Pharmacological evaluation of hydro-ethanol and hot water leaf extracts of *Bauhinia galpinii* (Fabaceae): A South African ethnomedicinal plant

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Highlights

- *Bauhinia galpinii* is used to treat gastrointestinal disorders, inflammation and infectious diseases.
- Hot water and ethanol–water extracts were prepared to mimic traditional use.
- Strong activity was detected against *Salmonella* Typhimurium and *Candida albicans*.
- Moderate anti-inflammatory and antioxidant activity support traditional uses of *B. galpinii*.
- Three potentially bioactive flavonoids were identified by UPLC-MS.

ABSTRACT

Bauhinia galpinii is one of several plant species native to South Africa that has been reported to be used traditionally to manage ailments including gastrointestinal disorders, infectious diseases and inflammation. This study was designed to evaluate the *in vitro* antibacterial, antifungal, antioxidant, cytotoxicity and anti-inflammatory activities of the hydro-ethanol (70% ethanol) and hot water extracts of *Bauhinia galpinii* leaves. The antibacterial and antifungal activities of the extracts were determined using the serial microplate dilution technique. The minimum inhibitory concentrations

(MIC) of seven bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), *Salmonella enterica* Serotype Dublin (*S.* Dublin), *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and three clinical fungal isolates (*Aspergillus fumigatus, Candida albicans* and *Cryptococcus neoformans*) were determined. The extraction yield and MIC values were used to determine the total activity (TA) of the extracts. Radical scavenging potential of the extracts was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay while the lipoxygenase (LOX) enzyme inhibitory assay was used to ascertain the anti-inflammatory activity. The safety of the extracts was determined using the colorimetric mitochondrial viability assay against Vero, Caco-2 and C3A cells. UPLC-MS analysis was also conducted to detect and identify the compounds present in the plant. Results from the study showed that the extracts had inhibitory activities against the growth of all tested pathogens with MIC values ranging from 0.04 to 1.25 mg/ml. Both extracts were most active against *S.* Typhimurium with MIC = 0.16 mg/ml, while the hot water extract was very active at 48 h incubation against *Candida albicans* with MIC = 0.04 mg/ml. The hydro-ethanol and hot water extracts had high total antibacterial activity (TAA) of 1000 and 937 ml/g against *Salmonella* Typhimurium respectively. The highest total antifungal activity of 3750 ml/g was recorded against C*andida albicans* by the hot water extract. The extracts showed strong DPPH radical scavenging activity with the hydro-ethanol extract displaying higher scavenging activity (IC_{50} = 3.72 mg/ml) than the hot water extract (IC_{50} = 10.82 mg/ml). Both extracts had moderate lipoxygenase inhibitory activity. The extracts had relatively low cytotoxic to non-cytotoxic effects with LC_{50} values of 0.094 to >1.000 mg/ml and selectivity index values of 0.075 to 22.698. Three compounds with known biological activity (2"-*O*-rhamnosylvitexin, myricetin 3-*O*-galactopyranoside and quercetin 3-*O*-galactopyranoside) were tentatively identified from UPLC-MS analysis. Therefore,

this study supports the folkloric use of the plant in treating gastrointestinal disorders, inflammation and infectious diseases.

Keywords: Antimicrobial; Anti-inflammatory; *Bauhinia galpinii*; Cytotoxicity; Fabaceae; UPLC-MS

1. Introduction

Herbal medicines comprise one of the ancient therapies used by man (Li and Vederas, 2009). In recent times, the demand for these traditional therapies has soared, in part owing to their relatively lower complications and fewer side effects than synthetic drugs (Boyd et al., 2013). Reports show that in developing countries, up to 80% of people living in rural areas rely on traditional plant-based medicines for their primary healthcare (United Nations, 2018). Similar assertions have been made in the past where, in a myriad of developing countries, traditional medicine is one of the primary healthcare systems (Farnsworth, 1993; Houghton, 1995). Herbs have been exploited to a great extent in traditional medical systems, and their healing potential is acknowledged and documented (Dubey et al., 2004). Additionally, the therapeutic efficacy of several indigenous plants for numerous disorders has been described by many traditional medicine practitioners (Mabogo, 1990; Rabe and Van Staden, 1997; Ramasamy and Manoharan, 2004; Van Wyk and Gericke, 2000; Watt and Breyer-Brandwijk, 1962).

Plants form an integral part of South Africa's rich culture and biodiversity, with many species serving as a cornerstone of traditional medicine for symptomatic treatment and alleviation of various diseases. Many South Africans rely on medicinal plants for their health care needs, including some patients who access conventional drugs (Ahmed et al., 2012). South Africa has approximately 10% of the vascular plant species diversity of the world, a potential source of undiscovered secondary metabolites with

activity against a variety of bacterial and fungal pathogens (Shai et al., 2008). One such indigenous plant in South Africa with reported ethnomedical uses is *Bauhinia galpinii* N.E.Br. *B. galpinii,* commonly called "Pride of De Kaap" or Pride of the Cape, which is endemic to parts of eastern and southern Africa. It is an attractive shrub or creeper belonging to the family Fabaceae. It is a common garden plant, particularly in the warmer areas, and originates from South Africa (Aderogba et al., 2007; Hankey, 2001). Ethnomedically, the leaves are used to treat epilepsy and convulsions (Risa et al., 2004), the bark and leaves to manage diarrhoea and infertility (Mabogo, 1990; Samie et al., 2010), and the seeds to treat amenorrhoea (Van Wyk and Gericke, 2000). The bark and roots are used against stomach spasms, and the root is also used to treat infertility (Arnold and Gulumian, 1984). The tuber is used to treat pneumonia, venereal disease and diarrhoea (Van Wyk and Gericke, 2000). Generally, *Bauhinia* species, including those native to South Africa, are used traditionally in medicine across the world to treat diabetes, inflammation, gastrointestinal tract (GIT) disorders and infectious diseases (Ahmed et al., 2012; Filho, 2009).

Pharmacologically, several experiments have been done on the leaves and other parts of *B. galpinii*. The fresh, dried leaf resulted in toxicity in a sheep feeding test (Watt and Breyer-Brandwijk, 1962). In 2004, Risa and colleagues tested the water and ethanol leaf extracts of the plant for their antiepileptic properties (Risa et al., 2004). Reid et al. (2006) reported the antimutagenicity of the methanol and dichloromethane leaf extracts. The antibacterial activity of the acetone extract of the bark against 110 clinical strains of *Campylobacter* was carried out by Samie et al. (2009). Aderogba et al. (2007) assessed the methanol and solvent fractions (ethyl acetate, hexane, dichloromethane and butanol) of the leaf extract for antioxidant potential and cytotoxicity. The antimicrobial, antioxidant, anti-inflammatory and cytotoxicological properties of the acidified 70% acetone crude extract and fractions of the leaf were also investigated (Ahmed et al., 2012).

Screening plants for bioactive compounds has become increasingly prominent in recent years (Rabe and Van Staden, 1997). One reason for this burgeoning interest in screening plants is the search for new lead compounds potentially useful in producing pharmaceuticals and phytomedicines (Eloff, 2004). Thus, given the above and the need to validate some of the reported uses of *B. galpinii*, we were motivated to investigate the antimicrobial, antioxidant, anti-inflammatory and cytotoxicological properties of the plant. The work was also designed to investigate the activity of widely used extractants by traditional medicine practitioners for possible herbal drug formulation of the extracts.

2. Materials and methods

2.1 Plant collection and authentication

Fresh leaves of *Bauhinia galpinii* were collected in February 2017 in Roodeplaat, Pretoria, South Africa. The plant was identified and authenticated at the H.G.W.J. Schweickerdt Herbarium of the Department of Plant and Soil Sciences, University of Pretoria, South Africa. The prepared voucher specimen was deposited at the herbarium with a voucher specimen number PRU123537. The collected leaves were stored in loosely woven bags for air drying. The dried leaves were blended to fine powder and kept in air-tight glass jars in the dark at room temperature prior to extraction.

2.2 Preparation of plant extracts

Distilled water and technical grade ethanol (70%) were used as extractants in a ratio of plant material: solvent of 1: 10 (w/v) (Eloff, 1998a). The finely powdered leaves (4 g and 3 g) were extracted with 40 ml of 70% ethanol and 30 ml of hot distilled water respectively. The resultant mixtures were sonicated for 10 min and filtered through Whatman No 1 filter paper after 48 h into pre-weighed sterile glass vials. It should be noted that before sonication, the extract prepared with distilled water was heated for 25 min at 55ºC to enhance the extraction of active metabolites. The extracts were concentrated to dryness under a stream of warm air and kept below 5ºC in air-tight glass jars. The extractants used

were chosen following their widely reported use in traditional medicine practice. The extract yield in percentage and grams was determined using the formula (% yield= mass of extract/mass of powdered material x 100).

2.3 Antioxidant assay

The protocol outlined by Brand-Williams et al. (1995) with modifications by Ahmed et al. (2014) was used to determine the radical scavenging of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) by the extracts. Forty microliters (40 µl) of methanol was initially added to the wells of 96-well microtitre plates with an equal volume of the extracts (2.5 mg/ml) added to designated wells and serially diluted. An aliquot of 160 µl of DPPH was added to the selected wells, and 160 µl of methanol also added to respective wells. The plates were then incubated for 30 min in the dark and absorbance was read with the aid of a microplate reader (Epoch™ Microplate Spectrophotometer) at 517 nm. Ascorbic acid and Trolox at 1 mg/ml were used as positive controls while the negative control was the absorbance of the DPPH in methanol without antioxidant. The radical scavenging potential of the extracts was calculated using the equation below-

$$\% \text{ inhibition of DPPH radical} = [(A0 - A1 / A0) \times 100],$$

Where A0 is the absorbance of the negative control, and A1 is the absorbance of the extract or standard sample.

The IC_{50} (50% inhibitory concentration) values were determined using the non-linear regression curve of the percentages of inhibition of DPPH radical against the logarithm of concentrations tested.

2.4 Antimicrobial activity

The investigated bacterial strains included *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), *Salmonella* Typhimurium (ATCC 39183), *Salmonella enterica* Dublin (ATCC 15480), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella*

pneumoniae (clinical isolate). The fungal strains which were all clinical isolates included *Candida albicans, Aspergillus fumigatus* and *Cryptococcus neoformans*. All strains were sourced from the collection of the Phytomedicine Programme, University of Pretoria, South Africa. The antibacterial and antifungal activity of the extracts was investigated following the microdilution technique described by Eloff (1998b). The minimum inhibitory concentrations (MIC) and total antimicrobial activity (TAA) of the extracts were determined. The total activity is the total mass in mg extracted from 1 g of the powdered leaf material divided by the MIC in mg/ml. The total activity (ml/g) is an indication of the volume to which the extract from 1 g of plant material can be diluted and still hinder the growth of the microorganism (Eloff, 2000).

2.5 The 15-lipoxygenase (15-LOX enzyme) inhibitory assay

The spectrophotometric assay with slight modifications, according to del Carmen Pinto et al. (2007) was used in determining the inhibitory activity of the extracts against the 15-LOX enzyme. The assay assessed the inhibition of 15-lipoxygenase activity by the extracts in the presence of linoleic acid, based on the formation of the complex Fe^{3+}/xylenol orange with absorption at 560 nm. The substrate linoleic acid (final concentration 140 μM) was prepared in Tris-HCl buffer (50 mM, pH 7.4). The 70% ethanol extract (10 mg/mL) was suspended in DMSO and further diluted initially to 2 mg/mL in Tris-HCl buffer, and the water extract was prepared at 2 mg/mL in Tris-HCl buffer. Forty microliters of the enzyme (15-LOX), diluted in ice-cold Tris-HCl buffer (final concentration, 0.2U/mL), was mixed with 20 μL of different concentrations (100 to 0.78 μg/mL) of test samples as well as quercetin (positive control) at 25°C for 5 min. Linoleic acid (40 μL) was added to the mixture and was further incubated at 25°C for 20 min in the dark. The assay was terminated by the addition of 100 μL of freshly prepared FOX reagent [Sulfuric acid (30 mM), xylenol orange (100 μM), iron (II) sulfate (100 μM) in methanol/water (9:1)]. The negative control was a mixture of the 15-LOX enzyme

solution, Tris-HCl buffer, substrate and FOX reagent while the blanks (background) contained the 15-LOX enzyme and buffer, but the substrate was added after the FOX reagent. The lipoxygenase inhibitory activity was determined by computing the percentage of the inhibition of hydroperoxide production from the changes in absorbance values at 560 nm after 30 min at 25°C as indicated in the formula below.

$$\% \text{ inhibition} = [(A_{control} - A_{blank}) - (A_{sample} - A_{blank}) / (A_{control} - A_{blank})] \times 100.$$

Where $A_{control}$ is the absorbance of the control wells, A_{blank} is the absorbance of blank wells, and A_{sample} is the absorbance of sample wells.

The 50% inhibitory concentrations (IC_{50}) were determined using the linear regression curve of the percentage (15-LOX) inhibition against the logarithm of concentrations tested.

2.6 UPLC/MS detection of active compounds of Bauhinia galpinii leaf extract

One milligram per millilitre (1 mg/ml) of the extract was dissolved in 500 µl of acetonitrile and 500 µl water (UPLC grade) and sonicated for 5 min. From the mixture, 100 µl was pipetted into Eppendorf tubes and made up to 1 ml with acetonitrile and water and vortexed for 2 min. From the solution 100 µl was transferred into UPLC grade p-vials and made up to 1 ml. A blank containing acetonitrile and water was prepared. Compound detection was performed using a Waters® Synapt G2 high definition mass spectrometry (HDMS) system (Waters Inc., Milford, Massachusetts, USA). Samples were analysed using flow injection analysis (FIA). The system comprises a Waters Acquity Ultra Performance Liquid Chromatography (UPLC®) system hyphenated to a quadrupole-time-of-flight (QTOF) instrument. The system was operated with MassLynxTM (version 4.1) software (Waters Inc., Milford, Massachusetts, USA) for data acquisition and processing.

2.7 Toxicological evaluation of extracts of B. galpinii leaf

The toxicity of the extracts was assessed against a normal cell line (African green monkey kidney cell) and two cancer cell lines (C3A human liver and Caco-2-colon cancer cell). The MTT reduction assay, as previously described by Mosmann (1983) with minor modifications (McGaw et al., 2007), was adopted in ascertaining the cytotoxic levels of the extracts. The density of cells seeded was 0.1×10^6 cells/ml while the varying final concentration of extracts ranged from 0.025 to 1 mg/ml. The cytotoxic drug used as reference was doxorubicin (0.2 - 40 µM). The MTT reduction was assessed by measuring absorbance using a microplate reader (SpectraMax 190) at 570 nm. The cell growth inhibition as a percentage was computed via comparison with untreated cells. The LC_{50} values were determined as the concentration of the extracts that lead to a 50% reduction in absorbance compared to the untreated cells. The selectivity index (SI) values were calculated by dividing the LC_{50} values from the cytotoxicity by the MIC values ($SI = LC_{50}/MIC$).

2.8 Statistical analysis

The mean and standard error of means of the appropriate assays were determined. Percentages were also calculated. The IC_{50} and LC_{50} were determined using linear and non-linear regression curves where applicable. One Way ANOVA was done to compare means of different groups as well as a Tukey post hoc test to analyze differences among various means and the interaction between the variables. Differences at $P<0.05$ were considered statistically significant. SPSS 25.0 computer software package was used in all the analysis.

3. Results

3.1 Extract yield

The extractants, hydro-ethanol and hot water, gave different yields. The hydro-ethanol extract had a slightly higher yield of 16% with the hot water extract yielding 15%.

3.2. Antioxidant assay

The DPPH radical scavenging activity results of the hydro-ethanol and hot water extracts of *B. galpinii* leaves are presented in Table 1. The hydro-ethanol extract had an IC_{50} value of 3.72±0.30, whereas the hot water extract had IC_{50} = 10.82±0.58 (Table 1). The inhibitory activity of both extracts against the DPPH radical was significantly different from each other and the controls ($P<0.05$)

Table 1. DPPH radical scavenging activities of extracts of *B. galpinii* leaf expressed as IC_{50} (mg/ml)

Plant extracts	**IC_{50}**	**R^2**
Hydro-ethanol	3.72^{a} ±0.30	0.76
Hot water	10.82^{b} ±0.58	0.85
Trolox	$0.33^{c, e}$ ±0.14	0.85
Vitamin C	$1.65^{d, e}$ ±0.04	0.83

Values are mean±SEM, n = 2, Positive controls: Trolox and Vitamin C; Values with same superscript letters show no significant difference between means at $P<0.05$

3.3. 15-LOX enzyme inhibition assay

The potential of the hydro-ethanol and hot water extracts of *B. galpinii* leaf to inhibit the 15-LOX enzyme expressed in IC_{50} is presented in Table 2. Both extracts had an IC_{50} greater than 50 µg/ml compared to 24.61 µg/ml recorded for the positive control (quercetin) (Table 2). The inhibitory activity of the hydro-ethanol extract against the 15-LOX enzyme was not statistically different from that of the hot-water extract and control at $P<0.05$ (Table 3)

Table 2. Estimated IC_{50} (µg/ml) for 15-LOX enzyme of the extracts of *B. galpinii* leaf

Plant extracts	IC_{50}	R^2
Hydro-ethanol	$61.32^{a}\pm10.92$	0.9306
Hot water	$88.77^{a, b}\pm 9.73$	0.8863
Quercetin	$24.61^{a, c}\pm1.73$	0.9871

Values are mean±SEM, n=3; Different superscript letters show significant difference between means at P<0.05.

Table 3. Antibacterial activities of extracts of *B. galpinii* leaf.

Test organisms	Gentamicin (mg/ml)	Hydro-ethanol extract		Hot water extract	
		MIC(mg/ml)	TAA (ml/g)	MIC(mg/ml)	TAA (ml/g)
Escherichia coli	0.016	1.25	128.00	1.25	120.00
Enterococcus faecalis	0.500	1.25	128.00	1.25	120.00
Staphylococcus aureus	0.008	**0.31**	516.13	1.25	120.00
Salmonella Typhimurium	0.008	**0.16**	**1000.00**	**0.16**	**937.50**
Salmonella Dublin	0.004	0.63	253.97	1.25	120.00
Klebsiella pneumoniae	0.500	1.25	128.00	1.25	120.00
Pseudomonas aeruginosa	0.004	**0.31**	516.13	**0.31**	483.87

TAA- Total antimicrobial activity; values in bold indicate good activity

3.4. Antimicrobial activity

The antibacterial and antifungal potential of the extracts expressed in minimum inhibitory concentrations (MIC) and total antimicrobial activity (TAA) are presented in Tables 3 and 4 respectively. Both extracts had the best antibacterial activity against *Salmonella* Typhimurium at an MIC of 0.16 mg/ml and a TAA range of 938 to 1000 ml/g. The hot water extract had the best antifungal activity against *Candida albicans* after 48 hours incubation with an MIC of 0.04 mg/ml and a corresponding TAA of 3750 ml/g.

Table 4. Antifungal activities of extracts of *B. galpinii* leaf after 48 and (72) hours

Test organisms	Amphotericin B **(mg/ml)**	**Hydro-ethanol extract**		**Hot water extract**	
		MIC(mg/ml)	**TAA (ml/g)**	**MIC(mg/ml)**	**TAA(ml/g)**
Candida albicans	0.031(0.500)	**0.31**(0.63)	516.13(253.97)	***0.04**(0.63)	**3750.00**(238.10)
Aspergillus fumigatus	0.008(0.125)	0.63(**0.31**)	253.97(516.13)	0.63(1.25)	238.10(120.00)
Cryptococcus neoformans	0.031(0.063)	**0.31**(0.63)	516.13(253.97)	**0.31**(0.63)	483.87(238.10)

TAA- Total antifungal activity, values in bold indicate good and *excellent activity

3.5. Ultra-performance liquid chromatography- mass spectrometry (UPLC/MS) detection of active compounds of Bauhinia galpinii

Ultra-performance liquid chromatography-mass spectrometry electrospray ionization (UPLC-MS-ESI) chromatogram of the hydro-ethanol extract of *B. galpinii* indicated three compounds (molecular weight at m/z 577.1552 $[M-H]^-$, 479.0816 $[M-H]^-$ and 463.0872 $[M-H]^-$), as the major constituents

(Fig. 1). These compounds are 2''-*O*-rhamnosylvitexin, myricetin 3-*O*-galactopyranoside and quercetin 3-*O*-galactopyranoside, respectively (Table 5).

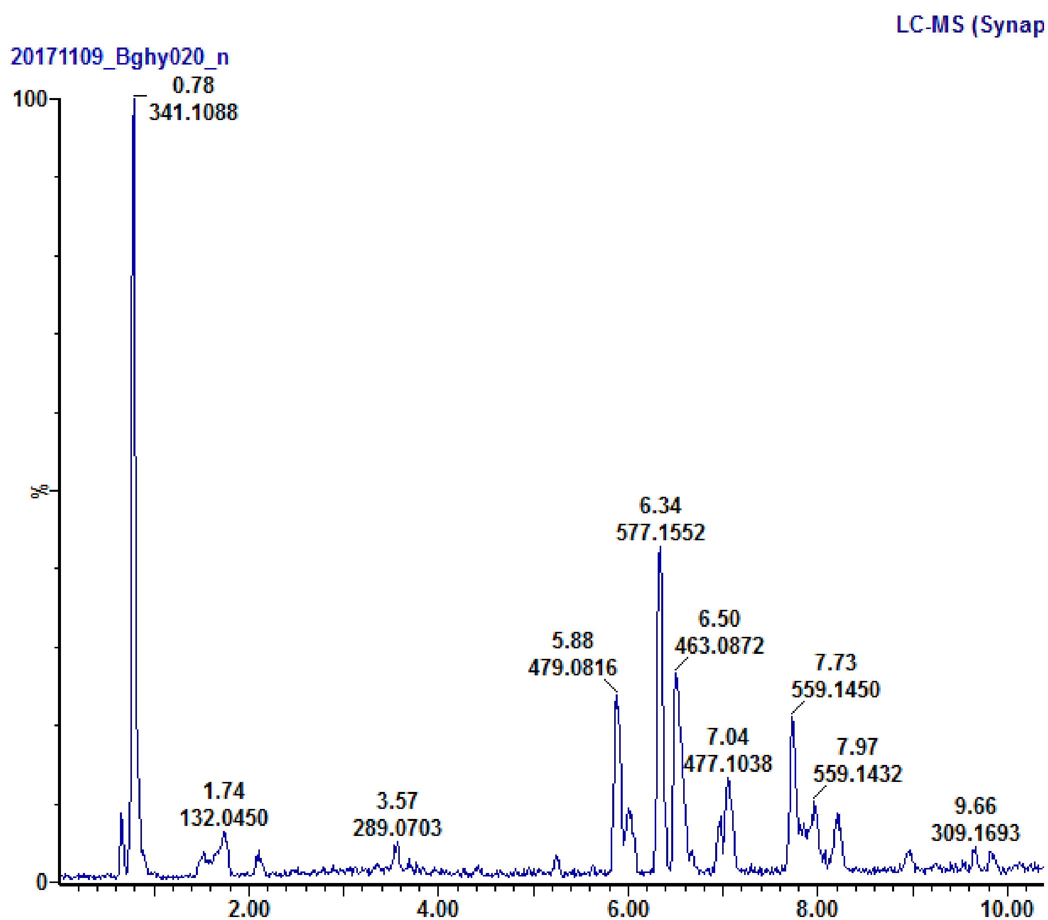

Fig. 1. UPLC-MS-ESI chromatogram fingerprints of the hydro-ethanol extract of *B. galpinii* leaf

Table 5. Detected compounds and characteristic peaks of UPLC-MS-ESI fingerprints of the hydro-ethanol extract of *B. galpinii* leaf.

Compound names	Retention time (min)	$[M-H]^-$ (m/z)	Molecular weight
2''-*O*-rhamnosylvitexin	6.34	577.1552	578
Myricetin 3-*O*-galactopyranoside	5.88	479.0816	480
Quercetin 3-*O*-galactopyranoside	6.50	463.0872	464

3.6. Cytotoxicity assay

The results of the *in vitro* cytotoxicity assessment of the extracts expressed in LC_{50} and selectivity index on the cell viability of three cell lines (Vero kidney, Caco-2 and C3A) are reported in Table 6. The hot water extract had higher LC_{50} values of 0.107 mg/ml and greater than 1.000 mg/ml (the highest concentration tested) against Vero kidney and C3A cells than the hydro-ethanol extract, with LC_{50} = 0.094 and 0.3935 mg/ml respectively. For Caco-2 cells, the hydro-ethanol extract had a higher LC_{50} of greater than 1.000 mg/ml compared to 0.9079 for the hot water extract. The hot water extract had the highest selectivity index of 22.698 against *Candida albicans* and Caco-2 cells.

Table 6. Cytotoxic effects and selectivity index values of *Bauhinia galpinii* leaf extracts against Vero kidney, Caco-2 and C3A cells.

Cell lines	**Plant extracts**	**LC_{50} (mg/ml)**	**Selectivity index (LC_{50}/MIC)**									
			Ec	**Ef**	**Sa**	**ST**	**SD**	**Kp**	**Pa**	**Ca**	**Af**	**Cn**
Vero cells	**Hydro-ethanol**	0.094	0.075	0.075	0.304	0.589	0.150	0.075	0.304	0.304	0.150	0.304
	Hot-water	0.107	0.086	0.086	0.086	0.669	0.075	0.086	0.346	**2.678**	0.170	0.346
	*Doxorubicin	0.002	Na	Na	Na	Na	Na	Na	Na	Na	Na	Nd
Caco-2 cells	**Hydro-ethanol**	>**1.0000**	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	**2.929**
	Hot-water	0.9079	0.726	0.726	0.726	**5.674**	0.726	0.726	**2.929**	**22.698**	**1.441**	Na
	*Doxorubicin	0.0004	Na	Na	Na	Na	Na	Na	Na	Na	Na	
C3A cells	**Hydro-ethanol**	0.3935	0.315	0.315	**1.269**	**2.459**	0.625	0.315	**1.269**	**1.269**	0.625	**1.269**
	Hot-water	>**1.0000**	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	*Doxorubicin	<0.0001	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na

*Positive control; Ec- *Escherichia coli*; Ef- *Enterococcus faecalis; Sa- Staphylococcus aureus; ST- Salmonella* Typhimurium*; SD- Salmonella enterica* Dublin*;* *Kp- Klebsiella pneumonia; Pa- Pseudomonas aeruginosa; Ca- Candida albicans; Af- Aspergillus fumigatus; Cn- Cryptococcus neoformans*; Nd- not determined; Na- not applicable*;* Values in bold indicate extracts with low to no cytotoxicity and best SI values.

4. Discussion

4.1 Antioxidant properties of the extracts

Generally, plant extracts with low IC_{50} or high percentage inhibition are said to have good antioxidant activity. In this study, we observed that the hydro-ethanol extract with an IC_{50} of 3.72 mg/ml was more active in scavenging the DPPH radical than the hot water extract with an IC_{50} of 10.82 mg/ml. The positive controls (Trolox and Vitamin C) had IC_{50} values of 0.33 and 1.65 mg/mL, respectively (Table 1). Our results further confirm the strong DPPH scavenging capacity of *B. galpinii* leaf extracts following previous reports on the antioxidant capacity of the acidic acetone (polyphenolic enriched) methanol, solvent fractions and isolated compounds (Aderogba et al., 2007; Ahmed et al., 2012). Thus, the strong antioxidant capacity of the extracts displayed in this study suggests that the plant can be used to reduce the excess free radicals produced in the body causing damage to cellular macromolecules like proteins (enzymes), lipids and DNA leading to oxidative stress and neurodegenerative disorders (Aderogba et al., 2013). This agrees with previous reports that antioxidants can be used to ameliorate conditions caused by excess free radicals (Reynolds et al., 2007).

4.2 Anti-inflammatory activity of the extracts

In this study, both extracts had moderate activity against the 15-LOX enzyme. The range of IC_{50} values of the hydro-ethanol and hot water extracts obtained in this study is similar to an earlier report on the activity of crude extracts of *B. galpinii* by Ahmed et al. (2012). Also, the extracts had IC_{50} values comparable to those of other LOX inhibitory crude plant extracts in the range of 1-100 μg/mL reported elsewhere (Chung et al., 2009; Dzoyem and Eloff, 2015; Ferhat et al., 2017; Motlhatlego et al., 2018; Schneider and Bucar, 2005). The anti-inflammatory activity of *B. galpinii* leaf extracts is an indication that it contains active secondary metabolites that can inhibit the LOX

enzyme. It further supports the use of species of the genus *Bauhinia* for managing inflammation and other related ailments traditionally.

4.3 Antimicrobial effects of the extracts

The hydro-ethanol and hot water extracts of *B. galpinii* leaves were active against all tested organisms with MICs ranging between 0.16 and 1.25 mg/ml, and 0.04 to 1.25 mg /ml for bacteria and fungi respectively. According to Rios and Recio (2005) plant extracts with MIC above 1 mg/ml are not active whereas MIC values below 0.1 mg/ml should be considered interesting. Other authors have also postulated what they consider to be excellent, significant/strong, good, moderate and weak antimicrobial activity (Cos et al., 2006; Eloff, 2004; Holetz et al., 2002; Kuete, 2010; Omokhua et al., 2018; Van Vuuren, 2008) (Supplementary Table 1). In this study we consider the antimicrobial activity of the extracts with MIC values of 0.02 -0.04 mg/ml as excellent, 0.08 mg/ml as very good, 0.16 -0.63 mg/ml as good and 1.25 – 2.5 mg/ml as weak. Both extracts had very good antibacterial activity against the Gram-negative bacteria (*Salmonella* Typhimurium) at an MIC of 0.16 mg/ml (Table 4). The hydro-ethanol extract had better antibacterial activity against *Staphylococcus aureus* at an MIC of 0.31 mg/ml and *S.* Dublin at an MIC of 0.63 mg/ml than the hot water extract with MIC= 1.25 mg/ml for the two organisms. However, *E. coli*, *E. faecalis* and *K. pneumoniae* were resistant to both extracts at an MIC of 1.25 mg/ml (weak activity) (Table 3). In a previous study, the polyphenolic-rich crude extracts and fractions of *B. galpinii* leaf showed various degrees of growth inhibition against similar organisms used in this study (Ahmed et al., 2012). This confirms that, although with varying extractants used, the plant contains antibacterial compounds, supporting its traditional use in managing infectious diseases such as diarrhoea. The hydro-ethanol extract had better total antibacterial activity (with TAA values ranging between 128 – 1000 ml/g) than the hot water extract (TAA = 120 – 938 ml/g) (Table 3). The highest TAA value

of 1000 ml/g was recorded against *Salmonella* Typhimurium for the hydro-ethanol extract. The TAA results imply that the extract from 1 g of dry plant material can be diluted to 1000 ml and still inhibit the growth of the microorganism (Eloff, 2000). Generally, a high TAA means the plant has good potential to be used as an antimicrobial agent (Eloff, 2000; Shai et al., 2013). The hot water extract had excellent or pharmacologically noteworthy antifungal activity against *Candida albicans* with an MIC of 0.04 mg/ml after 48h incubation (Table 4). Both extracts had good antifungal activities against *Aspergillus fumigatus* (MIC = 0.63 mg/ml after 48 h incubation), *Candida albicans* (MIC = 0.63 mg/ml after 72 h incubation) and *Cryptococcus neoformans* (MIC = 0.31 mg/ml and 0.63 mg/ml after 48 and 72 h incubation) (Table 4). Good activity was also noted for the hydro-ethanol extract against *Candida albicans* (MIC = 0.31 mg/ml after 48 h incubation) whereas *Aspergillus fumigatus* was most resistant to the hot water extract with an MIC of 1.25 mg/ml. The antifungal activity of the extracts against the tested fungal species corroborates previous investigations by Ahmed et al. (2012) on the same plant, but interestingly, the hot water extract used in this study with an MIC of 0.04 mg/ml had better activity than the polyphenolic-rich crude extracts and fractions (MIC between 0.08 – 0.63 mg/ml) previously explored. This is most likely due to the extractant or method of extraction used, or the possible synergistic activity of bioactive compounds contained in the extract. The hot water extract had the highest total antifungal activity of 3750 ml/g against *Candida albicans*. This indicates the potential use of the plant in managing diseases/infections caused by *C. albicans*. The reference antibiotics (gentamicin and amphotericin B) had good to excellent antibacterial and antifungal activities against the tested bacterial and fungal strains with MIC values ranging between 0.004 to 0.500 mg/ml and 0.008 to 0.500 mg/ml for bacteria and fungi respectively. The antimicrobial profile of the extracts reveals that the plant had activity against both bacteria (Gram-positive and Gram-negative) and fungi

(moulds and yeasts). The low resistance of the bacterial and fungal isolates against the extracts may be an indication of the broad-spectrum bioactive secondary metabolites they contain (Ahmed et al., 2012). Additionally, the antimicrobial activity of the extracts can be described as biologically noteworthy. This is based on previous work (Holetz et al., 2002; Rios and Recio, 2005) that antimicrobial activity of extracts with MIC below 1 mg/ml are pharmacologically significant. Therefore, the extracts can be good candidates for exploring antimicrobial compounds to manage infectious diseases.

4.4 Ultra Performance Liquid Chromatography-Mass Spectrometry analysis

From the chromatogram results (Fig. 1), it can be affirmed that 2''-*O*-rhamnosylvitexin, myricetin 3-*O*-galactopyranoside and quercetin 3-*O*-galactopyranoside are the major antimicrobial and antioxidant agents responsible for the activity of *Bauhinia galpinii* which displayed antimicrobial and antioxidant activity (Aderogba et al., 2007; Ahmed et al., 2012). The identified compounds in this study from the crude extracts are similar to the compounds earlier identified from the methanol and solvent fractions (ethyl acetate and butanol) from the leaf of *B. galpinii* (Aderogba et al., 2007). Unfortunately, there was not enough material to run UPLC/MS analysis on the hot water extract. Previous studies elsewhere (Ahmed et al., 2012) confirmed the antimicrobial potential (MIC between 7.8 and 125 µg/ml) of the detected and identified compounds in this study against selected microorganisms (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922). Aderogba et al. (2007) also reported the antioxidant activity (EC_{50}:11.33 µM and EC_{50}:20.52µM) of myricetin-3-O-galactopyranoside and quercetin-3-O-galactopyranoside.

4.5 Cytotoxicity of the extracts

The safety of traditional medicines, particularly those of plant origin as well as from other natural sources, is often trivialized. These natural products with traditional medicinal uses are generally deemed safe. However, studies have shown that chemicals from natural sources like plants can be cytotoxic, genotoxic and carcinogenic when consumed without caution or in excess (Ahmed et al., 2012). Generally, it has been shown that plants of the genus *Bauhinia* are non-toxic (Barata, 2005). This has been supported by several studies in animal models. For example, Pepato et al. (2004) showed that *Bauhinia forficata* did not affect toxicological biomarkers (amylase, creatine kinase, lactate dehydrogenase, bilirubin and angiotensin-converting enzyme). Similarly, the orally administered stem-bark extract of *Bauhinia racemosa*, at doses of 100 and 200 mg/kg had no untoward effect on haematological parameters, liver and kidney functions (Kumar et al., 2005). In another study, Reid et al. (2006) using the Ames assay reported the antimutagenic properties of *Bauhinia galpinii* leaf extract in the presence of the S9 metabolic activator without causing any mutagenic response against *Salmonella* Typhimurium. These observations corroborate our results on the non-toxic effects of the extracts of *B. galpinii* against the three cell lines with LC_{50} ranging from 0.094 to greater than 1.000 mg/ml (Table 6). In a previous study on the leaf of *B. galpinii*, Ahmed et al. (2012) reported an LC_{50} value of 0.036 mg/ml compared to the LC_{50} range of 0.094 to 0.107 mg/ml obtained in this study. Despite the disparity in LC_{50} values, the extracts can be deemed non-toxic to cells. This takes into consideration the assertion that plant extracts with LC_{50} values greater than or equal to 0.02 mg/ml (20 µg/ml) are safe to cells (Zirihi et al., 2005). The selectivity index (SI) was also explored as a safety parameter. It is generally recognized that the higher the SI values the safer the extract. Extracts with SI value greater than one are less toxic to the host cell than the bacteria (Makhafola et al., 2012). In this study, the hot water extract had the

best SI of 22.698 against *Candida albicans*. The hydro-ethanol extracts had an LC_{50} of greater than 1 mg/ml against the Caco-2 cells. Similar results were also obtained for the hot water extract against the C3A cell line. This resulted in the non-determination of the SI values against the corresponding organisms since the LC_{50} value is higher than the highest concentration used in this study. It indicates an SI value greater than one since the LC_{50} is above 1 mg/ml. Therefore, it can be deduced from the cytotoxic results and using the SI values that the extract is selectively toxic against some of the isolates. This toxicity may be due in part to general metabolic toxins affecting microorganisms and animal cells (Elisha et al., 2017) but the extracts were more toxic to the bacteria than to the cells. This supports the therapeutic use of the plant in treating various ailments.

5. Conclusions

The *B. galpinii* extracts (70% ethanol and hot water) used in this study were relatively effective against bacteria and fungi, notwithstanding the widely-held notion that these solvents are ineffective in extracting biologically active compounds. Conclusively, *B. galpinii* leaf extracts had a strong antioxidant capacity as well as broad-spectrum antimicrobial potential with a relatively safe margin as they were generally not toxic to cells, and anti-inflammatory activity. The data obtained therefore support the traditional medicinal use of this plant. The anti-biofilm potential of the plant extracts and permeability studies are currently being explored to unravel the possible mechanism of action and bioavailability of the extracts.

Conflict of interest

The authors declare no conflict of interest.

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