Comparison of the Antibacterial Activity of Australian Terminalia Spp. Extracts Against *Klebsiella Pneumoniae*: Novel Treatment for Ankylosing Spondylitis

Ian Cock (✉ I.Cock@griffith.edu.au)

Griffith University <https://orcid.org/0000-0002-8732-8513>

Reece Courtney

Griffith University School of Science: Griffith University School of Natural Sciences

Research Article

Keywords: Australian plants, Combretaceae, autoimmune inflammatory disease, complementary and alternative therapies, *Klebsiella pneumoniae*, *Kakadu plum*, gubinge

Posted Date: November 22nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1090934/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Traditional medicines prepared using *Terminalia* species have been used globally to treat inflammation and pathogenic infections. Recent studies have demonstrated that multiple Asian and African *Terminalia* spp. inhibit bacterial triggers of some autoimmune inflammatory disease, including ankylosing spondylitis. Despite this, the effects of Australian *Terminalia* spp. on a bacterial trigger of ankylosing spondylitis (*K. pneumoniae*) remain unexplored. Fifty-five extracts from five Australian *Terminalia* spp. were investigated for *K. pneumoniae* growth inhibitory activity. Methanolic, aqueous and ethyl acetate extracts of most species and plant parts inhibited *K. pneumoniae* growth, with varying potencies. Methanolic leaf extracts were generally the most potent bacterial growth inhibitors, with MIC values of 66 µg/mL (*T. ferdinandiana*), 128 µg/mL (*T. carpenteriae*) and 83 µg/mL (*T. petiolaris*). However, the aqueous leaf extract was the most potent *T. grandiflora* extract (MIC = 87 µg/mL). All *T. catappa* extracts displayed low growth inhibitory activity. The *Terminalia* spp. methanolic leaf extracts were examined by LC-MS and GC-MS. All contained a relative abundance of simple gallotannins (particularly gallic and chebulic acids), the flavonoid luteolin, as well as the monoterpenoids cineole and terpineol. Notably, all *Terminalia* spp. were non-toxic or of low toxicity in ALA and HDF toxicity assays, highlighting their potential for preventing the onset of ankylosing spondylitis and treating its symptoms once the disease is established.

Introduction

Ankylosing spondylitis (AS) is a relatively common autoimmune inflammatory disease that afflict approximately 0.9% of the world's population, with a significantly higher incidence in males (Rashid and Ebringer 2007). The onset is most common in middle to older age, although it can also occur in younger individuals (Braun and Sieper 2007; Rashid and Ebringer 2007). AS is a debilitating autoimmune inflammatory disease that affects spinal and pelvic sacroiliac joints of genetically susceptible individuals and is characterized by inflammation of the spinal joints, resulting in chronic back pain and reduced mobility. Chronic AS inflammation may induce ossification of the annulus fibrosis, resulting in the formation of syndesmophytes, which may form bone bridges between the vertebrae, thereby fusing them together. There currently are no effective cures for AS and current treatments instead target the symptoms of the disease (particularly pain and local tissue swelling) using analgesic and anti-inflammatory therapies rather than addressing the etiology or progression of the disease. However, these treatments only address the immediate symptoms of the disease and do not significantly affect progression of the disease or the significant tissue damage that results from chronic AS (Saag et al. 1994). Furthermore, prolonged usage of anti-inflammatory drugs and analgesics often results in toxicity and unwanted side effects (Saag et al. 1994).

Safe and effective AS chemotherapeutics are urgently required. Ideally, new AS drugs should prevent AS disease progression (rather than just blocking the symptoms), thereby inhibiting the formation of spinal syndesmophytes and subsequent vertebrae fusion. Notably, some causes of AS have been identified. It may be triggered by molecular mimicry in genetically susceptible individuals (individuals with the MHC class 1 allele HLA-B27) who contract *K. pneumoniae* infections (Cock and Cheesman 2018; Rashid and Ebringer 2007). *Klebsiella pneumoniae* infections induce the production of antibodies that target bacterial surface proteins, which also cross-react with host HLA-B27 QTDRED and DRED epitopes, as well collagens I, III and IV in genetically susceptible people (Rashid and Ebringer 2007). Binding of these bacterial-targeted antibodies to antigens in the host's tissues triggers inflammatory cascades, resulting in increased production of acute phase proteins, complement proteins, vasoactive amines, as well as multiple cytokines and chemokines (Rashid and Ebringer 2007). Furthermore, binding of the host-reactive antibodies to self-tissue also activates cellular processes, including activating natural killer cells, thereby stimulating further damage to the host tissue (Braun and Sieper 2007).

Multiple antibiotics are already effective inhibitors of *K. pneumoniae* growth, including the aminoglycosides, chloramphenicol, fluoroquinolones and tetracyclines. However, as AS is a chronic condition, preventative therapy would entail ongoing administration of antibiotics. This mode of therapy would be counter-productive as prolonged exposure to antibiotics induces the production of antibiotic resistant bacterial strains, thereby reducing (or even totally negating) the efficacy of the therapy. Indeed, several super-resistant bacterial strains of *K. pneumoniae* have already been reported, including a strain that is resistant to all common clinical antibiotics (Cheesman et al 2017; Ashayeri-Panah et al 2014). New safe and effective anti-*K. pneumoniae* therapies are urgently required. A re-examination of traditional medicines is an attractive option for antibiotic drug discovery as the anti-bacterial properties of some medicinal plants have been known for hundreds or (in some cases) thousands of years. Furthermore, due to the established use of traditional medicines, toxicities and side effects are often well documented. Plant-based preparations also often contain multiple compounds with antibacterial activity, as well as potentiating compounds, and are therefore essentially combinational therapies. This is noteworthy because the presence of multiple molecules targeting different aspects of bacterial growth substantially reduces the possibility of the bacteria developing resistance to the therapy. Indeed, we are unaware of any bacteria that have previously developed resistance to crude plant extracts.

Recent studies from our group have identified extracts prepared from several *Terminalia* spp. as being effective against the bacterial triggers of rheumatoid arthritis (RA) (Sirdaarta et al, 2015a; Cock and Van Vuuren 2014). Additionally, several other studies have also demonstrated that African and Indian *Terminalia* spp. are also good inhibitors of *K. pneumoniae* and are therefore promising leads for novel AS prevention and treatment therapies (Cock and Van Vuuren 2015; Cock 2015). In contrast, Australian *Terminalia* spp. are yet to be screened for growth inhibitory activity against bacterial triggers of AS, despite their taxonomic relationship with African and Indian species with noteworthy anti-*K. pneumoniae* activity and their inhibitory effects against bacterial triggers of RA (Cock 2015; Cock and Van Vuuren 2014), multiple sclerosis (MS) (Sirdaarta et al 2015b) and rheumatic fever (RF) (Wright et al 2016a). This study aimed to address this gap in the literature by screening several Australian *Terminalia* spp. for their inhibitory activity against *K. pneumoniae* using *in vitro* assays.

Materials And Methods

Plant sources and extraction

All *Terminalia ferdinandiana* Exell., *T. carpenteriae* C.T.White and *T. grandiflora* Benth. plant material was provided and identified by David Boehme of Northern Territory Wild Harvest, Australia and were sourced from wild trees in Kakadu Park, Australia under government collection licence. The *Terminalia catappa* L.

bark, fruit and leaves were obtained from the Mt Cootha Botanical Gardens in Brisbane and were identified by the gardens botanist. *Terminalia petiolaris* Benth. fruit and leaves were a gift from Jacinta Monck of Kimberley Wild Gubinge, Australia. Voucher specimens of all plant materials were prepared and are stored at the School of Environment and Science, Griffith University, Australia. All plant materials were dried using a Sunbeam food dehydrator and the desiccated material was subsequently ground into a fine powder. Methanol, deionised water, ethyl acetate, chloroform or hexane were added individually to 1 g masses to give a final volume of 50 mL and extracted for 24 h at 4 °C with gentle mixing on an orbital shaker. All solvents were AR grade and were purchased from Ajax Fine-Chemicals, Australia. The mixtures were filtered through Whatman No. 54 filter paper to remove the solid plant material. The organic solvents were dried at 50 °C in a vacuum oven, whilst the aqueous extracts were lyophilised by at -50 °C. The resultant dry pellets were weighed to determine extraction yield and were subsequently dissolved in 10 mL deionised water (containing 0.5% DMSO), filtered through a 0.22 µm syringe driven filter (Sarstedt) and stored at 4°C until use.

Qualitative phytochemical studies

Alkaloids, cardiac glycosides, flavonoids, phenolic compounds, phytosterols, saponins, tannins and triterpenoids were detected using standard qualitative phytochemical assays (Cheesman et al 2019).

Antibacterial screening

Test bacterial strains

All media was supplied by Oxoid Ltd., Australia. The reference *Klebsiella pneumoniae* strain (ATCC31488) was purchased from American Type Culture Collection (ATCC). The clinical isolate *Klebsiella pneumoniae* strain was obtained from the science teaching laboratory at Griffith University, Australia. Stock cultures were subcultured and maintained in Mueller-Hinton broth at 4 °C. Culture purity was intermittently monitored by 16S rRNA sequencing to ensure culture purity and homogeneity.

Evaluation of antimicrobial activity

The growth inhibitory properties of the *Terminalia* spp. extracts against *K. pneumoniae* were determined using standard disc diffusion assays (Cheesman et al 2019; Sirdaarta et al 2015a) on Mueller-Hinton agar plates. Each antimicrobial assay was performed three times, each with internal triplicates and mean values (± SEM) are reported in this study. Standard discs of ampicillin (10 µg) and chloramphenicol (10 µg) were obtained from Oxoid Ltd., Australia and included on each agar plate as positive controls. Filter paper discs infused with 10 µL of sterile deionised water were also included as a negative control.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration for each extract was determined using liquid dilution MIC assay and solid phase (agar) MIC assays. Liquid dilution MIC assays are a common method for quantifying antibacterial activity and are considered sensitive compared to other assay systems (Ilanko and Cock 2019; Hübsch et al 2014). Furthermore, antibacterial evaluations using this method allows for comparison of the efficacy with other treatments and between studies. Solid phase agar disc MIC assays were also used in this study as a confirmatory assay, and as a more realistic approximation of bacterial infections on solid surfaces.

Microplate liquid dilution MIC assay

The MICs of the extracts were evaluated by previously described standard methods (Ilanko and Cock 2019; Hübsch et al, 2014). A 0.2 mg/mL volume of p-iodonitrotetrazolium violet (INT; Sigma, Australia) solution was used to indicate bacterial growth as a colour change (from clear to red). The MIC was visually determined as the lowest dose at which colour development was inhibited.

Disc diffusion MIC assay

The MICs of all extracts was also evaluated by disc diffusion assay across a range of extract concentrations as previously described (Cock et al 2015; Winnett et al 2014). Graphs of the zone of inhibition versus Ln concentration were plotted and linear regression was used to determine MIC values.

Toxicity screening

The toxicity of all extracts was assessed using the *Artemia* nauplii lethality assay (ALA) for rapid toxicity screening, as well as an MTS cellular proliferation assay for an evaluation of cytotoxicity.

Artemia franciscana Kellogg nauplii toxicity screening

Standard *Artemia franciscana* nauplii lethality assays were used as an initial evaluation of the toxicity of the *Terminalia* spp. extracts (Ruebhart et al 2009). Potassium dichromate (1 mg/mL) (AR grade, Chem-Supply, Australia) was included with each assay as a reference toxin. The LC₅₀ with 95% confidence limits for each treatment was calculated using Probit analysis.

Cellular viability assay

All *Terminalia* spp. extracts were also screened for cytotoxicity against normal human primary dermal fibroblasts (HDF ATCC PCS-201-012) purchased from American Type Culture Collection. All extracts were screened at 200 µg/mL following 24 h exposure using standard protocols and absorbances were monitored at 540 nm and a blank wavelength of 690 nm using a Molecular Devices, Spectra Max M3 plate reader (Shalom and Cock, 2018). All tests were performed three times in triplicate (n=9) and triplicate controls were included on each plate and the % cellular viability (compared to the untreated control) was

recorded. Cellular viability $\leq 50\%$ of the untreated control was classified as toxic, whereas extracts that yielded cell viability $>50\%$ untreated control were deemed to be nontoxic.

HPLC-MS QTOF fingerprint analysis

Separation, identification of extract components and determination of the relative abundance was achieved using an Agilent 1290 HPLC system equipped with a Zorbax Eclipse C18 column (2.1 x 100 mm, 1.8 μm particle size) linked to an Agilent 6530 quadrupole time-of-flight (QTOF) mass spectrometer. The chromatographic separation utilised parameters previously optimised for the identification of phytochemical components in related *Terminalia* spp. extracts (Sirdaarta et al 2015a). Mass spectra were obtained in the electron ionisation mode and analytes were recorded for 45 mins in total ion count (TIC) mode.

GC-MS head space analysis

The *Terminalia* spp. extract components were separated, identified and their relative abundance was analysed using a Shimadzu GC-2010 plus (USA) chromatography system linked to a Shimadzu MS TQ8040 (USA) mass selective detector system using previously developed parameters (Sirdaarta et al 2015b). For analysis, the mass spectrometer's ionisation mode was set to 70eV and the mass signals were recorded as total ion count (TIC) mode for 45 mins across a mass range of 45-450 m/z.

Statistical analysis

Data is expressed as the mean $\pm$ SEM of three independent experiments, each with three internal replicates (n=9). Statistical significance was calculated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis. *P* values <0.01 were considered significant.

Results

Liquid extraction yields

Extraction of the *Terminalia* spp. plant materials with solvents of varying polarity yielded dried plant extracts ranging from 32 mg (*T. grandiflora* leaf ethyl acetate extract) to 426 mg (aqueous *T. ferdinandiana* leaf extract) from 1 g of dried, powdered plant material. These dried extracts were resuspended in 10 mL deionised water (containing 1 % DMSO) for further testing. The aqueous and methanolic extracts generally had substantially higher yields of extracted material compared to the corresponding chloroform, ethyl acetate and hexane extracts (Table 1). Additionally, the higher polarity methanol and water solvent extractions yielded the greatest relative abundance and widest diversity of phytochemical classes for all *Terminalia* spp. across all plant materials examined. In general, all of the methanolic and aqueous *Terminalia* spp. extracts contained high levels of phenolics, flavonoids and tannins, as well as moderate levels of saponins. The ethyl acetate extracts also contained similar phytochemical classes, although in substantially lower relative abundances compared to the methanolic and aqueous extracts.

Antibacterial activity

To evaluate the growth inhibitory activity of the Australian *Terminalia* spp. extracts against *K. pneumoniae*, all extracts were initially screened by disc diffusion assays and the zones of inhibition (ZOI) were measured. The methanolic, aqueous and ethyl acetate *T. ferdinandiana* fruit (Figure 1a), leaf (Figure 1b) and seed (Figure 1c) extracts all inhibited *K. pneumoniae* growth. Notably, the *T. ferdinandiana* leaf extracts were substantially stronger than the extracts produced from either the fruit or seeds of that species (as evaluated by size of the ZOI). Indeed, ZOIs of approximately 18 mm were measured for the methanolic leaf extract against both the reference and clinical isolates strains of *K. pneumoniae* (Figure 1b). Furthermore, this extract was a substantially better inhibitor of *K. pneumoniae* growth than both control antibiotics. Both strains of *K. pneumoniae* were completely resistant to the ampicillin control, although both bacterial strains were highly susceptible to chloramphenicol (ZOIs ~ 14 mm). Interestingly, the ZOI measured for the methanolic *T. ferdinandiana* leaf extract was substantially larger than the chloramphenicol control disc, indicating that the extract is a substantially more potent anti-*K. pneumoniae* treatment than the pure antibiotic. Additionally, a relatively high dose (10 μg) of pure chloramphenicol was infused into the control antibiotic disc. In contrast, the extracts tested herein contain complex mixtures of compounds, with the antibacterial components expected to account for a relatively low percentage of the total extracted material. Therefore, the inhibitory activity of the individual methanolic *T. ferdinandiana* leaf extract components are particularly noteworthy. In contrast, the methanolic *T. ferdinandiana* fruit and seed extracts produced ZOIs of approximately 10 and 7 mm respectively.

The methanolic *T. carpentariae* leaf extract was also a strong inhibitor of both *K. pneumoniae* strains (ZOIs ~ 12 mm) (Figure 2). Indeed, the *K. pneumoniae* growth inhibition by this extract compares favourably to the inhibition by the antibiotic controls (as judged by the ZOI size). Similarly, the methanolic *T. grandiflora* fruit (Figure 3a) and leaf (Figure 3b) extracts were also good inhibitors of the growth of both *K. pneumoniae* strains, with ZOIs of approximately 11 and 13 mm respectively. However, a notable difference was evident for the *T. grandiflora* leaf extracts compared to fruit extract (and to the other *Terminalia* spp. screened in our study). Specifically, the aqueous *T. grandiflora* leaf extract was a substantially stronger inhibitor of both *K. pneumoniae* strains than the methanolic extract was, with ZOIs of 16 and 16.7 mm against the reference and clinical isolate strains respectively.

The methanolic *T. petiolaris* fruit (Figure 4 a) and leaf extracts (Figure 4b) were also strong inhibitors of both *K. pneumoniae* strains (ZOIs of approximately 13.5 and 17 mm respectively). The aqueous and ethyl acetate *T. petiolaris* fruit and leaf extracts were also good inhibitors of bacterial growth, albeit with substantially smaller ZOIs than measured for the corresponding methanolic extracts. In contrast, relatively weak anti-*K. pneumoniae* activity was noted for the *T. catappa* bark (Figure 5a), fruit (Figure 5b) and leaf extracts (Figure 5c). Indeed, weak growth inhibition was only seen for the methanolic *T. catappa* extracts (ZOIs ≤ 7.5 mm against both *K. pneumoniae* strains), whilst all other *T. catappa* extracts were completely ineffective inhibitors of *K. pneumoniae* growth.

Quantification of minimum inhibitory concentration (MIC)

The relative level of *K. pneumoniae* growth inhibitory activity was also quantified and further evaluated by determination of MIC values of each extract (Table 2). MIC values were only determined for those extracts that displayed activity in the disc diffusion screening assays. With some noteworthy exceptions, the methanolic and ethyl acetate leaf extracts were generally substantially more potent inhibitors of *K. pneumoniae* growth than extracts produced from other plant parts, or using other solvents. The *T. ferdinandiana* methanolic (66 and 53 µg/mL against the reference and clinical strains respectively) and ethyl acetate leaf extracts (78 and 75 µg/mL against the reference and clinical strains respectively) were particularly good inhibitors of both *K. pneumoniae* strains. Similarly, the *T. petiolares* leaf methanolic (83 and 69 µg/mL against the reference and clinical strains respectively) and ethyl acetate extracts (105 and 92 µg/mL against the reference and clinical strains respectively) were also noteworthy. As *K. pneumoniae* can trigger ankylosing spondylitis in genetically susceptible people (Cock and Cheesman 2018), these extracts may be particularly promising for the prevention and control of that disease, as well as other diseases caused by *K. pneumoniae* infections.

The growth inhibitory activity of the methanolic and ethyl acetate extracts of most parts of most of the other *Terminalia* spp. was also noteworthy, with MIC values generally substantially below 1000 µg/mL. The *T. catappa* extracts (prepared using all plant parts) were a notable exception to this trend, with high MIC values (1500->5000 µg/mL) determined for all *T. catappa* extracts against both *K. pneumoniae* strains. Similarly, MIC values substantially >1000 µg/mL were also determined for all of the *T. ferdinandiana* seed extracts against these bacteria. Thus, the *T. catappa* extracts and the *T. ferdinandiana* seed extracts are likely to be of limited use for the prevention and treatment of ankylosing spondylitis. A further trend was also evident: The leaf extracts were generally substantially more potent inhibitors of *K. pneumoniae* growth than the corresponding fruit, bark or seed extracts. Additionally, the anti-*K. pneumoniae* activity of the aqueous extracts of most *Terminalia* spp. and plant parts studied were also noteworthy, albeit with substantially higher MIC values than determined for the methanolic and ethyl acetate extracts.

Quantification of toxicity

All of the Australian *Terminalia* spp. extracts were initially screened across a range of concentrations in the *Artemia* nauplii toxicity assay and LC₅₀ values were quantified (Table 2). As LC₅₀ values >1000 µg/mL have been defined as nontoxic in this assay (Ruebhart et al 2009), the majority of the Australian *Terminalia* spp. extracts were classified as nontoxic. Indeed, only the methanolic *T. ferdinandiana* fruit (LC₅₀= 925 µg/mL), *T. grandiflora* fruit (LC₅₀= 934 µg/mL) and leaf (LC₅₀= 884 µg/mL), as well as the *T. petiolares* fruit extracts (LC₅₀= 925 µg/mL) displayed toxicity. Furthermore, the relatively high LC₅₀ values calculated for those extracts indicate that they are only of low toxicity. Additionally, it is noteworthy that whilst *Artemia* nauplii are generally robust, they are sensitive to acidic pH (Kwast, 1996) and extracts containing high levels of ascorbic acid may provide falacious results (Wright et al., 2016b; Kwast, 1996). Notably, high ascorbic acid content is a characteristic observed in all *Terminalia* spp. (Cock, 2015) and previous studies have reported that the apparent toxicity of Indian (Mandeville and Cock, 2018), Australian (Shalom and Cock, 2018; Lee et al., 2016; Wright et al., 2016b; Sirdaarta et al., 2015a, b) and African *Terminalia* spp. (Cock and van Vuuren 2015; Cock and van Vuuren 2014) correlates with their high ascorbic acid content. Thus, this assay may have overestimated the toxicity of these extracts.

Cellular cytotoxicity assays are generally considered to provide a more relevant evaluation of the toxicity of plant extracts. Notably, acidic phytochemicals (including ascorbic acid) generally do not significantly influence the viability of eukaryotic cell lines and thus we also evaluated the toxicity of the Australian *Terminalia* spp. extracts using normal human dermal fibroblasts HDF viability assays. Notably, exposure of HDFs to all of the *Terminalia* spp. extracts (including those classified as low toxicity in the *Artemia* nauplii assay) resulted in substantially >50% for all tests (compared to untreated control viability). Thus, all extracts were determined to be nontoxic to HDFs.

Phytochemical fingerprint analysis

The *T. ferdinandiana*, *T. carpentariae*, *T. grandiflora* and *T. petiolares* methanolic leaf extracts generally displayed substantially greater bacterial inhibitory activity than the other solvent extractions (or the *T. catappa* extracts). Thus, those extracts were analysed by metabolomic phytochemical fingerprint analysis. In recent studies, LC-MS analyses have been used to profile the phytochemical composition of multiple *Terminalia* spp. extracts for the presence of specific compounds, and to quantify their relative abundances in those extracts (Nel et al 2020; Rayan et al 2015; Sirdaarta et al 2015a; Sirdaarta et al 2015b). Notably, a number of compounds were detected in high relative abundance in the leaf extracts of multiple *Terminalia* spp. leaf extracts. In particular, the low molecular mass tannins ellagic acid and ellagic acid dehydrates, gallic acid, chebulic acid, and exifone were present in relative abundance. Additionally, the higher molecular mass tannins castalagin, chebulagic acid, chebulinic acid and corilagin were also previously reported in relative abundance in the leaf extracts of other *Terminalia* spp. leaf extracts. The flavonoid luteolin was also previously reported to be in relative abundance in multiple *Terminalia* spp. leaf extracts (Nel et al 2020; Rayan et al 2015; Sirdaarta et al 2015a; Sirdaarta et al 2015b).

Notably, all of these compounds were also identified in relative abundance in the Australian *Terminalia* spp. leaf extracts examined in our study (Table 3). Gallotannins were particularly prevalent across all of the Australian *Terminalia* spp. methanolic leaf extracts, with the levels of gallic acid (5.8-6.9 % relative abundance), chebulic acid (4-6.9 % relative abundance), corilagin (5-6.9 % relative abundance), chebulinic acid (3.1-4.5 % relative abundance) and exifone (5.7-6.6 % relative abundance) particularly noteworthy. The percentages cited in Table 3 correspond to the chromatographic percent peak area compared to the total area for all peaks in the chromatogram. The relative abundances of ellagitannins was also notable, albeit generally at lower levels than noted for the gallotannins. In particular, ellagic acid (1.3-1.7 % relative abundance), ellagic acid dehydrates (2.1-2.8 % relative abundance) and castilagin (2.1-2.8 % relative abundance) were present across the extracts of all of the Australian *Terminalia* spp. studied herein. The flavonoid luteolin was also present in particularly high relative abundance (5.8-7.4 %) across all of the extracts tested. Notably, the relative abundances of the tannin and flavonoid compounds in the methanolic *T. ferdinandiana*, *T. carpentariae* and *T. grandiflora* leaf extracts in our study correlate closely with the previously reported values (Cock et al 2019). To the best of our knowledge, the presence and abundance of these compounds has not previously been reported in *T. petiolares*. Interestingly, the LC-MS fingerprint of the *T. petiolares* leaf extract reported herein is similar in both identity and relative percentage abundance to the other Australian *Terminalia* spp. leaf extracts.

Previous studies have also identified a relative abundance of monoterpenoids in *Terminalia* spp. leaf extracts using GC-MS headspace techniques (Wright et al., 2016b; Sirdaarta et al., 2015b). In particular, cineole, terpineol, borneol, limonene have all been reported in relative abundance, with camphor, isomenthol, and isomycorene reported in lower relative abundance. Therefore, our study also screened for these compounds using GC-MS headspace fingerprint analysis to identify and quantify the relative abundance these compounds in the *T. ferdinandiana*, *T. carpentariae*, *T. grandiflora* and *T. petiolares* methanolic leaf extracts. Interestingly, cineole (1.6-2.2 % relative abundance), terpineol (1.3-1.6 % relative abundance), borneol (0.9-1.5 % relative abundance), limonene (~0.4 % relative abundance) and camphor (0.3-0.5 % relative abundance) were identified in particularly high relative abundance in all of the extracts screened herein. Notably, these relative abundances are also similar to the levels previously reported for other *Terminalia* spp. leaf extracts using similar analytical methods (Wright et al 2019).

Discussion

The current repertoire of therapies to treat AS are not ideal and only target the symptoms of the disease, whilst allowing irreversible tissue degradation to continue in the vertebral and sacroiliac joints. Therapies targeting the etiological events would prevent the onset of the disease, which would not only alleviate the symptoms of AS, but would also prevent tissue damage. The role of *K. pneumoniae* infections in the etiology of AS is well established and is supported by a wealth of evidence (Cock and Cheesman 2018; Rashid and Ebringer 2007). Despite this, there are currently no clinical therapeutics for AS that target *K. pneumoniae*. Inhibiting the growth of *K. pneumoniae* using prophylactic antibiotic therapies may be effective in the prevention and treatment of AS, and other autoimmune inflammatory diseases. However, prophylactic usage of conventional antibiotics would also induce the development of further antibiotic resistance, rendering the target bacterium (and other microbial species) unsusceptible to the effects of the antibiotic. The use of combinations of antibiotics (or antibiotics and potentiator compounds) may be more effective than antibiotic monotherapies and may also decrease the possibility of the bacteria developing further resistance.

Traditional medicines and herbal therapies are promising targets for antibiotic drug discovery as they have often been used for long periods (in some cases, thousands of years) and are perceived (often erroneously) to be safer than synthetic drugs (Moreira et al 2014). The development of multiple-drug resistant strains of many clinically relevant bacterial pathogens has resulted in the failure of many current antibiotics and has highlighted the need to develop new safe and effective antibiotic chemotherapeutics. Indeed, the World Health Organisation (WHO) considers the development of super-resistant bacteria to be one of the biggest challenges currently facing medical science (WHO, 2014). Previously, new antibiotic therapies have readily been obtained from microbial sources, and from chemical modification of natural product scaffolds. However, for reasons discussed elsewhere (Cheesman et al 2017), it is unlikely that these methods of drug development will be as fruitful in the future and new development methods are needed.

The genus *Terminalia* is an attractive target for the development of novel antibacterial therapies as multiple *Terminalia* spp. have been used traditionally to treat both inflammation and bacterial infections (Cock 2015). Furthermore, extracts produced from several *Terminalia* spp. have been reported to have noteworthy activity against a wide range of bacterial pathogens (Nel et al 2020; Mandeville and Cock 2018; Courtney et al 2015), including the *K. pneumoniae* trigger of AS (Mandeville and Cock 2018; Cock and Van Vuuren 2015). Whilst the bacterial growth inhibitory properties of several Australian *Terminalia* spp. have been reported in recent years (Wright et al 2019; Sirdaarta et al 2015a; Sirdaarta et al 2015b), they have yet to be tested for anti-*K. pneumoniae* activity. Methanolic extracts prepared from all of the *Terminalia* spp. screened in our study displayed noteworthy *K. pneumoniae* growth inhibitory activity. Additionally, the aqueous and ethyl acetate extracts of all *Terminalia* spp. tested (except *T. catappa*) also inhibited *K. pneumoniae* growth. Notably, for all of the Australian *Terminalia* spp., the leaf extracts had substantially greater potency than the corresponding fruit, bark or seed extracts. Therefore, the methanolic *T. ferdinandiana*, *T. carpentariae*, *T. grandiflora* and *T. petiolares* leaf extracts were further analysed to determine their phytochemical composition using both LC-MS and GC-MS headspace analysis.

Metabolomic fingerprint analyses of the methanolic *T. ferdinandiana*, *T. carpentariae*, *T. grandiflora* and *T. petiolares* leaf extracts confirmed the presence of some noteworthy compounds with therapeutically relevant properties. In particular, our study targeted the tannins, flavonoids and volatile terpenoids. All of the methanolic leaf extracts evaluated contained a diversity of tannins in relative abundance. Notably, tannins inhibit the growth of multiple bacterial pathogen growth by disrupting bacterial cell walls (Hogg and Embery 1982; Wu-Yuan et al 1988), binding and inactivating specific bacterial cell surface proteins, as well as functioning as inhibitors of bacterial enzymes (Wu-Yuan et al 1988; Buzzini et al 2008). Thus, it is likely that the Australian *Terminalia* spp. tannins components detected in our study may contribute to the anti-*K. pneumoniae* activity reported herein. Additionally, the flavonoid luteolin was also identified in all of the Australian *Terminalia* spp. methanolic leaf extracts examined in our study. Interestingly, multiple flavonoids including luteolin have been reported to have potent broad-spectrum antibacterial activity (Cushnie and Lamb 2005; Resende et al 2018) and may therefore also contribute to the noteworthy antibacterial activity reported herein.

Another feature of the Australian *Terminalia* spp. phytochemistry reported herein was the diversity and relative abundance of volatile terpenoids identified in the methanolic leaf extracts. In particular, the monoterpenoids cineole, terpineol and linalool were present in relative abundance. Interestingly, each of these compounds has potent broad-spectrum bacterial growth inhibitory activity against both gram positive and gram negative pathogens (Zenegin and Baysal, 2014; Paduch et al, 2007). Their antibacterial activities are linked to their small sizes and lipophilic characteristics (Paduch et al, 2007). Cineole, terpineol and linalool (as well as other monoterpenoids) can insert into cytosolic membranes, thereby altering bacterial membrane fluidity and permeability, as well as changing the conformation and function of bacterial membrane proteins. These membrane changes dramatically alter bacterial respiration, as well as several other crucial cellular processes (Cristani et al 2007; Di Pasqua et al 2006). Furthermore, modulation of bacterial membrane fluidity by small volatile terpenoids may also alter the permeability of the membrane to other compounds with growth inhibitory properties, thereby potentiating their antibiotic effects (Brem-Stecher and Johnson 2003).

The diversity and abundance of small volatile monoterpenoids in the Australian *Terminalia* spp. extracts is also particularly noteworthy as their non-specific antibacterial mechanisms are inherently difficult for bacteria to adapt to and to develop resistance against. Indeed, we were unable to find any reports of the

development of resistance to monoterpenoids by any bacterial species. Furthermore, multiple terpenoid compounds that may function via several antibacterial mechanisms were identified in these extracts. These combinations may enhance the growth inhibitory potency of the combination relative to the individual components, and may also substantially reduce the development of antibiotic resistance in the target bacteria. It is unlikely any of these compounds is solely responsible for the bacterial growth inhibitory activity reported in our study. Instead, it is likely that multiple components with several antibacterial properties may collectively interact to produce the noteworthy activity reported herein. Furthermore, synergistic interactions may potentiate the anti-*K. pneumoniae* activity, thereby increasing their efficacy of the individual components.

Our study was limited to examining the inhibitory activity of the Australian *Terminalia* spp. extracts towards a bacterial trigger of AS. However, these are crude extracts, which contain complex phytochemical mixtures. It is therefore possible that these extracts may also modulate other inflammatory processes such as cytokine release and therefore effect several aspects of AS disease progression. Indeed, several of the compounds identified in our study have been reported to have pleuripotent anti-inflammatory effects by acting on both the initiator and downstream inflammatory stages of the disease. For example, several of the terpenoids that we have identified herein can suppress NF- κ B signalling, and may therefore also directly inhibit inflammation (Salminen et al, 2008). Further studies are required to evaluate Australian *Terminalia* spp. extracts for direct anti-inflammatory properties, and for additional combinational effects that may enhance the therapeutic properties of individual components of these extracts.

Conclusions

The results of this study demonstrate the potential of *T. ferdinandiana*, *T. carpentariae*, *T. grandiflora* and *T. petiolares* as inhibitors of *K. pneumoniae*, a bacterial trigger of AS. The methanolic and ethyl acetate extracts prepared from *T. ferdinandiana* and *T. petiolaris* leaves were particularly good inhibitors of *K. pneumoniae* growth. Additionally *T. ferdinandiana*, *T. grandiflora* and *T. petiolaris* fruit extracts, as well several aqueous extracts, also had noteworthy activity. Whilst the extracts examined in this report are promising as anti-AS agents, caution is needed before these compounds can be applied to medicinal purposes. In particular, further toxicity studies using human cell lines are needed to verify the suitability of these extracts for these purposes.

Declarations

ACKNOWLEDGEMENTS

We are grateful to David Boehme of Northern Territory Wild Harvest, Australia for providing the *T. ferdinandiana*, *T. carpentaria* and *T. grandiflora* plant material used in these studies. We also thank Jacinta Monck of Kimberley Wild Gubinge, and the Mount Cootha Botanical Gardens for providing the *T. petiolaris* and *T. catappa* plant materials respectively. Financial support for this work was provided by the Centre for Planetary Health and Food Security, and the School of Environment and Science, Griffith University, Australia.

References

1. Ashayeri-Panah M, Feizabadi MM, Eftekhari F (2014) Correlation of multi-drug resistance, integron and blaESBL gene carriage with genetic fingerprints of extended-spectrum β -lactamase producing *Klebsiella pneumoniae*. Jundishapur J Microbiol 7(2):e8747
2. Braun J, Sieper J (2007) Ankylosing spondylitis. Lancet 369: 1379-1390
3. Brem-Stecker B, Johnson EA (2003) Sensitisation of *Staphylococcus aureus* and *Escherichia coli* to antibiotics by the sesquiterpenoids nerolidol, farnesol, bisabolol, and apritone. Antimicrob Agents Chemother 47(10):3357-3360
4. Buzzini P, Arapitsas P, Goretti M et al (2008) Antimicrobial and antiviral activity of hydrolysable tannins. Mini Rev Med Chem 8: 1179–1187
5. Cheesman MJ, Ilanko A, Blonk B, et al (2017) Developing new antimicrobial therapies: Are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution? Pharmacogn Rev 11: 57-72
6. Cheesman MJ, White A, Matthews B, et al (2019) *Terminalia ferdinandiana* fruit and leaf extracts inhibit methicillin-resistant *Staphylococcus aureus* growth. Planta Med 85(16): 1253-1262
7. Cock IE, Wright MH, Matthews B, et al (2019) Bioactive compounds sourced from *Terminalia* spp. in bacterial malodour prevention: an effective alternative to chemical additives. Int J Cosmet Sci 41(5): 496-508
8. Cock IE (2015) The medicinal properties and phytochemistry of plants of the genus *Terminalia* (Combretaceae). Inflammopharmacol 23(5): 203-229
9. Cock IE, Cheesman MJ (2018) The potential of plants of the genus *Syzygium* (Myrtaceae) for the prevention and treatment of arthritic and autoimmune diseases. In Bioactive Foods as Dietary Interventions for Arthritis, Osteoarthritis, and related Autoimmune Diseases, 2nd Edition. Editors Preedy VR, Watson RR. Elsevier Publishing
10. Cock IE, Winnett V, Sirdaarta J, et al (2015) The potential of selected Australian medicinal plants with anti-*Proteus* activity for the treatment and prevention of rheumatoid arthritis. Pharmacogn Mag 11(42): S190-S208
11. Cock IE, van Vuuren SF (2015) The potential of selected some South African plants with anti-*Klebsiella* activity for the treatment and prevention of ankylosing spondylitis. Inflammopharmacol 23(1): 21-35
12. Cock IE, van Vuuren SF (2014) Anti-*Proteus* activity of some South African medicinal plants: Their potential for the treatment and prevention of rheumatoid arthritis. Inflammopharmacol 21(1): 23-36
13. Courtney R, Sirdaarta J, Matthews B, et al (2015) Tannin components and inhibitory activity of Kakadu plum leaf extracts against microbial triggers of autoimmune inflammatory diseases. Pharmacogn J 7(1): 18-31

14. Cristani M, D'Arrigo M, Mandalari G, et al (2007) Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. *J Agric Food Chem* 55: 6300-6308
15. Cushnie TT, Lamb AJ (2005) Antimicrobial activity of flavonoids. *Int J Antimicrob Agents* 26: 343-356
16. Di Pasqua R, Hoskins N, Betts G, et al (2006) Changes in membrane fatty acid composition of microbial cells induced by addition of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. *J Agric Food Chem* 54: 2745–2749
17. Hogg S, Embery G (1982) Blood-group-reactive glycoprotein from human saliva interacts with lipoteichoic acid on the surface of *Streptococcus sanguis* cells. *Arch Oral Biol* 27: 261–268
18. Hübsck Z, Van Zyl RL, Cock IE, et al (2014) Interactive antimicrobial and toxicity profiles of conventional antimicrobials with Southern African medicinal plants. *S Afr J Bot* 93: 185-197
19. Ilanko A, Cock IE (2019) The interactive antimicrobial activity of conventional antibiotics and *Petalostigma* spp. extracts against bacterial triggers of some autoimmune inflammatory diseases. *Pharmacogn J* 11(2): 292-309
20. Kwast KE, Hand SC (1996) Oxygen and pH regulation of protein synthesis in mitochondria from *Artemia franciscana* embryos. *Biochem J* 313: 207–213
21. Lee CJ, Wright MH, Arnold MSJ, et al (2016) Inhibition of *Streptococcus pyogenes* growth by native Australian plants: New approaches towards the management of impetigo, pharyngitis and rheumatic heart disease. *Pharmacogn Commun* 6(3): 164-173
22. Mandeville A, Cock IE (2018) *Terminalia chebula* Retz. fruit extracts inhibit bacterial triggers of some autoimmune diseases and potentiate the activity of tetracycline. *Indian J Microbio.* 58: 496–506
23. Moreira DD, Teixeira SS, Monteiro MH, et al (2014) Traditional use and safety of herbal medicines. *Rev Bras Farmacogn* 24: 248-257
24. Nel AL, Murhekar, S, Matthews, B, et al (2020) The interactive antimicrobial activity of *Terminalia sericea* Burch ex DC. leaf extracts and conventional antibiotics against bacterial triggers of selected autoimmune inflammatory diseases. *S Afr J Bot* 133: 17-29.
25. Paduch R, Kandefer-Szerszen M, Trytek M et al (2007) Terpenes: substances useful in human healthcare. *Arch Immunol Ther Exp* 55: 315–327
26. Rashid T, Ebringer A (2007) Ankylosing spondylitis is linked to *Klebsiella* – the evidence. *Clin Rheumatol* 26: 858-864
27. Rayan P, Matthews B, McDonnell A, et al (2015) *Terminalia ferdinandiana* extracts as inhibitors of *Giardia duodenalis* proliferation: a new treatment for giardiasis. *Parasitol Res* 114: 2611-2620.
28. Resende FA, Nogueira LG, Bauab TM, et al (2018) Antibacterial potential of flavonoids with different hydroxylation patterns. *Eclat Quim* 40: 173-179
29. Ruebhart DR, Wickramasinghe W, Cock IE (2009) Protective efficacy of the antioxidants vitamin E and Trolox against *Microcystis aeruginosa* and microcystin-LR in *Artemia franciscana* nauplii. *J Toxicol Environ Health Part A* 72: 1567-1575.
30. Saag KG, Koehnke R, Caldwell JR, et al (1994) Low dose long-term corticosteroid therapy in rheumatoid arthritis: an analysis of serious adverse events. *Am J Med* 96(2): 115-123
31. Salminen A, Lehtonen M, Suuronen Tet al (2008) Terpenoids: Natural inhibitors of NF-κB signalling with anti-inflammatory and anticancer potential. *Cell Mol Life Sci* 65(19): 2979-2999
32. Shalom J, Cock IE (2018) *Terminalia ferdinandiana* Exell. fruit and leaf extracts inhibit proliferation and induce apoptosis in selected human cancer cell lines. *Nutr Cancer* 70(4): 579-593.
33. Sirdaarta J, Matthews B, Cock IE (2015a) Inhibitory activity of Kakadu plum fruit extracts against microbial triggers of rheumatoid arthritis: Identification of stilbene and tannin components. *J Funct Food* 17: 610-620
34. Sirdaarta J, Matthews B, White A, et al (2015b) GC-MS and LC-MS analysis of Kakadu plum fruit extracts displaying inhibitory activity against microbial triggers of multiple sclerosis. *Pharmacogn Commun* 5(2): 100-115
35. WHO (2014) The Evolving Threat of Antimicrobial Resistance: Options for Action. World Health Organization 2014. http://apps.who.int/iris/bitstream/10665/44812/1/9789241503181_eng.pdf. Accessed 14 March 2017.
36. Winnett V, Boyer H, Sirdaarta J, et al (2014) The potential of *Tasmannia lanceolata* as a natural preservative and medicinal agent: antimicrobial activity and toxicity. *Pharmacogn Commun* 4(1): 42-52
37. Wright MH, Shalom J, Matthews B, et al (2019) *Terminalia ferdinandiana* Exell: extracts inhibit *Shewanella* spp. growth and prevent fish spoilage. *Food Microbiol* 78: 114-122
38. Wright MH, Arnold MSJ, Lee CJ, et al (2016a) Qualitative phytochemical analysis and antibacterial activity evaluation of Indian *Terminalia* spp. against the pharyngitis causing pathogen *Streptococcus pyogenes*. *Pharmacogn Commun* 6(2): 85-92
39. Wright MH, Sirdaarta J, White A, et al (2016b) GC-MS headspace analysis of *Terminalia ferdinandiana* fruit and leaf extracts which inhibit *Bacillus anthracis* growth. *Pharmacogn J* 9: 73–82
40. Wu-Yuan C, Chen C, Wu R (1988) Gallotannins inhibit growth, water-insoluble glucan synthesis, and aggregation of *Mutans streptococci*. *J Dent Res* 67: 51–55
41. Zenegin H, Baysal AH (2014) Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. *Molecules* 19: 17773–17798.

Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Table 2: Disc diffusion (DD) and liquid dilution (LD) MIC values ($\mu\text{g/mL}$) for Australian *Terminalia* spp. extracts against *K. pneumoniae*, and LC_{50} ($\mu\text{g/mL}$) in the ALA and HDF assays.

Plant Species	Extract	<i>K. pneumoniae</i> (ATCC31488)		<i>K. pneumoniae</i> (clinical strain)		LC_{50}	
		DD MIC	LD MIC	DD MIC	LD MIC	ALA	HDF
<i>T. ferdinandiana</i>	Fruit M	653	416	825	508	925	-
	Fruit W	584	345	620	482	1164	-
	Fruit E	406	227	463	286	1083	-
	Leaf M	253	66	220	53	1172	-
	Leaf W	584	290	459	221	1428	-
	Leaf E	285	78	224	75	1026	-
	Seed M	1434	1346	1235	828	1863	-
	Seed W	1146	922	1422	1139	2295	-
	Seed E	1287	1046	1046	1047	1940	-
<i>T. carpentariae</i>	Leaf M	558	128	492	98	1185	-
	Leaf W	1458	983	1288	725	1385	-
	Leaf E	825	260	610	164	1553	-
<i>T. grandiflora</i>	Fruit M	1235	983	1492	1146	934	-
	Fruit W	1150	884	1280	1003	1387	-
	Fruit E	1635	1220	1587	1322	1876	-
	Leaf M	854	526	527	392	884	-
	Leaf W	399	87	304	198	2564	-
	Leaf E	883	600	488	274	-	-
<i>T. petiolaris</i>	Fruit M	733	427	651	376	916	-
	Fruit W	946	669	827	658	1257	-
	Fruit E	870	364	693	274	1073	-
	Leaf M	304	83	228	69	1120	-
	Leaf W	725	342	655	253	1362	-
	Leaf E	382	105	327	92	1225	-
<i>T. catappa</i>	Bark M	4385	3760	4140	3406	1473	-
	Fruit M	>5000	>5000	>5000	>5000	1088	-
	Leaf M	2856	1685	2540	1455	1227	-
Antibiotic and toxin controls	Penicillin-G	NT	-	NT	-	-	-
	Erythromycin	NT	-	NT	-	-	-
	Tetracycline	NT	1.25	NT	1.25	-	-
	Chloramphenicol	NT	2.5	NT	2.5	-	-
	Potassium dichromate	NT	NT	NT	NT	58	NT
	Quinine	NT	NT	NT	NT	NT	87

M = methanol extract; W = water extract; E = ethyl acetate extract; DD = disc diffusion; LD = liquid dilution; NT = not tested; - indicates no inhibition or not toxic at any dose tested.

Table 3: Qualitative HPLC-MS QTOF and GC-MS headspace analysis of the most promising *Terminalia* spp. methanolic leaf extracts extract, elucidation of empirical formulas and putative identification (where possible) of the compounds.

	Retention time (min)	Empirical formula	Molecular Mass (Da)	Putative identification	Relative abundance (%)							
	<i>T. ferdinandiana</i> leaf				<i>T. carpenteriae</i> leaf		<i>T. grandiflora</i> leaf		<i>T. petiolaris</i> leaf			
	Positive mode				Negative mode	Positive mode	Negative mode	Positive mode	Negative mode	Positive mode	Negative mode	
LC-MS Evaluation	1.065	C14 H10 O10	338.029	Ellagic acid dihydrate	2.13	-	2.78	-	2.63	-	2.44	-
	1.383	C4 H8 O5	136.037	Gallic acid	-	6.73	-	5.82	-	6.55	-	6.9
	1.54	C14 H12 O11	356.039	Chebolic acid	4.41	6.93	2.94	5.33	4.37	5.99	4.04	6.9
	4.374	C14 H6 O8	302.007	Ellagic acid	1.22	1.3	1.39	1.42	1.66	1.42	1.55	1.7
	7.669	C41 H26 O26	934.072	Castalagin	2.06	0.83	2.77	1.26	2.1	0.92	2.22	0.9
	7.774	C27 H22 O18	634.082	Corilagin	2.49	6.81	1.86	4.96	2.38	6.5	2.56	6.9
	8.501	C34 H22 O22	782.062	Punicalin	-	-	0.36	-	0.18	-	-	-
	8.604	C21 H20 O11	448.102	Luteolin	0.47	6.58	0.41	5.79	0.49	6.44	0.45	7.5
	8.728	C41 H32 O27	956.113	Chebulinic acid	1.27	4.46	0.69	3.14	0.89	3.76	1.18	4.5
	9.312	C13 H10 O7	278.043	Exifone	5.69	1.4	6.62	1.47	6.02	1.39	5.82	1.4
	9.846	C41 H30 O27	954.098	Chebulagic acid	2.08	1.93	1.66	1.52	1.83	1.77	2.04	1.8
Headspace GC-MS Evaluation	Retention time (min)	Empirical formula	Molecular Mass (Da)	Putative identification	<i>T. ferdinandiana</i> leaf		<i>T. carpenteriae</i> leaf		<i>T. grandiflora</i> leaf		<i>T. petiolaris</i> leaf	
	14.09	C10 H16	136	Limonene	0.38		0.43		0.36		0.41	
	14.58	C10 H16	136	Isomyocorene	0.04		0.07		0.03		0.05	
	15.5	C10 H18 O	154	Cineole	2.16		1.62		2.11		2.2	
	17.71	C10 H16 O	152	Camphor	0.3		0.46		0.38		0.27	
	18.41	C10 H18 O	154	Borneol	0.92		1.46		1.26		0.96	
	19.16	C10 H18 O	154	Terpineol	1.58		1.31		1.4		1.55	
	36.52	C16 H22 O4	278	Diisobutyl phthlate	0.01		0.13		0.07		0.04	
	39.46	C10 H20 O	165	Isomenthol	0.09		0.14		0.11		0.16	

The relative abundance expressed in this table is a measure of the area under the peak expressed as a % of the total area under all chromatographic peaks.

Figures

Figure 1

Growth inhibitory activity of the *Terminalia ferdinandiana* (a) fruit, (b) leaf and (c) seed extracts measured as zones of inhibition (mm) against reference (ATCC31488) and clinical isolate strains of *K. pneumoniae*. Inhibition zones are represented as the means of three independent experiments, each with internal triplicates ($n=9$) $\pm$ SEM. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin control (10 μ g); Chl = chloroform control (10 μ g); ATCC and CI refer to the reference and clinical bacterial strains respectively; NC = negative control.

Figure 2

Growth inhibitory activity of the *Terminalia carpentariae* leaf extract measured as zones of inhibition (mm) against reference (ATCC31488) and clinical isolate strains of *K. pneumoniae*. Inhibition zones are represented as the means of three independent experiments, each with internal triplicates ($n=9$) $\pm$ SEM. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin control (10 μ g); Chl = chloroform control (10 μ g); ATCC and CI refer to the reference and clinical bacterial strains respectively; NC = negative control.

Figure 3

Growth inhibitory activity of the *Terminalia grandiflora* (a) fruit and (b) leaf extracts measured as zones of inhibition (mm) against reference (ATCC31488) and clinical isolate strains of *K. pneumoniae*. Inhibition zones are represented as the means of three independent experiments, each with internal triplicates ($n=9$) $\pm$ SEM. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin control (10 μ g); Chl = chloroform control (10 μ g); ATCC and CI refer to the reference and clinical bacterial strains respectively; NC = negative control.

Figure 4

Growth inhibitory activity of the *Terminalia petiolaris* (a) fruit and (b) leaf extracts measured as zones of inhibition (mm) against reference (ATCC31488) and clinical isolate strains of *K. pneumoniae*. Inhibition zones are represented as the means of three independent experiments, each with internal triplicates ($n=9$) $\pm$ SEM. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin control (10 μ g); Chl = chloroform control (10 μ g); ATCC and CI refer to the reference and clinical bacterial strains respectively; NC = negative control.

Figure 5

Growth inhibitory activity of the *Terminalia catappa* (a) bark, (b) fruit and (c) leaf extracts measured as zones of inhibition (mm) against reference (ATCC31488) and clinical isolate strains of *K. pneumoniae*. Inhibition zones are represented as the means of three independent experiments, each with internal triplicates ($n=9$) $\pm$ SEM. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin control (10 μ g); Chl = chloroform control (10 μ g); ATCC and CI refer to the reference and clinical bacterial strains respectively; NC = negative control.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.docx](#)