

Antioxidant and antibacterial activity studies of the stem-bark and leaf extracts of Nigerian *Antiaris toxicaria* Lesch

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Abstract

A tropical medicinal plant *Antiaris toxicaria* Lesch, has been used in the treatment and management of various diseases. *In-vitro* antioxidant and antibacterial activity of ethanol, ethyl acetate and n-hexane leaf and stem-bark extracts of *A. toxicaria* were investigated. Results showed that total phenolic content expressed as gallic acid equivalents ranges from 42.88 ± 0.16 to 337.57 ± 0.09 mg g⁻¹ of dry weight of the extracts while the flavonoids contents varied from 98.38 ± 0.29 to 233.34 ± 0.43 mg g⁻¹ expressed as quercetin equivalents for all extracts tested. The extracts exhibited concentration and solvent polarity dependent DPPH radical scavenging activity comparable to the standard ascorbic acid used. 500 µg mL⁻¹ ethanolic extract of the stem bark of *A. toxicaria* inhibited radicals up to 87.50 % and IC₅₀ value of 5.98 µg mL⁻¹ was recorded. Antimicrobial susceptibility was observed with all extracts tested at 100 µg mL⁻¹ against the four Gram-negative and two Gram-positive strains. However, the ethyl acetate extract was most active against sensitive and resistant Gram-positive *staphylococcus aureus* with zone of inhibition of 30mm. This study reveals that *A. toxicaria* may be a potential natural source of antioxidants in the management of bacterial related diseases.

Keywords: *Antiaris toxicaria*, antioxidant, antibacterial, ethnomedicine.

Introduction

Over the years, continuous and enormous research has been conducted on medicinal plants for the exploration of natural resources which exhibit antioxidant and antibacterial properties to serve as a safer source for the management and treatment of various ailments with little or no side

effects. Approximately 80% of the world populace relies on various medicinal plants for healing and treatment of many disorders and more than 30% of pharmaceutical raw materials are plants derived (WHO, 1996; Street and Prinsloo, 2013).

Pathogenesis of a variety of diseases have been ascribed to free radicals and reactive oxygen species (ROS) involved in oxidative damage (Aniya et al., 2002; Valko et al., 2007). Therefore, current studies in finding naturally sourced antioxidants are been focused on in the bid to minimize oxidative damage. Of these, the phenolic group of compounds are one of the most notable natural source. Positive correlation has also been established in literature between the high phenolic content and the strong antioxidant property of phenolic compounds from medicinal plants (Cai et al., 2004; Djeridane et al., 2006). Hence, treatment of various disorders can be combatted with medicinal plants that have antioxidant principles. Pharmacological research of active medicinal plants is also primarily centered on antimicrobial activity as a result of drug resistance to a number of pathogenic bacteria (Römling and Balsalobre, 2012).

Antiaris toxicaria Lesch. a monoecious tree of up to 45-60 m in height belongs to the Moraceae (Mulberry) family and the only species documented in the genus *Antiaris* with smooth greyish white to greyish green bark. It is geographically distributed in the tropical regions of the world including Africa (Senegal, Zambia, Angola, Ethiopia and Madagascar), Asia, Northern Australia, Indonesia, Philippines and Tonga. It is commonly known in English as bark cloth tree, false iroko or upas tree (Gill, 1992; Bosu and Krampah, 2005; Kemila and Krishnaveni, 2016). The fruits are edible. The different parts of the tree have varying applications from making clothing, sacks, mats, furniture and drum making, light constructions, commercial applications to folk medicine (Bosu and Krampah, 2005). The latex from the plant is used for dart and arrow poisons; it also facilitates cuts and wound healings. It serves as a purgative and is used in the treatment of eczema and leprosy. The seed, leaves, bark and root are used severally as anodyne, vermifuge as well as as febrifuge, fodder, analgesic and anticonvulsant, astringent, antidiarrhetic and for mental illness treatment. The bark is used to treat hepatitis and is also engaged in dyeing. (Mshana et al., 2000; Bosu and Krampah, 2005; Orwa et al., 2009).

Pharmacologically, *A. toxicaria* has been described for its anticonvulsant, antimicrobial, antioxidant and antiproliferative properties (Liu et al., 2013; Kemila and Krishnaveni, 2016; Mante et al., 2017). The aqueous stem bark extract of the plant has neuropharmacological activity (Mante et al., 2012) and ethanolic trunk bark extract showed potent cardiogenic effect on isolated guinea pig atria (Shi et al., 2010). Additionally, various isolated phytochemical compounds have been reported from different parts of the plant which include flavonoids, sesquiterpenoid glycosides, cardenolides, and cardiac glycosides (Que et al., 2009; Dai et al., 2009; Shi et al., 2010; Dong et al., 2011; Liu et al., 2013; Wu et al., 2014). Two of the compounds isolated, *toxicarioside F* and *toxicarioside G* showed good cytotoxic activity against the K562, SGC-7901, SMMC-7721, and HeLa cell lines (Dai et al., 2009). Thus far, despite several documented pharmacological studies on *A. toxicaria* from different geographical locations of the world, there is no detailed report on the antibacterial and antioxidant studies of the Nigerian species of *A. toxicaria*. We herein report the antibacterial and antioxidant activity studies of the stem bark and leaf extracts of *A. toxicaria*.

Materials and method

Chemicals and Reagents

2,4-dinitrophenylhydrazine (DPPH), ferric chloride, sodium hydrogen trioxocarbonate, gallic acid, quercetin, aluminium chloride, sulphuric acid, aqueous sodium hydroxide, hydrochloric acid, ammonia solution, glacial acetic acid and benzene were purchased from Sigma Aldrich, Germany. All reagents used were analytical grade.

Collection of plant materials

Fresh leaves and stem-bark of *A. toxicaria* were collected from the Forestry Research Institute of Nigeria (FRIN) Ibadan. The plant samples were authenticated at the herbarium of the Department of Plant Biology, Faculty of Life sciences, University of Ilorin (voucher No. UILH/002/1222). The fresh leaves were air dried, pulverized and kept in clean air tight containers prior to analysis.

Preparation of the plants extracts

The powdered stem bark (1.4 kg) and leaves (840 g) material of *A. toxicaria* were sequentially extracted with organic solvents of increasing

polarity; hexane (Hex), ethyl acetate (EtOAc) and ethanol (EtOH) for 48 hours. The extracts were combined, filtered and concentrated to afford 8.58 g (Hex), 10.41 g (EtOAc) and 11.49 g (EtOH) stem bark extracts while for the leaves 15.05 g (hex), 7.06 g (EtOAc) and 9.97 g (EtOH) were obtained.

Determination of total phenolic content (TPC)

The method used for the determination of total phenolic content of each extract was modified from the method cited by McDonald *et al.* (2001). Briefly, 200 μ l of the extract ($240 \mu\text{g mL}^{-1}$) was incubated with 1 ml of 10 times diluted Folin ciocalteu reagent and 800 μ l of 0.7 M Na_2CO_3 for 30 min at room temperature. The absorbance values were recorded at 765 nm on a Deckmann GU750 spectrophotometer. All measurements were carried out in triplicates. The results were calculated as mean values $\pm$ standard deviations and expressed as the gallic acid equivalents (GAE).

Determination of Flavonoid Concentrations in the Plant Extracts

The total flavonoid in the plant extracts were determined using the spectrophotometric method as described by Quetter *et al.* 2000. 1 mL of methanolic solution of the extracts (1, 5 and 10 mg/mL concentration) were added to 1 mL of 2% AlCl_3 solution prepared in methanol. The samples were incubated for an hour at room temperature and the absorbance read at the wavelength of 415 nm using Deckmann GU750 spectrophotometer. The samples were analysed in triplicates and the mean values determined. A similar procedure was repeated for the quercetin standard solution and the calibration line was constructed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line, then, the content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of Q/g of extract).

Evaluation of Antioxidant Activity of the Plant Extract

The ability of the plant extracts to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals was evaluated using standard methods with slight modifications (Kumarasamy *et al.*, 2007). 1 mg/mL of the stock solution of extracts were prepared in methanol and dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.99 and 0.97 $\mu\text{g/mL}$. Diluted solutions (1 mL each) were mixed with 1 mL of 1 mg/mL concentration of methanolic solution of DPPH. After 30 minutes of incubation in the dark at room temperature, the absorbance was

measured at 517 nm against blank samples.

The DPPH radical scavenging activity was calculated using the following formula:

$$(\%) \text{ DPPH Scavenging Inhibition} = [(A_0 - A_1 / A_0) \times 100],$$

where A_0 is the absorbance of the control (DPPH solution) and A_1 is the absorbance of the tested extracts /standard (DPPH solution + tested extracts/standard).

IC_{50} (inhibitory concentration at 50%) was estimated from the % inhibition versus log concentration plot, using a non-linear regression algorithm. The data were presented as mean values $\pm$ standard deviation ($n = 3$).

Microbial strains

A total of six cultures of human pathogenic bacteria were used in this study: four Gram-negative strains; *Salmonella typhi* (UCH 4801), *Escherichia coli* (UCH 0026), *Pseudomonas aeruginosa* (UCH 1102) and *Klebsiella pneumoniae* (UCH 2894); and two Gram-positive strains; *Bacillus subtilis* (UCH 7423) and *Staphylococcus aureus* (UCH 2473). All the microorganisms used were clinical strains from the Medical Microbiology laboratory of the University College Hospital, University of Ibadan, Ibadan, Nigeria.

Antibacterial susceptibility testing (AST)

Using the agar-well diffusion assay, the antibacterial susceptibility and antifungal activity of the crude leaf and stem bark extracts from *A. toxicaria* was determined (CLSI, 2007). Stock solutions, i.e., 100 mg mL^{-1} for the crude extracts were prepared using appropriate solvents (hexane, ethyl acetate, ethanol or DMSO). By serial dilution, subsequent concentrations of 50, 25, 12.5, 6.25 and $3.13 \mu\text{g/ml}$ were prepared. Bacterial clinical isolates were grown overnight by taking a loop full of the organism from stock which was inoculated into the sterile nutrient broth of 5 ml incubated for 24 h at 37°C . From overnight culture, 0.1 mL of the organism was taken and put into 9.9 mL of sterile distilled water to get 1:100 (10^{-2}) of the suspension. From the cell suspensions (10^{-2}), 0.2

98.38±0.29 to 181.01±0.52 mg Q /g for the leaves extract respectively.

Table 2. Concentration of flavonoids in various solvent extracts of *A. toxicaria* expressed in terms of quercetin equivalent (mg of Q/g of extract)

Sample	(mg Q/g of extract)
<i>Stem bark</i>	
Hex	112.63±0.15
EtOAc	210.81±0.76
EtOH	233.34±0.43
<i>Leaves</i>	
Hex	98.38±0.29
EtOAc	174.85±0.73
EtOH	181.01±0.52

Hex: Hexane extract; EtOAc: ethyl acetate extract; EtOH: ethanol extract. Q: quercetin equivalent. Data are presented as the mean ± SD values of triplicate determinations.

The highest flavonoid concentration was measured in the ethanol fraction (233.34 mg Q/ g) of the stem bark extract while the lowest was in hexane fraction (98.38 mg Q/ g) of the leaf extract. Our findings were in line with the observation of Min and Chun-Zhao (2005) that reported concentration of flavonoids in plant extracts were dependent on the polarity of solvents used in the extract preparation. Furthermore, flavonoids have also been reported isolated from the roots of *A. toxicaria* (Que et al., 2009).

The simplicity, rapidness and sensitivity of DPPH radical scavenging assay explain its prevalent use for regular analysis in the assessment of its antioxidant potential (Prior et al., 2005). The percentage inhibition for the different extracts ranged from 29.17 to 87.50% (Table 3).

Table 3. DPPH scavenging activities (% inhibition) of extracts from parts of *A. toxicaria* and Ascorbic Acid (Standard)

Sample	Concentration ($\mu\text{g/ml}$)									
	0.97	1.95	3.90	7.81	15.25	31.25	62.50	125.0	250.0	500.0
Stem bark										
Hex.	29.65	31.74	36.32	45.79	55.56	56.06	56.94	59.72	86.29	63.68
EtOAc	34.23	37.99	40.42	49.79	59.23	67.85	69.65	77.98	84.51	85.42
EtOH	40.76	44.44	46.32	53.26	60.21	68.75	74.79	83.33	85.90	87.50
Leaves										
Hex.	29.17	33.54	36.81	42.15	48.13	52.08	54.65	59.51	64.58	68.75
EtOAc	32.43	35.63	38.89	47.43	51.60	55.07	61.81	68.54	75.69	81.25
EtOH	34.72	37.50	41.67	49.51	52.78	59.61	69.44	72.99	79.38	83.13
AA (standard)	18.57	21.66	38.26	64.18	75.55	80.76	84.26	85.79	87.26	89.01

Hex: Hexane extract; EtOAc: ethyl acetate extract; EtOH: ethanol extract and AA: ascorbic acid.

The DPPH radical scavenging activity and IC_{50} values of solvent extracts from stem bark, leaf of *A. toxicaria* and ascorbic acid (standard) occurred in a concentration-dependent manner as shown in Table 3, Table 4, Figure 1 and Figure 2. The comparable high DPPH radical scavenging activities recorded for the polar solvent extracts with standard ascorbic acid also suggests that the extracts contain phytoconstituents with high proton donating ability and could aid as free radical inhibitors. With data from DPPH activity, the IC_{50} values were determined for the extracts and ascorbic acid, implying the lower the IC_{50} value, the greater the scavenging activity.

Table 4. IC₅₀ values of different solvent extracts of parts of *A. toxicaria*.

Sample	IC ₅₀ (µg/ml)
<i>Stem bark</i>	
Hex	11.02
EtOAc	7.98
EtOH	5.98
<i>Leaves</i>	
Hex	22.93
EtOAc	12.41
EtOH	8.95
AA	5.67
(standard)	

Hex: Hexane extract; EtOAc: ethyl acetate extract; EtOH: ethanol extract and AA: ascorbic acid.

The largest capacity to neutralise DPPH radicals was found for ethanol stem bark extract (87.50 %) which also had the lowest IC₅₀ value of 5.98 µg/ml while the lowest capacity was recorded for the non-polar hexane extract at the lowest concentration used. Čanadanović-Brunet *et al.*, (2008) reported high concentrations of phenolics in extracts obtained using polar solvents and this is in accordance to this study where the ethanol stem bark extract had the highest ability to inhibit DPPH radicals (Table 3) with the highest total phenolic content been recorded for the extract (Table 1) with the lowest IC₅₀ (Table 4). Due to hydroxyl groups of phenolic compounds they possess the ability to scavenge free DPPH radicals and therefore are known as important plant constituents and these compounds may probably contribute to its antioxidant action. (Tosun *et al.*, 2009). The high total phenolic content, flavonoid concentration being observed for the ethanolic stem bark extract is in correlation with the intense antioxidant activity of these extracts (Figure 1 and Figure 2).

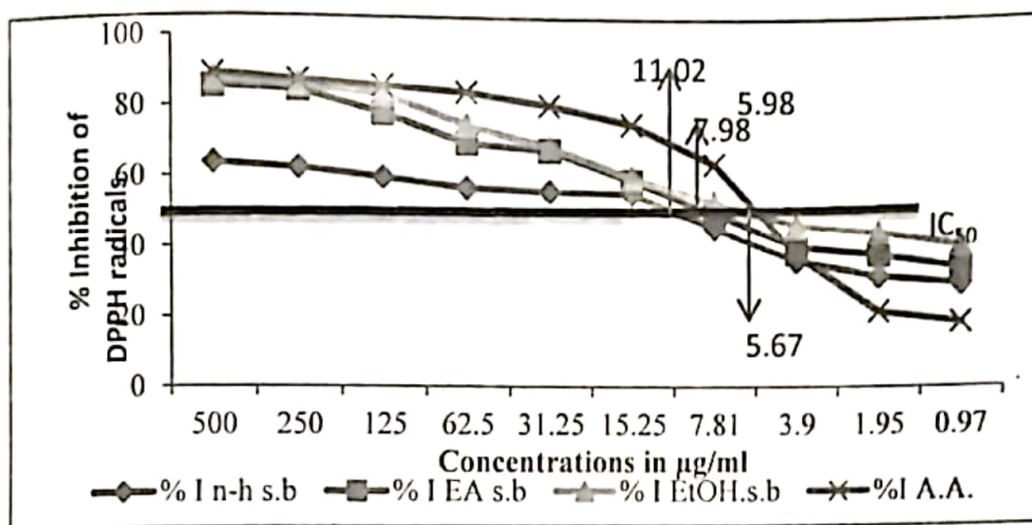


Figure 1. Antioxidant (DPPH) scavenging activity of stem bark extracts presented as percentage inhibition and IC_{50} value ($\mu\text{g/ml}$)

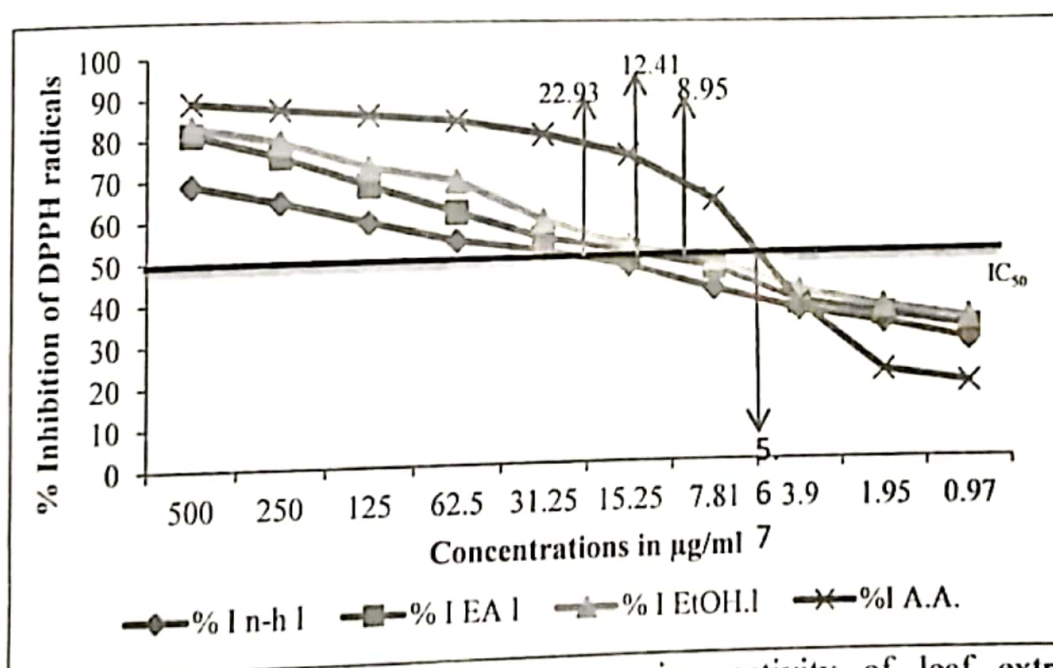


Figure 2. Antioxidant (DPPH) scavenging activity of leaf extracts presented as percentage inhibition and IC_{50} value ($\mu\text{g/ml}$)

Key: % I = percentage inhibition, A.T = *Antiaris toxicaria*, n-h = n-hexane leaf extract, EA = ethyl acetate leaf extract, EtOH = ethanol leaf extract, A.A = Ascorbic acid, l = leaf, s = stem bark.

The crude hexane, ethyl acetate and ethanol extracts of the stem bark and leaves of *A. toxicaria* were tested against both Gram-negative and Gram-positive bacterial strains to determine whether or not the crude extracts gotten from Nigeria have any antibacterial activity to substantiate the reported use of the plant and other species of *Antiaris* as antibacterial agents (Pamio and Mann, 2008; Kemala and Krishnaveni, 2016).

In general, the stem bark and leaves extracts were active against several of the Gram-negative and Gram-positive bacterial strains at higher concentrations (Table 5 and Table 6). Several of the extracts had solvent specific activity at up to 100 µg/ml, *E. coli* (UCH 0026) was susceptible to both the EtOAc and EtOH extracts of the stem bark and leaves as well as *S. typhi* (UCH 4801) and *K. pneumoniae* (UCH 2894). The Gram-positive strain, *S. aureus* (UCH 2473) was more susceptible to the EtOAc extract of the stem bark with the highest zone of inhibition of 30 mm. This extract also showed intermediate activity against *B. subtilis* (UCH 7428). The EtOAc extract of the stem bark was thus the most active.

The selective activity toward polar solvents was consistent with a study by Kemala and Krishnaveni (2016) on different plant extracts from Indian forest. In their study only polar extracts showed activity against the bacterial strains used and this was also reported for another species from the genus *Antiaris* (Pamio and Mann, 2008). The poor activity observed in our study for non-polar extracts was in accordance with the report of Kemala and Krishnaveni (2016). This suggests that polar solvents maybe effective solvents for extraction when carrying out antibacterial activity and it also justifies the use of *A. toxicaria* in the management of bacterial related diseases.

Table 5. Antibacterial activity of stem bark extracts (zones of inhibition in mm) of *A. toxicaria*

Test strains	Zones of Inhibition (mm)						
	Extracts	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.13 µg/ml
Stem bark							
<i>E. coli</i> UCH 0026	Hex	16	14	12	1	-	-
	EtOAc	27	22	18	14	12	10
	EtOH	27	24	20	18	14	11
	Gentamycin (10µg/ml)	39					
<i>P. aeruginosa</i> UCH 1102	Hex	18	16	14	12	10	-
	EtOAc	23	20	17	14	12	10
	EtOH	27	24	21	18	14	11
	Gentamycin (10µg/ml)	39					
<i>K. pneumoniae</i> UCH 2894	Hex	19	17	15	13	10	-
	EtOAc	27	23	20	18	14	11
	EtOH	27	24	20	17	14	10
	Gentamycin (10µg/ml)	37					
<i>S. typhi</i> UCH 4801	Hex	20	18	16	14	10	-
	EtOAc	25	21	18	14	12	10
	EtOH	29	25	21	17	14	11
	Gentamycin (10µg/ml)	37					
<i>B. subtilis</i> UCH 7428	Hex	18	16	14	12	10	-
	EtOAc	24	20	18	16	14	10
	EtOH	27	24	20	16	13	10
	Gentamycin (10µg/ml)	39					
<i>S. aureus</i> UCH 2473	Hex	18	16	13	11	5	-
	EtOAc	30	25	21	18	14	10
	EtOH	29	25	21	18	14	11
	Gentamycin (10µg/ml)	38 ± 0					

Hex: Hexane extract; EtOAc: ethyl acetate extract; EtOH: ethanol extract

Table 6. Antibacterial activity of leaves extracts (zones of inhibition in mm) of *A. toxicaria*

Test strains	Zones of Inhibition (mm)						
Extracts	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.13 µg/ml	
	Leaves						
<i>E. coli</i> UCH 0026	Hex	17	14	12	10	-	-
	EtOAc	23	19	17	14	10	-
	EtOH	23	19	17	14	12	10
	Gentamycin (10µg/ml)	39					
<i>P. aeruginosa</i> UCH 1102	Hex	15	13	10	-	-	-
	EtOAc	21	18	16	14	11	-
	EtOH	22	18	16	14	12	10
	Gentamycin (10µg/ml)	37					
<i>K. pneumoniae</i> UCH 2894	Hex	15	13	10	-	-	-
	EtOAc	21	18	15	13	11	-
	EtOH	23	20	18	15	1	10
	Gentamycin (10µg/ml)	38					
<i>S. typhi</i> UCH 4801	Hex	17	14	10	-	-	-
	EtOAc	23	19	17	14	10	-
	EtOH	24	20	18	14	12	10
	Gentamycin (10µg/ml)	37					
<i>B. subtilis</i> UCH 7428	Hex	17	14	12	10	-	-
	EtOAc	21	18	15	13	10	-
	EtOH	23	19	17	14	12	10
	Gentamycin (10µg/ml)	37					
<i>S. aureus</i> UCH 2473	Hex	17	14	12	10	-	-
	EtOAc	23	19	16	13	10	-
	EtOH	25	23	19	17	14	10
	Gentamycin (10µg/ml)	38					

Hex: Hexane extract; EtOAc: ethyl acetate extract; EtOH: ethanol extract

Conclusion

The antioxidant and antibacterial studies of *A. toxicaria* suggest the relevant use of the plant in phytotherapy. Based on the total phenolic content, flavonoids concentration and DPPH scavenging activity of the hexane, ethyl acetate and ethanol stem bark and leaves extracts, the polar extracts can serve as a natural source of antioxidants. Selective solvent-specific antibacterial activities were reported for the extracts with greater activity been observed for the polar extracts against Gram-positive, albeit at high concentration. It is quite possible from our findings that the reported high total phenolic contents and flavonoids concentration of the polar extracts from *A. toxicaria* indicative of phenolic compounds in the extracts could also be responsible for the antibacterial activity experienced by those who use the plant for medicinal purposes.

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