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DETERMINATION OF ANTIOXIDANT POTENTIALS OF *Acacia seyel* STEM BARK EXTRACTS

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ABSTRACT

Acacia seyel is a plant species distributed throughout most parts of tropical regions of the world and has a number of medicinal uses; antidiabetic, antioxidant, immunomodulatory, antiinfalammatory, antiulcer, wound healing, antibacterial, antimalarial and anticarcinogenic properties. This work is to investigate the antioxidant potentials of n-hexane, ethyl acetate and methanol extracts of A. seyel. Air-dried powdered plant material was successively extracted with n-hexane, ethyl acetate and methanol using soxhlet extractor for 12 hours each. The antioxidant activities of the extracts from the stem bark A. seyel were evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and phosphomolybdate techniques. The phenolic content of the plant extracts were investigated based on Folin-Ciocaltaeu method. The extracts demonstrate significant antioxidant activity against DPPH radical and phosphomolabdate technique. Mathanol extract showed highest phenolic content. In conclusion, A. seyel extract demonstrate presence of antioxidant compounds with radical scavenging and electron transfer potentials.

Key words: Acacia seyel, Antioxidant, phenolics, DPPH, phsphomolybdate

INTRODUCTION

Free radicals are continuously produced in our body and they are important for the maintenance of normal physiological function (Singal & Kirshenbaum, 1990). They are generally highly reactive and participate in hydrogen abstraction, radical addition, bond scission and annihilation reactions damaging the macromolecules such as proteins, DNA and lipids (Evans & Halliwell, 1999). Antioxidants prevent the oxidative reactions that occur naturally in tissues by scavenging free radicals, chelating metal ions and acting as electron donors. A search of naturally occurring antioxidant compounds from plant sources might provide leads for the development of novel drugs, which may reduce the risk of chronic diseases caused by free radicals (Kim et al., 2003).

Acacia seyal Del. (homotypic synonym: *Vachellia seyal* Del.; another synonym: *Acacia stenocarpa* Hochst.; English name: Whistling thorn; Hausa Name: Dinshe) (Hussein, 2017). *A. seyal* is a well-known traditional medicinal plant that has a wide range of medicinal applications related to its different phytoconstituents from organized parts, e.g., fruits, barks, stem, and roots, and unorganized parts (gum acacia) (Thiele *et. al.*, 2011).

Acacia gum has been reported to possess several pharmacological activites, including, antidiabetic, antioxidant, immunomodulatory, and cytoprotective antiulcer, wound healing, antibacterial, antimalarial and anticarcinogenic properties (Aloqbi, 2020; Ahmed, 2018; Samy et al, 2014; Lattimer & Haub, 2010; Smolinske, 1992). The stem bark of *A. seyal* has been reported to contain flavonoids, saponins, terpenoids, steroids, alkaloids, phenols, coumarin, and tannins (Garba *et. al.*, 2018; Abdllha *et. al.*, 2016). The present study was carried out to investigate the free radical scavenging and electron donation potentials of *A. seyel* extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and phosphomolybdate electron transfer models.

EXPERIMENTAL

Plant material

Acacia seyel stem bark was collected from Dawakin Tofa, local government area, Kano State, Nigeria in February, 2023. Identification and authentication of the plant was carried out at the Plant Biology Department, Bayero University Kano by Mr. Bahauddeen Adam. The

sample was deposited in the departmental herbarium with voucher number BUKHAN 0476.

Extraction

Air dried powdered stem bark of *A. seyel* (50 g) was extracted successively with n-hexane (200 mL), ethyl acetate (200 mL) and methanol (200 mL) in a soxhlet extractor each for 12 hours. The samples were concentrated using rotary evaporator to give n-hexane (2.00 g, 4.0%), ethyl acetate (0.69 g, 1.4%) and methanol (2.90 g, 5.8%) extracts.

Infrared Spectroscopic Analysis of the Extracts

Infrared (IR) absorptions of the extracts were measured on ATR-FTIR spectrophotometer using attenuated total reflectance (ATR) technique. The crystal area was cleaned up and the background was collected. The extract is then placed onto the small crystal area for the IR measurement (Coates, 2000).

Free radical-scavenging activity

The free radical scavenging activity of the plant extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined according to the method described by Idris *et al.* (2021a) with slight modification. Each sample of stock solution (20 mg/L) was diluted to final concentration of 1000, 500, 250, 125, 62.5, 31.3, 15.6 and 7.8 µg/mL. Then, a total of 50 µM DPPH methanolic solution (160 µL) was added to sample solution (40 µL) and allowed to react at room temperature for 30 min in dark. The absorbance of the mixtures was measured at 517 nm. Ascorbic acid was used as positive control. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and vice versa. Inhibitions of DPPH radical in percent (I%) were calculated using the formula:

$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$

Where,

A_{blank} is the absorbance value of the control reaction (containing all reagents except the test compound) and

A_{sample} is the absorbance values of the test compounds.

The sample concentration that provides 50% inhibition (IC_{50}) was determined using MS Excel.

Total antioxidant activity by phosphomolybdanum assay

Total antioxidant activities of samples were determined according to the method of Idris *et al.* (2021a) with slight modification. An aliquot of 20 µL of sample solution (0.1 mg/mL) was mixed with 180 µL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 min in a water bath. Absorbance of all the sample mixture was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalence of gallic acid. A calibration curve of gallic acid was prepared and the total antioxidant activity was standardized against gallic acid and was expressed as mg gallic acid equivalents per gram of sample on a dry weight (DW) basis *(* Raja *et al.*, 2010).

Determination of Total Phenolic Content

The total phenolics content of the plant extracts were determined using Folin-Ciocalteau reagent and Gallic acid as standard (Idris *et al* 2021b) with a slight modification. The plant extract (1 mg/ml, 20µl) was mixed with Folin-Ciocalteau reagent (20µl) in a 96 well micro plate. After 5 minutes, sodium carbonate (0.01M, 20µl) was added to each sample and allowed to stand for 5 minutes before adding 125 µl of distilled water. The mixture was measured at the absorbance at 765nm using Thermo-scientific multiskan GO spectrophotometer (Thermofisher Scientific, Vartaa, finland). The blank is the same reaction mixture with water instead of the extracts or standard. The total phenolic content was expressed as mg gallic acid equivalent per gram.

RESULTS AND DISCUSSION

Dried powdered stem bark of *Acacia seyel* was subjected to a successive sohxlet extraction using n-hexane, ethyl acetate and methanol. Methanol extract (ACME, 5.8%) was observed to be higher than n-hexane (ACHX, 4.0%) and ethyl acetate (ACEA, 1.4%) extracts as gummy greenish, oily green and gummy dark green, respectively. Dapkevicius et al. (1998) reported that, the amount of material extracted using different solvents could be associated with composition of each particular herb, differences in the solubility of extractives and their polarity as well as extraction technique employed.

Infrared spectroscopic analysis of the extracts (ACHX, ACEA and ACME) from the stem bark of *A. seyel* was performed to reveal functional groups present in the extracts. The IR spectra (Figures 1 to 3) show absorptions in the ranges of 2840-2948; cm^{-1} (C-H), 1655-1741 cm^{-1} (C=O) in all the three extracts. The O-H and C-O stretching vibration were observed in the respective absorption range 3335-3372 cm^{-1} and 1019-1037 cm^{-1}, for ACEA and ACME.

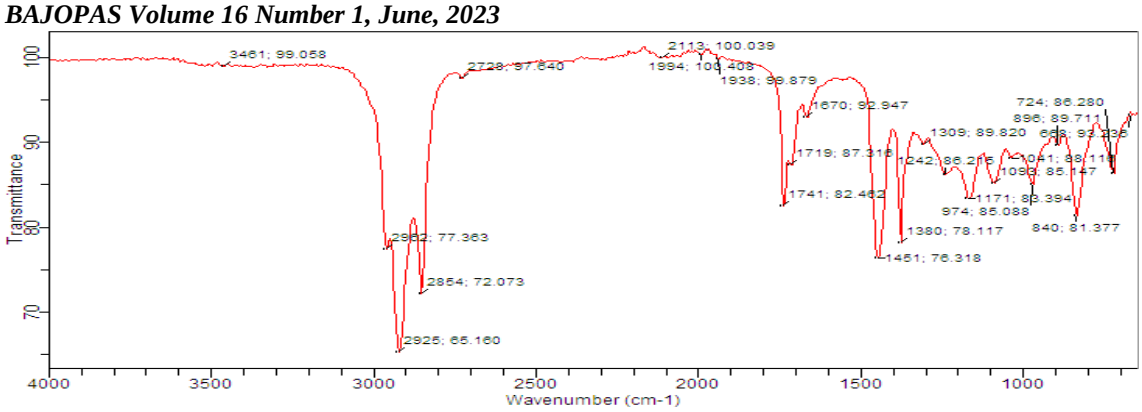

Figure 1: IR spectrum of the n-hexane extract (ACHX)

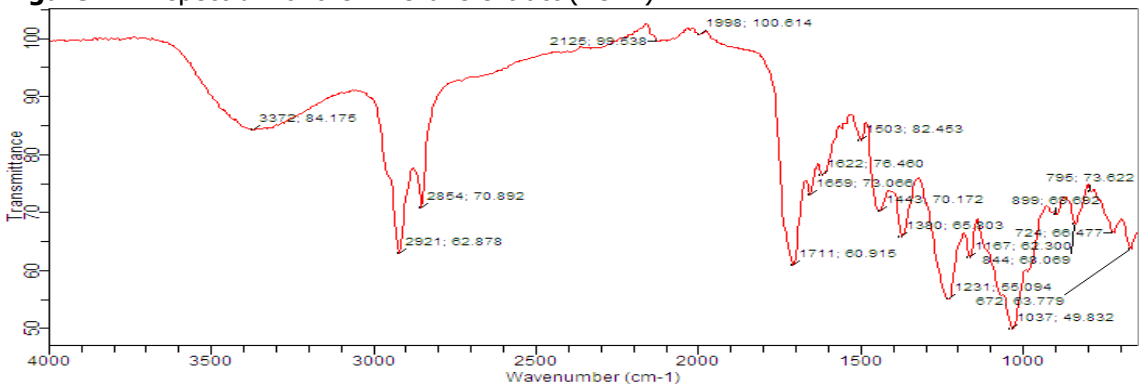

Figure 2: IR spectrum of the ethyl acetate extract (ACEA)

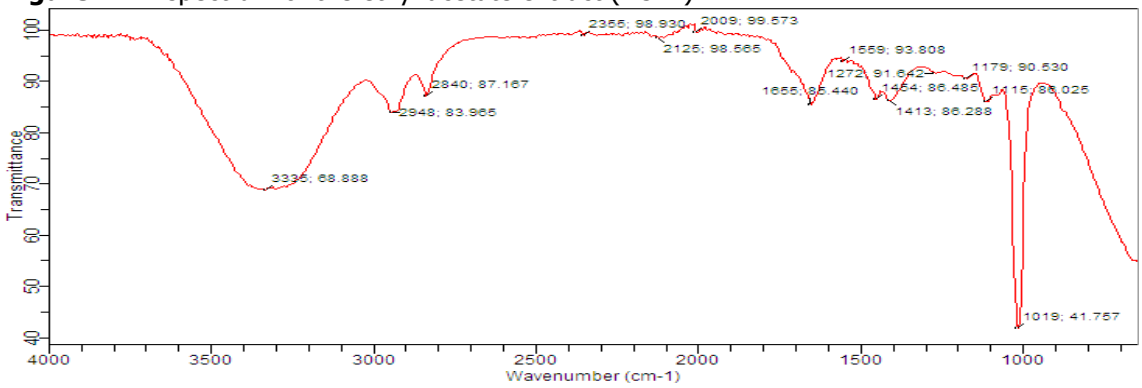

Figure 3: IR spectrum of the methanol extract (ACME)

Antioxidants are substances that can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and/or electron tranfers resulting in diminishing oxidative stress (Dai & Mumper, 2010). The DPPH scavenging activities of *A. seyel* stem bark extracts were measured using 2-fold serial dilution of the concentrations 1000-15.63 µg/mL expressed as percent inhibition (Figure), and also amount of antioxidant needed to decrease the concentration of DPPH by 50%, statistically significant at $p<0.05$ samples compared with the ascorbic acid (Table 1).

The scavenging activity of ASHX is higher than that of ASEA and ASME in both percent inhibition and the IC_{50} values (Figure 4 and Table 1). Elmi *et. al.*, (2020) reported the scavenging activity methanol extract (150±2.2 µg/mL) from the stem bark of *A. seyel* with higher activity than the one recorded in this work (235.52±3.102 µg/mL). This could be attributed to the successive extraction of the plant material with solvent in gradient of n-hexane, ethyl acetate and methanol in this work, whereas, methanol was the only solvent used in Elmi's and co-workers reported work. The extracts from *A. seyel* in this work contain comparable antioxidants to that of positive control, and suggest the presence of secondary metabolites with abstractable hydrogen in the extract.

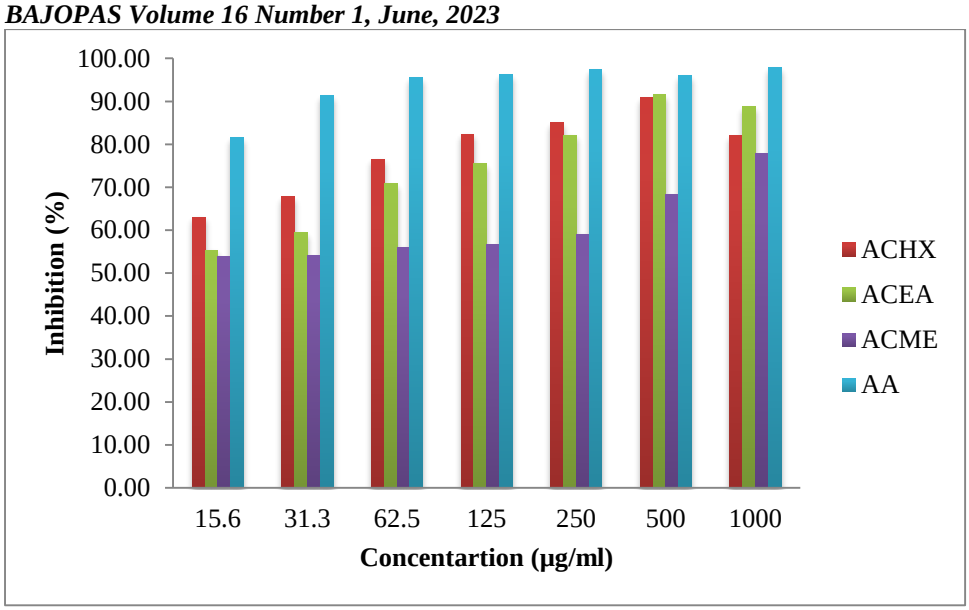

Figure 4: Radical scavenging activity of test samples from *Acacia seyel*

Table 1: Antioxidant activities of test samples from *Acacia seyel*

Extract	IC_{50} (µg/mL)	TAC (mg GAE/g)	TPC (mg GAE/g)
ASHX	66.48±1.968	66.48±0.003	88.42±0.000
ASEA	229.14±2.695	229.14±0.005	242.42±0.000
ASME	235.52±3.102	235.52±0.001	249.43±0.001
AA	0.63±0.034	-	-

AS=*A. seyel*; HX=n-hexane; EA=ethyl acetate; ME=methanol; AA=ascorbic acid; TAC=total antioxidant capacity; TPC=total phenolic content

Phospho-molybdate antioxidant activities of the extracts from the stem bark of *A. seyel* were also investigated based on milligram gallic acid equivalence per gram (mg GAE/g; $y=0.0017x+0.05$; $r^2=0.94$) presented in Table 1. Highest antioxidant power among the samples was observed in ASME with 235.52 mg GAE/g, whereas lowest activity was exhibited by ASHX with estimation of 66.48 µg GAE/mg.

Phenolic content of the *A. seyel* extracts were investigated based on Folin-Ciocaltaue's technique, and expressed as mg gallic acid/g of extract (mg GAE/g; $y=0.0019x$; $r^2=0.91$) with a significant of $P<0.05$ samples compared with 1000 mg GAE/g (Table). The amount of phenolic content in ASME (249.43 mg GAE/g) and ASEA (242.42 mg GAE/g) are three times higher than that of ASHX (88.42 mg GAE/g) as observed in Table 1. Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants through radical scavenging potentials, and their metals chelating abilities in the free radical production (Dai & Mumper, 2010).

CONCLUSION

It can be concluded that *A. seyel* contains antioxidant compounds with radical scavenging and electron transfer ability as evident from respective antioxidant power of ASHX and ASME. The ASME antioxidant ability was supported by its highest phenolic contents.

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