International Journal of Chemistry; Vol. 10, No. 3; 2018
ISSN 1916-9698 E-ISSN 1916-9701
Published by Canadian Center of Science and Education

South African Invasive Tree: Studies of the Chemical and Biological Profiles of *Acacia Decurrens* (Wild)

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Received: June 2, 2018 Accepted: July 12, 2018 Online Published: July 17, 2018

doi:10.5539/ijc.v10n3p60 URL: https://doi.org/10.5539/ijc.v10n3p60

Abstract

In this study, we aimed to profile the stem bark of *Acacia decurrens* biochemically. Extracts obtained by maceration were phytochemically screened, spectroscopically analysed with the aid of UV-visible, FT-IR, GC-MS, and, ICP-OES and subjected to primary biological assay. Spectra obtained from the UV-visible and FT-IR confirm the presence of $n \rightarrow \pi^*$ which are characteristic of compounds with oxygenated backbones such as the glycoside, phenols, terpenoids, tannins, and flavonoids. On further analysis, the chromatogram revealed the presence of thirty-five major compounds of which eight bioactive compounds had previously been isolated. The metal profile of the stem bark registered high concentrations of Cr, K, and Fe. Quantitative phytochemical evaluation showed, large amount of tannins (30.87 – 55. 81 mgTAE/g), steroids (13.92-41.2%), and phenols (40.6 - 65.5 mgGAE/g) in all fractions. The ethyl acetate and methanol fractions were found to be rich sources of antibacterial compounds with MIC value of 12.5 µg/mL while the chloroform fraction is a potent antioxidant fraction with IC_{50} values of 37.00 ± 0.06 µg/mL and 42.20 ± 0.72 µg/mL against DPPH and ABTS radicals, respectively. The presence of these secondary metabolites and the hyper-tolerance capacity for metals can be exploited pharmaceutically and for phytoremediation purpose, respectively.

Keywords: antioxidant, chromatogram, hyper-tolerance, inhibitory, inoculum, phytochemical

1. Introduction

The earliest mention of Acacia was in the Book of Exodus, referring to *Acacia raddiana*, in regards to the construction of the Tabernacle (Hutton Balfour, 1866). *Acacia decurrens* (Wild), commonly known as black wattle, is a perennial *Mimosoideae* tree of the *Fabaceae* family. They survive all terrestrial habitats, including alpine, rainforests, woodlands, grasslands, coastal dunes and deserts (Quattrocchi, 2006); and are classified as invasive in some countries due to their prolific nature. The flora parts such as the flowers, seed pods, and gum are edible, while the trees are grown for firewood, windbreaker, shelter or dyes stuff (Elliot & Jones, 2002). In South Africa, *A. decurrens* is a non-native species (Cororaton, Orden, & Peterson, 2009), threatening local vegetations and wildlife (Wallace & Bargeron, 2014). The ecological dominance of *A. decurrens* is due to their biological resilience and ability to compete better for water and soil minerals than native flora. These unique biological attributes can be exploited for their pharmacological activities against autochthonous infections.

Some of the major chronic and degenerative diseases indigenous to South Africa such as atherosclerosis, ischemic heart disease, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases are initiated or caused by undesirable oxidative reactions (Halliwell, 1994; Young & Woodside, 2001). A large population of South Africans is dependent on natural therapeutic agent for combating oxidative diseases. Furthermore, the recent upsurge in the demand of plants with pharmacological potentials and low toxicity has led to the continuous search for ‘‘novel green medication’’ (Cao, Sofic, & Prior, 1996; Pourmorad, Hosseinimehr, & Shahabimajd, 2006). In this study, our investigation focuses on the evaluation of the chemical composition and in vitro biological potentials of the *A. decurrens* stem bark.

2. Materials and Methods

2.1 Plant Materials

Fresh stem bark of *A. decurrens* was harvested around summer from Heidelberg (26.5033◦S, 28.4397◦E), South Africa, diced, and dried at ambient temperature and relatively low humidity. The stem bark was authenticated by the South Africa National Biodiversity Institute, Pretoria, and voucher specimen number: 1200-1, was deposited.

2.2 Digestion of Stem Bark and Metal Ion Analysis

According to the protocol of Mwita (2011), 1.0 g of diced stem bark prior to maceration was digested for 3 h at 60 ℃ in 10 mL of Aqua Regia. After cooling, the content of the digestion flask was filtered, transferred into 50 mL volumetric flask and diluted to mark with deionized water. The clear solution of the reagent blank and sample solution was obtained by diluting 10 times before analysis. The blank reagent solution contained 1% HNO_3. Standard curtail solution containing seven metal ions (Cr, Ni, Zn, Fe, Co, K and Ca) were used for calibration (Chiou & Martin, 1980), and metal ion concentration (ppm) of the stem bark was estimated from the standard calibration curve.

2.3 Extraction and Yield Estimation

The stem bark was diced to facilitate drying at a relative humidity of 55% and ambient temperature. The fractions were obtained by serial maceration of 2 kg pulverized stem bark with 4 L of hexane, chloroform, ethyl acetate, and methanol with slight agitation of 111 rpm for seven days. Afterward, the fractions were filtered through cotton wool then Whatman filter paper No. 42 (125 mm) and dried to constant weight in open air.

2.4 Characterisation of the Fractions

The functional group composition of the fractions was determined on FT-IR (PerkinElmer Spectrum 400, Waltham, MA) scanned between 4000 and 500 cm^{-1}. The metal ion concentration was analysed using an inductively coupled plasma spectrometer (ICP-OES, PerkinElmer, Billerica, MA). The chromophores present in the fractions of *A. decurrens* were analysed within the UV-visible range of 200 to 900 nm (Agilent Technologies Cary 60 UV-Vis, Santa Clara, CA).

2.5 GC–MS Analysis of the Fractions

The GC analysis was carried out on Clarus 500 PerkinElmer Gas Chromatograph with an Elite -5 (100% Dimethylpolysiloxane) column coupled to a mass spectrometer detector. The GC method involves adjusting the helium flow rate to 1 mL/min with the injection port temperature at 250°C. Solutions of the fractions were injected in 10:1 split mode at an initial column temperature of 110°C, held for 2 min. The oven temperature increased at the rate of 5°C/min, to 280°C, and held for 9 min. The mass spectral scan range was set at 45-450 (m/z).

2.6 Qualitative and Quantitative Phytochemical Screening

Phytochemical screening of the fractions was carried out using standard qualitative protocols. The total phenolic content (TPC) was determined as described by Padmavathi (2013), the results were expressed as mg of gallic acid equivalents per g of sample (mgGAE/g). Total flavonoid content (TFC) was estimated by the method of Zhishen, et al. (1999), the results were expressed as mg of Rutin equivalents per g of sample (mgRUE/g). The tannin content (TC) was determined as described by Singh (2012), the results were expressed as mg of tannin equivalents per g of sample (mg TAE/g), while the alkaloids, saponins, and terpenoids contents were determined as described by Fazel et al. (2010), Makkar et al. (2007) and Tejavathi et al. (2013), respectively and expressed in percentage.

2.7 Antimicrobial Assay

2.7.1 Test Micro-Organisms

Micrococcus luteus (ATCC 26883) *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (NCTC 11954), *Salmonella typhi* (ATCC 29692), *Klebsiella pneumonia* (BAA 1706), *Shigella sonnei* (ATCC 25931), *Staphylococcus epidermis* (ATCC 12228), *Listeria monocytogenes* (ATCC(R) BAA- 751TM) and Enterococcus faecalis (ATCC 22735)

2.7.2 Preparation of Inoculum

Stock bacteria culture obtained from the Department of Biotechnology, Vaal University of Technology, was maintained at 4℃ on slants of nutrient agar. The active stock culture was inoculated into a fresh tube of Muller-Hinton Broth medium, and the bacteria were incubated for 24 h at 37℃.

2.7.3 Evaluation of the Antimicrobial Activity

Bacteria cultures were grown on nutrient broth liquid medium at 37℃. Afterward, each microorganism, at a concentration of 10^6 cells/mL, was inoculated on the surface of nutrient agar plates. Impregnated 6 mm diameter disk of fractions with various concentrations, were placed on the surface of the inoculated nutrient agar dish and incubated at 37°C for 24 h. The zones of inhibition (ZI) were measured after 24 h, and fractions with ZI more than 7 mm were

subjected to further antimicrobial assay to determine the minimal inhibitory concentration (MIC) (Wahid, Ahmad, Nor, & Rashid, 2017). The MIC of each fraction and ampicillin sodium salt (positive control) were reported. DMSO (2%) and Tween 20 were used as negative controls, and all tests were performed in triplicate.

2.8 Antioxidant Assay

2.8.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

The antioxidant activity of the fractions was examined on the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. A freshly prepared ethanolic solution of (300 µL, 0.05 mM) DPPH was mixed with 40 µL of each fraction. To the mixture, 2.7 mL of ethanol (96%) was added, vortexed and left to incubate for 5 min at ambient temperature. The absorbance of the blank and sample was measured spectrophotometrically at 517 nm. The radical scavenging activities of the fractions expressed as percentage inhibition were calculated according to equation (**1**) (Lee, Hwang, Ha, Jeong, & Kim, 2003).

$$\%\ DPPH\ inhibition\ = \left(\frac{A_B - A_A}{A_B}\right) 100 \quad (1)$$

Where A_A and A_B are the absorbance values of the fractions and the blank, respectively. The percent inhibition versus concentration curve was plotted, and the IC_{50} (μg/mL) of the fractions were determined by linear regression analysis. All determinations were carried out in triplicate.

2.8.2 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) Assay

The assay was performed according to the method of Re et al. (1999), with slight modifications. Solutions of 7 mM ABTS and 2.45 mM potassium persulfate prepared in distilled water and mixed. The mixture was left in the dark at ambient temperature for 16 h to generate the radical ($ABTS^{\cdot+}$). The $ABTS^{\cdot+}$ solution was adjusted to an absorbance of 1.00 at 734 nm, and the fractions of various concentrations added. Further, the absorbance of the mixture was taken after 6 min of incubation at 734 nm. The total proton-donating potential (PDP) of the fractions is expressed as half maximal inhibitory concentration (IC_{50}) (μg/mL) were determined by linear regression analysis. All analyses were carried out in triplicate and the % PDP was estimated using the formula (Eq **2)**:

$$\%\ \mathrm{PDP} = 100\left[\frac{(\text{Abs. of ABTS control} - \text{Abs. of the fraction})}{(\text{Abs. of ABTS control})}\right] \quad (2)$$

Ascorbic acid was used as a positive control.

3. Results and Discussion

3.1 Elemental Composition of A. Decurrens Stems Bark

The concentration of nine metals was analysed by ICP-OES and presented in Figure 1. The stem bark was found to have significant levels ($p < 0.05$) of Fe, K, and Cr, due to high bioaccumulation and phyto-tolerance capacities of most tannin-rich plants (Antonovics, Bradshaw, & Turner, 1971; Singh, Parihar, Singh, Singh, & Prasad, 2015). The hyper-accumulation capacity of *Acacia* is an adaptive evolution to hostile environments, acquired over many generations, and most particularly as earned it the category 2 invasive reputation in South Africa.

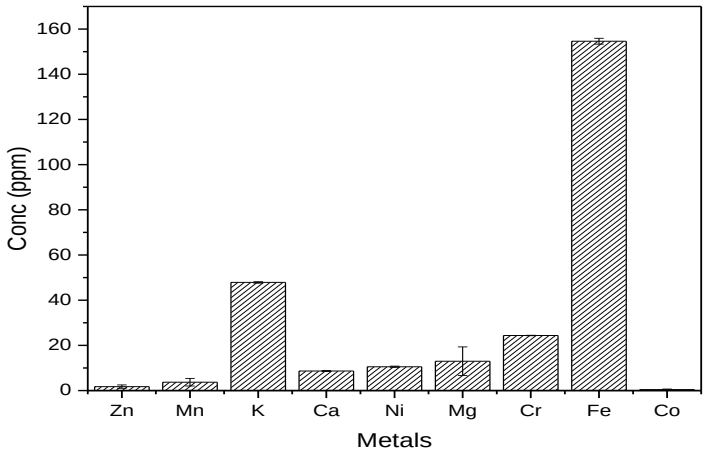

Figure 1. Elemental composition of *A decurrens* stem bark

This high sorption potential also accounts for the dominance of *A. decurrens*, consequently taking up more minerals and water than native flora. The high metal uptake and bioaccumulation may be due to the metal chelating bioactive principles, as reported by Török et al. (2015) in their study on phytoremediation capacity of aquatic plants associated with phyto-chelation.

3.2 Influence of Solvent on the Recovery Yield of Secondary Metabolite

The secondary metabolites recovered by the four solvents all differ in nature, and yield (Table 1). There was a significant difference ($p < 0.05$) in the extraction yield of the methanol compared to the hexane fraction with the lowest yield. The yield of the methanol fraction indicated that most of the secondary metabolites are soluble in the polar solvent, which is consistent with the report of a previous study on the effect of extraction solvents on the recovery yields of secondary metabolites (Ghasemzadeh, Jaafar, Rahmat, Wahab, & Halim, 2010). Also, according to Dhanani et al. (2017), polar solvents are effective for the extraction most phytochemical compound.

Table 1. Nature and yield% of the Secondary Metabolite from the Stem Bark Fractions.

Fractions	Physical Nature	Colour	Yield%
HAD	Oily	Lemon	0.20 ± 0.02
CAD	Solid	Light brass gold	0.98 ± 0.05
EAD	Solid	Dark brown	0.60 ± 0.01
MAD	Solid	Chocolate	4.70 ± 0.03

Key: HAD: Hexane *A. decurrens* fraction*;* CAD: Chloroform *A. decurrens* fraction*;* EAD: Ethylacetate *A. decurrens* fraction*;* MAD: Methanol *A. decurrens* fraction

Another significant factor which further justifies the high extractability of methanol is the dielectric constants (ε); the higher the constant, the higher the efficiency of extraction.

3.3 Chromophores and Functional Groups Classification of the Fractions

Similar absorption maxima were observed on the spectra of hexane and ethyl acetate fractions with slight differences in the visible region at 410 (0.116) and UV region 390 (0.648) and 345 (0.663) nm. There is a remarkable difference in the absorption pattern of the methanol fraction compared to the other fractions as a result of the compositional differences. This observation is in agreement with a study by Longair (2008), on the close relationship between the UV-visible absorption of an organic compound and its structure.

The FT-IR spectra of the fractions are presented in Figure 2 for comparison of the functional group compositions. The spectrum of *A. decurrens* hexane fraction indicated the presence of N–H vibrations (an asymmetric stretch of secondary amine) at 3385 cm^{-1}, C=O vibrations of saturated acid at 1712 cm^{-1} and C–O vibrations of acid at 1462 and 1376 cm^{-1}.

Peaks at 3614 and 1741 cm^{-1} corresponding to O-H and C=O vibrations of phenolic and ketonic compounds (such as flavonoids and terpenoids), respectively were recorded on the spectrum of the chloroform fraction. The ethyl acetate fraction contains majorly all the vibrational peaks observed in the other fractions, while the spectrum of the *A. decurrens* methanol fraction, presents a broadband at 3256 cm^{-1} representing the stretching vibrations of O–H (from phenolic or flavonoid compound).

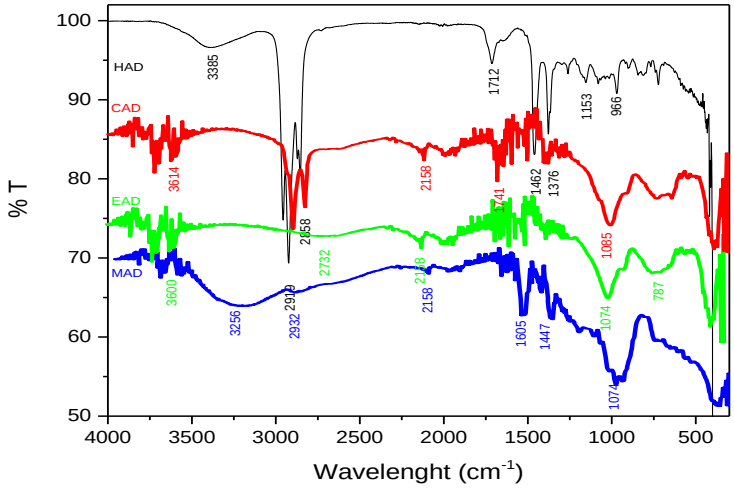

Figure 2. The UV-vis and FT-IR spectral data of the fractions

The presence of a band at 2158 cm^{-1} in all the fractions except hexane could be due to carboxyl group stretching vibrations (Can, Bulut, Ornek, & Ozacar, 2013). Furthermore, the peaks in the vicinity of 1047-1153 cm^{-1} are indication of C-O single bond found in all fractions, confirming the presence of oxygenated compounds such as glycoside, phenols, terpenoids, tannins, and flavonoids.

3.4 GC-MS Analysis of the A. Decurrens Fractions

The GC-MS analysis of the stem bark fractions clearly showed the presence of thirty-five major compounds, with the retention time (T_R) and m/z values presented in Table 2. The hexane, chloroform, and ethyl acetate fractions contained eight, fourteen, and thirteen major compounds, respectively. Constituent compounds of the methanol fractions are too polar to be analysed using the adopted GC method and used column.

Table 2. Retention times and MS of the compounds in the fractions

HAD				CAD				EAD			
Major Compounds	**T_R (min)**	**m/z**	**Ref**	**Major Compounds**	**T_R (min)**	**m/z**	**Ref**	**Major Compounds**	**T_R (min)**	**m/z**	**Ref**
1	3.22	142		9	3.76	388		23	4.19	264	
2	10.78	182	(Joseph & Sekomeng, 2017)	10	7.18	142		24	6.47	318	
3	11.3	298		11	8.75	199		25	9.09	328	
4	12.26	387		12	9.44	207	(Seneviratne & Fowden, 1968)	26	9.39	341	(Uchiyama et al., 2003)
5	12.59	374		13	11.16	222		27	11.22	350	
6	13.99	328		14	16.01	218	(Joseph & Sekomeng, 2017)	28	12.44	378	(Anam, 1998)
7	20.07	370		15	19.43	290	(Nyila, Leonard, Hussein, & Lall, n.d.)	29	19.73	320	
8	21.25	253		16	20.59	308		30	21.57	396	
				17	23.01	327		31	25.39	252	
				18	24.21	347		32	26.45	232	(Tewari & Jindal, 2010)
				19	25.05	371		33	27.4	326	(Aaby, Ekeberg, & Skrede, 2007; Seeram, Lee, Scheuller, & Heber, 2006)
				20	26.79	295		34	28.53	347	
				21	29.19	260		35	29.11	247	
				22	29.81	265					

Key: HAD: Hexane *A. decurrens* fraction*;* CAD: Chloroform *A. decurrens* fraction*;* EAD: Ethylacetate *A. decurrens* fraction*;* MAD: Methanol *A. decurrens* fraction

A literature search of the thirty-five major compounds detected on the chromatogram confirmed the previous isolation and elucidation of eight principles in the past decade. The previously isolated compounds were 2-methyl-octahydro-indene-4-carboxylic acid (**2**), **S**-carboxyisopropyl-cysteine (**12**), 6-methyldecahydro-1*H*-phenanthren-9-one (**14**), epicatechin (**15**), icetexon (**26**), 1,2,3,4,4',9,10,10'-octahydro-5,8-dihydroxy-7-(2-hydroxypropan-2-yl)-6-methoxy-1,1-dimethyl-9-oxophenanth rene-4-carbaldehyde **(28)**, 2,3,4-tri-O-methyl-D-glucuronic acid (**32**) and *p*-coumaroyl-glucoside (**33**).

3.5 Qualitative and Quantitative Phytochemical Screening

Results of the qualitative and quantitative phytochemical screening of *A. decurrens* fractions were presented in Tables 3 and 4. Evidence of terpenoids, phenols, tannins, flavonoids, saponins, alkaloids were found in the various stem bark fractions. The primary outcome of the investigation revealed a high level of health-enhancing phytochemical constituents (Harris, 2003), an indication of the medicinal value of *A. decurrens* contrary to the sole invasive classification by Department of Environment, South Africa (Cororaton, Orden, & Peterson, 2009). Velásquez et al. (2007) also reported the ethnomedicinal usage of *A. decurrens* stem bark as an astringent and anti-diarrhoea decoction.

Table 3. Preliminary Phytochemical investigation

	Phytochemical Composition						
Fractions	**Alkaloids**	**Glycosides**	**Phenols**	**Saponins**	**Terpenoids**	**Tannins**	**Flavonoids**
HAD	+	+	-	-	+++	++	-
CAD	++	+++	+++	-	++	+++	-
EAD	-	+++	+++	++	+++	+++	+++
MAD	-	+++	++	+++	+++	+++	+++

Key: HAD: Hexane *A. decurrens* fraction*;* CAD: Chloroform *A. decurrens* fraction*;* EAD: Ethylacetate *A. decurrens* fraction*;* MAD: Methanol *A. decurrens* fraction*;* -, absent; +, low in abundance; ++, moderate in abundance; +++, high in abundance

All the fractions contain glycosides, steroids, and tannins, which can mediate cardiotonic and are known insecticidal, antioxidant, and antimicrobial phyto-compounds (Arts & Hollman, 2005). Antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, anti-inflammatory and antimutagenic secondary metabolite such as phenols and flavonoids were found in all the fractions except hexane (Manjunatha, 2006; Soobrattee, Neergheen, Luximon-Ramma, Aruoma, & Bahorun, 2005). There is a significant difference ($p < 0.05$) in the amount of tannins and flavonoids extracted as the solvent changes from a less polar (hexane) to a more polar (methanol) (Table 4), implying the presence of more bioactive principles with polar functional units in the methanol fraction (De Amorim, de Castro, de Melo, Correa, & Sobrinho, 2012; Gonçalves, Carlos, Rodrigues, Aparecido, & Padovese, 2012). Phenolic compounds, glycosides, and flavonoids are better extracted with ethyl acetate and methanol while any of the terpenoids and tannins compounds can be extracted with any of the four solvents. The best extractant for the various bioactive principles varies with polarity and dielectric constant.

Table 4. Compositional analysis of the bioactive principles

	Phytochemical Composition					
Fractions	**TPC** (mgGAE/g)	**TFC (mgRUE/g)**	**TC (mgTAE/g)**	**Steroids (mg/100 g)**	**Saponins (mg/100 g)**	**Alkaloid (mg/100 g)**
HAD	-	-	30.87 ±0.12	34.62 ±0.21	-	0.61 ±0.01
CAD	65.61 ±0.02	-	47.52 ±0.39	31.50 ±0.07	-	3.30 ±0.03
EAD	40.60 ±0.14	210.24 ±0.37	55.81 ±0.46	41.23 ±0.17	8.64 ±0.09	-
MAD	45.82 ±0.32	281.52 ±0.51	55.80 ±0.15	13.92 ±0.16	5.12 ±0.05	-

Key: HAD: Hexane *A. decurrens* fraction*;* CAD: Chloroform *A. decurrens* fraction*;* EAD: Ethylacetate *A. decurrens* fraction*;* MAD: Methanol *A. decurrens* fraction

3.6 Evaluation of the Antimicrobial Potential of Plant Fractions

The antimicrobial potential of the fractions is presented in Table 5 and 6. All the fractions showed some level of antimicrobial activity to at least one of the tested microorganisms. Ethyl acetate and methanol fractions were the most active fraction inhibiting 60.3% and 68.2% of the screened microorganisms, respectively. Some microorganisms which are known to show resistance to different antibiotics, had their growth inhibited by the ethyl acetate and methanol fractions. *M luteus* was sensitive to all the fractions, despite its ability to survive most oligotrophic environments for extended periods of time (Greenblatt et al., 2004).

On the other hand, the hexane and chloroform fractions showed < 50% anti-bacterial activity against the tested organisms. *E coli* and *K pneumonia* were not sensitive to any of the fractions, as a result of the organisms' ability to produce penicillinase rendering the fractions ineffective (Hudson, Bent, Meagher, & Williams, 2014) or the absence of a potent anti-coli and anti-pneumonia principle in the fractions.

MIC data of the ethyl acetate and methanol fractions showed potent antibacterial activity against the same test organisms (Table 6) with the MIC value of 12.5 μg/mL. The MIC for the polar fractions (ethyl acetate and methanol) is comparable with the MIC value of ampicillin, an indication that the polar extracts can revert transpeptidase; an enzyme needed by the bacteria to cross-links the peptidoglycan chains to form rigid cell-walls.

Table 5. Sensitivity of the fractions against the test organisms.

	Fractions				
Bacteria	**HAD**	**CAD**	**EAD**	**MAD**	**Ampicillin**
S. aureus	+	-	+	+	+
E. coli	-	-	-	-	-
S. typhi	+	-	+	-	+
K. pneumoniae	-	-	-	-	-
M. luteus	+	+	+	+	+
S. sonnei	-	-	+	+	+
S. epidermis	-	-	+	+	+
L.monocytogenes	-	-	+	+	+
E. faecalis	-	+	+	+	+

Key: HAD: Hexane *A. decurrens* fraction*;* CAD: Chloroform *A. decurrens* fraction*;* EAD: Ethylacetate *A. decurrens* fraction*;* MAD: Methanol *A. decurrens* fraction*;* (+) Susceptibility (inhibition zone ≥ 7mm); (-) Absence of susceptibility

Ampicillin mechanism of action involves two critical stages of bacterial cell-wall breach ultimately leading to cell lysis; therefore, ampicillin, ethyl acetate, and methanol fractions can be referred to as being bacteriolytic (Bratzler et al., 2013). The hexane and chloroform fractions showed poor but similar activity most likely as a result of the presence of closely related phytochemical composition and GC-MS compound peaks. Hence, decoction of *A. decurrens* stem bark can be used for the treatment of opportunistic infection such as recurrent bacteraemia, septic shock, septic arthritis, endocarditis, meningitis, intracranial suppuration, and cavitating pneumonia in immunosuppressed patients.

Table 6. Antibacterial screening (μg/mL) of the isolated compound on test microorganisms

	Fractions				
Bacteria	**HAD**	**CAD**	**EAD**	**MAD**	**Ampicillin**
S. aureus	-	-	12.5	12.5	+
E. coli	50	-	-	-	-
S. typhi	50	-	12.5	-	+
K. pneumoniae	-	-	-	-	-
M. luteus	25	50	12.5	12.5	+
S. sonnei	-	-	12.5	12.5	+
S. epidermis	-	-	12.5	12.5	+
L.monocytogenes	-	-	12.5	12.5	+
E. faecalis	-	-	12.5	12.5	+

Key: HAD: Hexane *A. decurrens* fraction*;* CAD: Chloroform *A. decurrens* fraction*;* EAD: Ethylacetate *A. decurrens* fraction*;* MAD: Methanol *A. decurrens* fraction

3.7 Antioxidant Assay

The fractions exhibited moderate radical scavenging activity, and the result is presented in Table 7. Ethyl acetate and methanol *A. decurrens* fractions showed comparative antioxidant activities and were less potent compared than chloroform fraction. The IC_{50} values revealed that antioxidant strength is in the order of ascorbic acid > chloroform > ethyl acetate > methanol > hexane fractions. Similarly, the proton donating potentials of the fractions assayed by the ABTS revealed that the potential decreases in the order chloroform > methanol > ethyl acetate > ascorbic acid > hexane fractions.

The electron and proton donating potentials observed for the different fractions are a function of the secondary metabolites present. The phytochemical screening of the chloroform fraction indicated the presence of phenols (65.5 mg GAE/g) and tannins (47.5 mg TAE/g) which significantly impacted the antioxidant activity, enhancing facilitating the free radical scavenging activities. Chandrashekar and Rao (2013), reported the direct correlation between the phenolic and tannin contents, and the antioxidant activity of the ethanolic extract of *Leucas indica* leaves.

Table 7. Radical scavenging activities of the fractions from the Stem Bark of *A. decurrens*.

Fractions	IC_{50} (μg/mL)	
	DPPH	ABTS
HAD	75.00 ± 0.57	54.70 ± 0.21
CAD	37.00 ± 0.06	42.20 ± 0.72
EAD	46.00 ± 0.88	49.60 ± 0.70
MAD	48.60 ± 0.92	44.60 ± 0.95
Ascorbic acid	31.70 ± 0.19	54.70 ± 0.35

Key: HAD: Hexane *A. decurrens* fraction*;* CAD: Chloroform *A. decurrens* fraction*;* EAD: Ethylacetate *A. decurrens* fraction*;* MAD: Methanol *A. decurrens* fraction

Also, *A. decurrens* ethyl acetate and methanol fractions contain potent antioxidant compounds such as flavonoids, phenols, and tannins, while the *A. decurrens* hexane fraction showed poor antioxidant activity due to the absence of primary antioxidant metabolites (Beyer, 1994).

4. Conclusion

Findings from this study confirm chloroform as an effective solvent for extracting sterols, flavonoids, alkaloids, tannins, and phenols; which are known antioxidant class of compounds. Also, active antimicrobial compounds were extracted with ethyl acetate and methanol due to the solubility of bioactive phenols, tannins, flavonoids, saponins, and glycosides in these solvents. The GC-MS chromatogram of the fractions confirms the presence of thirty-five significant compounds excluding compounds in the methanol fraction due to the poor peak resolution of the polar laden biologically active consistent.

The search for potent antimicrobial compounds should be focused on the ethyl acetate and methanol fractions, while the antioxidant-rich bioactive principles are chloroform soluble. The stem bark was found to have hyperaccumulation capacity for heavy metals because of the significant amount of metal-chelating secondary metabolites present. Regulations can be put in place to carefully control the application of *A. decurrens* as a phytoremediator of heavy metals from contaminated industrial sites.

Conflict of Interest Statement

We declare that we have no conflict of interest.

References

Aaby, K., Ekeberg, D., & Skrede, G. (2007). Characterization of Phenolic Compounds in Strawberry (*Fragaria ananassa*) Fruits by Different HPLC Detectors and Contribution of Individual Compounds to Total Antioxidant Capacity. *Journal of Agricultural and Food Chemistry*, *55*(11), 4395–4406. https://doi.org/10.1021/jf0702592

Anam, E. (1998). Novel Diterpenoids from *Acacia decurrens* (Fabaceae). *Indian J. Chem*, *37*(12), 1307–1309.

Antonovics, J., Bradshaw, A. D., & Turner, R. G. (1971). Heavy Metal Tolerance in Plants. *Advances in Ecological Research*, *7*, 1–85. https://doi.org/10.1016/S0065-2504(08)60202-0

Arts, I. C. W., & Hollman, P. C. H. (2005). Polyphenols and disease risk in epidemiologic studies. *The American Journal of Clinical Nutrition*, *81*(1 Suppl), 317S–325S. https://doi.org/10.1093/ajcn/81.1.317S

Beyer, R. E. (1994). The role of ascorbate in antioxidant protection of biomembranes: Interaction with vitamin E and coenzyme Q. *Journal of Bioenergetics and Biomembranes*, *26*(4), 349–358. https://doi.org/10.1007/BF00762775

Bratzler, D. W., Dellinger, E. P., Olsen, K. M., Perl, T. M., Auwaerter, P. G., Bolon, M. K., … Weinstein, R. A. (2013). Clinical practice guidelines for antimicrobial prophylaxis in surgery. *American Journal of Health-System Pharmacy*, *70*(3), 195–283. https://doi.org/10.2146/ajhp120568

Can, M., Bulut, E., Örnek, A., & Özacar, M. (2013). Synthesis and characterization of valonea tannin resin and its interaction with palladium (II), rhodium (III) chloro complexes. *Chemical Engineering Journal*, *221*, 146–158. https://doi.org/10.1016/j.cej.2013.01.043

Cao, G., Sofic, E., & Prior, R. L. (1996). Antioxidant Capacity of Tea and Common Vegetables. *Journal of Agricultural and Food Chemistry*, *44*(11), 3426–3431. https://doi.org/10.1021/jf9602535

Chandrashekar, R., & Rao. (2013). Phytochemical analysis of an ethanolic extract of leaves of *Leucas indicia* (EELLI). *International Journal of Pharma and Bio Sciences*, *4*(1), 33–38.

Chiou, G. C. Y., & Martin, M. K. (1980). Chemotherapy of neuroblastoma in mice with anticancer agents. *Journal of Pharmaceutical Sciences*, *69*(5), 592–594. https://doi.org/10.1002/jps.2600690532

Cororaton, C. B., Orden, D., & Peterson, E. (2009). *A review of the literature on the economics of invasive species*.

De Amorim, E. L., de Castro, V. T., de Melo, J. G., Correa, A. J. C., & Sobrinho, T. J. (2012). Standard Operating Procedures (SOP) for the Spectrophotometric Determination of Phenolic Compounds Contained in Plant Samples. *Latest Research into Quality Control*, 47–66. https://doi.org/10.5772/51686

Dhanani, T., Shah, S., Gajbhiye, N. A., & Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry*, *10*, S1193–S1199. https://doi.org/10.1016/j.arabjc.2013.02.015

Elliot, W. R., & Jones, D. L. (2002). *Encyclopedia of Australian plants suitable for cultivation*. *Encyclopedia of Australian Plants* (2nd ed., Vol. 8). Port Melbourne: Lothian Press.

Fazel, S., Hamidreza, M., Rouhollah, G., & Verdian-Rizi, M. (2010). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Journal of Applied Horticulture*, *12*(1), 69–70.

Ghasemzadeh, A., Jaafar, H. Z. E., Rahmat, A., Wahab, P. E. M., & Halim, M. R. A. (2010). Effect of different light intensities on total phenolics and flavonoids synthesis and anti-oxidant activities in young ginger varieties (*Zingiber officinale* Roscoe). *International Journal of Molecular Sciences*, *11*(10), 3885–3897. https://doi.org/10.3390/ijms11103885

Gonçalves, C., Rodrigues, A., & Padovese, L. (2012). Identification of lubricant contamination by biodiesel using vibration analysis and neural network. *Industrial Lubrication and Tribology*, *64*(2), 104–110. https://doi.org/10.1108/00368791211208714

Greenblatt, C. L., Baum, J., Klein, B. Y., Nachshon, S., Koltunov, V., & Cano, R. J. (2004). *Micrococcus luteus* - Survival in Amber. *Microbial Ecology*, *48*(1), 120–127. https://doi.org/10.1007/s00248-003-2016-5

Halliwell, B. (1994). *Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? Lancet* (Vol. 344). Lancet. https://doi.org/10.1016/S0140-6736(94)92211-X

Harris, R. (2003). Trease and Evans' Pharmacognosy. *International Journal of Aromatherapy*, *13*(1), 55. https://doi.org/10.1016/S0962-4562(03)00051-1

Hudson, C. M., Bent, Z. W., Meagher, R. J., & Williams, K. P. (2014). Resistance Determinants and Mobile Genetic Elements of an NDM-1 Encoding *Klebsiella pneumoniae* Strain. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0099209

Hutton Balfour, J. (1866). Plants of the Bible.

Joseph, B., & Sekomeng, J. (2017). New Pharmacophore from the Stem Bark Fractions of *Acacia decurrens* (Wild) an Invasive South Africa Tree. *Journal of Applied Chemistry*, *2017*, 1–28. https://doi.org/10.1155/2017/1815278

Lee, S. E., Hwang, H. J., Ha, J. S., Jeong, H. S., & Kim, J. H. (2003). Screening of medicinal plant extracts for antioxidant activity. *Life Sciences*, *73*(2), 167–179. https://doi.org/10.1016/S0024-3205(03)00259-5

Longair, M. S. (2008). Maxwell and the science of colour. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *366*(1871), 1685–1696. https://doi.org/10.1098/rsta.2007.2178

Makkar, H. P., Siddhuraju, P., & Becker, K. (2007). Methods in molecular biology: Plant Secondary Metabolites. *Totowa: Human Press*, *393*, 93–100.

Manjunatha, B. (2006). Antibacterial activity of *Pterocarpus santalinus*. *Indian Journal of Pharmaceutical Sciences*, *68*(1), 115. https://doi.org/10.4103/0250-474X.22982

Mwita, C. (2011). Determination of Heavy Metal Content in Water, Sediment and Microalgae from Lake Victoria, East Africa. *The Open Environmental Engineering Journal*, *4*(1), 156–161. https://doi.org/10.2174/1874829501104010156

Nyila, M. A., Leonard, C. M., Hussein, A. A., & Lall, N. (n.d.). Activity of South African medicinal plants against Listeria monocytogenes biofilms, and isolation of active compounds from. Acacia karroo. *S. Afr. J.*, *78*, 220–227.

Padmavathi, M. (2013). International Journal of Herbal Medicine. *International Journal of Herbal Medicine*, *1*(3), 56–60.

Pourmorad, F., Hosseinimehr, S. J., & Shahabimajd, N. (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology*, *5*(June), 1142–1145.

Quattrocchi, U. (2006). *CRC World Dictionary of grasses. Volume I, A-D*. 1 A-C. CR C, Press. https://doi.org/10.1201/9781420003222

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying

an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Seeram, N., Lee, R., Scheuller, S., & Heber, D. (2006). Identification of phenolic compounds in strawberries by liquid chromatography electrospray ionization mass spectroscopy. *UCLA, 97,* 1–11. https://doi.org/10.1016/j.foodchem.2005.02.047

Seneviratne, A. S., & Fowden, L. (1968). The amino acids of the genus Acacia. *Phytochemistry*, *7*(7), 1039–1045. https://doi.org/10.1016/S0031-9422(00)88249-7

Singh, R. (2012). Total phenolic, flavonoids and tannin contents in different extracts of Artemisia absinthium. *Journal of Intercultural Ethnopharmacology*, *1*(2), 101–104. https://doi.org/10.5455/jice.20120525014326

Singh, S., Parihar, P., Singh, R., Singh, V. P., & Prasad, S. M. (2015). Heavy Metal Tolerance in Plants: Role of Transcriptomics, Proteomics, Metabolomics, and Ionomics. *Frontiers in Plant Science*, *6*, 1143.

Soobrattee, M. A., Neergheen, V. S., Luximon-Ramma, A., Aruoma, O. I., & Bahorun, T. (2005). Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. *Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis*, *579*(1–2), 200–213. https://doi.org/10.1016/j.mrfmmm.2005.03.023

Tejavathi, D., & Jayashree, D. (2013). Phytochemical Screening of Selected Medicinal Herbs Inoculated With *Arbuscular mycorrhizal* Fungi. *Ijbpas*, *2*(211), 2090–2106.

Tewari, A., & Jindal, V. K. (2010). Studies on uronic acid materials and structure of Acacia decurrens gum polysaccharide. *J. Chem. Pharm. Res*, *2*(2), 233–239.

Török, A., Gulyás, Z., Szalai, G., Kocsy, G., & Majdik, C. (2015). Phytoremediation capacity of aquatic plants is associated with the degree of phytochelatin polymerization. *Journal of Hazardous Materials*, *299*(299), 371–378. https://doi.org/10.1016/j.jhazmat.2015.06.042

Uchiyama, N., Kiuchi, F., Ito, M., Honda, G., Takeda, Y., Khodzhimatov, O. K., & Ashurmetov, O. A. (2003). New icetexane and 20-norabietane diterpenes with trypanocidal activity from *Dracocephalum komarovi*. *Journal of Natural Products*, *66*(1), 128–131. https://doi.org/10.1021/np020308z

Velásquez, J. O., Jiménez, G. J., & Sepúlveda, M. (2007). Determinación de la calidad ambiental de la Ciénaga Colombia. *Gestión y Ambiente*, *10*(4), 187–200.

Wahid, H., Ahmad, S., Nor, M. A. M., & Rashid, M. A. (2017). *Prestasi kecekapan pengurusan kewangan dan agihan zakat: perbandingan antara majlis agama islam negeri di Malaysia*. *Jurnal Ekonomi Malaysia* (Vol. 51).

Wallace, R. D., & Bargeron, C. T. (2014). Identifying invasive species in real time: early detection and distribution mapping system (EDDMapS) and other mapping tools. In *Invasive Species and Global Climate Change* (pp. 219–231). https://doi.org/10.1079/9781780641645.0219

Young, I. S., & Woodside, J. V. (2001). Antioxidants in health and disease Antioxidants in health and disease. *J Clin Pathol*, *54*, 176–186. https://doi.org/10.1136/jcp.54.3.176

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*. https://doi.org/10.1016/S0308-8146(98)00102-2