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Research Article
Bioactive Compounds, Antioxidant Activity, Minerals Composition and Antimicrobial Activity of *Acacia nilotica* Fruit Flesh and Seeds

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Abstract

Background and Objective: Plant extracts can be used as alternatives for controlling food poisoning and preserving foods. Many studies have been carried out on *Acacia nilotica* fruit flesh, but the seeds have not been investigated. Therefore, a comparative study on bioactive compounds, antioxidant activity, minerals composition, fatty acid profile and antimicrobial activity of fruit flesh and seeds, was conducted. **Materials and Methods:** In the present study, bioactive compounds, antioxidant activity, minerals composition, fatty acid profile and antimicrobial activity were determined using standard methods and analyzed by using Student's t-test. **Results:** The *A. nilotica* fruit flesh methanolic extract (ANFF) was rich in phenolic compounds compared to that of the seed (ANS). Phenolic compounds in ANFF significantly ($p \leq 0.01$) higher than ANS. However, the antioxidant activity of ANS (92.63%) was significantly ($p \leq 0.05$) higher than that of ANFF (89.88%). The fruit seed had significantly higher amounts of macro-minerals ($p \leq 0.05$) and micro-minerals ($p \leq 0.01$) than those in the fruit flesh. Linoleic acid in the seed and was significantly ($p \leq 0.01$) higher than that of the flesh, but the flesh had significantly higher ($p \leq 0.05$) oleic acid than that in the seeds. The ratio of unsaturated to saturated fatty acids exhibited considerable variation among the samples. SME significantly ($p \leq 0.05$) inhibited the growth of Gram-negative (*E. coli*) and Gram-positive bacteria comparable to that of penicillin than do ANFF. **Conclusion:** The findings suggested that the flesh and seeds of *A. nilotica* are excellent sources of bioactive compounds, with the seeds having superior results.

Key words: *Acacia nilotica*, bioactive compounds, antioxidant activity, minerals, fatty acids, antimicrobial activity, secondary metabolites

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food poisoning in developing countries is considered a serious cause of illness and death[1]. Bacteria, mainly Gram-negative ones are causative organisms for food poisoning[2]. Gram-positive bacteria have been found to cause food spoilage[3]. Prevention of food contamination and spoilage can be achieved using chemical preservatives[4]. Such chemicals are efficient in preventing the outbreak of food poisoning diseases, but when used repeatedly will become accumulated in food and feed chains, which may not only result in deleterious effects on human health but also assist microorganism to become resistant to such chemicals[5]. Therefore, attempts are being made to develop alternate natural food preservatives which in addition to being effective are considered as safe and healthy. This has encouraged utilization of plant extracts, having antimicrobial potential, for the preservation of foodproducts[6].

Acacia belongs to the Leguminosae family where it is the second largest genus, comprise of approximately 1,350 species and found mainly in sub-arid and arid parts of the world[7]. Different kinds of secondary metabolites, including cyanogenic glycosides, condensed tannins, terpenes, cyclitols, alkaloids and gums have been detected in *Acacia*. The seeds of some *Acacia* species are rich in nutrients, such as oil, minerals and soluble carbohydrates. One species of this genus, *Acacia nilotica*, grows naturally near riverbeds at lowland sites or seasonally flooded black cotton soils[7].

Acacia nilotica is used for several purposes, including livestock feed because of its high nutritional value[8], ability to be used as a natural fertilizer[9], gum production and its importance in fuel-wood and charcoal production[10], multipurpose medicinal and pharmaceutical plant[11] and traditionally it has been utilized as remedy to cure different diseases including small pox, tuberculosis, gonorrhea and pneumonia and also been studied to demonstrate antibacterial and antifungal activities[12].

The antibacterial activities of *A. nilotica* leaves and stem bark methanolic extracts have been demonstrated against gram-positive and Gram-negative bacteria[13]. Saini *et al.*[12] reported that *A. nilotica* showed the maximum antifungal effects against *Aspergillus niger* and *Candida albicans*. Additionally, methanolic extracts prepared from *A. nilotica* leaf demonstrated high inhibitory effects against *Aspergillus flavus* growth[13]. Singh *et al.*[14] reported that *A. nilotica* bark extract could cause inhibition of liver injury and hepatic malondialdehyde formation.

Methanol and water extracts of the fruit flesh are reported to cause inhibition of viral proteases from HIV-1[15] and hepatitis C[16]. Phytochemical analysis of shoots from this plant has resulted in the identification and detection of numerous phenolic compounds including catechin derivatives[16,17]. These derivatives have been associated with different biological properties that include their roles as antioxidant, anti-inflammatory and anti-carcinogenic agents[18]. Another study by Maldini *et al.*[19] also reported appreciable amounts of phenolic compounds galloylated derivatives of catechin and gallocatechin in *A. nilotica*.

Despite all of the investigations of *Acacia* species and fruit flesh extracts being used for medicinal and preservative purposes; the seeds have not yet been studied. This study investigated the bioactive compounds, antioxidant activity, mineral composition and microbiological activity of *A. nilotica* seed extract and powder compared to that of the fruit flesh.

MATERIALS AND METHODS

Acacia nilotica fruits were donated by a farm located in Khartoum, Sudan. Part of this work was carried out in Sudan, Faculty of Agriculture, University of Khartoum during the period from November, 2018 to March, 2019. The rest was carried out at national Research center, Khartoum, Sudan during the period from May, 2019 to July, 2019. All analytical chemicals were purchased from Sigma-Aldrich Co., St. Louis, USA.

Use value (UV) and extraction yield (EY): The formula of Phillips and Gentry[20] to determine the relative importance of *A. nilotica* used is presented:

$$UV = \frac{\sum Ui}{n}$$

where, Ui represents the number of uses reported by each informer for a specific plant species i and n is the number of informants. Ethnobotanical data for UV were collected from 200 people using a random survey (females, males, herbalist males and herbalist females). The EY percentages were calculated according to the following equation[21]:

$$EY\ (\%) = \frac{R}{S} \times 100$$

where, R represents the weight of extract residues and S reflects the weight of raw sample.

Preparation of samples: Extraneous materials, such as leaves and dirt, were removed from the *A. nilotica* fruits and the seeds were removed from the flesh manually. Flesh and seeds were separately ground in an electrical grinder to a fine powder. Part of the powder was used to determine minerals and oil contents and the rest was used to prepare individual flesh and seed extracts[22].

Preparation of sample extract: About 500 g of *A. nilotica* seeds or flesh powder were extracted separately using 200 mL methanol in each case and 24 h stirring. The filtration of slurry was carried out using Whatman No. 1 filter paper. The final methanolic extract was freeze-dried and stored at -20°C before further analysis[22].

HPLC analysis of phenolic and flavonoid compounds: Analyses of the individual phenolic compounds of the *A. nilotica* flesh and seed extracts were determined according to the manufacturer manual using HPLC system from Shimadzu Corporation, Kyoto, Japan. The system consisted of a PDA detector and Inertsil ODS-3 (5 μm, 4.6×250 mm) column. The gradient profile was measured with a temperature set at 30°C and was 0-0.10 min, 8% B: 0.10-2 min, 10% B: 2-27 min, 30% B: 27-37 min, 56% B: 37-37.10 min, 8% B: 37.10-45 min, 8% B and 20 mL acetic acid. The PDA detector wavelengths were set at 280 and 330 nm, which were used for peak detection and measurement after a 1 h sample time.

Evaluation of antioxidant activity: The evaluation of antioxidant activity of extracts from fruit flesh and seed was carried out using free radical scavenging method[23]. Sample (1 mL) from extracts was mixed with to 2 mL solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in methanol and the mixture was shaken well followed by 30 min incubation at 37°C. The absorbance values were determined at 517 nm using spectrophotometer and the antioxidant activity was quantified using following relation:

$$\text{Antioxidant activity}\ (\%) = \left(1 - \frac{A_1}{A_0}\right) \times 100$$

where, A_0 and A_1 represent the absorbance values as determined for the control and sample extracts, respectively.

Determination of mineral content: Approximately 20 mg of *A. nilotica* fruit flesh or seeds were ashed at 210°C in a burning cup that contained of pure HNO_3(15 mL) and 30% w/v H_2O_2 (2 mL). Followed by filtration using Whatman no. 42 filter paper, the filtrates from digested samples were subjected to a concurrent analysis using ICP-AES Varian-Vista (Varian, Springvale, Australia) and the mineral contents in *A. nilotica* were quantified using standard solutions. According to Skujins method[24], the ICP-AES system was set to work at RF power of 0.7-1.5 kw (1.2-1.3 kw for Axial), viewing height of 5-12 mm, copy and reading time of 3 sec (max 100 sec) and copy time of 1-5 sec (max 60 sec), Plasma gas was used at a flow rate (Ar) of 10.5-15 L min^{-1} (radial), 15" (Axial) and the auxiliary gas flow rate was set at 1.5.

Composition of oil and fatty acid: The analysis for estimating oil composition was carried out using AOAC method[25]. The estimation and quantification of *A. nilotica* flesh and seed oil fatty acid profile was carried out through evaluation of the chromatographic retention times of their methyl esters and comparison with those of standards[26]. The fatty acids in oil samples were first esterified and then injected to agas chromatography (GC) system (5890, Varian). The GC system was equipped with a capillary column (CP-Sil 88) which had 0.25 mm internal diameter, 100 m length and a film thickness of 0.2 μm.

Antimicrobial activity of acacia fruit flesh and seed extracts: The method of Boyanova *et al.*[27] was used to determine the antimicrobial activity of the *A. nilotica* fruit flesh and seed extracts. Pure cultures of indicator microorganisms (*E. coli* ATCC 10536, *S. typhimurium* ATCC 14028, *Yersinia enterocolitica* ATCC 27729, *Klebsiella pneumonia* ATCC 10031, *B. cereus* ATCC 14579 and *S. aureus* ATCC 29737) were grown on nutrient agar plates. Saturated filter papers with *A. nilotica* fruit flesh and seed extracts in discs (10 μg mL^{-1}) were placed on cultures surface and incubated at 37°C for 24 h. Next, the inhibition zones around the discs were determined. Penicillin (10 μg/discs) was used as a standard antibiotic and control.

Statistical analysis: The analysis of the samples were conducted in triplicate and the data were reported as mean±standard deviation (MSTAT-C) of the independent *A. nilotica* samples. Comparisons of the parameter means for each sample were calculated using Student's t-test at $p \leq 0.01$ and 0.05.

RESULTS AND DISCUSSION

Ethnobotanical data and extract yield: The ethnobotanical data (not shown) of *A. nilotica* parts used in Sudan were collected from 200 people using a random survey (females, males, herbalist males and herbalist females). The UV of the plant parts was found to be 1.5 for whole fruit, which indicates that a large proportion of people use the tree fruit for medicinal purposes. However, many of them did not consider using the seeds (UV = 0.5). Generally, a high UV indicates that people are highly likely to disperse information about the medicinal effects and practices using plants. In many regions of Sudan, the commonly used parts included fruit (flesh and seeds) or just the flesh, where the entire fruit would be macerated in different ways and used as oral malaria treatment, the flesh would made into a powder for external use (as liniments or poultices) for treating furuncles, or smoke created from the whole fruit would be inhaled for treating phlegmatic coughing. In other regions, *A. nilotica* fruits are used extensively by fumigation or maceration as an antiseptic and for the cure of different diseases that include cold, flu, pharyngitis, cough, pustular diseases, catarrh, fever, measles and tonsillitis. Crushed fruit flesh is also used for hypertension treatment.

The 500 g dried plant extracts mixed with methanol had EY values of 6.56% from the flesh and 8.87% from the seeds. The seed extracts had higher EY percentages that the flesh extracts. This could be related to the presence of more bioactive compounds in seed than in the flesh.

Bioactive compounds and antioxidant activity of flesh and seed extract: A comparison of phenolic and flavonoid compounds and antioxidant activity between *A. nilotica* flesh and seed extracts is presented in Table 1. The present findings revealed that *A. nilotica* seed and flesh extracts had 16 phenolic compounds that were categorized into four groups; flavones (quercetin, (+)-catechin, naringenin, kaempferol and isorhamnetin), phenolic acids (3,4-dihydroxybenzoic, syringic, caffeic, p-coumaric, gallic, trans-ferulic and trans-cinnamic acids), polyphenols (1,2-dihydroxybenzene and resveratrol) and glycosylated flavonoids (Rutin trihydrate and apigenin-7-glucoside). Among these, the most abundant bioactive compounds in *A. nilotica* flesh and seed extracts were phenolic acids (1580.59 mg/100 g) and flavones (57.53 mg/100 g).

The observed phenolic compound profile from the *A. nilotica* flesh extract had significantly ($p \leq 0.01$) higher phenolic acids, flavones, glycosylated flavonoids and polyphenols than the seed extract. However, significantly ($p \leq 0.01$) higher amounts of gallic acid were found in the seed extract than the flesh extract. Moreover, within the four groups the most dominant phenolic compound in both seed and flesh extracts was (+)-catechin (1524.27 and 32.65 mg/100 g, respectively), whereas gallic acid (1.09 mg/100 g) and 3, 4-dihydroxybenzoic acid (0.14 mg/100 g) exhibited the smallest values in *A. nilotica* flesh and seed extract, respectively.

Table 1: Comparative study of phenolic and flavonoid compounds and antioxidant activity of *Acacia nilotica* fruit flesh (ANFF) and seeds (ANS) methanolic extracts

Parameters	ANFF	ANS	Mean difference
Phenolic compounds (mg/100 g)			
Gallic acid	1.09±0.02	20.51±0.88	-19.42**
3,4-dihydroxybenzoic acid	305.28±6.53	0.14±0.01	305.14**
Syringic acid	233.36±1.67	3.57±0.52	229.79**
Caffeic acid	422.78±4.18	1.58±0.15	421.2**
p-coumaric acid	62.01±0.72	0.53±0.19	61.48**
Trans-ferulic acid	553.26±1.95	2.72±0.48	550.54**
Trans-cinnamic acid	2.81±0.45	0.97±0.16	1.84*
Phenolic acids	1580.59±2.87	30.02±0.96	1550.57**
(+)-Catechin	1524.27±8.68	32.65±0.31	1491.62**
Naringenin	4.33±0.56	1.96±0.27	2.37**
Kaempferol	5.39±0.29	3.51±0.28	1.88**
Isorhamnetin	5.48±0.33	5.28±0.51	0.20
Quercetin	20.80±0.37	14.13±0.27	6.67**
Flavones	1560.27±2.18	57.53±0.85	1502.74**
Rutin trihydrate	178.06±1.23	6.59±0.48	171.47**
Apigenin 7 glucoside	159.02±1.82	13.50±0.25	145.52**
Glycosylated flavonoids	337.08±2.22	20.09±0.49	316.99**
1,2-dihydroxybenzene	808.84±6.94	11.05±0.19	797.79**
Resveratrol	14.64±0.87	4.29±0.26	10.35**
Polyphenols	823.48±0.99	15.34±0.78	808.14**
Antioxidant activity (%)	89.88±1.02	92.63±0.93	-2.75*

Values are Mean±SD of 3 samples, **$p \leq 0.01$, *$p \leq 0.05$

Acacia nilotica seed extract had significantly higher ($p \leq 0.01$) antioxidant activity (92.63%) than that of the flesh extract (89.88%). The values obtained for both flesh and seeds were higher than the range of antioxidant activity (39.16-66.67%) reported in leaves, flowers and seed flesh obtained from three *Acacia* species[28]. The difference in antioxidant activity between flesh and seed extracts in this study could be attributed to variation in the ability of flesh or seed phenolic compounds to scavenge the DPPH free radical. The high antioxidant activities of both the flesh and seeds make them potentially suitable plant materials for applications as natural antioxidants. Moreover, the explanation for the difference in antioxidant activity between the two extracts may be related with the differences in composition and quantities of phenolics in these samples. These possible chemical differences could lead to different responses to low concentrations of phenolics, which were not below concentrations measured in this study.

Table 2: Minerals contents (mg/100 g) of *Acacia nilotica* fruit flesh (ANFF) and seed (ANS) powders

Minerals	ANFF	ANS	Mean difference
Macro-minerals			
Ca	6297.31±5.09	5355.01±8.96	942.30**
K	13121.01±15.61	13157.82±13.41	-36.81*
Mg	557.61±3.95	2813.72±18.33	-2256.11**
P	778.32±4.37	2675.74±8.38	-1897.42**
S	527.01±2.48	11409.31±6.59	-10882.3**
Micro-minerals			
Zn	11.31±0.67	33.22±1.08	-21.91**
B	10.02±0.75	42.33±0.69	-32.31**
Cu	5.22±0.79	5.51±0.23	-0.29
Fe	107.83±1.19	49.62±0.83	58.21**
Mn	7.14±0.53	27.84±0.97	-20.7**

Values are Mean±SD of 3 samples, **p≤0.01, *p≤0.05

Table 3: Oil and fatty acid composition (%) of *Acacia nilotica* fruit flesh (ANFF) and seeds (ANS)

Oil/fatty acids	ANFF	ANS	Mean difference
Oil	1.80±0.43	8.12±0.76	-6.32**
Saturated			
Myristic (C14:0)	0.17±0.01	0.11±0.01	0.06**
Palmitic (C16:0)	12.15±0.43	13.29±0.42	-1.14*
Stearic (C18:0)	7.18±0.32	4.15±0.31	3.03**
Arachidic (C20:0)	1.300±0.01	0.02±0.001	1.28**
Behenic (C22:0)	1.61±0.12	0.01±0.001	1.6**
Monounsaturated			
Oleic (C18:1)	41.99±0.87	27.17±0.91	14.82**
Elaidic (trans Δ9-C18:1)	0.10±0.001	0.01±0.001	0.09**
Polyunsaturated			
Linoleic (C18:2)	34.17±0.68	54.48±0.84	-20.31**
Linolelaidic (trans Δ9, 12-C18:2)	0.00±0.00	0.01±0.001	-0.01**
Linolenic (C18:3)	0.19±0.02	0.14±0.03	0.05
Arachidonic (C20:4)	0.31±0.18	0.01±0.001	0.30
SFA	22.41±0.84	17.58±0.39	4.83*
MUFA	42.09±0.65	27.18±0.46	14.91**
PUFA	34.67±0.93	54.64±0.52	-19.97**
U/S	3.43±0.48	4.65±0.38	-1.22

Values are Mean±SD of 3 samples, **p≤0.01, *p≤0.05, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, U/S: Ratio of unsaturated fatty acids to saturated fatty acids

The phytochemicals already detected in *Acacia* species and the phenolic compounds detected in the current study are attributed to carry antimicrobial and antioxidant activities. The (+)-catechin, the most abundant phenolic compound in both *Acacia* flesh and seed extracts, have been observed to carry antioxidant and antimicrobial activities comparable to common synthetic chemicals used as antioxidants and antimicrobials[18,29,30].

Minerals content of flesh and seed powder: Plants are the main sources of minerals required in human nutrition. Table 2 shows the comparison of mineral content between *A. nilotica* flesh and seed powder. The results showed that potassium and iron are the predominant macro and micro minerals, respectively, in both flesh and seed powder. The high potassium content agrees with the findings of Embaby and Rayan[31] and Vijayakumari *et al.*[32] for seed samples from different *Acacia* species. The *A. nilotica* seed powder had significantly (either p≤0.05 or p≤0.01) higher macro and micro minerals than those in the flesh powder, with the exception of calcium and iron, which were significantly (p≤0.01) higher in the flesh powder.

The mineral content of both *A. nilotica* flesh and seeds were higher than found in *Acacia* species reported by Aganga *et al.*[33] and *Acaciatortilis* seeds reported by Embaby and Rayan[31]. The differences may be due to varietal differences, geographical location, fruit maturity, or methods used for the analyses. Based on these results, *Acacia* flesh and seed extracts are a good source of minerals. Some of the commercial baking flours lower contents of one or more elements; hence they might be fortified with *Acacia* seed flour for enhancing their nutritional status.

Oil and fatty acid composition of flesh and seed powder: The data presented in Table 3 demonstrate that *A. nilotica* seeds had significantly (p≤0.05) higher oil and protein content than did the flesh. The seeds oil content was comparable to that reported for *A. tortilis* seeds[31] and *Acacia tumida*[34] and *Acacia cyclops*, *Acacia cyanophylla* and *Acacia mollissima* reported by Jelassi *et al.*[35]. The relative higher oil contents in *A. nilotica* seed may qualify it as good energy source.

The physico-chemical characteristics and nutritional attributes of oils might be related with the fatty acids' contents and types and their location on the glycerol moiety. A comparison of the oil fatty acid contents from *A. nilotica* flesh and seeds is shown in Table 3. An analysis of the oil from the samples found 11 fatty acids, 5 being saturated (SFA) and 6 were unsaturated and being either monounsaturated (MUFA) or polyunsaturated (PUFA). *Acacia nilotica* flesh has a greater amount of saturated fatty acids (22.41%) than that of the seeds (17.58%), with palmitic and stearic acids being the predominant SFA in both flesh and seeds.

Similar trends were observed in the MUFA content and it should be noted that oleic acid (41.99%) was the major fatty acid identified in *A. nilotica* flesh. However, analysis of the unsaturated fatty acids showed that oils from the seeds are rich in PUFA (54.64%) as compared with the flesh (34.67%). The most abundant unsaturated fatty acid detected in the seeds was linoleic acid (54.48%) which significantly (p≤0.01) higher than that of the flesh (34.17%). The PUFA values reported in this study for both flesh and seeds are lower than

Table 4: Antimicrobial activity of *Acacia nilotica* fruit flesh (ANFF) and seed (ANS) methanolic extracts compared to penicillin

	Inhibition zone (mm)		
Bacterial strains	ANFF (10 µg µL^{-1})	ANS (10 µg µL^{-1})	Penicillin (10 µg µL^{-1})
Escherichia coli ATCC 10536	18.7±0.23^{c}	20.0±0.15^{b}	32.0±0.09^{a}
Salmonella typhimurium ATCC 14028	12.4±0.12^{b}	10.0±0.05^{c}	19.0±0.13^{a}
Yersinia enterocolitica ATCC 27729	7.3±0.41^{b}	6.0±0.02^{c}	12.0±0.04^{a}
Klebsiella pneumoniae ATCC 10031	7.5±0.32^{b}	6.0±0.05^{c}	16.0±0.08^{a}
Bacillus cereus ATCC 14579	10.4±0.21^{c}	12.2±0.07^{b}	18.0±0.18^{a}
Staphylococcus aureus ATCC 29737	17.6±0.10^{c}	19.7±0.11^{b}	24.0±0.12^{a}

Values presented as Mean±SD of 3 samples, means not sharing a common superscript(s) a and b in a row are significantly different at $p \leq 0.05$ as assessed by Duncan's multiple range test

those found for *A. cyclops* and *A. mollissima*[36]. However, the results of *A. nilotica* seeds in the present study showed higher PUFA than that of *A. cyanophylla* reported by Akhtar *et al*.[37]. Also, flesh oleic acid was greater than that found in the seeds of most *Acacia* species, such as *A. cyclops*, *A. cyanophylla* and *A. mollissima*[34,37] and was comparable to values reported by Jelassi *et al*.[35].

Acacia nilotica oils demonstrated a unsaturated/saturated (U/S) ratio of 3.43 for flesh and 4.65 for seeds, with the seeds being superior to other oilseeds, such as soybean oil (3.69)[37]. In addition, the U/S ratios of both *A. nilotica* flesh and seeds were higher than *A. cyanophylla* (1.91). A higher U/S ratio contributes favorably in the reduction of serum cholesterol and the prevention of atherosclerosis and heart diseases[38].

The high oleic acid content in *A. nilotica* flesh is desirable because of its significance in nerve cell construction and in the prevention of heart related problems[37]. Both flesh and seeds are rich in PUFA and the high linoleic acid in *Acacia* species seeds is important as it is needed for human growth and health[39]. The results of this study indicated that *A. nilotica* is a promising oilseed with high levels of unsaturated fatty acids, which makes them desirable as a nutritional oil source that could be potentially used as an oil source for human diets.

Antimicrobial activity of flesh and seed extracts: In the present study the effect of flesh and seed extracts of *A. nilotica* on Gram-negative (*E. coli*, *S. typhimurium*, *Y. enterocolitica* and *K. pneumonia*) and Gram-positive (*B. cereus* and *S. aureus*) bacteria was evaluated and compared the results with penicillin, which was used as an antibiotic control. As presented in Table 4, *A. nilotica* extracts (flesh and seed) affected the pathogenic bacteria in significantly ($p \leq 0.05$) different way for both Gram-negative and Gram-positive, with seeds having inhibition zone comparable to that of the flesh. However, flesh had a larger inhibition zone for the Gram-negative bacteria, with the exception of *E. coli* ATCC 10536, than the seeds. Both extracts had significantly ($p \leq 0.05$) lower inhibition zones compared to penicillin for both Gram-positive and gram-negative bacteria. The high antimicrobial potential of the *A. nilotica* seeds extract, compared to the flesh extract, could be attributed to seed phenolic and mineral contents, especially sulfur, which has been reported to be used as a remedy of some diseases[40] as well as the fatty acid composition. Shan *et al*.[4] and Nakamura and Hatanaka[41] reported that gram-positive bacteria were more susceptible to antibacterial as compared to Gram-negative ones. This variation in susceptibility could be attributed to the resistance of the outer membrane of Gram-negative bacteria, which contains high amounts of lipopolysaccharide molecules, which is a buffer against different antibiotic molecules. Moreover, the outer membrane of Gram-negative bacteria also contains perivascular enzymes that can breakdown molecules crossing into a cell. The cell walls and the cytoplasmic membranes of some bacteria can still be destroyed by some antibiotics, resulting in the cytoplasm being released[4]. Mahesh and Satish[13] have shown high antibacterial and antifungal activities of leaf and bark extracts from other *Acacia* species. Thus, the high antibacterial activity of flesh and seeds extracts of *A. nilotica* we found indicates that it can be a useful ingredient for prolonging the shelf-life of foods and can be used as remedy for microbial poisonings.

CONCLUSION

The seed extract of *Acacia* exhibited significantly higher antioxidant activity, which indicates that bioactive compounds in it had increased antioxidant activity. The high antioxidant activity, plus the high levels of minerals and fatty acids, in the seed extract indicate its inhibitory effects against gram-negative and gram-positive bacteria. The present findings suggested that the flesh and seeds of *A. nilotica* are excellent sources of bioactive compounds, with the seeds having superior results and can be exploited as potential sources of healthier antioxidants.

SIGNIFICANCE STATEMENT

This study discovers the flesh and seeds extracts of *Acacia nilotica* that can be beneficial as natural antioxidants. This study will help the researcher to uncover the critical areas of diseases remedy that many researchers were not able to explore. Thus a new theory on such extracts may be arrived at.

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