nyiwa et al., 2017; Shadman et al., 2021). The egg-shaped or ovoid-shaped fruits begin as green and turn yellow or yellowish-orange on ripening (Theophine et al., 2012; Koto-te-Nyiwa et al., 2017). The fruits have pleasant odour, they are edible, and they have jelly like flesh, which is sweet in taste (Theophine et al., 2012; Koto-te-Nyiwa et al., 2017). *A. senegalensis* is distributed in many countries in the African continent which include the Kingdom of Eswatini (formerly Swaziland), South Africa, Lesotho, Botswana, Mozambique, Zimbabwe, Zambia, Kenya, Tanzania, Democratic Republic of Congo, Guinea, Ethiopia, Sudan, Nigeria, Mali, Gambia, Cameroon Uganda,, Senegal, Ivory Coast, Sierra Leone, Burkina Faso, Congo, Angola, and Madagascar (Theophine et al., 2012; Shadman et al., 2021).

A. senegalensis finds several therapeutic applications in the traditional medicine. It has been used to treat various diseases and conditions which include sleeping sickness, joint disease, respiratory disease, fever, headaches, diarrhea, stomach aches, dysentery conjunctivitis, burns, jaundice, bleeding, seizures, muscle relaxant, sedative effects, **s**yphilis, asthenia, cancer, malarial infection, sterility, insect bites, scorpion bites and snack bites (Igwe and Onabanjo, 1989; Graham et al., 2000; Ajaiyeoba et al., 2006; Abubakar et al., 2007; Freitas et al., 2008; Okoye and Akah, 2010; Saha, 2011; Zakari et al., 2018; Ansori et al., 2021; Leite et al., 2021; Sulaiman et al., 2022; Ahirwar et al., 2023). Different parts of *A. senegalensis* collected at various locations have previously been investigated for their biological and pharmacological activities which include antimalarial, anti-diabetic, hepatoprotective, antitumor, antibacterial, antifungal, wound healing, antiulcer, anthelmintic, anti-arthritic, anti-inflammatory, analgesic, anticonvulsant, trypanocidal, anti-snake venom, anti HIV, cytotoxic, anticancer, anti-infertility, anti-platelet, anti-diarrhea, neuroprotective, hypolidemic, immunomodulatory, nephroprotective, antiulcer and antioxidant activities (Adzu et al., 2003; Atawodi at al., 2003; Ezugwu and Odoh, 2003; Odoh et al., 2004; Adzu et al., 2005; Samie et al., 2005; Apak and Olila, 2006; Ogbadoyi, et al., 2007; Freitas et al., 2008; Suleiman et al., 2008; Saha, 2011; Barros et al., 2016; Zakari et al., 2018; Ansori et al., 2021; Leite et al., 2021; Sulaiman et al., 2022; Ahirwar et al., 2023). The presence of several classes of phytochemicals such as terpenoids, flavonoids, phenolics, fatty acids, alkaloids, steroids, coumarins, anthocyanins, quinones, carotenoids, saponins, carbohydrates, tannins, acetogenins, and ent-kaurenoides has previously been reported from *A. senegalensis* (Freitas et al., 2008; Saha, 2011; Tshimenga et al., 2018; Zakari et al., 2018; Omeke et al., 2019; Ansori et al., 2021; Leite et al., 2021; Sulaiman et al., 2022; Ahirwar et al., 2023). Additionally, many pure compounds have also been reported from different parts of this plant, which include scetogenins, quercetin, α-pinene, limonene, *E*-caryophyllene, bicyclogermacrene, caryophyllene oxide, germacrene D, spathulenol, etc. (Freitas et al., 2008; Saha, 2011; Tshimenga et al., 2018; Zakari et al., 2018; Omeke et al., 2019; Ansori et al., 2021; Leite et al., 2021; Sulaiman et al., 2022; Ahirwar et al., 2023). Generally, several factors contribute towards the efficacy of the biological and pharmacological activities of any plant species under investigation, which include environmental factors such as climatic conditions, temperatures, seasonal variations, the stages of plant maturation, and the geographic location at which the plant parts are collected, and experimental conditions such as methods of extraction and plant parts used for extraction. Overall, the above mentioned factors contribute to the availability of various classes of phytochemicals, and their proportions. Furthermore, the availability of these phytochemicals and their proportions decide the potency of the biological and pharmacological activities of a particular plant. The antioxidant activity of various extracts obtained from different parts of *A. senegalensis* collected at multiple locations has previously been reported. However, our literature search revealed that the antioxidant activity of 100% methanolic extract obtained from the stem-bark of *A. senegalensis* and fractions obtained from this methanolic extract has not been reported so far. Additionally, the phytochemical

analysis, biological, and pharmacological activities of *A. senegalensis* collected from the Kingdom of Eswatini has not been investigated so far, including antioxidant activity. As discussed previously that the geographic locations at which the plants collected are play important role not only in the availability of variation in the phytochemical compositions but also their proportions. Additionally, the same factors would reflect in the efficacy of the biological and pharmacological activities of the plant. It is this research gap that prompted us to investigate the antioxidant activity of *A. senegalensis* collected from the Kingdom of Eswatini. In the current study, we set our objectives to evaluate the antioxidant activity by DPPH assay, to determine the half-minimal inhibition concentration (IC_{50}) values, to determine the total phenolic contents (TPCs), and to determine the total flavonoid contents (TFCs) of 100% methanolic crude extract and fractions obtained from this methanolic crude extract, viz. n-hexane, dichloromethane, ethyl acetate, and methanol-water fractions of the stem-bark of *A. senegalensis* collected from the Kingdom of Eswatini. The results are discussed in this article. This report is first of its kind on the antioxidant activity of the stem-bark of *A. senegalensis* collected in the Kingdom of Eswatini.

2. MATERIALS AND METHODS

Collection of plant materials

A mass of 9.75kg of fresh stem-bark of *A. senegalensis* was collected in March-April 2025 at Manzini region in the Kingdom of Eswatini. The plant material was identified by Dr. M. N. Dludlu, Department of Biological Sciences, University of Eswatini (UNESWA), Kwaluseni Campus, Kingdom of Eswatini. Voucher specimen for the stem-bark (LYH/SB/2025) was deposited at the Chemistry Research Laboratory for Postgraduate (CRL-PG) Programme.

Processing of plant materials

The plant materials were chopped into small pieces, and they were allowed for air-drying for eight weeks at CRL-PG. The air-dried plant materials were then ground into coarse powder using a laboratory based mechanical grinder (MRC Laboratory Equipment, Model KM 1500) and 6.59kg powdered stem-bark was obtained.

Preparation of plant extracts

The powdered stem-bark that obtained in the above process was macerated for 72 hours at room temperature at 28-30°C using methanol as a macerating solvent while shaking the mixture occasionally. The methanol solvent was removed from the mixture by decantation, and then filtered using a Whatman No. 1 filter paper. The solvent methanol was removed by vacuum distillation using a Buchi-Rotavapor and thus, a methanol crude extract was obtained, which was taken in a previously weighed dry and clean beaker. The maceration process was repeated for two more times, and the crude extract thus obtained was combined. The plant materials were recovered from the above maceration process and were extracted again with methanol for 8 hours, but under reflux conditions. The methanol solvent was removed from the mixture by decantation, and then filtered using a Whatman No. 1 filter paper as previously. The methanol crude extract obtained from this hot solvent extraction technique was combined with the previously obtained crude extract from the maceration technique. A total mass of 33.02g of combined methanolic crude extract was obtained from the powdered stem-bark, and this methanolic crude extract was labeled as CrE. Approximately 20.00g of this crude extract was dissolved in a solvent mixture of methanol-water (8:2, *v*/*v*). The solution of this crude extract was transferred into a separating funnel and then subjected to solvent-solvent partition using solvents of increasing polarity such as n-hexane, dichloromethane, and ethyl acetate. A volume of 250mL of n-hexane was first added as a partitioning solvent to the solution of this solvent mixture (methanol-water) and the mixture was shaken well shaken well. The mixture was allowed to undergo solvent-solvent partition, and then put aside for a while. An organic layer (upper layer, i.e. n-hexane layer) was formed and it was collected separately in a clean weighed beaker. This solvent-solvent partition with n-hexane was repeated two more times. The organic layer was collected and combined with the previously obtained organic layer. Approximately 20g of anhydrous sodium sulphate was added as a drying agent to this combined organic layer and the solution was swirled well, and then it was kept aside for few minutes. The organic layer was then filtered using a Whatman No. 1 filter paper, and it was distilled in *vacuo* using the Buchi-Rotavapor. A total mass of 7.10g of n-hexane fraction was obtained after removal of the n-hexane solvent, and it was labeled as Fr1. The aqueous layer (methanol-water layer) was recovered from the above process and then 250 mL of dichloromethane (DCM) was added to it as a partitioning solvent and then allowed to undergo solvent-solvent partition as previously. An organic layer (the lower layer, i.e. DCM layer) was formed and it was collected separately in another clean weighed beaker. The above process was repeated two more times with DCM. The organic layer was collected and combined with the previously obtained organic (DCM) layer. Approximately 20g of anhydrous sodium sulphate was added as a drying agent to this combined

organic layer and the solution was swirled well, and then it was kept aside for few minutes. The organic layer was then filtered using a Whatman No. 1 filter paper, and vacuum distilled as previously. A total mass of 6.16g of DCM fraction was obtained after removal of the DCM solvent, and it was labeled as Fr2. The aqueous layer was recovered from the above process and an additional quantity of water was added to it, and the methanol-water proportion was now kept to a ratio of 7:3 (*v*/*v*). A volume of 250 mL of ethyl acetate was added as a partitioning solvent to this methanol-water solvent mixture and then allowed to undergo solvent-solvent partition. An organic layer (the upper layer, i.e. ethyl acetate layer) was formed and it was collected separately in another clean weighed beaker. The above process was repeated two more times with ethyl acetate. The organic layer was collected and it was combined with the previously obtained organic layer. Approximately 20g of anhydrous sodium sulphate was added as a drying agent to this organic layer, and the solution was swirled and then it was kept aside for few minutes. The solution was filtered using a Whatman No. 1 filter paper, and it was vacuum distilled as previously. A total mass of 2.36g of ethyl acetate fraction was obtained after removal of solvent, and it was labeled as Fr3. The aqueous layer (methanol-water layer) recovered from the above process was distilled over the Buchi-Rotavapor. The solvent methanol was removed first followed by as much as of water and a thick liquid was obtained. This thick liquid was heated in a water bath to evaporate as much water as possible and then it was kept aside for drying at room temperature at 28-30°C. A total mass of 1.39g of methanol-water fraction was obtained after drying, and it was labeled as Fr4. The methanolic crude extract (CrE) and the four fractions, viz. Fr1, Fr2, Fr3, and Fr4 were utilized to evaluate their DPPH radical scavenging activity, and to determine the IC_{50} values, total phenolic contents (TPCs), and total flavonoid contents (TFCs).

Solvents, reagents, and chemicals

AR grade of solvents, reagents, and chemicals were used in this study. Solvents such as hexane, dichloromethane, ethyl acetate, acetone, and methanol were obtained from Promark chemicals. Chemicals and reagents such as ascorbic acid, tris-hydrochloric acid buffer, and 2,2- diphenyl-1-picrylhydrazyl were purchased from Promark chemicals. Quercetin, tannic acid, Folin-Ciocalteu reagent, and sodium carbonate were purchased from Associated Chemical Enterprises (ACE). Sodium phosphate was obtained from Glass World. Sodium nitrite was purchased from Rochelle Chemicals. Sodium hydroxide was purchased from MCB Laboratory and Medical Suppliers.

DPPH-free radical scavenging activity and determination of IC_{50} values

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical activity was performed as per procedures described in literature (Pillai et al., 2019; Mpopo et al., 2021; Pillai and Mthimkhulu, 2024; Pillai and Samkelisiwe, 2024; Pillai and Simelane, 2025). The purple colored methanolic solution of DPPH radical turns (reduces) to yellow colored DPPH-H neutral molecule, when DPPH radical receives hydrogen radical from donor compounds such as flavonoids, phenolics, etc. The DPPH radical acts as an oxidant and the plant extract acts as an antioxidant. Stock solutions of methanolic crude extract, fractions obtained from this crude extract (n-hexane, dichloromethane, ethyl acetate, and methanol-water fractions), and positive control (ascorbic acid) were prepared separately. Briefly, stock solutions of crude extract, various fractions, and ascorbic acid were prepared independently. A mass of 3.0mg of each one of the above was dissolved separately in 1.0mL of 50% methanol. Dilutions such as 3000, 2000, 1500, 1000, 800, 500, and 20µg/mL were prepared separately from each one of the above stock solutions. These dilute solutions served as antioxidants. A mass of 3.94mg of DPPH dissolved in methanol and it was made up to 100mL, and this 0.1mM DPPH solution served as an oxidant. A concentration of 50mM phosphate-buffered saline (PBS) solution was prepared, and it served as a buffer with pH 7.4. The buffer solution ensures the solubility of DPPH radicals in the reaction mixture, and it maintains a stable pH. Therefore, a maximum absorbance could be achieved (Pillai et al., 2025a; Pillai et al., 2025b; Pillai et al., 2025c). The reaction mixture consisted of 20µL of crude extract solution or solution from fraction or positive control (oxidant), 200µL of 0.1 mM DPPH solution (antioxidant), and 90µL of 50mM PBS buffer at pH 7.40. The reaction mixture was incubated in a dark-cabinet for 30 minutes. The absorbance of the reaction mixture was then measured using a UV-Vis spectrometer (Infinite M200) at 517nm. Similarly, the absorbance of the negative control was also measured. Since the negative control does not encounter any hydrogen donors, it must exhibit maximum absorbance due to the availability of a large number of DPPH radicals. In the presence of solutions of extract or fractions or positive control, the DPPH radicals are reduced to DPPH-H (neutral molecules) due to the availability of hydrogen donors from those test solutions. Therefore, lower values of absorbance are observed in the presence of those test solutions. The values of absorbance depend on the concentrations of those test solutions. In other words, a lower value of absorbance indicates a higher concentration of test solutions or positive control and *vice versa*. In other words, a lower value of absorbance indicates a higher free radical scavenging (antioxidant) activity of a solution, and

vice versa. The experiment was performed in triplicates and the average of the triplicate values were used for calculation. The percentage inhibition of radical scavenging potential was calculated using the following equation (Matamane et al., 2020; Mokoroane et al., 2020).

DPPH Radical Scavenged (%) = [(Abs. of control – Abs. of test)/Abs. of control] × 100

Abs. of test = Absorbance of extract solution or positive control.
Abs. of control = Absorbance of negative control.

The concentration (in µg/mL) of an extract or pure natural product or positive control that inhibits the formation of DPPH radical by fifty percent is called fifty percent inhibition concentration (IC_{50} value) (Pillai et al., 2018; Pillai et al., 2019) and it could be determined using a graph by plotting extract concentrations (in x-axis) *versus* the percentage inhibition of DPPH radical (in y-axis).

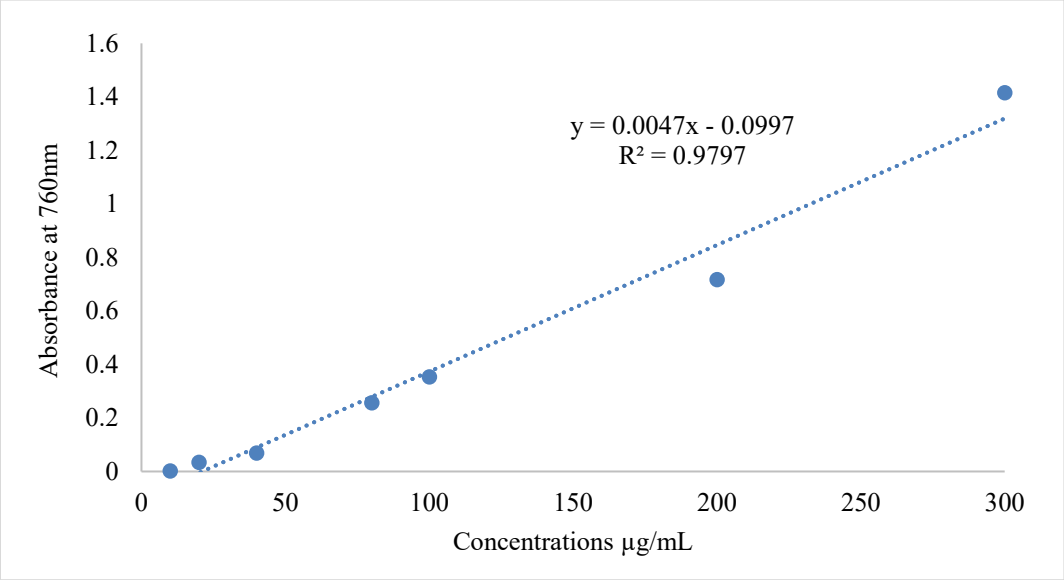

Figure 1: The calibration curve of gallic acid used to estimate the TPCs of methanolic crude extract and its four fractions obtained from the stem-bark of *A. senegalensis*.

Determination of total phenolic contents (TPCs)

The total phenolic contents (TPCs) of methanolic crude extract obtained from the stem-bark and its four fractions, viz. n-hexane, dichloromethane, ethyl acetate, and methanol-water fractions, were determined using the Folin-Ciocalteu colorimetric method as per the details given in the literature (Selepe and Pillai, 2022; Pillai et al., 2023a; Pillai et al., 2023b). A calibration curve was obtained using gallic acid as a standard. A mass of 1.0mg of gallic acid was dissolved in 1.0mL of 50% methanol *(v/v)*, and this solution served as a stock solution of standard (gallic acid). Solutions such as 10, 20, 40, 80, 100, 200, and 300µg/mL were prepared from the above stock solution and they served as test solutions of standard. Similarly, the test solutions of methanolic crude extract and its fractions were prepared separately by dissolving 1.0mg of each sample in 1.0mL of 50% methanol (*v/v*). A negative control was prepared, which consisting of only 50% methanol (*v/v*). Additionally a solution of Folin-Ciocalteu reagent and water was prepared at a ratio of 1:4 (*v/v*). A mixture of 50µL of each sample and 50µL of Folin-Ciocalteu reagent served as a test solution. The test solution was incubated for 5 minute and then a volume of 5.0mL of 7.5% sodium carbonate solution was added to this test solution. The resulting solution was then incubated for 30 minutes at 40°C in a dark cabinet. The absorbance of the resulting mixture was measured at 760nm using a UV-Vis spectrometer (Infinite M200) against the negative control. The experiments were conducted in triplicate for each one of the test solutions, and the average of the three values was used to determine the TPCs of crude extract or each fraction. The absorbance of the standard was also measured using the same procedure as detailed previously, and a calibration curve ($y = 0.0047x - 0.0997$; $R^2 = 0.9797$) was obtained by plotting various concentrations (x-axis) of standard versus their absorbance (y-axis) (Figure 1). This calibration curve

was employed to estimate the total phenolic contents (TPCs) of the methanolic crude extract and its fractions, and it was expressed in milligrams of gallic acid equivalent per gram dry weight of the extract (mg GAE/g DW).

Determination of total flavonoid contents (TFCs)

The total flavonoid contents (TFCs) of methanolic crude extract from the stem-bark and its four fractions were determined using the aluminium chloride colorimetric method as per the details given in the literature (Selepe and Pillai, 2022; Pillai et al., 2023a; Pillai and Thebe, 2023b). Quercetin served as a standard and a calibration curve for quercetin was obtained as described in the literature. A mass of 1.0mg of quercetin dissolved in 1.0mL of 50% methanol served as a stock solution. Solutions of 10, 40, 80, 100, and 200µg/mL were prepared from this stock solution. Similarly, 1.0mg of methanolic crude extract or each one of the fractions dissolved separately in 1.0mL of 50% methanol, which served as test solutions. A negative control was also prepared, which consisting of only 50% methanol (*v*/*v*). Test solution consisted of 150µL of methanolic crude extract or each one of the fractions and 150µL of sodium nitrite. The test solution was allowed undergo reaction for five minutes. A volume of 150µL of 10% aluminium chloride, 200µL of 1.0M sodium hydroxide, and 600µL of water were then added to this test solution, and it was incubated for 30 minute in a dark cabinet at room temperature. The absorbance of each one of the above reaction mixtures was then measured at 510nm using a UV-Vis spectrometer (Infinite M200) against the negative control. For each one of the test solutions, the experiment was conducted in triplicates, and the average of the three absorbance values was used for calculation. The absorbance of the standard was measured using the same procedure detailed as previously. A calibration curve ($y = 0.0011x - 0.2142$; $R^2 = 0.9570$) was obtained by plotting various concentrations of standard (x-axis0 versus their absorbance values (y-axis) (Figure 2). This calibration curve was employed to estimate the total phenolic contents (TFCs) of the methanolic crude extract and its fractions, and it was expressed in milligrams of quercetin equivalence per gram dry weight of the extract (mg QE/g DW).

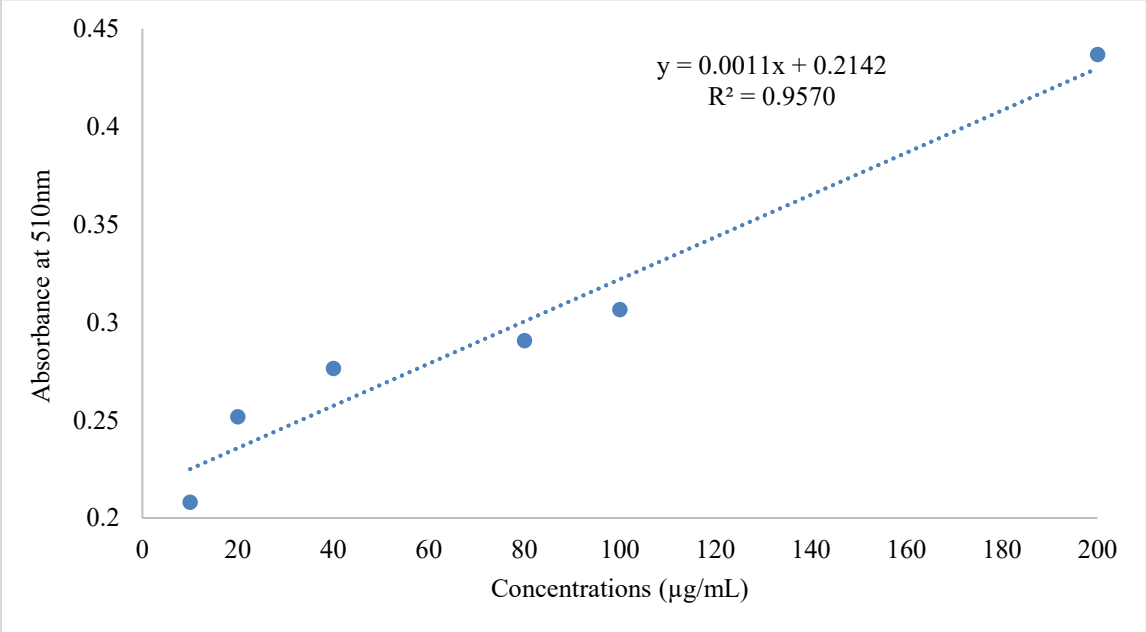

Figure 2: The calibration curve of quercetin used to estimate the TFCs of methanolic crude extract and its four fractions obtained from the stem-bark of *A. senegalensis*.

Statistical analysis

STATISCA software version 8.0 was used for statistical analysis. The difference between means was statistically significant when $p \leq 0.05$.

3. RESULTS AND DISCUSSION

A methanolic crude extract was obtained from the stem-bark of *A. senegalensis*, and this crude extract was labeled as CrE. Fractions such as n-hexane, dichloromethane, ethyl acetate, and methanol-water fractions were obtained from this methanolic crude extract and these

fractions were labeled as Fr1, Fr2, Fr3, and Fr4, respectively. The radical scavenging potential CrE and F1-F4 was evaluated at concentrations of 200, 500, 800, 1000, 1500, 2000, and 3000µg/mL using the DPPH radical scavenging assay. Ascorbic acid served as positive control. The radical scavenging potential of positive control was also evaluated at the same concentrations of 200, 500, 800, 1000, 1500, 2000, and 3000µg/mL. The results of the radical scavenging potential of CrE and F1-F4 are summarized in Table 1. The radical scavenging potential of CrE and Fr1-Fr4 showed a linear relationship with concentrations. In other words, the percentage of radical scavenging potential of CrE and Fr1-Fr4 increased with increasing in concentrations. At a concentration of 3000µg/mL, CrE (crude extract) showed scavenging potential of 74.60±0.18%. At a concentration of 3000µg/mL, Fr1-Fr4 showed scavenging potential of 87.05±0.18, 76.90±0.36, 94.80±0.04, and 85.97±0.10%, respectively. The positive control exhibited a scavenging potential of 89.61±0.18% at the same concentration of 3000µg/mL (Table 1). Therefore, at a concentration of 3000µg/mL, Fr3 (ethyl acetate fraction) showed the highest scavenging potential (94.80±0.04%) followed by Fr1, Fr4, Fr2, and CrE. The scavenging potential of Fr1 (n-hexane fraction) and Fr4 (methanol-water fraction) were comparable to each other and their scavenging potential were 87.05±0.18 and 85.97±0.10%, respectively at a concentration of 3000µg/mL. The radical scavenging potential of CrE (crude extract) and Fr2 (dichloromethane fraction) were also comparable to each other and their scavenging potential were 74.60±0.18 and 76.90±0.36%, respectively at a concentration of 3000µg/mL. This result showed that fraction Fr3 (ethyl acetate fraction) showed the highest scavenging potential and CrE (crude extract) showed the lowest scavenging potential among all (Table 1 & Figure 3).

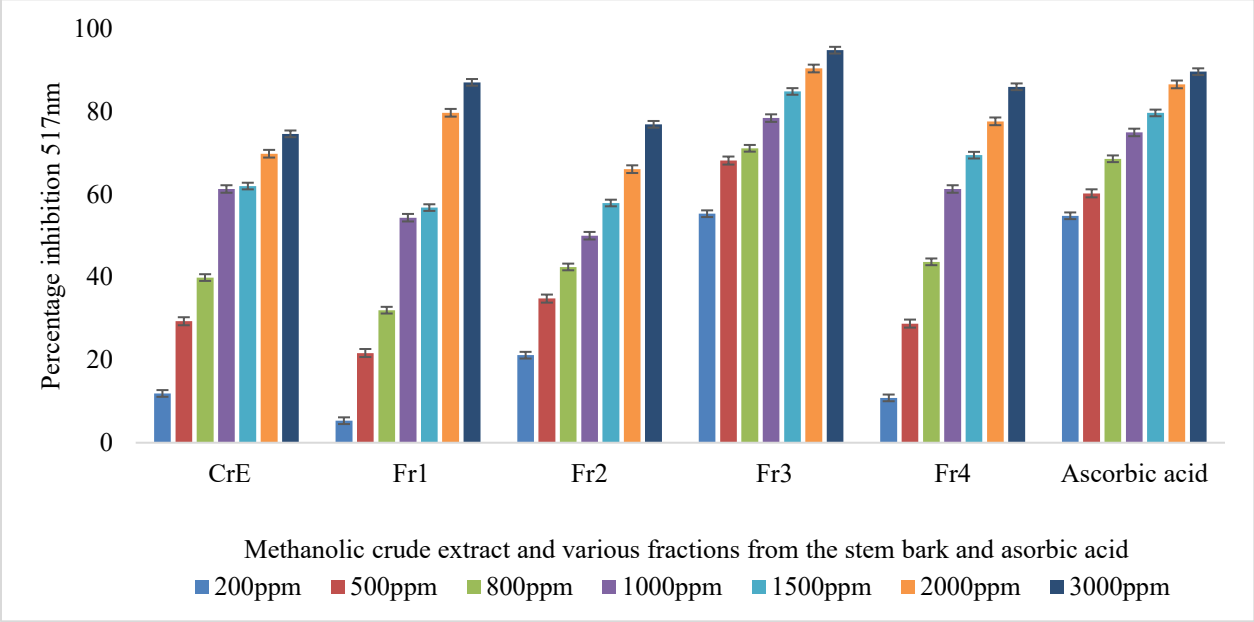

Figure 3: DPPH radical scavenging potential of methanolic crude extract and its four fractions obtained from the stem-bark of *A. senegalensis*. CrE and Fr1-Fr4 = Refer to the footnote of Table 1.

The IC_{50} values CrE, Fr1-Fr4, and the positive control (ascorbic acid) were determined and are also listed in Table 1. The IC_{50} value of the positive control was <200µg/mL. The IC_{50} values of CrE and Fr1-Fr4 were determined as 945.80, 905.54, 1040.00, <200, and 828.58, respectively. The result revealed that the methanolic crude extract (CrE) and fractions Fr1, Fr2, and Fr4 showed relatively higher IC_{50} values compared to the positive control. Additionally, the Fraction Fr3 (ethyl acetate fraction) showed an IC_{50} value similar to the positive control and this Fr3 showed the lowest IC_{50} value compared to CrE and the other three fractions (Fr1, Fr2, and Fr4). In other words, Fr3 showed the highest scavenging potency compared to CrE and other three fractions (Fr1, Fr2, and Fr4). The CrE (crude extract) and Fr1 (n-hexane fraction) showed comparable IC_{50} values of 945.80 and 905.54µg/mL, respectively. Fraction Fr4 (methanol-water fraction) showed slightly lower IC_{50} value of 828.58µg/mL compared to CrE, Fr1 and Fr2. Fraction Fr2 showed highest IC_{50} value compared to CrE and other three fractions (Fr1, Fr3, and Fr4) and its IC_{50} value was 1040.00µg/mL.

Table 1: The percentage inhibition of DPPH radical scavenging potential of methanolic crude extract and its fractions obtained from the stem-bark of *A. senegalensis*.

Extract/ Fractions	Concentrations (µg/mL)/Inhibition (%)							IC_{50} (µg/mL)
	200	500	800	1000	1500	2000	3000	
CrE	11.90 ±0.36	29.34 ±0.21	39.89 ±0.10	61.28 ±0.10	61.99 ±0.45	69.80 ±0.10	74.60 ±0.18	945.80
Fr1	5.32 ±0.45	21.67 ±0.74	32.01 ±0.54	54.35 ±0.13	56.89 ±0.10	79.69 ±0.18	87.05 ±0.18	905.54
Fr2	21.14 ±0.27	34.81 ±0.37	42.45 ±0.54	55.05 ±0.37	57.90 ±0.18	66.07 ±0.21	76.90 ±0.36	1040.00
Fr3	55.31 ±0.19	68.15 ±0.18	71.11 ±0.36	78.40 ±0.19	84.84 ±0.19	90.38 ±0.12	94.80 ±0.04	<200
Fr4	10.83 ±0.53	28.77 ±0.80	43.69 ±0.18	61.28 ±0.10	69.45 ±0.27	77.62 ±0.81	85.97 ±0.10	828.58
Asc. acid	54.81 ±0.36	60.23 ±0.53	68.58 ±0.18	74.94 ±0.36	79.65 ±0.46	86.54 ±0.45	89.61 ±0.18	<200

CrE = Methanolic crude extract obtained from the stem-bark of *A. senegalensis*, Fr1-Fr4 = n-hexane fraction, dichloromethane fraction, ethyl acetate fraction, and methanol-water fraction, respectively obtained from the methanolic crude extract. Asc. acid = Ascorbic acid.

Table 2: Determination of TPCs and TFCs of methanolic crude extract and its four fractions obtained from the stem-bark of *A. senegalensis*.

Extracts/ Fractions	TPCs (mg GAE/g DW)	TFCs (mg QE/g DW)
CrE	144.17±7.60	130.27 ± 7.78
Fr1	62.55±3.84	44.09 ± 5.14
Fr2	82.3 ±5.74	46.36 ± 0.71
Fr3	100.30±4.50	115.18 ± 5.09
Fr4	157.04±1.70	139.54 ± 8.69

CrE and Fr1-Fr4 = Refer to the footnote of Table 1.
TPCs = Total phenolic contents, TFCs = Total flavonoid contents.

The results of the total phenolic contents (TPCs) of CrE and Fr1-Fr4 are summarized in Table 2. The calibration curve of standard (gallic acid) is given in Figure 1. This calibration curve was used to estimate the TPCs of CrE and Fr1-Fr4. The TPCs of CrE and Fr1-Fr4 were determined as 144.17±7.60 and 62.55±3.84, 82.30±5.74, 100.30±4.50, and 157.04±1.70mg of gallic acid equivalent per gram dry weight of the extract (mg GAE/g DW), respectively (Table 2).This result revealed that Fr4 (methanol-water fraction) exhibited the highest TPCs followed by CrE, Fr3, Fr2, and Fr1 (Table 2). The crude extract (CrE) exhibited relatively higher TPCs compared to Fr1, Fr2, and Fr3 but showed lower TPCs than Fr4. Fraction Fr1 (hexane fraction) showed the lowest TPCs; its TPCs was relatively lower than CrE and the other three fractions (Fr2, Fr3, and Fr4). Fraction Fr2 (dichloromethane fraction) showed relatively lower TPCs compared to CrE and other two fractions (Fr3 and Fr4) but showed higher TPCs than Fr1. Fraction Fr3 (ethyl acetate fraction) showed lower TPCs than CrE and Fr4 but showed higher TPCs than the other two fractions (Fr1 and Fr3). Fraction Fr4 (methanol-water fraction) showed the highest TPCs and its TPCs was higher than CrE and the other three fractions (Fr1, Fr2 and Fr3). Fractions Fr1-Fr4 showed gradual increase in this TPCs and this could be due to increasing polarity nature of solvents. Fraction Fr1 was obtained from the n-hexane solvent and this non-polar solvent must have poor extraction power of the polar phenolic compounds. Therefore, it showed lowest TPCs among all fractions and CrE. Fraction Fr2 was obtained from dichloromethane as a solvent and this less polar solvent must have better extraction power than n-hexane. Therefore, it showed higher TPCs than Fr1. Fraction Fr3 was obtained from ethyl acetate as a solvent and this medium polar solvent must have relatively higher extraction power than n-hexane and dichloromethane. For the same reason, Fr3 showed relatively higher TPCs than Fr1 and Fr2. Fraction Fr4 was a methanol-water fraction and both methanol and water are high polar solvents and it must have more extraction power of phenolic compounds and for the same reason, fraction Fr4 showed relatively higher TPCs compared to other three fractions (Fr1, Fr2, and Fr3). It is noted that the crude

extract (CrE) showed relatively lower TPCs than Fr4 (methanol-water fraction). It might be because, since the crude extract must have many other phytochemicals in addition to phenolic compounds, and the relative availability of the phenolic compounds in this crude extract might be lower than the phenolic compounds available in fraction Fr4.

Similarly, the results of TFCs of CrE and Fr1-Fr4 are summarized in Table 2. The calibration curve of standard (quercetin) is given in Figure 2. The TFCs of CrE and Fr1-Fr4 was estimated using this calibration curve. The TFCs of CrE and Fr1-Fr4 were determined as 130.27±7.78 and 44.09±5.14, 46.36±0.71, 115.18±5.09, and 139.54±8.69mg of quercetin equivalent per gram dry weight of the extract (mg QE/g DW), respectively. Fraction Fr4 (methanol-water fraction) exhibited highest TFCs followed by CrE, Fr3, Fr2, and Fr1 (Table 2). The CrE (crude extract) exhibited slightly lower TFCs than Fr4 but showed relatively higher TFCs compared to Fr1, Fr2, and Fr3. Fraction Fr1 (n-hexane fraction) showed the lowest TFCs and its TFCs were comparable to Fr2 (dichloromethane fraction). However, Fr2 showed relatively lower TFCs compared to CrE and the other three fractions (Fr2, Fr3, and Fr4). Fraction Fr2 (dichloromethane fraction) showed slightly higher TFCs than Fr2 but showed relatively lower TFCs compared to CrE and the other two fractions (Fr3 and Fr4). Fraction Fr3 (ethyl acetate fraction) showed lower TFCs than CrE and Fr4 but showed higher TFCs than the other two fractions (Fr1 and Fr2). Fraction Fr4 (methanol-water fraction) showed the highest TFCs and it showed higher TFCs than CrE, and the other three fractions (Fr1, Fr2 and Fr3). Again, the TFCs of Fr1-Fr4 showed gradual increase, and this could be due to the polarity of solvents as discussed previously. Fraction Fr1 was obtained from the non-polar n-hexane as a solvent. n-Hexane must have poor extraction power of polar flavonoid compounds. Therefore, Fr1 showed the lowest TFCs among all fractions and CrE. Fraction Fr2 was obtained from the less polar dichloromethane as a solvent. Dichloromethane must have better extraction power of flavonoid compounds than n-hexane. Therefore, Fr2 showed slightly higher TFCs than Fr1. Fraction Fr3 was obtained from the medium polar ethyl acetate as a solvent. Ethyl acetate must have relatively higher extraction power of TFCs than n-hexane and dichloromethane. Therefore, Fr3 showed higher TFCs than Fr1 and Fr2. Fraction Fr4 was a methanol-water fraction and both methanol and water are high polar solvents and it must have more extraction power of flavonoid compounds than the other three solvents. Therefore, Fr4 showed relatively higher TFCs than the other three fractions (Fr1, Fr2, and Fr3). It is noted that the crude extract (CrE) showed relatively lower TFCs than Fr4 (methanol-water fraction). It might be because, since the phytochemical compositions of crude extract was contributed not only by flavonoid compounds but also by many other phytochemicals, and the relative availability of the flavonoid compounds in this crude extract might be lower than fraction Fr4.

In a previous report, 70% methanolic and ethyl acetate extracts were obtained from the leaves of *A. senegalensis* collected separately from Burkina Faso and Togo (Potchoo et al., 2008). These leaf extracts have been evaluated for their antioxidant activity using the DPPH radical scavenging assay. The 70% methanolic and ethyl acetate leaf extracts obtained from the *A. senegalensis* of Burkina Faso origin showed IC_{50} values of 8.51±0.66 and 21.08±0.31µg/mL, respectively. However, the 70% methanolic and ethyl acetate leaf extracts obtained from the *A. senegalensis* of Togo origin showed IC_{50} values of 12.46±1.05 and 29.22±2.03µg/mL, respectively (Potchoo et al., 2008). The lowest IC_{50} values (or the highest antioxidant potential) of extracts from the Burkino Faso origin might be due to the environmental factors such as climatic condition, the stages of plant maturation, temperature, etc. (Potchoo et al., 2008). Additionally, the TPCs and TFCs of 70% methanolic extract obtained from the leaves of *A. senegalensis* collected in Burkina Faso exhibited 2.66±0.08 milligram tannic acid/100mg dry extract (mg TAE/100mg DE) and 1.64±0.04 milligram quercetin/100mg dry extract (mgQE/100mg DE), respectively (Potchoo et al., 2008). On the other hand, the ethyl acetate extract obtained from the leaves of *A. senegalensis* collected in Burkina Faso did not exhibit any TPCs. Still it exhibited TFCs of 40.38±1.08mg TAE/100mg DE (Potchoo et al., 2008). The TPCs and TFCs of 70% methanolic extract obtained from the leaves of *A. senegalensis* collected in other location (Togo) exhibited 3.47±0.03 mg TAE/100mg DE and 2.33±0.17 mgQE/100mg DE, respectively (Potchoo et al., 2008). However, the ethyl acetate extract obtained from the leaves of *A. senegalensis* collected at this Togo location did not exhibit any TPCs but exhibited TFCs of 13.27±0.20mg TAE/100mg DE (Potchoo et al., 2008). In another report, an essential oil was obtained using the hydro-distillation method from the leaves of *A. senegalensis* collected from Burkina Faso, and this essential oil has been evaluated for antioxidant activity using the DPPH radical scavenging assay (Zenabou et al., 2018). This essential oil showed an IC_{50} value of 10.64µL (Zenabou et al., 2018). In another report, a hydro-ethanolic extract was obtained from the leaves of *A. senegalensis* collected in Benin (Emmanuel et al., 2024). This hydro-ethanolic extract was evaluated for antioxidant activity using the DPPH radical scavenging assay, and it showed an IC_{50} value of 0.22mg/mL (Emmanuel et al., 2024). In another report, seed oil was obtained from the fruits of *A. senegalensis* collected in Burundi (Jonathan et al., 2021). The seed oil was dissolved in n-hexane and subjected to solvent-solvent partition using methanol-water (8:2, v/v). The methanol-water and the seed oil extracts have been evaluated for antioxidant activity using the DPPH radical scavenging assay (Jonathan et al.,

2021). Both extract showed lower scavenging potential of less than 0.1mmol ascorbic acid equivalent per gram extract (Jonathan et al., 2021). The TPCs of methanol-water fraction has been determined as <3.0 mg GAE/g of oil (Jonathan et al., 2021).

In another report, 95% ethanolic extracts were obtained separately from the leaves, twigs, and bark of *A. senegalensis* collected in Cameroon (Kengni et al., 2017). Additionally, these 95% ethanolic extracts were subjected to solvent-solvent partition and obtained different fractions (Kengni et al., 2017). The 95% ethanolic extracts and the various fractions have been evaluated for their antioxidant activity using DPPH radical scavenging assay, ferric reducing antioxidant power (FRAP), and nitric oxide inhibition (NO) (Kengni et al., 2017). The IC_{50} values of these extracts were found as 1.50±0.10-167.00±8.20µg/mL in the DPPH assay, 0.53±0.0007->200µg/mL in the NO scavenging assay, and 2.07±0.86->200µg/mL in the FRAP assay (Kengni et al., 2017). In another report, dichloromethane/methanol (1:1, *v*/*v*) extract was obtained separately from the leaves and bark of *A. senegalensis* collected from Cameroon (Viviane et al., 2019). These extracts showed IC_{50} values of 48.04±0.10 and 7.40±0.00µg/mL, respectively in the DPPH radical scavenging assay (Viviane et al., 2019). The extract obtained from the leaves showed 92.59±0.33% of scavenging potential at a concentration of 100µg/mL with an IC_{50} value of 33.83±2.34µg/mL in the nitric oxide assay (Viviane et al., 2019). Similarly, the extract from the bark showed 57.63±0.61% at a concentration of 100µg/mL of scavenging potential with an IC_{50} value of 86.77±7.54µg/mL in the nitric oxide assay (Viviane et al., 2019). Additionally, the TPCs and TFCs of the leaf extract were determined as 90.45 ± 0.21mg GAE/g extract and 3.46 ± 0.00mg QE/g extract (Viviane et al., 2019). Similarly, the TPCs and TFCs of the bark extract were determined as 100.00 ± 1.28mg GAE/g extract and 2.95 ± 0.01 mg QE/g extract (Viviane et al., 2019). In another report, fruits were obtained from the *A. senegalensis* collected in Cameroon and extracted the powders of the dry fruits using 80% methanol, and this extract was termed as free fraction (Koubala et al., 2021). Similarly, powders of the dry fruits was extracted using 95% methanol by applying some chemical processes and this extract was termed as bound fraction (Koubala et al., 2021). Both the free and bound fractions were subjected to TPCs and TFCs analysis. The TPCS and TFCs of free fraction were determined as 246.02± 0.12 mg GAE/100g and 19.4 ± 0.05 mg QE/100g, respectively (Koubala et al., 2021) and the TPCS and TFCs of bound fraction were determined as 216.57±0.12mg GAE/100g and 95.8±0.09mg QE/100g, respectively (Koubala et al., 2021).

In a previous report, leaves of *A. senegalensis* were collected in the Democratic Republic of Congo. Ethanolic and dichloromethane extracts were obtained separately from the dried leaves (Gedeon et al., 2017). Both ethanolic and dichloromethane extracts have been evaluated for antioxidant activity by DPPH assay and 2,2'-azino-bis-3-ethylbenz-thiazoline-6-sulphonic acid (ABTS) assay. The IC_{50} values of ethanolic and dichloromethane extracts were determined as 112.46±4.22 and 53.20±0.97µg/mL, respectively in DPPH assay (Gedeon et al., 2017). The IC_{50} values of ethanolic and dichloromethane extracts were determined as 46.88±1.43 and 381.01.20±154.73µg/mL, respectively in the ABTS assay (Gedeon et al., 2017). In another report, an aqueous extract was obtained from the leaves of *A. senegalensis* collected from the Ivory Coast (Nanti et al., 2019). This water extract showed an IC_{50} value of 95.4mg/mL in the DPPH radical scavenging assay (Nanti et al., 2019). Additionally, the TPCs and TFCs of this aqueous extract were determined as 61.73 ± 0.40 mg GAE/g extract and 3.63 ± 0.03 mg QE/g extract, respectively (Nanti et al., 2019). In another report, aqueous and 80% methanolic extracts were obtained from fruits of *A. senegalensis* collected from Malawi (Bonface et al., 2023). Both the aqueous and 80% methanolic extracts have been evaluated for their antioxidant activity using DPPH radical scavenging assay and they showed scavenging potential of 83.17 and 36.01%, respectively (Bonface et al., 2023). Additionally, the aqueous and 80% methanolic extracts exhibited 108:08 ± 1:70 and 99:05 ± 7:56 mg TEAC/100 g FW of ferric reducing antioxidant power, respectively (Bonface et al., 2023). Furthermore, the TPCs and TFCs of aqueous extract were determined as 578.67 milligram gallic acid equivalent per 100 gram fresh weight (mg GAE/100 g FE) and 649.67 milligram quercetin equivalent per 100 gram fresh weight (mg QE/100 g FE), respectively (Bonface et al., 2023). The TPCs and TFCs of 80% methanolic extract were determined as 925.33 mg GAE/g FE and 71.67 mg QE/100 g FE, respectively (Bonface et al., 2023).

In another report, dichloromethane/methanol (1:1, *v*/*v*) extract was obtained from the stem-bark of *A. senegalensis* collected in Nigeria (Chukwudi et al., 2019). Hexane, ethyl acetate, and methanol fractions were obtained separately from this crude extract. The crude extract and all three fractions have been evaluated for their radical scavenging activity using DDPH radical scavenging assay, hydrogen peroxide scavenging assay, hydroxyl radical scavenging assay, superoxide radical scavenging assay, ABTS scavenging assay, and ferric reducing power assay (FRAP) (Chukwudi et al., 2019). The crude extract, n-hexane, ethyl acetate, and methanol fractions showed IC_{50} values of 752.67±15.60, 55.67±4.49, 121.67±2.40, and 293.67±16.91µg/mL, respectively in DPPH radical scavenging assay (Chukwudi et al., 2019). The IC_{50} values of the crude extract, n-hexane, ethyl acetate, and methanol fractions have been determined as 390.33±0.88, 437.00±29.05, 256.33±0.88, and 404.00±37.70µg/mL, respectively in the hydrogen peroxide scavenging assay (Chukwudi et al., 2019). The IC_{50} values of the crude extract, n-hexane, ethyl acetate, and methanol fractions have been determined as 418.00±5.29,

371.67±36.25, 75.67±6.33, and 370.67±7.80µg/mL, respectively in hydroxyl radical scavenging assay (Chukwudi et al., 2019). The IC_{50} values of the crude extract, n-hexane, ethyl acetate, and methanol fractions have been determined as 333.67±2.40, 140.67±7.97, 79.33±1.45, and 235.33±0.88µg/mL, respectively in superoxide radical scavenging assay (Chukwudi et al., 2019). The IC_{50} values of the crude extract, n-hexane, ethyl acetate, and methanol fractions have been determined as 372.00±3.46, 391.00±14.57, 126.67±1.20, and 217.00±4.04µg/mL, respectively in the ABTS scavenging assay (Chukwudi et al., 2019). The IC_{50} values of the crude extract, n-hexane, ethyl acetate, and methanol fractions have been determined as 63.00±12.22, 281.67±15.34, 81.00±1.00, and 275.67±6.33µg/mL, respectively in FRAP assay (Chukwudi et al., 2019). Additionally, the TPCs and TFCs of dichloromethane/methanol (1:1, *v/v*) extract have been determined as 866.67±8.41mg GAE/g extract and 845.67±93.62 milligram rutin equivalent per gram extract (mg RE/g extract), respectively (Chukwudi et al., 2019). The TPCs and TFCs of the hexane fraction obtained from this crude extract were determined as 88.64±8.33mg GAE/g extract and 78.45±6.54mg RE/g extract, respectively (Chukwudi et al., 2019). The TPCs and TFCs of ethyl acetate fraction obtained from the crude extract have been determined as 582.00±1.73mg GAE/g extract and 587.33±50.83mg RE/g extract, respectively (Chukwudi et al., 2019). The TPCs and TFCs of methanol fraction obtained from the crude extract have been determined as 115.33±3.84mg GAE/g extract and 113.67±9.28mg RE/g extract, respectively (Chukwudi et al., 2019).

In another report, a methanolic extract was obtained from the roots of *A. senegalensis* collected from Abuja, Nigeria (Idris et al., 2020). Fractionation of this methanolic extract by preparative TLC method gave eleven fractions (Idris et al., 2020). All eleven fractions were evaluated for antioxidant activity using DPPH radical scavenging assay and they showed IC_{50} values ranging from 2.2267±0.02-6.2890±0.01mg/mL (Idris et al., 2020). In another report, a methanolic extract was obtained from the fruits of *A. senegalensis* collected in Nigeria (Hamzah et al., 2018). This methanolic extract showed scavenging potential of 38.43-77.15% in the DPPH radical scavenging assay (Hamzah et al., 2018). In another report, a 70% ethanol extract was obtained from the roots of *A. senegalensis* collected from Nigeria (Jajere et al., 2024). This 70% ethanolic extract showed 1.1-25% scavenging potential with an IC_{50} value of 3.68mg/mL in the DPPH assay (Jajere et al., 2024). In another report, methanol extract was obtained from the leaves of *A. senegalensis* collected from Nigeria (Thagriki et al., 2017). This methanolic extract has been evaluated for antioxidant activity using DPPH radical scavenging assay, ABTS radical scavenging assay, and hydrogen peroxide radical scavenging assay (Thagriki et al., 2017). This extract showed 78.73±0.38-80.75±0.33% scavenging potential at a concentration range of 20-100 µg/mL with an IC_{50} value of 45.72µg/mL in the DPPH assay. This extract also showed 52.93±0.91-80.34±0.06% scavenging potential at a concentration range of 20-100 µg/mL with an IC_{50} value of 48.98µg/mL in the ABTS assay (Thagriki et al., 2017). Additionally, this extract showed 26.60.93±0.57-49.90±0.85% scavenging potential at a concentration range of 20-100 µg/mL with an IC_{50} value of 82.67µg/mL in the hydrogen peroxide assay (Thagriki et al., 2017). The percentage ferric reducing power of this methanolic extract at concentrations of 20, 40, 60, 80, and 100µg/mL has been determined as 18.80 ± 0.14, 31.80 ± 0.42, 38.95 ± 0.10, 45.68 ± 0.39, and 53.40 ± 0.57%, respectively and the IC_{50} value has been determined as 84.75µg/mL (Thagriki et al., 2017). In another report, an aqueous extract was obtained from the stem-bark of *A. senegalensis* collected in Nigeria (Lopez-Soriano et al., 2020). This aqueous extract has been evaluated for antioxidant activity using DPPH radical scavenging assay and nitric oxide radical scavenging assay, and showed effective concentration (EC_{50}) values of 17.15±4.76 and 39.50±4.45µg/mL, respectively (Lopez-Soriano et al., 2020).

In another report, leaves and stem-bark of *A. senegalensis* were collected separately in Senegal at three different locations, viz. Kolda, Ziguinchor, and Thies. Hydro-ethanolic extracts (8:2, v/v) were obtained separately from these leaves and stem-bark and were subjected to DPPH radical scavenging assay (Diawo et al., 2024). The leaf hydro-ethanolic extracts collected from these three locations showed scavenging potential of 94.41±0.11, 94.54±0.53, and 95.06±0.10%, respectively (Diawo et al., 2024). Similarly, the stem-bark hydro-ethanolic extracts collected from these three locations showed scavenging potential of 89.47±0.64, 89.36±0.25, and 92.87±0.10%, respectively (Diawo et al., 2024). Additionally, the hydro-ethanolic extract obtained from the leaves collected at Kolda showed TPCs and TFCs of 49.363 ± 0.018 grams of gallic acid equivalent per 100 grams of dry matter (g GAE/100 g DM) and 0.095 ± 0.001grams of catechin equivalent per 100 grams of DM (g CE/100 g DM), respectively (Diawo et al., 2024). The TPCs and TFCs of hydro-ethanolic extract obtained from the stem-bark have been determined as 42.018 ± 0.013g GAE/100 g DM and 0.194 ± 0.002 g CE/100 g DM, respectively (Diawo et al., 2024). The hydro-ethanolic extract obtained from the leaves collected at Ziguinchor showed TPCs and TFCs of 89.931 ± 0.018 g GAE/100 g DM and 0.099 ± 0.004g CE/100 g DM, respectively (Diawo et al., 2024). Whereas, the hydro-ethanolic extract obtained from the stem-bark collected at Ziguinchor showed TPCs and TFCs of 77.886 ± 1.001 g GAE/100 g DM and 0.195 ± 0.004g CE/100 g DM, respectively (Diawo et al., 2024). The hydro-ethanolic extract obtained from the leaves collected at Ziguinchor showed TPCs and TFCs of 89.931 ± 0.018 g GAE/100 g DM and 0.099 ± 0.004g CE/100 g DM, respectively (Diawo et al., 2024). On the other hand, the hydro-ethanolic extract obtained from the leaves collected at Thies showed TPCs and TFCs of 39.118 ± 0.020 g GAE/100

g DM and 0.096 ± 0.004g CE/100 g DM, respectively (Diawo et al., 2024). The hydro-ethanolic extract obtained from the stem-bark collected at Thies showed TPCs and TFCs of 31.393 ± 1.020 g GAE/100 g DM and 0.194 ± 0.001g CE/100 g DM, respectively (Diawo et al., 2024).

Analysis of the previously reported results discussed above revealed the fact that various extracts, fractions, and essential oils obtained from different parts of *A. senegalensis* collected at multiple locations exhibited a wide range of antioxidant activity and they also possessed TPCs and TFCs at various levels. Analysis of our results on the antioxidant potential and TPCs and TFCs of *A. senegalensis* revealed the fact that there was a significant variation in these parameters compared to the previous reports. This variation might be due to two reasons in present study i) we used the stem-bark of *A. senegalensis* collected in a different geographic location, i.e. from the Kingdom of Eswatini, and ii) additionally, we used a different extraction technique, i.e. we used methanol to obtain a crude extract, and four fractions were obtained from this methanolic crude extract. We discussed previously that the collection of plant materials at different geographic locations and methods of extraction contribute significantly to the variation in phytochemicals and their compositions and this contribution would reflect to the biological and pharmacological activities. Our result, therefore, substantiated this fact, and to the best of our knowledge, this is the first report of this kind on the antioxidant activity, TPCs and TFCs of methanolic crude extract and its fractions obtained from the stem-bark of *A. senegalensis* collected in the Kingdom of Eswatini.

4. CONCLUSION

A methanolic crude extract was obtained from the stem-bark of *A. senegalensis*. n-Hexane, dichloromethane, ethyl acetate, and methanol-water fractions were also obtained from the methanolic crude extract. The methanolic crude extract and the four fractions were evaluated for their DPPH radical scavenging activity. Ascorbic acid served as a positive control. At a concentration range of 200-3000 μg/mL, the radical scavenging potential of the methanolic crude extract, the four fractions, and the positive control was determined as 11.90±0.36-74.60±0.18%, 5.32±0.45-94.80±0.04%, and 54.81±0.36-89.61±0.18%, respectively. Additionally the IC_{50} values of the methanolic crude extract and its four fractions were determined as 945.80, 905.54, 1040.00, <200, and 828.58μg/mL, respectively, and the IC_{50} value of the positive control was determined as <200 μg/mL. The ethyl acetate fraction was identified as the most potent and exhibited higher scavenging activity compared to the methanolic crude extract and the rest of the other fractions. Furthermore the TPCs of the methanolic crude extract and its four fractions were determined as 144.17±7.60, 62.55±3.84, 82.30 ±5.74, 100.30±4.50, and 157.04±1.70mg GAE/g DW, respectively. Similarly, the TFCs of the methanolic crude extract and its four fractions were determined as 130.27±7.78, 44.09±5.14, 46.36±0.71, 115.18±5.09, and 139.54±8.69QE/g DW, respectively. From this study, we concluded that the methanolic crude extract and the four fractions obtained from this crude extract of stem-bark of *A. senegalensis* showed significant radical scavenging activity. Notably, the ethyl acetate fraction was identified as the potent with higher scavenging potential. Additionally, the methanolic crude extract and all four fractions possessed moderate to high TPCs and TFCs. *A. senegalensis* has been used in the traditional Swazi medicine, and our findings also support its uses in traditional medicine. Further studies on this plant to develop antioxidant-based therapeutics are recommended as a future perspective.

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Authors' Contributions

Manoharan Karuppiah Pillai: Proposed idea, supervised the research work, oversaw the research progress, drafted, reviewed and edited the manuscript.

Yende Lugile Happiness: Conceived the idea, collected the data, performed the experiments and involved in drafting the manuscript.

Ethical Approval

In this article, as per the plant regulations followed in the Department of Chemistry, Faculty of Science & Engineering, University of Eswatini, Kwaluseni Campus, Private Bag 4, Kwaluseni, M201, The Kingdom of Eswatini, Southern Africa; the authors observed the antioxidant activity of *Annona selegalensis* - from the Kingdom of Eswatini. The ethical guidelines for plants & plant materials are followed in the study for observation, identification & experimentation.

Informed Consent

Not applicable.

Conflicts of interests

The authors declare that they have no conflicts of interests, competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Data and materials availability

All data associated with this work are present in the paper.

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