Isolation and characterization of antimicrobial and anti-inflammatory triterpenoids from the acetone extract of *Grewia flava* DC. (Malvaceae) roots

N.I. Mongalo[a,b], L.J. McGaw[c], J.F. Finnie[a], J. Van Staden[a]*

[a]*Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa*

[b]*University of South Africa, College of Agriculture and Environmental Sciences, Private Bag X6, Florida1710, South Africa*

[c]*University of Pretoria, Phytomedicine Programme, Department of Paraclinical Sciences, Private Bag X04, Onderstepoort 0110, South Africa*

**Corresponding author: rcpgd@ukzn.ac.za (J. Van Staden)*

Tel: +27332605130

Highlights

- *Grewia flava* root extracts and fractions had good antimicrobial activity.
- Two triterpenoids, taraxerol and lupeol, were isolated and characterised.
- Lupeol had MIC as low as 10 µg/ml against a range of bacterial species.
- The compounds had good anti-inflammatory effect, inhibiting 15-LOX and nitric oxide.

Abstract

Grewia flava root acetone extracts were assessed for antimicrobial activity against a range of pathogenic microorganisms using the broth microdilution and bioautography assays. Cytotoxicity against human dermal fibroblast (HDF) and bovine dermis (BD) cell lines was also investigated. Solvent-solvent fractionation was carried out on the acetone extract to yield fractions of different polarities. Bioautograms from fractions showed four antimicrobial compounds in the carbon tetrachloride fraction, with activity against *Escherichia coli*. Two compounds from carbon tetrachloride, hexane and butanol fractions were active against *Candida albicans*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Escherichia coli*. The aqueous fraction had the lowest minimum inhibitory concentration (MIC) values of 40 µg/ml against *Cryptococcus neoformans*, *Staphylococcus aureus*, *K. pneumoniae* and *Pseudomonas aeruginosa.* Two triterpenoid compounds, namely taraxerol and lupeol, were isolated and had potent antimicrobial activity. Lupeol had better antimicrobial activity than vancomycin (control drug) with MIC of 10 µg/ml against *Mycobacterium smegmatis*, *Mycoplasma hominis* and *Escherichia coli*. Both taraxerol and lupeol exhibited better anti-inflammatory activity than quercetin (positive control) in the soybean lipoxygenase (15-LOX) inhibition assay and by inhibiting nitric oxide release from RAW264.7 macrophages. Although fractions and isolated compounds exhibited potent antimicrobial, antioxidant and anti-inflammatory activity, there is a need to explore the modes of action thereof. The study supports the use of the plant species in the treatment and management of various microbial infections posing danger to human health.

Key words: *Grewia flava*, taraxerol, antimicrobial activity, free radicals, cytotoxicity

1. Introduction

Antimicrobial resistance of microorganisms to common antibiotics, particularly in developing countries, poses a serious threat to both human and animal life. Such resistance is well reported in major hospitals and within many communities worldwide (Tadesse et al., 2017). The situation is further compounded by opportunistic infections which may be associated with weakened immune systems in patients suffering from HIV-AIDS and various forms of cancers (Cobucci et al., 2012).

Besides microbes, many degenerative diseases in humans may be caused by free radicals and these may also give rise to inflammation. Excessive free radicals are capable of damaging genetic material, causing lipid peroxidation in cell membranes and inactivating membrane-bound enzymes (Florence, 1995), hence resulting in many illnesses such as cancer, kidney infections and other degenerative diseases. Inflammation may be triggered by several factors, including damage to living tissues resulting from bacterial, viral and fungal infections, as well as physical agents and defective immune responses (Oguntibeju, 2018). The occurrence of uncontrolled inflammation and excess free radicals may easily impact a human body, particularly in immunocompromised patients.

The genus *Grewia* belongs to the family Malvaceae and comprises approximately 400 species, mainly shrubs and trees, distributed in the warmer parts of the world, mostly in Africa, Asia and Australia (Mulholland et al., 2002). Members of the genus are generally known to possess promising antimicrobial activity (Shangal et

al., 2012; Kaigongi et al., 2014; Khanal et al., 2016) as well as antifungal activity (Arora, 2011; Uddin et al., 2011). This may be the reason that they are commonly used in traditional medicine to manage and treat a variety of life-threatening human and animal infections. Furthermore, the species have some important pharmacological properties which include anti-inflammatory, antioxidant, antimalarial, hyperglycaemic, analgesic, antiplatelet and anti-parasitic and neuroprotective activity (Paviaya et al., 2013; Adebiyi et al., 2016, Akhtar et al., 2016; Sharma et al., 2016; Nguyen-Pouplin *et al*., 2007).

Grewia flava DC. is a shrub or small tree that can grow up to 4 m in height, with yellow flowers and elliptic or oblanceolate leaves, which are silvery grey-green, alternate, simple and toothed. Medicinally, the roots are used to treat sexually transmitted infections and ethno-veterinary related illnesses (Van der Merwe et al., 2001; McGaw and Eloff, 2008), while the fruits are used to brew beer and manufacture other processed products including jam (Van Wyk, 2011). Previous studies showed that the acetone extract from *Grewia flava* roots had noteworthy antimicrobial activity against *Candida albicans*, *Mycoplasma hominis*, *Mycobacterium smegmatis* and other pathogenic microorganisms (Mongalo et al., 2017; Lamola, 2015). In the current study, the antimicrobial and anti-inflammatory activities of fractions and isolated compounds from *G. flava* root acetone extract were investigated.

2. Materials and methods

2.1 Plant material, extraction, fractionation, isolation and characterization of compounds from Grewia flava roots

2.1.1 Plant material and extraction

Grewia flava DC. roots were collected from Pickum Farm, Limpopo Province, South Africa in June 2018. A voucher specimen was prepared and lodged at the Bews Herbarium (NU, voucher number MongaloNI 24), University of KwaZulu-Natal, Pietermaritzburg Campus. Roots were dried on a laboratory bench at room temperature and ground into 2 mm mesh size using a Scientec Hammer mill. About 2 kg of finely ground plant material was immersed in acetone (AR grade, Merck) at a ratio of 1:5 w/v and placed into a mechanical shaker (Already Enterprise Inc., Taiwan, Model LM-600 RD) at 120 rpm for 4 days. The plant material was filtered through Whatman's No. 1 filter paper and the resulting liquid was reduced to dryness using a rotary evaporator at 50 °C. This yielded 14.2 g plant extract.

2.1.2 Fractionation, isolation and characterization of compounds

The resulting extract was further subjected to solvent-solvent fractionation as proposed by the United States National Cancer Institute (Suffness and Duros, 1979) as shown in Figure 1. The fractions were further evaporated under reduced pressure using a rotary evaporator. The butanol (C_4H_9OH) and aqueous (Aq) fractions yielded 0.68 and 1.6 g respectively after drying, while the hexane fraction yielded 3.8 g.

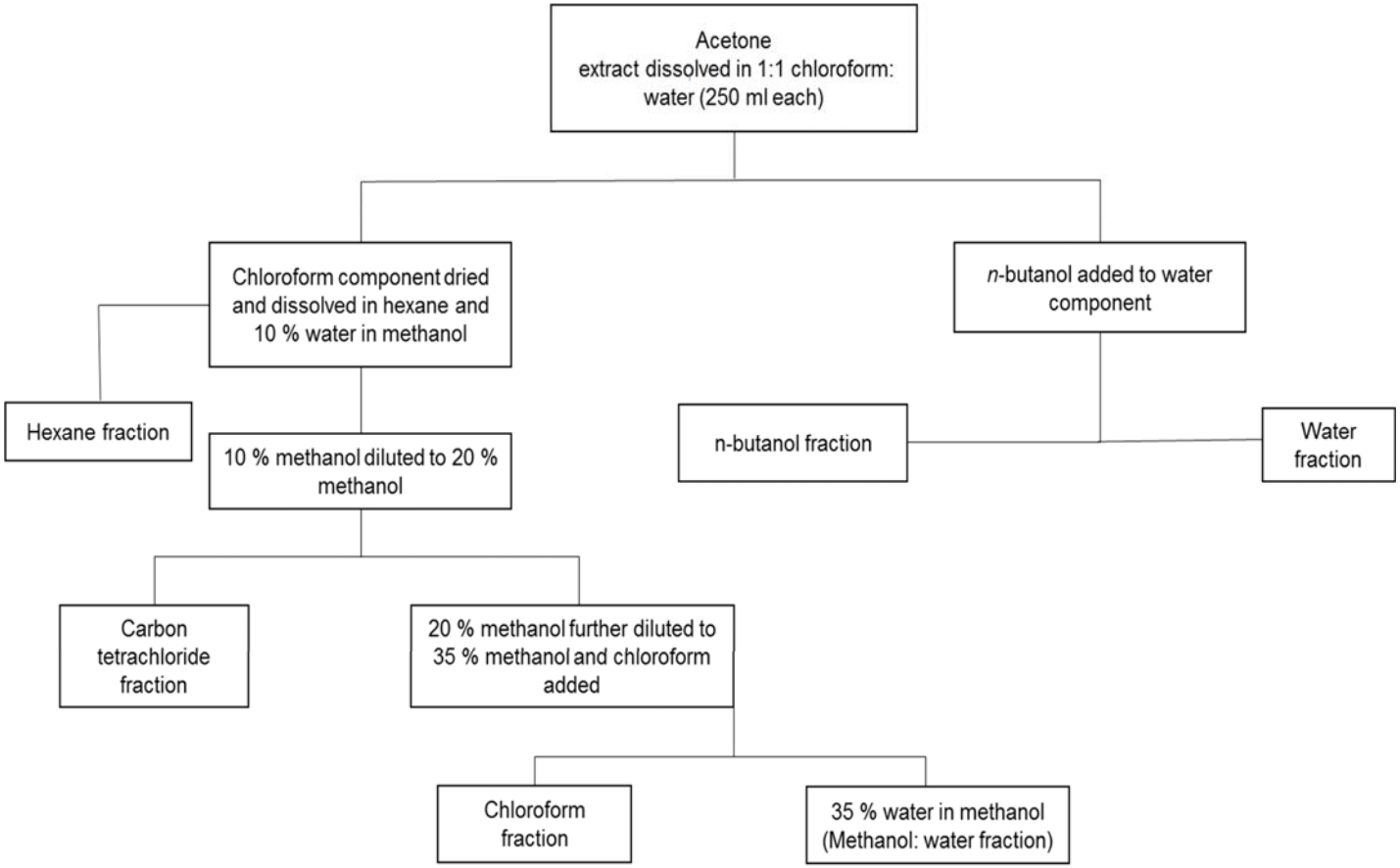

Fig. 1. Solvent-solvent fractionation of *Grewia flava* roots acetone extract

Carbon tetrachloride (CCl_4), chloroform ($CHCl_3$) and aqueous-methanol (MW) fractions yielded 0.75, 6.54 and 4.52 g respectively.

The CCl_4, $CHCl_3$ and MW fractions exhibited noteworthy antimicrobial activity in the bioautography assay and showed some of the similar compounds, hence combined and subjected to column chromatography. The column was eluted slowly using hexane and ethyl acetate at a starting ratio of 95:5, with the eluent gradually increasing in polarity and monitored using thin layer chromatography (TLC). The two pure compounds were further characterised and identified using 1H and ^{13}C NMR (Agilent Unity Inova 600 NMR spectrometer), Agilent Technologies-USA, with a 1H frequency of 600 MHz and a 13C frequency of 150 MHz. A 5mm dual channel IDpfg probe was used to collect the spectra. Fractions 23-45 resulted in **compound 1** (pure whitish crystals) of about 0.94 g. Fractions 61 to 67 yielded 0.41 g of creamy-brownish to brown crystals of **compound 2**. For the conclusive interpretation of the isolated compounds, the NMR data was compared to the other compounds in the literature. NMR data for **compound 1** matched that reported by Saritha and Prakash (2018) while that of **compound 2** matched Shwe et al (2019) and were identified as taraxerol and lupeol respectively. Mass Spectrophotometry was also performed using a Waters Synapt (G2) ESI PROBE, ESI Pos and Cone Voltage of 15V. Fourier-transform infrared spectroscopy (FTIR) technique was used to obtain an infrared spectrum of absorption or emission of the powders of the isolated compounds while Correlation Spectroscopy (COSY) was also used to determine the correlations through the chemical bonding and which proton resonances are mutually coupled on the structures obtained. **Compound 1** and **compound 2** exhibited melting points of 283.6 and 214.4 °C respectively.

2.2 Antimicrobial activity

2.2.1 Selection and maintenance of microorganisms

The clinical isolates of *Candida albicans*, *Cryptococcus neoformans*, *Staphylococcus aureus*, *Proteus mirabilis*, *Moraxella catarrhalis* and *Klebsiella pneumonia*e were sourced from an HIV-AIDS patient presenting with wounds, lesions and excessive coughing. The isolates organisms were identified using both Gram stain and culturing techniques as per the National Health Laboratory Services (NHLS) protocols by their staff members. Other selected organisms included *Bacillus cereus* (ATCC 10702), *Proteus vulgaris* (ATCC 29906), *Mycobacterium smegmatis* (14468), *Mycoplasma hominis* (ATCC 15488), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 10031). All the fungal and bacterial strains were maintained as slants on their respective growth mediums and kept in a refrigerator at -4 °C.

2.2.2 Bioautography

The method of Begue and Kline (1972) was adopted for carrying out bio-autography of the fractions. Each of the fractions, about 30 µl of 10 mg/ml stock solution of the fractions dissolved in acetone were separately spotted in the form of a band 1 cm from the bottom of a silica gel coated TLC plate and separated with 1:1 hexane: ethyl acetate. The plates in the TLC tank were monitored and the solvent front was marked by a pencil. The plates were allowed to dry in a laminar flow cabinet for three days and then sprayed with a fresh liquid culture of microorganisms of choice and incubated in a moist environment overnight at 37 °C. The next day, plates were sprayed with 2 mg/ml of *p*-iodonitro-tetrazolium chloride (INT, Sigma Aldrich,

Germany) and then incubated again until a pinkish colour appeared on the plates. Clear spots indicating activity of the fractions and patterns of antimicrobial compounds were marked and compared.

2.2.3 Microdilution assay

The antibacterial activity of the selected fractions was investigated using the broth microdilution assay described by Eloff (1998) with slight modification while the antifungal activity was determined using the method adopted from Masoko *et al*. (2005). The overnight cultures were diluted with fresh broth to a concentration of $1.1x10^7$cfu/ml. The selected microorganisms were separately grown in their respective growth mediums as in 2.2.1.

In brief, a volume of 100 µl of fractions and compounds (20 mg/ml in 5% DMSO) were added to wells of a 96-well micro-titre plates containing 100 µl of sterile distilled water and then two-fold serially diluted. Bacterial culture of known concentration was added (100 µl) to each well. Streptomycin and vancomycin were used as a positive control for all the bacterial strains while amphotericin B was used as the control for fungal strains. Plates were then incubated overnight at 37 °C. Plates for *Mycobacterium smegmatis* were grown for 72 h, those of *Mycoplasma hominis* were incubated for 24 h while those of other strains were incubated overnight. To each of the wells, 40 µl of 0.2 mg/ml freshly prepared *p*-iodo-nitrotetrazolium chloride (INT) was added and incubated for 30 min at the same temperature. The MIC was defined as the lowest concentration of the fraction that inhibit the bacterial growth. For the fungal strains, results were read after 48 h incubation.

2.3 Cytotoxicity studies against human dermal fibroblast (HDF) and bovine dermis (BD) cells

The cytotoxicity of fractions and isolated compounds from *Grewia flava* roots was evaluated against adult human dermal fibroblast (HDF) and bovine dermis (BD) cell lines (Sigma Aldrich, Germany). Viable cell growth after incubation of cells with the fractions dissolved in DMSO was determined using the tetrazolium-based colorimetric (MTT) assay described by Mosmann (1983). Cells of a sub-confluent culture of each cell line were harvested and then centrifuged (Eppendorf AG, Hamburg, Germany) at 2.0 rpm for 5 min, and re-suspended in a growth medium to $5x10^4$ cells per millilitres. The growth medium used was Minimal Essential Medium (MEM, Whitehead Scientific) supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Highveld Biological). A cell suspension of 200 µl was pipetted into each well of columns 2 to 11 of a sterile 96-well micro-titre plate in a sterile laminar flow cabinet. MEM (200 µl) was added to wells of columns 1 and 12 to minimize the "edge effect" and maintain humidity.

The plates were incubated for 24 h at 37 °C in a 5% CO_2 incubator, until the cells were in the exponential phase of growth. The MEM was aspirated from the cells, which were then washed with 150 µl phosphate buffered saline (PBS, Whitehead Scientific) and replaced with 200 µl of the selected test fractions and isolated compounds at different concentrations ranging from 7.5 to 1000 µg/ml. The serial dilutions of the fractions were prepared in MEM. The microtitre plates were incubated at 37 °C in a 5% CO_2 incubator for 48 h. Untreated cells and positive control (doxorubicin chloride, Pfizer Laboratories) were included in the assay. Each

experiment was repeated three times independently. The IC_{50} (concentration of the plant extract that inhibited 50% of cell growth) values were determined from the graphs of the concentration vs % inhibition using the formula below. Percentage cell inhibition = 100 -Abs (Sample) / Abs (Control) x 100, while the selectivity index values of both fractions and isolated compounds was calculated using the formula SI= IC_{50} in µg/ml/ MIC in µg/ml (Sreejaya and Santhy, 2013; Fadipe et al., 2015).

2.4 Anti-inflammatory activity

2.4.1 Soybean lipoxygenase (15-LOX) inhibition assay

The anti-inflammatory activity of the fractions and isolated compounds at a stock solution of 5 mg/ml was evaluated against the 15-LOX enzyme using the method explained by Pinto et al. (2007). The 15-LOX (Sigma-Aldrich, Germany) was made up to a working solution of 200 units per millilitres and kept on ice. A volume of 12.5 µl of each of the test sample or control (dissolved in DMSO) was added to 487.5 µl of 15-LOX in a 24-well microtitre plate and incubated at room temperature for approximately 5 min. After incubation, 500 µl substrate solution (10 µl linoleic acid dissolved in 30 µl ethanol, made up to 120 ml with 2 M borate buffer at pH 9.0) was added to the solution.

After 5 min of incubation at room temperature, the absorbance was measured using a microplate reader at 234 nm (SpectraMax 190, Molecular Devices, Germany). Quercetin (1 mg/ml) was used as a positive control, while pure DMSO was used as the negative control in the assay. The percentage enzyme inhibition of each extract compared with negative control as 100% enzyme activity was calculated using the equation;

% Inhibition = ODextract-ODblank/ ODnegative control-ODblank x 100%

The results were expressed as IC_{50}±SE, where IC_{50} is the concentration of plant extract that inhibits 50% of the enzyme calculated from the graphs.

2.5.2 Determination of cell viability in LPS-activated RAW 264.7 macrophages

The cell culture studies were carried out according to the method described by Mosman (1983). The RAW 264.7 macrophage cells murine obtained from the American Type Culture Collection (Rockville, USA) were cultured in a plastic culture flask in DMEM containing L-glutamine supplemented with 10% FCS and 1% PSF solution under 5% CO_2 at 37 °C. Cells were seeded in 96-well micro-titre plates and were activated by incubation in medium containing LPS (5 µg/ml) alone (control) or LPS with different concentrations (100, 50, 25, 12.5 and 5 µg/ml) of the extracts dissolved in DMSO. Quercetin served as a positive control in the assay.

Percentage cell inhibition =100 -Abs (Sample) / Abs (Control) x 100 (Sreejaya and Santhy, 2013). IC_{50} of each sample was obtained from extrapolated graphs of % inhibition vs concentration.

3. Statistical analysis

Each of the reported experimental techniques was repeated three times independently. For the antimicrobial assay, the results were reported as mean of three experiments. Data for cytotoxicity and cytotoxicity assays were analyzed using GraphPad Prism Version 7. The t-test was used to calculate the significance.

4. Results and Discussions

4.1 Antimicrobial activity

The resistance of virulent microorganisms poses a serious threat to both human and animal life. This is further compounded by HIV-AIDS infections, which leads to a weakened immune system resulting in susceptibility to various opportunistic infections including tuberculosis, skin infections and oral candidiasis (Khusro et al., 2018). The use of various fractions from medicinal plant species has been recommended as one of the possible ways to combat such resistance and further lead to isolation and identification of novel compounds which may possibly serve as alternative antibiotics in developing countries (Rajarathinam and Dronamraju, 2018; Pereira et al., 2018; Pham et al., 2018). In our earlier studies, the selected organisms, particularly Gram-negative bacterial strains, which includes *Pseudomonas aeruginosa, Escherichia coli*, *Moraxella catarrhalis* and *Klebsiella pneumoniae* exhibited some degree of resistance against variety of medicinal plant extracts and some common antibiotics (Soyingbe et al., 2018). Several literature sources have also corroborated that Gram-negative strains exhibit a notable degree of resistance (Mongalo et al., 2015; Mongalo et al., 2016).

The bioautograms of the fractions from *Grewia flava* root acetone extract against *Bacillus cereus*, *Candida albicans, E. coli* and *K. pneumoniae* are shown in Figure 2. The aqueous fraction showed antimicrobial inhibition of both *E. coli* and K. pneumoniae at the point of origin, while the carbon tetrachloride fraction exhibited four compounds actively inhibiting the growth of *E. coli*. Antimicrobial compounds with R_f values 0.29 and 0.57 were prevalent in the hexane, carbon tetrachloride and butanol

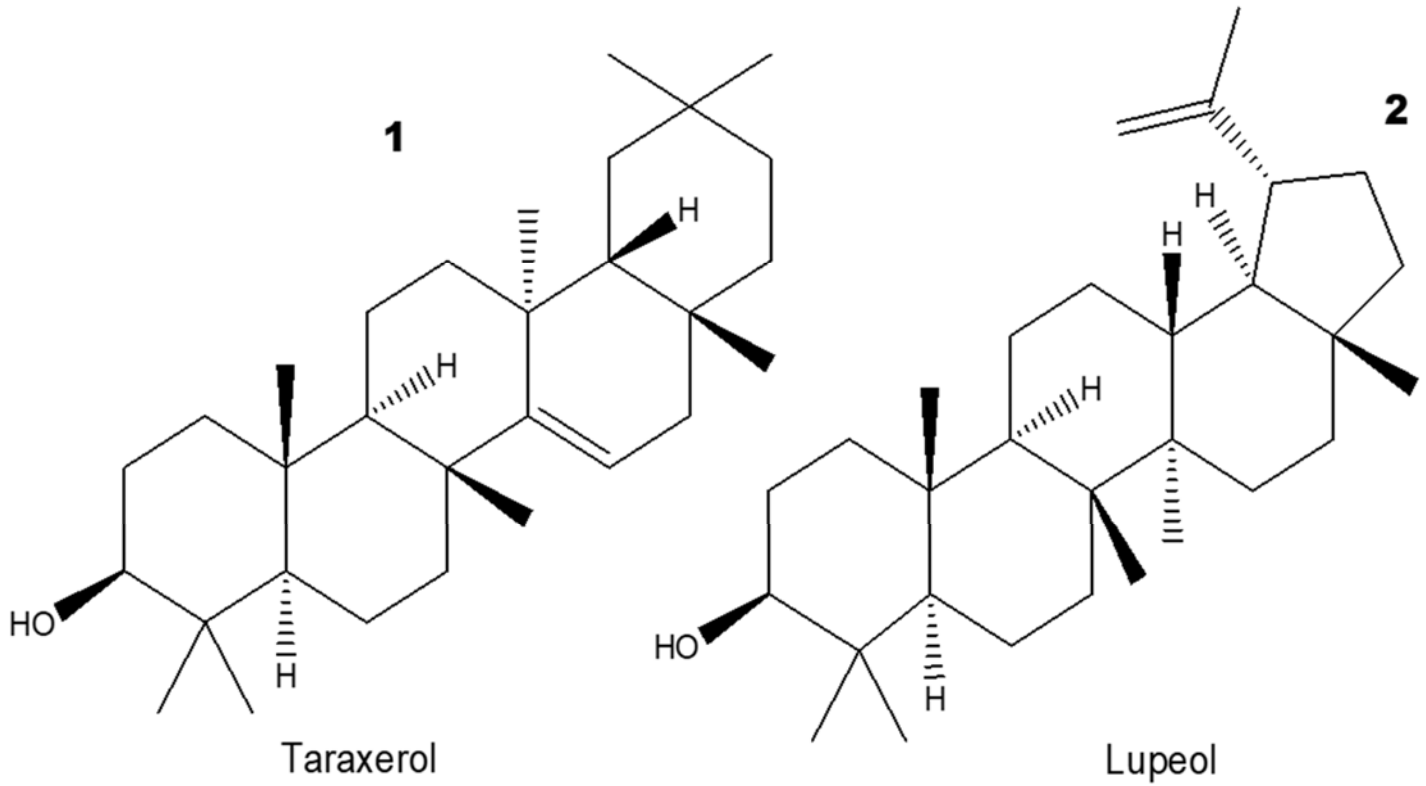

Fig. 2. Compounds isolated and characterised from *Grewia flava* roots acetone extract.

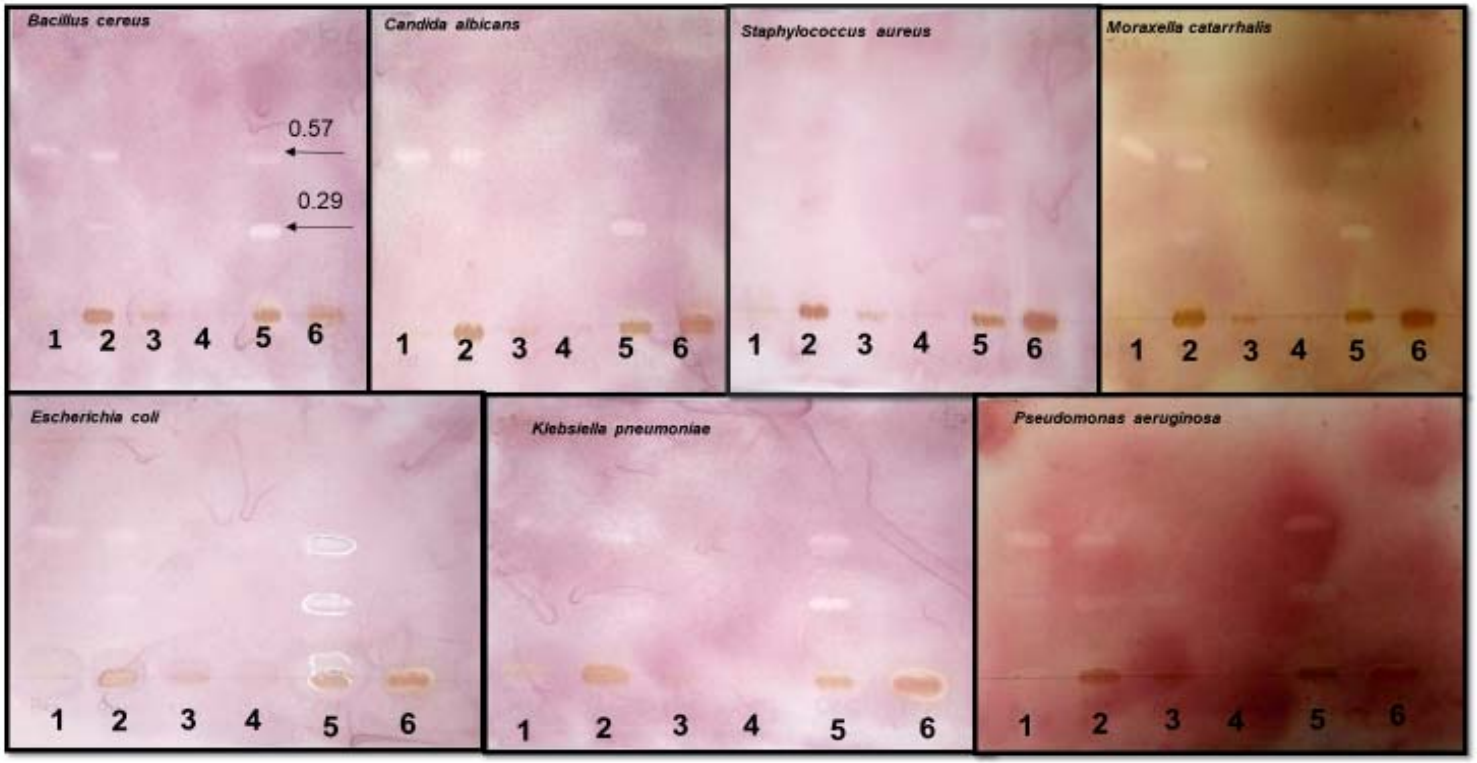

Fig. 3. Bio-autograms of fractions from *Grewia flava* roots against selected microorganisms.

(Key: 1, Hexane; 2, butanol; 3, methanol: water; 4, chloroform; 5, carbon tetrachloride, 6, water).

fractions and exhibited notable inhibition of *C. albicans*, *K. pneumoniae*, *E. coli and B. cereus.* The bioautogram showed that fractions inhibit *E. coli* much better than C. albicans, *Bacillus cereus* and *K. pneumoniae*. However, these organisms were resistant to both chloroform and methanol/water fractions. According to Suleimana et al. (2009), bioautography is a good method used in determining the antimicrobial activity of compounds from various plant-based sources and may well reveal the nature of the compounds with such activity even in a matrix of compounds. However, such activity may be attributed to similar compounds which have similar separation, hence resulting in synergistic effect. Furthermore, such compounds may be toxic to both animals and humans, and it is not so easy to determine such cytotoxic effect.

In the microdilution assay, the aqueous fraction had a noteworthy minimum inhibitory concentration (MIC) value of 40 µg/ml against four microorganisms, namely *Pseudomonas aeruginosa, Mycoplasma hominis, Staphylococcus aureus* and *Cryptococcus neoformans* (Table 1). A similar MIC value was exhibited by the methanol-water fraction against *E. coli.* It is interesting for the aqueous fraction to exhibit such a notable activity against pathogens as traditional medicine involves the use of water as a solvent mainly used in the extraction of secondary metabolites which may well inhibit the growth of pathogenic infections, hence curing the diseases.

The carbon tetrachloride fraction had better antimicrobial activity against yeasts and Gram-positive bacterial strains than Gram-negative strains, yielding an MIC value of 80 µg/ml against both *C. neoformans* and *S. aureus*. It is a general consensus that fractions and extracts exhibiting MIC values of 1.0 mg/ml or less are worth

Table 1. Antimicrobial activity (MIC in µg/ml) of fractions and isolated compounds from *Grewia flava* roots

Microorganisms	Fractions, isolated compounds and control drugs											
	Acetone extract	Hexane	Butanol	Methanol/ water	Chloroform	Carbon tetrachloride	Water	Taraxerol	Lupeol	Amp B	Strep	Vanc
Candida albicans	200	310	1250	**80**	1250	160	160	**30**	**30**	12.5	-	-
Cryptococcus neoformans	780	630	**80**	160	160	**80**	**40**	250	**30**	0.40	-	-
Staphylococcus aureus	390	1250	1250	310	310	**80**	**40**	250	250	-	13	200
Bacillus cereus	100	630	1250	310	1250	**80**	160	125	**60**	-	03	100
Moraxella catarrhalis	330	1250	1250	310	1250	630	**80**	125	**80**	-	03	13
Mycobacterium smegmatis	070	1250	1250	630	1250	310	630	**40**	**10**	-	06	25
Mycoplasma hominis	200	160	310	**80**	160	310	**80**	**40**	**10**	-	06	13
Klebsiella pneumoniae	100	1250	160	310	160	1250	**40**	125	125	-	03	25
Escherichia coli	100	1250	160	**40**	630	1250	310	130	**10**	-	25	100
Pseudomonas aeruginosa	200	1250	310	310	630	1250	**40**	125	125	-	06	25
Average MIC value	247	923.0	727.0	254.0	705.0	540.0	158.0	152.29	**72.10**	6.45	8.13	62.63

Key: Results were reported as a mean of three independent experiments. Amp B, Amphotericin B; Strep, Streptomycin; Vanc, Vancomycin; -, not done; Data in bold show noteworthy antimicrobial activity

investigating further for potential antimicrobial drugs (Aderogba et al., 2013; Desai et al., 2014; Mongalo et al., 2015; Mongalo et al., 2016) which may well serve as substitutes for common antibiotics used in developing countries (Pham et al., 2018). In the current work, the fractions with MIC values < 100 µg/ml are referred to as highly potent being in agreement with other authors (Bussman et al., 2010). Furthermore, fractions with MIC values ranging from 100 to 300 µg/ml possess moderate antimicrobial activity while fractions with MIC values >300 µg/ml are referred to as inactive. Judging by these criteria, the aqueous fraction had the most noteworthy and potent antimicrobial activity against the yeast, *C. neoformans*, four Gram-negative bacterial strains, namely *P. aeruginosa*, *M. catarrhalis, M. hominis* and *K. pneumoniae* and one Gram-positive bacterium *S. aureus* hence it had a broad-spectrum activity. In contrast, other authors reported fractions from plant species to possess better antimicrobial activity against only Gram-positive bacterial strains (Konaté et al., 2012) while others reported the antimicrobial activity to be prevalent in Gram-negative strains (Fadipe et al., 2015).

Lupeol had better antimicrobial activity than vancomycin (control drug) yielding a MIC value of 10 µg/ml against the microorganisms *Mycobacterium smegmatis*, *Mycoplasma hominis* and *E. coli*. Furthermore, lupeol had better antimicrobial activity than taraxerol, yielding an average MIC value of 72.10 µg/ml against the selected microorganisms. According to Naika et al. (2007), taraxerol isolated from other plant species exhibited broad spectrum antimicrobial activity with zones of inhibition ranging from 13.37 to 23.60 mm in diameter. These results may not be easily compared with our study as the methods used differ. The microorganisms were more susceptible to streptomycin (8.13 µg/ml) than vancomycin (62.63 µg/ml).

The resistance exhibited by *E. coli* and *K. pneumoniae* may be attributed to their morphological features compared to yeasts and Gram-positive bacterial strains. According to Kosanić et al. (2016), the cell wall of the Gram-negative bacteria consists of lipopolysaccharides and lipoproteins, which may well account for their resistance. The cell wall of Gram-positive bacteria consists of peptidoglycans (mureins) and teichoic acids, while that of fungi consists of polysaccharides such as chitin and glucan which may easily be penetrated by phytochemicals from plant species (Farkas, 2003). These results suggest that most active compounds from *Grewia flava* are of medium polarity. These corroborate those of other authors who reported intermediate polar fractions to possess the best antimicrobial activity (Ramadwa et al., 2017). However, in our study, there is a need to further explore the compounds which may be of higher and lower polarity alike as there are other compounds with activity that did not separate in the bioautography.

Recently, *M. hominis* was reported to be resistant to most fluoroquinolones and tetracyclines due to the presence of the tetM gene (Sonpar et al., 2018). In this study, the carbon tetrachloride fraction exhibited potent antimicrobial activity against yeast *C. neoformans* and two Gram-positive strains (*B. cereus* and *S. aureus*), suggesting that the fraction selectively inhibits the growth of Gram-positive bacterial strains and that of some of the fungal strains. Except for *S. aureus*, *K. pneumoniae* and *P. aeruginosa*, lupeol exhibited potent antimicrobial activity against the selected microorganisms with better MIC values than vancomycin. It is important to note that in the current study, vancomycin moderately inhibited the growth of *S. aureus*. Along with *K. pneumoniae* and *P. aeruginosa, S. aureus* is reportedly resistant to most antibiotics and therefore poses an enormous threat to both human and animal life (Gilbert et al.,

2001). Further, *S. aureus* may produce alpha toxins that may lead to co-infection with other microorganisms and may well enhance the lethality and bacterial proliferation of *K. pneumoniae* and *P. aeruginosa*, thereby rendering most healthcare facilities crippled and dysfunctional in treating such infections (Cheng et al., 2015; Cohen et al., 2016). Although the antibacterial activity of both root and leaf extracts of *G. flava* have earlier been reported (Lamola et al., 2017), it is not easy to compare results with the current work due to differences in type of solvents used and type of plant material extracted. In our earlier study, the acetone extract exhibited the lowest MIC value of 0.07 mg/ml against *M. smegmatis*. Furthermore, the extract showed MIC value of 0.20 mg/ml against both *C. albicans* and *M. hominis* (Mongalo et al., 2017).

4.2 Cytotoxicity studies and determination of selectivity index

The cytotoxic effects of fractions and isolated compounds from *Grewia flava* roots against bovine dermis (BD) and human dermal fibroblast (HDF) cell lines are reported (Figure 4). The fractions and compounds exhibited varying degrees of cytotoxicity towards the selected cell lines. The butanol fractions significantly ($P \leq 0.05$) revealed some degree of toxicity against HDF and BD yielding IC_{50} values of 88.99±0.05 and 139.23±0.03 µg/ml respectively. Taraxerol and lupeol had IC_{50} value of >1000 µg/ml against the bovine dermis cell suggesting that the compounds are not cytotoxic o the selected cell lines. According to the American National Cancer Institute (NCI), a plant-based compound or extract with a 50% lethal concentration (IC_{50}) of less than 30 µg/ml is referred to as toxic (Talib and Mahasheh, 2010). However, other authors refer to an IC_{50} of 100 µg/ml and lower as potentially toxic to cells (Hasibuan, 2014). This suggests that the butanol and chloroform fractions may be potentially toxic

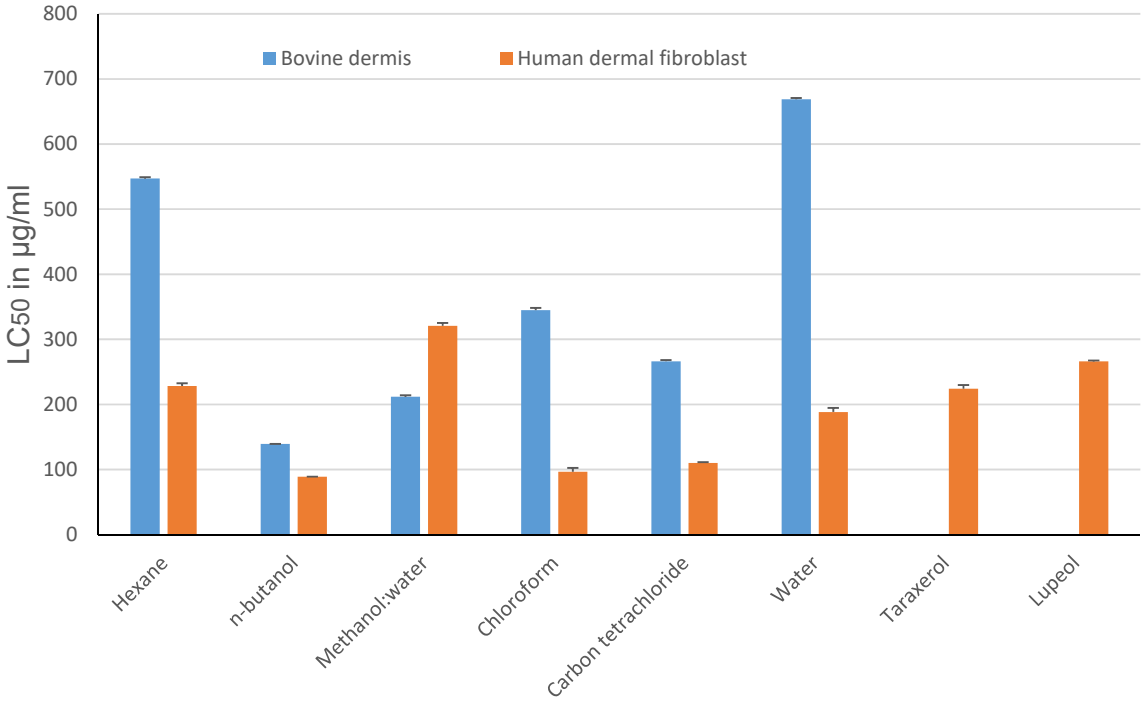

Fig. 4. Cytotoxicity of fractions and isolated compounds (IC_{50} in µg/ml ±SE) from *Grewia flava* roots against Human dermal fibroblast and Bovine dermis cell lines. Doxurubicin, control drug, exhibited IC50 values of 4.4±0.02 and 3.6±0.10 µM against Bovine dermis and human dermal fibroblast cell lines respectively.

to the human dermal fibroblast (HDF) cell line compared to other fractions, yielding IC_{50} values of 88.99±0.05 and 96.5±6.02 µg/ml respectively. However, such toxicity needs to be confirmed and validated against other human and animal cell lines. Also, toxicity *in vitro* does not necessarily translate to toxicity *in vivo*. In the current work, both fractions and isolated compounds revealed no cytotoxicity against bovine dermis (BD) cells.

Selectivity index (SI) values of the fractions and isolated compounds are reported in Table 2. SI values of fractions from *G. flava* ranged from 0.07 to 16.72, while those of the isolated compounds ranged from 0.30 to 26.62. The higher the SI value, the higher the safety margin. The fractions from *G. flava* roots exhibited higher selectivity index with bovine dermis cells than human dermal fibroblasts. When focusing on the bovine dermis cells, the aqueous fraction had the highest SI value of 16.72 against *Cryptococcus neoformans*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* compared to other fractions. Interestingly, the highest SI values were exhibited by the aqueous fraction against various Gram-negative and Gram-positive as well as yeast species, suggesting that this fraction had a higher general safety margin compared to the other fractions. This may well suggest that the use of aqueous extracts gives a higher safety margin, supporting their common use in African traditional medicine in the management and treatment of various pathogenic infections (Fadipe et al., 2015). The hexane fraction had an SI value of 3.42 against *Mycoplasma hominis* while the methanol-water fraction exhibited SI value of 3.48 against *Escherichia coli*. The carbon tetrachloride fraction had SI = 3.33 against *C. neoformans*, *Staphylococcus aureus* and *Bacillus cereus*. With the human dermal fibroblast cell line, the methanol-water fraction exhibited the highest SI

Table 2. Selectivity index values (SI) of fractions and isolated compounds from *Grewia flava* root acetone extract.

	Hexane	Butanol	Methanol/ water	Chloroform	Carbon tetrachloride	Water	Taraxerol	Lupeol
Bovine dermis								
Candida albicans	1.76	0.17	1.74	0.28	1.66	**4.18**	-	-
Cryptococcus neoformans	0.87	**2.64**	0.87	2.16	**3.33**	**16.72**	-	-
Staphylococcus aureus	0.44	0.17	0.45	1.11	**3.33**	**16.72**	-	-
Bacillus cereus	0.87	0.17	0.45	0.28	**3.33**	**4.18**	-	-
Moraxella catarrhalis	0.44	0.17	0.45	0.28	0.42	**8.36**	-	-
Mycobacterium smegmatis	0.44	0.17	0.22	0.28	0.86	1.06	-	-
Mycoplasma hominis	**3.42**	0.68	1.74	2.16	0.86	**8.36**	-	-
Klebsiella pneumoniae	0.44	1.32	0.45	2.16	0.21	**16.72**	-	-
Escherichia coli	0.44	1.32	**3.48**	0.55	0.21	**2.16**	-	-
Pseudomonas aeruginosa	0.44	0.68	0.45	0.55	0.21	**16.72**	-	-
Human dermal fibroblast								
Candida albicans	0.74	0.07	4.01	0.08	0.69	1.18	**7.47**	**8.87**
Cryptococcus neoformans	0.36	1.11	2.00	0.60	1.38	**4.71**	0.90	**8.87**
Staphylococcus aureus	0.18	0.07	1.03	0.31	1.38	**4.71**	0.90	1.06
Bacillus cereus	0.36	0.07	1.03	0.08	1.38	1.18	1.79	**4.44**
Moraxella catarrhalis	0.18	0.07	1.03	0.08	0.17	**2.35**	1.79	**3.33**
Mycobacterium smegmatis	0.18	0.07	0.51	0.08	0.36	0.30	**5.60**	**26.62**
Mycoplasma hominis	1.43	0.29	4.01	0.60	0.36	**2.35**	**5.60**	**26.62**
Klebsiella pneumoniae	0.18	0.56	1.03	0.60	0.09	**4.71**	1.79	**2.13**
Escherichia coli	0.18	0.56	**8.02**	0.15	0.09	0.61	1.72	**26.62**
Pseudomonas aeruginosa	0.18	0.29	1.03	0.15	0.09	**4.71**	1.79	**2.13**

Key: -, Not calculated, Bold data show noteworthy SI values

value of 8.02 against *Escherichia coli* compared to other fractions, while the aqueous fraction exhibited SI value of 4.71 against four pathogens such as *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *C. neoformans*. Lupeol exhibited higher SI values compared to taraxerol, yielding 26.62 against *Mycobacterium smegmatis*, *E. coli* and *M. hominis.* These may well suggest that lupeol had a higher safety margin compared to taraxerol against these organisms. Elsewhere, lupeol exhibited higher antimycobacterial activity and lower SI values compared to other isolated compounds (Fadipe et al., 2015; Fadipe et al., 2017). Taraxerol exhibited an SI value of 4.71 against *Pseudomonas aeruginosa*, *Cryptococcus neoformans*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Lupeol exhibited a higher SI value of 26.62 than taraxerol in a human dermal fibroblast cell line against *Mycoplasma hominis*, *Mycobacterium smegmatis* and *Escherichia coli.* According to Vonthron-Sénécheau et al. (2003), an SI value ≥10 means that biological efficacy is not due to *in vitro* cytotoxicity but there is a possible therapeutic use, so only the aqueous fractions and lupeol against *C. neoformans*, *E. coli*, *M. hominis*, *M. smegmatis*, *K. pneumoniae* and *S. aureus* satisfies that criterion. However, it is important to note that these results may not necessarily translate into *in vivo* studies as metabolic activities and other pharmacokinetic parameters may vary from one individual to the other in living systems (Makhafola et al., 2014). However, other authors promote the use of *in vitro* assays to reduce the need for acute toxicity animal studies as potentially toxic candidates may be discarded at an early stage (Nardone, 1989).

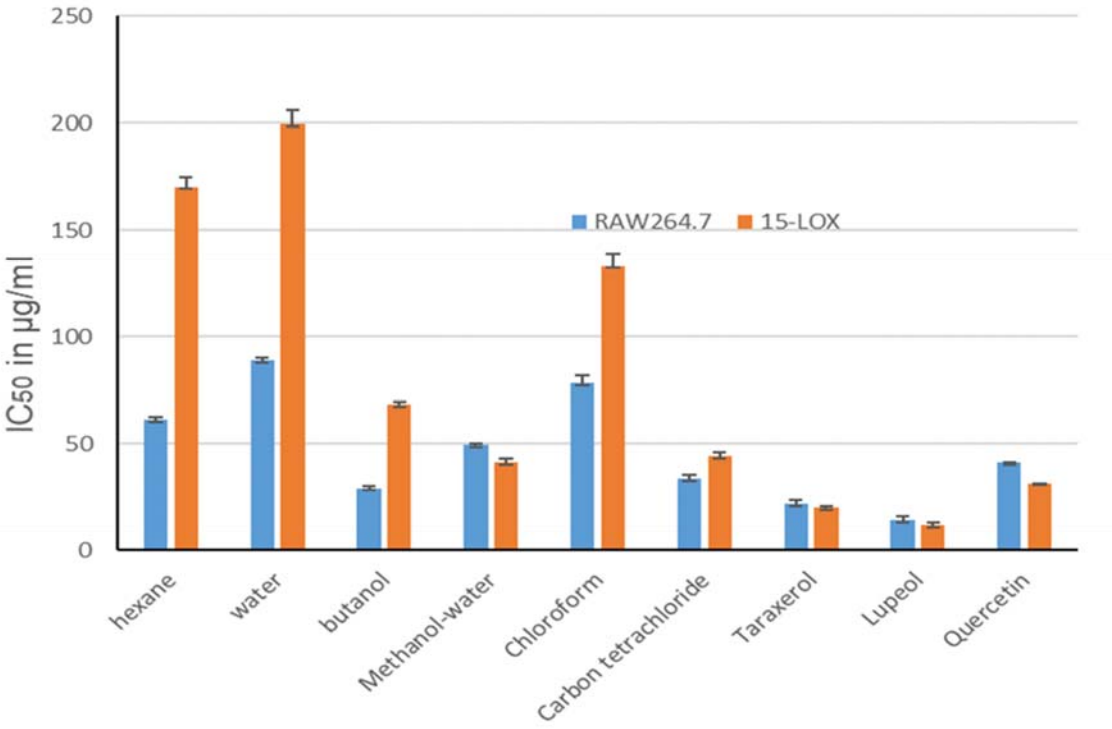

Fig. 5. Anti-inflammatory activity (IC50 in µg/ml ±SE) of fractions and isolated compounds from *Grewia flava* root acetone extract

4.3 Anti-inflammatory activity

The results for the anti-inflammatory activity of both fractions and isolated compounds from *Grewia flava* roots acetone extract are reported (Figure 5). The butanol and carbon tetrachloride fractions had noteworthy IC_{50} values of 29.31±0.02 and 33.56±1.99 µg/ml respectively against RAW264.7 cells compared to quercetin which exhibited 41.44±0.09 µg/ml. Both taraxerol and lupeol exhibited better anti-inflammatory activity in both assays compared to quercetin, the control drug. Except for lupeol, all fractions and taraxerol yielded better inhibition of NO release from RAW264.7 cells compared to the 15-LOX hydrogenase enzyme.

Taraxerol has been isolated and characterised in abundance in other medicinal plants world-wide and is reported to possess promising anti-tumour, anti-inflammatory, anti-allergic, anti-diabetic and antioxidant activities (Sharma and Zafar, 2015; Maiti et al., 2016; Aruwa et al., 2018). Lamola (2015) reported the presence of lupeol from *G. flava* leaves, suggesting that the compound is largely accumulated in leaves and not the roots. To the best of our knowledge, this is the first work to report the presence of lupeol and taraxerol in roots of *Grewia flava*. Furthermore, compound(s) that appear in abundance, particularly alnulin, may well explain the biological activity reported, hence supporting the use of the plant species in African Traditional Medicine. The mechanisms of action of these compounds against microorganisms need to be elucidated. They may work through adsorption and disruption of microbial membranes, interaction with relevant enzymes and metal ion deprivation (Elechi and Igboh, 2017).

Taraxerol and lupeol exhibited much better anti-inflammatory activity compared to quercetin, a control drug, against both RAW264.7 and 15-LOX enzyme. Taraxerol and lupeol yielded promising IC_{50} values of 21.88±0.02 and 14.2±0.01 µg/ml against nitric oxide production in RAW264.7 cells respectively and 19.96±0.009 and 11.88±0.04 µg/ml against 15-LOX enzyme. Quercetin yielded IC_{50} values of 31.55±0.02 and 41.44±0.004 µg/ml against 15-LOX enzyme and nitric oxide production by RAW264.7 cells respectively. The methanol-water fraction had the lowest IC_{50} value of 41.32±0.08 µg/ml against 15-LOX enzyme. Except for the methanol-water fraction, all the fractions exhibited better inhibition of nitric oxide production in RAW264.7 cells than inhibition of 15-LOX. It is important to notice that 15-LOX is an important enzyme involved in the synthesis of leukotriene from arachidonic acid. Leukotrienes are mediators of many pro-inflammatory compounds and targeting 15-LOX is considered as one of the therapeutic strategies in the management of inflammatory conditions (Ondua et al., 2019). Although the fractions and compounds exhibited promising anti-inflammatory activity against both 15-LOX and RAW264.7 cells, the mode of action still needs to be explored. Furthermore, *in vivo* studies need to be carried out as *in vitro* results may not always translate into similar results.

5. Conclusions

Fractions from *G. flava* roots had varying degrees of antimicrobial, anti-inflammatory and antioxidant activities *in vitro.* The mechanism of action of such fractions and isolated active compounds, taraxerol and lupeol, against microorganisms remains unknown and needs to be explored. Furthermore, the fractions were not cytotoxic

against both human dermal fibroblasts and bovine dermis cells. The good antimicrobial activity of the aqueous fraction is important and may well explain that some compounds in the plant responsible for such activities are soluble in water. Although the fractions were not cytotoxic, there is a need to explore the safety profile of the plant species against other internal normal human cell lines as medicine derived from the plant is reportedly drunk to treat a variety of infections, including sexually transmitted infections (STIs). Biological activity against pathogens causing STIs still needs to be explored. The antioxidant and anti-inflammatory activity of fractions and isolated compounds *in vitro* is of importance. However, the mode of action of such fractions and compounds needs to be explored, including *in vivo* studies. Furthermore, the isolation of other compounds from the plant species should be investigated.

Acknowledgements

The authors are grateful to the Research Centre for Plant Growth and Development, University of KwaZulu-Natal for financial assistance.

References

Adebiyi, O.E., Olopade, J.O., Olayemi, F.O., 2016. Neuroprotective effect of *Grewia carpinfola* extract against vanadium induced behavioural impairment. Foli Veterinaria 60, 5-13.

Aderogba, M.A., Ndhlala, A.R., Rengasamy, R.R.R., Van Staden, J., 2013. Antimicrobial and selected *in vitro* enzyme inhibitory effects of leaf extracts, flavonols and índole alkaloids isolated from *Croton menyharthii*. Molecules 18, 12633-2644.

Akhtar, B., Ashraf, M., Javeed, J., Sharif, A., Akhtar, M.F., Saleem, A., Hamid, I., Alvi, S., Murtaza, G., 2016. Analgesic, antipyretic and anti-inflammatory activities of *Grewia asiatica* fruit extracts in albino mice. Acta Poloniae – Drug Research 73, 983-989.

Arora, S., 2011. Antibacterial, antifungal, antioxidant and phytochemical study on the leaves extracts of *Grewia optiva*. Journal of Pharmacy Research 4, 3110-3132.

Aruwa, C.E., Amoo, S.O., Kudanga, T., 2018. *Opuntia* (Cactaceae) plant compounds, biological activities and prospects- A comprehensive review. Food Research International 112, 328-344.

Begue, W.J., Kline, R.M., 1972. The use of tetrazolium salts in bioautographic procedure. Journal of Chromatography 88, 182-184.

Bussmann, R.W., Malca-García, G., Glenn, A., Sharon, D., Chait, G., Díaz, D., Pourmand, K. Jonat, B., Somogy, S., Guardado, G., Aguirre, C., Chan, R., Meyer, K., Kuhlman, A., Townesmith, A., Effio-Carbajal, J., Frías-Fernandez, F., Benito, M., 2010. Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. Journal of Ethnopharmacology 132,101-108.

Cobucci, R.N.O., Saconato, H., Lima, P.H., Rodrigues, H.M., Prudéncio, T.L., Junior, J.E., Giraldo, P.C., Da Silveira Goncalves, A.K., 2012. Comparative incidence of cancer in HIV-AIDS patients and transplant recipients. Cancer Epidemiology 36, e69-e73.

Cohen, T.S., Hilliard, J.J., Jones-Nelson, O., Keller, A.E., O'Day, T., Tkaczyk, C., DiGiandomenico, A., Hamilton, M., Pelletier, M., Wang, Q., Diep, B.A., Le, V.T.M., Cheng, L., Suzich, J., Stover, C.K., Sellman, B.R., 2016. *Staphylococcus aureus* a toxin potentiates opportunistic bacterial lung infections. Infectious Disease 8, 329ra31.

Desai, N.C., Maheta, A.S., Rajpara, K.M., Joshi, V.V., Vaghani, H.V., Satodiya, H.M., 2014. Green synthesis of novel quinoline based imidazole derivatives and evaluation of their antimicrobial activity. Journal of Saudi Chemical Society 18, 963-971.

Elechi, N.A., Igboh, O.T., 2017. Antibacterial activities of the methanol extract and fractions of the leaf of *Eriosema psoraleoides* (Lam.) G. Don (Leguminosae). International Journal of Pharmaceutical Sciences and Research 8, 698-705.

Eloff, J.N., 1998. A sensitive and quick micro plate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64, 711-713.

Fadipe, V.O., Mongalo, N.I., Opoku, A.R., Dikhoba, P.M., Makhafola, T.J., 2017. Isolation of anti-mycobacterial compounds from *Curtisia dentata* (Burm.f.) C.A.Sm Curtisiaceae. BMC Complementary and Alternative Medicine 17, 306.

Fadipe, V.O., Mongalo, N.I., Opoku, A.R., 2015. *In Vitro* evaluation of the comprehensive antimicrobial and antioxidant properties of *Curtisia dentata* (Burm.f) C.A. Sm: Toxicological effect on the Human Embryonic Kidney (HEK293) and Human Hepatocellular carcinoma (HepG2) cell lines. Experimental and Clinical Sciences Journal 14, 971-983.

Farkas, V., 2003. Structure and biosynthesis of fungal cell walls: Methodological approaches. Folia Microbiology 48, 469-478.

Florence, T.M., 1995. The role of free radicals in disease. Australian and New Zealand Journal of Ophthalmology 23, 3-7.

Gilbert, D.N., Kohlhepp, S.J., Slama, K.A., Grunkemeier, G. Lewis, G., Dworkin, R.J., Slaughter, S.E., Leggett, J.E., 2001. Phenotypic Resistance of *Staphylococcus aureus*, selected Enterobacteriaceae, and *Pseudomonas aeruginosa* after single and

multiple *In Vitro* exposures to Ciprofloxacin. Antimicrobial Agents and Chemotherapy 43, 883-892.

Hasibuan, R.P.A.Z., 2014. Cytotoxic effect of *n*-hexane, ethyl acetate and ethanol extracts of *Plectranthus amboinicus* (Lour.) Spreng.) on HeLa and Vero cells lines. International Journal of PharmTech Research 6, 1806-1809.

Kaigongi, M.M., Dossaji, S.F., Nguta, J.M., Lukhoba, C.W., Musila, F.M., 2014. Antimicrobial activity, toxicity and phytochemical screening of four medicinal plants traditionally used in Msambweni District, Kenya. Journal of Biology, Agriculture and Healthcare 4, 6-12.

Khanal, D.P., Raut, B., Kafle, M., 2016. A comparative study on phytochemical and biological activities of two *Grewia* species. Journal of Manmohan Memorial Institute of Health Sciences 2, 53-60.

Khusro, A., Aartia, C., Barbabosa-Pliego, A., Rivas-Cáceres, R.R., Cipriano-Salazard, M., 2018. Venom as therapeutic weapon to combat dreadful diseases of 21st century: A systematic review on cancer, TB, and HIV/AIDS. Microbial Pathogenesis 125, 96-107.

Konaté, K., Hilou, A., Mavoungou, J.F., Lepengué, A.N., Souza, A., Barro, N.,Datté, J.Y., M'Batchi, B., Nacoulma, O.G., 2012. Antimicrobial activity of polyphenol-rich fractions from *Sida alba* L. (Malvaceae) against cotrimoxazol-resistant bacteria strains. I. Annals of Clinical Microbiology and Antimicrobials 11, 5.

Kosanić, M., Ranković, B., Rančić, A., Stanojković, T., 2016. Evaluation of metal concentration and antioxidant, antimicrobial, and anticancer potentials of two edible mushrooms *Lactarius deliciosus* and *Macrolepiota procera*. Journal of Food and Drug Analysis 24, 477-484.

Lamola. S.M., 2015. Antimicrobial activity and cytotoxicity of extracts and an isolated compound from *Grewia flava* against four enteric pathogens. MSc Dissertation, University of Pretoria, Republic of South Africa.

Lamola, S.M., Dzoyem, J.P., Botha, F., Van Wyk, C., 2017. Antibacterial, free radical scavenging activity and cytotoxicity of acetone extracts of *Grewia flava*. African Health Sciences 17, 790-796.

Maiti, P., Nand, M., Kumari, M., Pant, R., Joshi, H., Chandra, S., 2016. Virtual screening of EGFR tyrosine kinase inhibitors associated with non-small cell lung cancer from phytochemical data set. Journal of Emerging Trends in Computing and Information Sciences 7, 229-236.

Makhafola, T.J., McGaw, L.J., Eloff, J.N., 2014. *In vitro* cytotoxicity and genotoxicity of five *Ochna* species (Ochnaceae) with excellent antibacterial activity. South African Journal of Botany 91, 9-13.

Masoko, P., Picard, P., Eloff, J.N., 2005. Antifungal activities of six South African *Terminalia* species (Combretaceae). Journal of Ethnopharmacology 99, 301-308.

McGaw, L.J., Eloff, J.N., 2008. Ethnoveterinary use of Southern African plants and scientific evaluation of their medicinal properties. Journal of Ethnopharmacology 119, 559-574.

Mongalo, N.I., McGaw, L.J., Finnie, J.F., Van Staden, J., 2017. Pharmacological properties of extracts from six South African medicinal plants used to treat sexually transmitted infections (STIs) and related infections. South African Journal of Botany 112, 290-295.

Mongalo, N.I., McGaw, L.J., Segapelo, T.V., Finnie, J.F., Van Staden, J., 2016. Ethnobotany, phytochemistry, toxicology and pharmacological properties of *Terminalia sericea* Burch ex DC (Combretaceae)- A review. Journal of Ethnopharmacology 194, 789-802.

Mongalo, N.I., McGaw, L.J., Finnie, J.F., Van Staden, J., 2015. *Securidaca longepedunculata* Fres. A review of its Ethnomedicinal uses, Toxicology, Phytochemistry and pharmacology. Journal of Ethnopharmacology 165, 215-226.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 65, 55-63.

Mulholland, D.A., Sewram, V., Raynor, M., Thornell, K., Raidoo, D.M., 2002. Coupling SFE to uterotonic bioassay: An online investigation of the uterotonic activity of compounds from *Grewia occidentalis* (Tiliaceae). South African Journal of Botany 68, 68-71.

Naika, H.R., Krishna, V., Harish, B.G., Ahamed, B.M.K., Mahadevan, K.M., 2007. Antimicrobial activity of bioactive constituents from the leaves of *Naravelia zeylanica* (L.) DC. International Journal of Biomedical and Pharmaceutical Sciences 1, 153-159.

Nardone, R.M., 1989. The LD_{50} test and *in vitro* toxicology strategies. Acta Pharmacologica et Toxicologica (Copenhagen) 1989; 52, 65-79.

Nguyen-Pouplin, J., Tran, H., Tran, H., Phan, T.A., Dolecek, C., Farrar, J., Tran, T.H., Caron, P., Bodo, B., Grellier, P., 2007. Antimalarial and cytotoxic activities of ethnopharmacologically selected medicinal plants from Vietnam. Journal of Ethnopharmacology 109, 417-427.

Oguntibeju O.O., 2018. Medicinal plants with anti-inflammatory activities from selected countries and regions of Africa. Journal of Inflammation Research 11, 307-317.

Ondua, M., Njoya, E.M., Abdalla, M.A., McGaw, L.J., 2019. Anti-inflammatory and antioxidant properties of leaf extracts of eleven South African medicinal plants used traditionally to treat inflammation. Journal of Ethnopharmacology 234, 27-35

Paviaya, U.S., Kumar, P., Wanjari, M.M., Thenmozhi, S., Balakrishnan, B.R., 2013. Analgesic and anti-inflammatory activity of root bark of *Grewia asiatica* Linn. in rodents. Ancient Science of Life 32, 150-155.

Pereira, A.O., Avila, J.M., Do Carmo, G., Siqueira, F.S., Campos, M.M.A., Back, D.F., Morel, A.F., Ionara I. Dalcola, I.I., 2018. Chemical composition, antimicrobial and antimycobacterial activities of *Aristolochia triangularis* Cham. from Brazil. Industrial Crops & Products 121, 461-467.

Pham, H.N.T., Sakoff, J.A., Vuong, Q.V., Michael C. Bowyer, M.C., Scarlett, C.J., 2018. Screening phytochemical content, antioxidant, antimicrobial and cytotoxic activities of *Catharanthus roseus* (L.) G. Don stem extract and its fractions. Biocatalysis and Agricultural Biotechnology 16, 405-411.

Pinto, M.D.C., Tejeda, A., Duque, A.L., Macias, P., 2007. Determination of lipoxygenase activity in plant extracts using a modified ferrous oxidation-xylenol orange assay. Journal of Agricultural Food and Chemistry 55, 5956-5959.

Rajarathinam, G., Dronamraju, S.V.L., 2018. *In vitro* and *in silico* antimicrobial activity of sterol and flavonoid isolated from *Trianthema decandra* L. Microbial Pathogenesis 101, 77-86.

Ramadwa, T.E., Elgorashi, E.E., McGaw, L.J., Ahmed, A.S., Eloff, J.N., 2017. Antimicrobial, anti-inflammatory activity and cytotoxicity of *Funtumia africana* leaf extracts, fractions and the isolated methyl ursolate. South African Journal of Botany 108, 126-131.

Saritha, S., Prakash, T., 2018. Comprehensive assignments of extraction, isolation and characterization of taraxerol from bark *Annona reticulata* L. and chemopreventive effect on human prostate cancer cell lines (Incap and pc-3). Carcinogenesis & Mutagenesis 9(1).

Shagal, M.H., Kubmarawa, D., Idi, Z., 2012. Phytochemical screening and antimicrobial activity of roots, stem bark and leave extracts of *Grewia mollis*. African Journal of Biotechnology 11, 11350-11353.

Sharma, K., Zafar, R., 2015. Occurrence of taraxerol and taraxasterol in medicinal plants. Pharmacognosy Review 9, 19-23.

Sharma, C., Malgaonkar, M., Sangvikar, S.G., Murthy, S.N., Pawar, S.D., 2016. *In vitro* evaluation of antimicrobial and antioxidant profile of *Grewia* L. roots extracts. Journal of Applied Life Sciences International 7, 1-9.

Sonpar, A., Brown, K., Chen, J., Megran, D., Sabo, M., Cervera, C., Girgis, S., Kabbani, D., 2018. Dual infection in pregnancy: Disseminated *Mycoplasma hominis* and necrotizing herpes simplex 2 hepatitis. International Journal of Infectious Diseases 71, 1-3.

Shwe, H.H., Win, K.K., Moe, T.T., Myint, A.A., Win, T., 2019. Isolation and structural characterization of lupeol from the stem bark of *Diospyros ehretioides* Wall. IEEE-SEM 7, 140-144.

Soyingbe, S.O., Mongalo, N.I., Makhafola, T.J., 2018. *In vitro* antibacterial and cytotoxic activity of leaf extracts of *Centella asiatica* (L.) Urb, *Warburgia salutaris* (Bertol. F.) Chiov and *Curtisia dentata* (Burm. F.) C.A.Sm -medicinal plants used in South Africa. BMC Complementary and Alternative Medicine 18, 315.

Sreejaya, S.B., Santhy, K.S., 2013. Cytotoxic properties of *Acorus calamus* in MCF-7 breast cancer cells. International Journal of Current Research & Academic Reviews 1, 106-111.

Suffness, M., Douros, J., 1979. Drugs of plant origin. Methods in Cancer Research 26, 73-126.

Suleimana, M.M., McGaw, L.J., Naidoo, V., Eloff, J.N., 2009. Detection of antimicrobial compounds by bioautography of different extracts of leaves of selected South African tree species. African Journal of Traditional, Complementary and Alternative Medicines 7, 64-78.

Shwe, H.H., Win, K.K., Moe, T.T., Myint, A.A., Win, T., 2019. Isolation and characterization of lupeol from the stem bark of *Diospyros ehretioides* Wall. IEEE-SEM 7, 140-144.

Tadesse, B.T., Ashley, E.A., Ongarello, S., Havumaki, J., Wijegoonewardena, M., González, I.J., Dittrich, S., 2017. Antimicrobial resistance in Africa: a systematic review. BMC Infectious Diseases 17, 616.

Talib, W.H., Mahasheh, A.M., 2010. Anti-proliferative activity of plant extracts used against cancer in traditional medicine. Scientia Pharmaceutica 78, 33-45.

Uddin, W.G., Rauf, A., Siddiqui, B.S., Rehman, T.U., Azam, S., Qaisar, M., 2011. Chemical constituents and biological screening of *Grewia optiva* Drummond ex Burret

whole plant. American-Eurasian Journal of Agriculture and Environmental Sciences 11, 542-546.

Van der Merwe, D., Swan, G.E., Botha, C.J., 2001. Use of ethnoveterinary medicinal plants in cattle by Setswana-speaking people in the Madikwe area of the North West Province of South Africa. Journal of the South African Veterinary Association 72, 189-196.

Van Wyk, B.-E., 2011. The potential of South African plants in the development of new food and beverage products. South African Journal of Botany 77, 857-868.

Vonthron-Sénécheau, C., Ouattara, M., Trabi, F., Kamenan, A., Anton, R., Weniger, B., 2003. *In vitro* antiplasmodial activity and cytotoxicity of extracts of ethnobotanically selected Ivorian plants. Journal of Ethnopharmacology 87, 221-225.