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Phytochemicals, polyphenols content, in vitro antioxidant and antibacterial activities of *Albizia coriaria* Welw ex. Oliver flowers

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Author contributions

Timothy Omara was responsible for sample collection, methodology, sample and data analyses, and original draft writing; Ambrose K. Kiprop and Viola J. Kosgei were responsible for reviewing, editing, and supervision.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

A. coriaria, *Albizia coriaria* Welw ex. Oliver; TFC, total flavonoid content; DPPH, 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl; IC_{50}, half-maximal inhibitory concentration; ZOI, zone of inhibition; TPC, total polyphenolic content.

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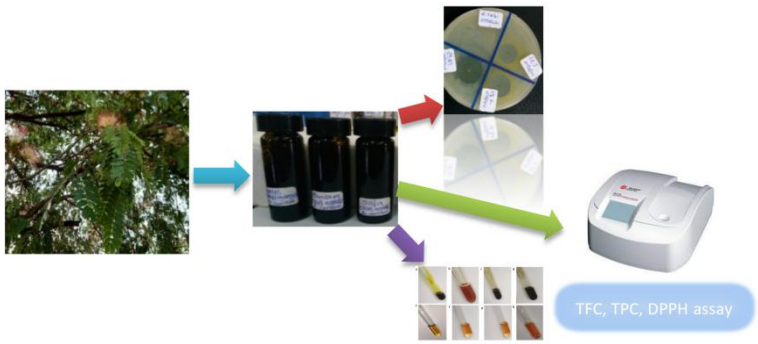
Abstract

Albizia coriaria Welw ex. Oliver (hereafter *A. coriaria*) is a revered medicinal tree whose organs are used by indigenous African societies for managing various ailments. Herein, we assessed the secondary metabolites, total polyphenolics, free radical scavenging and antimicrobial activities of *A. coriaria* flowers. The dry powdered sample was extracted serially with ethyl acetate, ethanol and distilled water. Results of classical phytochemical screening showed that saponins, phenols, alkaloids, flavonoids, tannins and terpenes were the dominant phytochemicals. The highest total polyphenolic content (10.37 ± 0.02 mg QE/g DW and 72.09 ± 0.09 mg GAE/g DW) and free radical scavenging potential (IC_{50} = 24.23 ± 0.04 mg/mL) were for the ethyl acetate extract. It also had a higher bacteriostatic activity than the ethanolic extract against *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* with diameters of inhibition ranging from 7.00 ± 0.00 to 22.00 ± 1.73 mm. The aqueous extract showed no appreciable antibacterial effect on the tested microorganisms. This is the first report which support the use of floral extracts of this species in the management of ailments in African traditional medicine.

Keywords: bacteriostatic; *Albizia coriaria*; DPPH assay; traditional medicine; oxidative stress



Highlights

For the first time, this study reports on the phytochemicals, polyphenols content, free-radical scavenging and antibacterial potential of *Albizia coriaria* floral extracts.

Medical history of objective

Albizia coriaria is a medicinal plant used across African communities for management of human and veterinary ailments. Its various parts are used for treating complex medical conditions such as ophidian bites, lameness (Butenge), meat allergy, barrenness (*Atengina ekolupana* in cows), bacterial and oxidative stress-mediated diseases.

Background

Challenges posed by multidrug-resistant pathogens, side effects and inaccessibility of conventional drugs have re-ignited interest in the utilization of natural products in primary health care [1]. As such, intensive research has focussed on the documentation of traditionally used natural products, their chemical composition, pharmacological activities and structure-bioactivity relationships. On the other hand, reactive oxygen and nitrogen species owing to their high reactivity induce oxidative stress, causing destructive and irreversible damages to cellular components including DNA, proteins, lipids and lipoproteins [2]. These events are known to trigger the onset of diseases and medical conditions such as malignancies, inflammation, reperfusion injury, cardiovascular dysfunction, cirrhosis, atherosclerosis, arthritis, diabetes and emphysema [3, 4]. Commercial antioxidants have been used to scavenge free radicals in the human body [5]. Plant materials possess a host of exogenous antioxidant complexes (inform of phytochemicals) that can boost endogenous antioxidant defences of the body [6]. This could explain the widespread use of herbal remedies in the treatment of oxidative stress-mediated complications [7, 8].

Albizia coriaria Welw ex. Oliver (*A. coriaria*) is a widely used medicinal tree in Africa. Its flowers, whole plant, seeds, roots, leaves, stem and root barks are employed by indigenous communities for prophylaxis and management of cancers, oral, digestive, skin, respiratory and venereal ailments [9]. Though the extracts from leaves, root and stem barks have been shown to exhibit some pharmacological actions supporting the traditional claims of using of *A. coriaria* [10–23], there is hardly any study pertaining to the phytochemicals and bioactivity of its flowers [9]. Herein, a study was carried out to establish the main secondary metabolites, total phenolic and flavonoid contents, free radical scavenging and antibacterial potential of floral extracts of *A. coriaria*.

Methods

Analytical grade reagents, ciprofloxacin and bacteria (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhi* 14028) were those used in our previous study [23], and were generously supplied by Centrihex Limited and Kobian Kenya Limited, Nairobi, Kenya.

Sampling and extraction procedure

A. coriaria flowers (2 kg) were collected from a flowering tree near Kyabazinga Way, Jinja, Uganda (0°26'34.3"N 33°12'27.3"E) [23] on Monday 8th February 2021. The sample was authenticated and a specimen (voucher No. 50997) was deposited at Makerere University Herbarium, Kampala, Uganda. The analytical sample was cleaned using water and then dried under shade for 21 days. The sample was then pulverized and 250 g of the powder was extracted serially by maceration in 500 mL of ethyl acetate, ethanol and distilled water for 2 days with intermittent agitation. The floral extracts were thereafter filtered. The organic solvent extracts were rotary evaporated under reduced pressure whereas the aqueous extract was freeze-dried [23].

Classical screening for secondary metabolites in the floral extracts

The crude extracts obtained were redissolved in ethyl acetate, water and ethanol and subjected to phytochemical screening [24], the results of which were expressed as abundant, moderate, traces or absent based on colour or foam intensities [23, 25, 26].

Quantification of total polyphenols in *A. coriaria* floral extracts

Spectrophotometric measurement of total phenolics followed the Folin-Ciocalteau method [27], as previously used with some modifications [23]. Briefly, 0.5 mL of 0.01 mg/mL of the extracts prepared using distilled in sample vials were added to 2.5 mL of Folin-Ciocalteau reagent and left for 7 minutes. Thereafter, 2.5 mL of 6% sodium carbonate was added to the vials and incubated in the dark. After 0.5 hours, the absorbances of the solutions were read at 725 nm on an ultraviolet-visible spectrophotometer (Beckham Coulter DU 720, Brea, CA, USA). The total phenolic contents (in mg gallic acid equivalents per gram of dry weight, mg GAE/g DW) were computed from a calibration curve prepared using absorbances of standard gallic acid solutions (10 to 80 mg/mL) [23].

Total flavonoids was established using the aluminum chloride method [28] with modifications [23, 29]. Methanolic solutions of the extracts (0.01 g in 5 mL) and standard quercetin solutions (5 to 100 ppm) were prepared and 0.6 mL of each were individually added to 0.6 mL of 2% methanolic aluminum chloride solution. After incubation for 60 minutes, the absorbances were read with methanol as a blank at 420 nm. The total flavonoid content (TFC) was computed from the quercetin calibration curve and reported in milligram quercetin equivalents per gram dry weight (mg QE/g DW) [23].

Total in vitro antioxidant activity of the floral extracts

Antioxidant potential of the floral extracts were determined using the 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) assay [30] with some modifications [23]. Pipetted 75.0 µL of 1.3 mg/mL methanolic DPPH solution was transferred to test tubes with 200 µL of 10 to 60 µg/mL methanolic solutions of the extracts and ascorbic acid standard. The absorbances of the set up at 517 nm were read after incubation for 0.5 hours. The half-maximal inhibitory concentration (IC_{50}) was computed from a plot of the percentage inhibition and concentration [23, 30].

Antimicrobial activity of *A. coriaria* floral extracts

The crude extracts were assessed for their inhibitory activity on *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* using culture-based agar disc diffusion assay as reported by Omara et al. [23]. Test extracts at 500 µg/mL were prepared in dimethyl sulfoxide and used to saturate paper discs which were subsequently air-dried. Bacterial cultures (1×10^8 CFU/mL) were subcultured onto prepared Muller Hinton broth in petri dishes. The impregnated discs were transferred carefully onto each plate in triangular formation, along with dimethyl sulfoxide and ciprofloxacin serving as negative control and positive controls, respectively. The plates were incubated at 37 ℃ for 24 hours after which the zone of inhibition (ZOI) was measured [23]. The minimum inhibitory and minimum bactericidal concentrations were determined for bacteria with ZOI > 12 mm after antibacterial screening following procedures used by previous authors [23, 31].

Statistical analysis

All analytical works were replicated at least once, and the data obtained were captured in Microsoft excel. The means of quantitative data were analyzed using one-way ANOVA followed by Tukey's post hoc test at 99% confidence interval. Further, Pearson's bivariate correlation was performed to establish any correlations between total polyphenolic content (TPC and TFC) and the IC_{50} values of the floral extracts. All analyses were executed using GraphPad Prism statistical software (v9.1.0, GraphPad Software, San Diego, CA, USA).

Results

Percentage yield of the floral extracts

The extraction yields of the extracts are shown in Figure 1. Ethyl acetate extract had the highest yield (5.00 ± 0.06%) vis-à-vis ethanol extract (2.61 ± 0.04%) and aqueous extract (1.11 ± 0.06%). One-way ANOVA confirmed that the yields differed significantly for the three solvents ($P < 0.01$).

Secondary metabolites in the floral extracts

Phenols, alkaloids, flavonoids, terpenes, saponins and tannins were the main secondary metabolites detected (Table 1). However, volatile oils, cardiac glycosides, steroids and quinones were absent. Alkaloids and flavonoids were not detected in the aqueous extract. Similarly, terpenes and tannins were absent in the ethanolic extract.

TPC of the floral extracts

The total phenolic content of the flower extracts was computed from gallic acid curve. The TPC followed the order: ethyl acetate (72.09 ± 0.09 mg GAE/g DW) > ethanol (21.30 ± 0.26 mg GAE/g DW) > aqueous (15.40 ± 0.29 mg GAE/g DW) (Table 2), with statistically significant differences as attested to by one-way ANOVA results ($P < 0.01$). Similarly, the TFC of the extracts established from the standard quercetin curve followed the sequence: ethyl acetate extract (10.37 ± 0.02 mg QE/g DW) > ethanolic extract (6.49 ± 0.10 mg QE/g DW) > aqueous extract (3.31 ± 0.05 mg QE/g DW).

Total in vitro antioxidant activity of *A. coriaria* floral extracts

In this study, ethyl acetate extract of *A. coriaria* flowers had the lowest IC_{50} value of 24.23 ± 0.04 mg/mL, followed by ethanolic extract (IC_{50} = 40.37 ± 0.19 mg/mL) and then aqueous extract (IC_{50} = 90.79 ± 0.16 mg/mL) (Table 3). However, the IC_{50} value of the standard antioxidant (ascorbic acid, IC_{50} = 0.17 ± 0.01 mg/mL) was lower than that of all the extracts. Statistical evaluation using one-way ANOVA indicated that significant differences ($P < 0.01$) existed between the IC_{50} values of the extracts.

Correlation between TPC and half maximal inhibitory concentration of *A. coriaria* floral extracts

Correlative analysis between the TPC and TFC indicated a positive correlation while only negative correlations were observed between TPC and IC_{50} values, and TFC and IC_{50} values obtained in DPPH assays (Table 4).

Antibacterial activity results

Only organic solvent extracts exerted bacteriostatic effect on the tested bacteria, with ethyl acetate extract having the highest ZOI of 22.00 ± 1.73 mm (Table 5). In regards to MIC and MBC, *Escherichia coli* had the lowest MIC (250 µg/mL) for the ethyl acetate extract compared to that of *Pseudomonas aeruginosa* (500 µg/mL). The MBC of the ethyl acetate extract was 500 µg/mL and greater than 500 µg/mL against *Escherichia coli* and *Pseudomonas aeruginosa*.

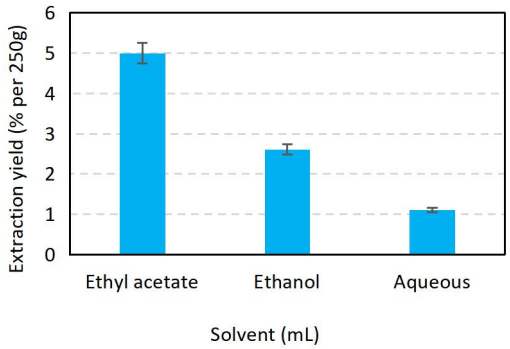


Figure 1 Percentage yield of different solvent extracts of *Albizia coriaria* Welw ex. Oliver flowers

Table 1 Phytochemicals screened in the organic solvent and aqueous floral extracts of *A. coriaria* sampled from Jinja, Uganda

Metabolite	Solution[1]	Result[2]
Phenols	EtAC	+ +
	EtOH	+
	AQ	+
Alkaloids	EtAC	+ + +
	EtOH	+ +
	AQ	−
Cardiac glycosides	EtAC	−
	EtOH	−
	AQ	−
Flavonoids	EtAC	+
	EtOH	+ +
	AQ	−
Saponins	EtAC	+
	EtOH	+ +
	AQ	+
Quinones	EtAC	−
	EtOH	−
	AQ	−
Steroids	EtAC	−
	EtOH	−
	AQ	−
Volatile oils	EtAC	−
	EtOH	−
	AQ	−
Terpenes	EtAC	+ +
	EtOH	−
	AQ	+
Tannins	EtAC	+
	EtOH	−
	AQ	+ +

[1]Extracts: EtAC = ethyl acetate, EtOH = ethanol, AQ = aqueous. [2]The symbols: $^{+++}$, $^{++}$, $^{+}$, and $^{-}$ denotes abundant, moderate, traces and absent, respectively. *A. coriaria*, *Albizia coriaria* Welw ex. Oliver.

Table 2 TPC of extracts of *A. coriaria* flowers from Jinja, Uganda

Extract	Total phenolic content (mg GAE/g DW)	Total flavonoid content (mg QE/g DW)
Ethyl acetate	72.09 ± 0.09	10.37 ± 0.02
Ethanolic	21.30 ± 0.26	6.49 ± 0.10
Aqueous	15.40 ± 0.29	3.31 ± 0.05

Results are presented as means ± standard deviations of triplicates. *A. coriaria*, *Albizia coriaria* Welw ex. Oliver; TPC, total polyphenolic content.

Table 3 DPPH results for inhibition concentration at 50% of *A. coriaria* flowers

Extract/standard	IC_{50} (mg/mL)
Ethyl acetate	24.23 ± 0.04
Ethanolic	40.37 ± 0.19
Aqueous	90.79 ± 0.16
Ascorbic acid	0.17 ± 0.01

Results are presented as means ± standard deviations of triplicates. *A. coriaria*, *Albizia coriaria* Welw ex. Oliver; DPPH, 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl; IC_{50}, half-maximal inhibitory concentration.

Table 4 Pearson's bivariate correlation coefficients between TPC and IC_{50} values of *A. coriaria* floral extracts

Parameter	Total flavonoid content	Antioxidant activity
Total phenolic content	R = 0.932, P = 0.118	R = −0.753, P = 0.457
Total flavonoid content	1.000	R = −0.941, P = 0.220

A. coriaria, *Albizia coriaria* Welw ex. Oliver; TPC, total polyphenolic content; IC_{50}, half-maximal inhibitory concentration.

Table 5 Mean diameters of inhibition by the floral extracts

Test solution	*Escherichia coli* (mm)[1]	*Staphylococcus aureus* (mm)	*Pseudomonas aeruginosa* (mm)	*Salmonella typhi* (mm)
Ethyl acetate extract	22.00 ± 1.73	11.00 ± 2.65	19.00 ± 2.10	7.00 ± 0.00
Ethanolic extract	8.00 ± 1.73	3.00 ± 1.00	9.00 ± 2.65	5.00 ± 0.02
Aqueous extract	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ciprofloxacin (positive control)	14.00 ± 2.10	12.00 ± 0.01	31.00 ± 0.11	20.00 ± 1.53

[1]Results are average ± standard error of three experiments. Dimethyl sulfoxide did not inhibit bacterial growth.

Discussion

Extraction of phytochemicals from *A. coriaria* flowers was done by maceration and ethyl acetate had the highest yield (5.00 ± 0.06%) vis-à-vis ethanol (2.61 ± 0.04%) and distilled water (1.11 ± 0.06%) (Figure 1). The yields obtained were comparable to 2.98% to 5.98% for *A. coriaria* leaf extracts [23] but lower than 8.3% to 12.7%, 11.9% and 10.88% for organic solvent extracts of its stem bark [31, 32] and leaves [23]. Statistical evaluation results indicated that the yields differed significantly for the three solvents (P < 0.01), implying that ethyl acetate was the best solvent for recovering bioactive compounds from the flowers. The low yield of aqueous extract is most likely due to the effect of serial extraction i.e., most bioactive compounds had already been extracted by the organic solvents. Nevertheless, the results attested to the fact that *A. coriaria* flowers possesses both polar and non-polar phytochemicals, which is concordant with reports on its leaves [23] and stem bark extracts [15, 31, 32]. On the other hand, phenols, alkaloids, flavonoids, terpenes, saponins and tannins were the main secondary metabolites detected (Table 1). These groups of secondary metabolites were previously detected in *A. coriaria* stem bark and leaves [14, 17, 20, 22, 23, 33, 34] and floral extracts of *Albizia lebbeck* (L.) Benth [35–37].

In regards to polyphenols, previous studies [15, 23] reported that TPC of ethanolic extracts of *A. coriaria* leaves (67.04 to 101.72 mg GAE/g DW) and stem bark (28.37 mg CAE/g E) were averagely higher than those of their corresponding ethyl acetate extracts (10.93 to 60.69 mg GAE/g DW and 28.36 mg CAE/g E). A plausible explanation for the higher TPC of the ethyl acetate extract than the ethanolic extract in this study could be due to extraction of majority of the polyphenols in the flower powder being non-polar and thus were extracted more by ethyl acetate than ethanol and water. In the case of water, the lower TPC could be because most of the polar polyphenols were already extracted by ethanol which is relatively polar. In addition, water is also reported to extract even non-active compounds in plant matrices, which may not constitute the TPC [23, 38]. In the same way, the TFC of the extracts followed the sequence: ethyl acetate extract > ethanolic extract > aqueous extract (Table 2). The results indicated that *A. coriaria* flowers have more flavonoid aglicones [39]. Omara et al. [23] reported that ethanolic extracts of *A. coriaria* leaves housed the highest quantity of total flavonoids (8.63 to 13.23 mg QE/g DW) vis-à-vis the ethyl acetate extracts (0.55 to 9.66 mg QE/g DW) and aqueous extracts (2.74 to 3.36 mg QE/g DW). As highlighted for the TPC, lower TFC of the aqueous extracts is reasonably due to the initial extraction of most flavonoids by the organic solvents. It is worth noting the TFC of the extracts were lower than their TPCs, corroborating the fact that most flavonoids are in essence phenolics [40]. Generally, extraction of polyphenols from plant matrices are influenced by the nature of the polyphenols, the extraction solvents' polarity, time and temperature used [39]. This explains, in part, the observed disparities in the polyphenolic content of the floral extracts.

Plant extracts elicit antioxidant activity in vitro owing to their reducing, chelating or oxygen quenching activities [41]. Antioxidants are compounds with characteristic potential to delay or inhibit oxidative damage to other molecules. This is primarily through trapping of free radicals. Phytocompounds such as polyphenols and flavonoids abundant in plants are known to scavenge free radicals, including hydroperoxides and peroxides [41]. In this study, ethyl acetate extract had the lowest IC_{50} value, followed by ethanolic and aqueous extracts (Table 3). These differences in the antioxidant potential may be attributed to the variations in the bioactive compounds in the extracts owing to the differences in the extracting power of the solvents used. In comparison with previous reports, the antioxidant potential obtained herein surpassed those reported for organic extracts of *A. coriaria* stem bark (with half effective concentrations of 0.02298 mg/mL and 0.01839 mg/mL) [15] but comparatively lower than those of organic solvent and aqueous extracts of *A. coriaria* leaves (IC_{50} = 18.65 to 29.66 mg/mL) [23]. The lower free radical scavenging activity (highest IC_{50} value) of aqueous extracts is because water solvates bioactive compounds along with inactive phytochemicals in matrices which may solely act as matrix interferences [38].

Pearson's correlative analysis between the TPC and TFC indicated a positive correlation (R = 0.932, P = 0.118). This is arguably owing to the chemistry that polyphenolic compounds are constituted by both flavonoid- and non-flavonoid compounds [23, 26, 42]. Further correlative analysis between the TPC and IC_{50} values gave a negative association (R = −0.753, P = 0.457), which was also observed between TFC and IC_{50} values (R = −0.941, P = 0.220). This result is concordant with a preceding report which observed poor and/or negative correlation between TPC or TFC and the corresponding free readical scavenging potential (EC_{50} and IC_{50} values) [15, 23]. Overall, poor and/or negative correlations between IC_{50} values of the extracts, and their TPC or TFC explains the chemistry that reduction in the DPPH radical progresses with decrement in scavenging action of the extracts. This suggests that polyphenols in the extracts are responsible for their DPPH radical scavenging potential [26, 42, 43].

In the antibacterial assay, only organic solvent extracts exerted bacteriostatic effect on the pathogens (Table 5). Based on Clinical and Laboratory Standards Institute Interpretive Criteria [44], only *Escherichia coli* and *Staphylococcus aureus* were susceptible to the ethyl acetate extract as their ZOI diameters lied within the one for conventional antibacterial drugs such as doxycycline, ampicillin and tetracycline. The results of antibacterial screening are similar to a previous observation wherein the ethyl acetate extract of *A. coriaria* stem bark elicited a higher bacteriostatic action against pathogenic bacteria vis-à-vis the methanolic extract [18]. However, Omara et al. [23] and Schultz et al. [15] reported higher bacteriostatic potency of ethanol extracts of *A. coriaria* leaves and stem bark than the corresponding ethyl acetate extracts. Interestingly, the aqueous extract in this study was inactive on all the tested pathogens, corroborating previous observations using the aqueous stem bark and leaf extracts of *A. coriaria* [18, 20, 23]. The secondary metabolites detected in the organic solvent extracts of *A. coriaria* flowers could be implicated for their bacteriostatic activities. Phytochemicals such as tannins, alkaloids, saponins, flavonoids and phenols are known antibacterial agents, either directly or through microbial proteins precipitation, intercalating microbial DNA or targeted deactivation of nucleic acid enzymes [45–47]. The bacteriostatic activities were in

correlation with phytochemical richness of the extracts. For instance, the extract obtained from maceration using ethyl acetate housed the highest levels of alkaloids, phenols, flavonoids and terpenes vis-à-vis other extracts (Table 1).

The lowest MIC was observed for *Escherichia coli* (250 µg/mL) while the MIC for *Pseudomonas aeruginosa* was 500 µg/mL. A previous report [18] reported that ethyl acetate extract of *A. coriaria* stem bark had MIC of 125 mg/mL for *Escherichia coli* and 250 mg/mL for *Pseudomonas aeruginosa*. A team investigating ethanolic extracts of *A. coriaria* stem bark had MIC > 256 µg/mL for *Pseudomonas aeruginosa* [19] while the same extract and its ethyl acetate extract had MIC of 250 and 500 µg/mL against *Staphylococcus aureus* and *Escherichia coli* [15] which are comparable to the MIC values obtained in this study. Omara et al. recently reported MIC values of 62.5–250 µg/mL for ethanol leaf extracts of this species against *Pseudomonas aeruginosa* [23].

The MBC of the ethyl acetate extract was 500 µg/mL and greater than 500 µg/mL against *Escherichia coli* and *Pseudomonas aeruginosa*. These were higher than the MBC of 125–250 µg/mL reported for the ethanolic extract of the leaves of *A. coriaria* against *Pseudomonas aeruginosa* [23]. A high MBC of 125 and >500 mg/mL were indicated for ethyl acetate stem bark extract of this species against *Escherichia coli* and *Pseudomonas aeruginosa*, respectively [18]. Overall, such higher minimum inhibitory and bactericidal concentrations emphasize that larger quantities of the floral extracts are necessary to traditionally treat the bacterial infections. This also reflected in the posology of this species as prescribed by herbalists in Uganda [48, 49].

Conclusion

The results obtained in this study demonstrated that *A. coriaria* flowers houses phytocompounds with free radical scavenging and bactericidal activities, which is in support of its traditional utilization in the treatment of ailments in Africa. In this context, further studies assessing the toxicity, structure and bioactivity of the phytocompounds are required.

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