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Anti-inflammatory Potential of South African Medicinal Plants used for the Treatment of Sexually Transmitted Infections

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Highlights

- Anti-inflammatory and antioxidant activity was observed in some plant extracts.
- A first report of 15-LOX and XO inhibition for most of these tested plants is presented.
- Proanthocyanidins were one of the major constituents in the active extracts.
- GC–MS analysis showed a likely synergistic constituent effect linked to activity.
- Extracts showed little or no cytotoxicity on murine macrophages and Vero cells.

ABSTRACT

Inflammation associated with sexually transmitted infection (STIs) often results from delayed diagnosis or treatment, and may result in life-threatening complications. The root or stem bark of plants selected in this study are used by traditional healers in the management of STIs and to treat the associated inflammation. This study evaluated the anti-inflammatory activities of

twelve South African medicinal plants used traditionally in the treatment of STIs and the associated inflammation. Acetone extracts were tested for anti-inflammatory activity against 15-lipoxygenase (15-LOX), xanthine oxidase (XO) and inducible nitric oxide production in lipopolysaccharide (LPS) stimulated RAW 264.7 macrophage cells. The free radical and nitric oxide scavenging capacity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide inhibition assays. The MTT assay was used to determine the cytotoxic effect of the extracts on RAW 264.7 macrophages and Vero cells. Preliminary phytochemical analysis was carried out using NMR spectroscopy, Gas Chromatography Mass Spectroscopy (GC-MS) analysis and the determination of total phenolic and flavonoid contents. The root bark extract of *Lannea schweinfurthii* had the highest inhibitory activity against 15-LOX (IC_{50} = 40 ± 3 µg/mL) while C. *abbreviata* best inhibited XO (IC_{50} = 46.8 ± 1.5 µg/mL). Extracts of *Ficus abutilifolia*, *Faurea saligna* and *Zanthoxylum capense* showed significant suppression of inducible nitric oxide production with IC_{50} values comparable to quercetin (IC_{50} < 30.0 µg/mL). The extracts of *C. abbreviata* and *F. abutilifolia* had significantly higher radical scavenging activities compared to ascorbic acid. Most of the extracts showed little or no cytotoxicity against the tested cell lines. Phytochemical analysis pointed to the presence of diverse classes of secondary metabolites as well as the presence of proanthocyanidins as the major constituents of the most active extracts in the anti-inflammatory assays. For most of the plants, this study provides the first report of 15-LOX inhibition and xanthine oxidase inhibition. The activity of most of the tested extracts supports the administration of these plants by traditional healers in the treatment of STIs and their associated inflammation.

Keywords: Medicinal plants; inflammation; 15-lipoxygenase; xanthine oxidase; nitric oxide; anti-oxidant; cytotoxicity.

1. Introduction

Inflammatory conditions and pain associated with sexually transmitted infections (STIs) usually develop as a result of delayed treatment or diagnosis. Both men and women are at risk of infection, however, the female anatomy increases chances of complications and reproductive health problems. These may result in infertility, ectopic pregnancy, miscarriages, preterm delivery, upper reproductive tract inflammation, gonococcal arthritis, pelvic inflammatory disease (PID), infection and or death of newborns (Kambizi and Afolayan, 2008; Mitchell and Prabhu, 2013; Château and Seifert, 2016). Despite the recent advances in science and therapeutics the incidence of STIs is still on the rise, particularly in sub-Saharan Africa which has the highest annual incidence (World Health Organization, 2016). Most reports of new STI cases in South Africa occur predominantly among youths and adults between the ages of 15 and 49 years (Shisana et al., 2012).

The pathophysiology of many disorders and diseases, including STIs, is manifested through inflammatory responses as the body recognizes the infection and sets in motion mechanisms to repair the damage (Viljoen et al., 2012). Inflammation, which has been suggested to be closely linked to carcinogenesis, plays a vital role in the aetiology of STIs. Inflammatory STIs such as gonorrhoea, chlamydia and trichomoniasis have been implicated in the later development of PID, prostate cancer and benign prostatic hyperplasia (Sutcliffe et al., 2006). Apart from the risk of onset of chronic inflammation, untreated STIs have also been reported to cause genital inflammation and predispose patients to a higher risk of HIV acquisition (Masson et al., 2015a).

Most STIs are associated with elevated levels of pro-inflammatory cytokines, which results in activation of various immune cells that serve as suitable target sites for HIV infection. They also reduce epithelial barrier integrity and promote viral replication through nuclear factor kappa-B

(NF-κB) activation. Therefore, this process highly predisposes STI patients to a risk of HIV infection (Mlisana et al., 2012). Although the inflammatory response plays a role in microbe clearance, it may facilitate tissue penetration by the pathogens which could result from destruction of infected epithelial cells by the body's immune cells (Masson et al., 2015b).

The inflammatory cascade is a highly regulated biochemical process whose successive progression is mediated by the synthesis of inflammatory mediators. The basic mechanism of the inflammatory response often involves localized increases in the number of leukocytes and a variety of complex mediator molecules such as cytokines, histamines, serotonin, leukotrienes and prostaglandins induced by phospholipases, cyclooxygenases (COX) and lipoxygenases (LOX) (Eldeen et al., 2005). These inflammatory mediators play a major role in the establishment of an effective inflammatory response as well as in clearance of the stimuli or infection (Niu et al., 2011). The uncontrolled activity of oxidative enzymes such as LOX, xanthine oxidase, COX and inducible nitric oxide synthase (iNOS) has been reported to increase oxidative stress as well as the over production of reactive oxygen and nitrogen (ROS and RNS) species. When present in excess, these free radicals results in an oxidative burst which leads to a non-resolution of inflammation which often persist when STIs are left untreated (Dobrian et al., 2011).

In the treatment of inflammation and related pain, non-steroidal anti-inflammatory drugs (NSAID) are often prescribed. However, reports suggest that almost 90% of drugs used against inflammation produce drug related toxicities and pose adverse effects (Jeengar et al., 2014; Adebayo et al., 2015; Jacob and Prakash Kumar, 2015). Their mechanism of action usually involves the inhibition of inflammatory mediators which in turn reduces the progression of inflammation (Allott et al., 2014). Plant bioactive secondary metabolites targeting multiple inflammatory pathways including the COX and LOX pathways or having dual COX/LOX

inhibitory activity are attractive targets for the treatment of inflammation (Sadeghian and Jabbari, 2016).

Medicinal plants have a long history of use in the management of STIs and its associated inflammatory conditions with their evidence of use also well documented (Buwa and Van Staden, 2006). Traditional folklores administer medicinal plants singly or in combination with the aim of eradicating STI causative pathogens, treat internal and externals sores, inflammation and pain associated with the infections (Naidoo et al., 2013). The anti-inflammatory activities and therapeutic potential of a large number of South African plant species used traditionally in the treatment of STIs are yet to be studied or fully exploited (Iwalewa et al., 2007).

South African medicinal plants used by traditional healers for the treatment of various STIs and related pain may possess multifunctional biological effects. Some of these plants have been validated through scientific studies to possess significant activity against some tested STI pathogens (Mulaudzi et al., 2011; Nair et al., 2013; Tshikalange et al., 2008). It is also highly plausible that when such plants are administered to STI patients, one of the modes of action may include attenuation of pain and reduction of excessive inflammatory mediators which are responsible for the initiation and resolution of infection pathogenicity. These possibilities serve as the pivotal basis for this study, as medicinal plants have a long history of use in the treatment of human ailments (Oguntibeju, 2018). This could have additional application in the management of pain and inflammation associated with STIs (Mulaudzi et al., 2013).

2. Materials and methods

2.1. Plant collection, authentication and extract preparation

Plant material from twelve plant species was collected from the Venda region in the Limpopo province of the Republic of South Africa. Selection of plants and parts was based on ethnobotanical

records for traditional use in the treatment of STIs (Table 1). An additional selection criterion was the paucity of scientific studies on their anti-inflammatory potential. Voucher specimens were prepared for identification and authentication at the H.G.W.J. Schweickerdt Herbarium, University of Pretoria.

Firstly, collected plant parts were cleaned, cut into small pieces, shade-dried and pulverized into fine powder. Since the main object of this study was to establish which plants contained anti-inflammatory substances, acetone was chosen as an extractant in order to maximize the quantity as well as diversity of compounds. Because of its polarity index, acetone is able to extract both non-polar as well as more polar substances from plant materials (Dzoyem and Eloff, 2015; Eloff, 1998). Six grams of plant material was extracted with 100 mL of acetone at room temperature for 48 h. The extracts were filtered using a Büchner funnel and the resultant filtrate was concentrated to dryness under vacuum, using a rotary evaporator. Dried extracts were stored in air-tight glass containers at 4°C. The percentage yield of each extract was also determined (Table 1).

2.2. Studies on crude extracts

2.2.1 Determination of total phenolic content

Total phenolic content of plant extracts was determined using the Folin-Ciocalteu method as described by Adebayo et al. (2015). In a spectrophotometer tube, 250 µL of Folin-Ciocalteu reagent was added to 25 µL of a 1 mg/mL extract (reconstituted in ethanol). The mixture was incubated for 5 min and the reaction was terminated by the addition of 750 µL of 20% anhydrous sodium carbonate. The volume in the tube was made up to 5 mL using distilled water and allowed to incubate in the dark at room temperature for 2 h.

After incubation, the absorbance of tubes containing solution was read at a wavelength of 760 nm. The total phenolic content in plant extract was determined by linear interpolation using a standard curve prepared with different concentrations of gallic acid. Results were expressed as milligram per gram gallic acid equivalent (mg/g GAE)

2.2.2. Determination of total flavonoid content

For determination of total flavonoid content, 300 µL of methanol was added to 100 µL of a 1 mg/mL extract (reconstituted in ethanol). To the above mixture, 20 µL of 10% aluminium chloride solution and 20 µL of 1.0 M sodium acetate solution was added. The resultant solution was made up to 1 mL with distilled water and allowed to incubate at room temperature for 30 min. After incubation, the cuvettes were placed in a spectrophotometer and the absorbance was read at a 450 nm wavelength. The total flavonoid content in the plant extracts was determined by linear interpolation using a standard curve prepared with different concentrations of quercetin. Results were expressed as milligram per gram quercetin equivalent (mg/g QE) (Adebayo et al., 2015).

2.3. Anti-inflammatory assays

2.3.1. In-vitro inhibition of 15-lipoxygenase (15-LOX)

Spectrophotometric assessment of the activity of 15-LOX (Soybean lipoxygenase; Sigma, St. Louis, MO, USA) was carried out using a previously described method (Lyckander and Malterud, 1992). The formation of a conjugated diene bond due to product formation from a suitable substrate (linoleic acid, final concentration 134 µM) was monitored at 234 nm in a Biochrom Libra S32 PC instrument (Biochrom, Cambridge, UK). Enzyme and substrate solutions were prepared in 0.2 M borate buffer with a pH of 9.00.

To preserve enzyme activity, the enzyme solution was placed on ice throughout the experimental period, and negative controls (without inhibitor) were measured at intervals during the measurement series. Extracts were tested in triplicate starting from stock solutions in DMSO (10 mg/mL) from which serial dilutions were made. To a solution of linoleic acid in buffer (2.9 mL), 50 µL of the DMSO solution of extract and 50 µL of 15-LOX enzyme (10,000 U/mL) were added and the mixture stirred briefly. The absorbance increase was recorded in quartz cuvettes from 30 to 90 seconds after enzyme addition. Quercetin, a good radical scavenger and a good inhibitor of 15-lipoxygenase and xanthine oxidase (Nguyen et al., 2016) was used as positive control in these experiments and DMSO alone was added in the place of extracts as negative control. Percentage enzyme inhibition was calculated as 100 x $[(\Delta A_1 - \Delta A_2) / \Delta A_1]$, where ΔA_1 and ΔA_2 represent changes in absorbance values from 30 to 90s for extracts and negative control respectively.

2.3.2. In vitro inhibition of xanthine oxidase (XO)

Inhibition of XO (xanthine oxidase from bovine milk; Sigma) was assessed according to Bräunlich et al. (2013). Briefly, 1.85 mL of sodium-potassium phosphate buffer (50 mM, pH 7.5), 50 µL test substance in DMSO (DMSO alone for samples without inhibitor) and 100 µL enzyme solution in buffer (1.8 U/mL) were mixed in quartz cuvettes. The reaction was initiated by adding 1.0 mL substrate solution (hypoxanthine in distilled water, 20 µg/mL). Absorbance increase at 290 nm was registered for a period of 5 min. Enzyme inhibition was calculated as: 100 x $[(\Delta A_1 - \Delta A_2)/ \Delta A_1]$, where, ΔA_1 and ΔA_2 are absorption increases for samples without and with inhibitor. Quercetin was used as positive control.

Table 1: Selected plant species, traditional uses and percentage yield of extracts.

Scientific Names	Family Name	Common Name	Herbarium Specimen Number	Part	Medicinal Uses	Yield (%)
Bridelia mollis Hutch.	Phyllanthaceae	Velvet Bridelia	JB120324	Stem bark	STIs, Cough, malaria (Shai et al., 2013)	4.5
Cassia abbreviata Oliv.	Leguminosae	Long-tail cassia	PKT0058	Stem bark	Venereal diseases (Chauke et al., 2015)	6.3
Diospyros mespiliformis Hochst ex A.DC.	Ebenaceae	Jackal-berry or Swamp ebony	PKT0094	Root bark	STIs and urinary tract infections (Tlakula, 2016)	2.5
Faurea saligna Harv.	Proteacea	Willow beech-wood	BCM 118700	Stem bark	Venereal diseases (Tlakula, 2016)	13.2
Ficus abutilifolia Miq.	Moraceae	Large-leaved Rock Fig	TE0211	Stem Bark	Boiled in mixtures for all STIs (Chauke et al., 2015)	11.2
Lannea schweinfurthii Engl.	Anacardiaceae	False marula	MPT0043	Root bark	Venereal and infectious diseases (Fernandes et al., 2008)	9.0
Obetia tenax Friis	Urticaceae	Rock tree-nettle	TE0217	Stem b ark	Venereal and infectious diseases (Fernandes et al., 2008)	1.5
Pseudolachnostylis maprouneifolia Pax.	Phyllanthaceae	Kudu berry	TE0218	Stem bark	Venereal diseases, bark for noisy stomach, root for pneumonia (Samie et al., 2010)	5.3
Terminalia prunioides M.A.Lawson	Combretaceae	Lowveld Cluster-Leaf	SAA120602/1	Stem bark	STIs, abdominal pains, backaches (Adebayo et al., 2015)	7.0
Solanum panduriforme E. Mey.	Solanaceae	Bitter Apple	PKT0165	Root bark	Used in mixture to treat gonorrhoea (Mulaudzi et al., 2015)	2.5
Strychnos spinosa Lam.	Loganiaceae	Green monkey orange	PKT0126	Root bark	Boiled in mixtures for all STIs (Watt and Breyer-Brandwijk, 1962)	3.8
Zanthoxylum capense (Thunb.) Harv.	Rutaceae	Small knob-wood	PKT058	Stem Bark	Decoction for syphilis (Mongalo, 2013)	3.2

2.3.3. Inhibition of nitric oxide production in LPS—stimulated RAW 264.7 macrophage cells

2.3.3.1. Cell culture and treatment

Murine RAW macrophage cells and Vero African green monkey kidney cells used in this study were initially obtained from the American Type Culture Collection (Rockville, MD, USA). RAW 264.7 cells were cultured in a plastic flask containing Dulbecco´s Modified Eagle Medium (DMEM) supplemented with L-glutamine, 10% foetal calf serum (FCS) and 1% penicillin/streptomycin/fungizone solution (PSF). The cells were maintained by incubating at 37°C in 5% CO_2 until about 70-80% confluency was achieved before splitting at least twice a week. Cells were seeded in a 96 well plate at a density of 40 000 cells per well and incubated for 24 h at 37°C in a humidified environment with 5% CO_2. The media was aspirated and the cells were activated with lipopolysaccharide (LPS) (1 µg/mL) before the addition of different concentrations of extracts (100, 50, 12.5 and 1.56 µg/mL) diluted in serum-free DMEM.

Quercetin, a good inhibitor of NO production in macrophages (Dzoyem et al., 2016, Ho et al., 2014, Mu et al., 2001) was also tested at the same concentration and served as the positive control while wells containing cells which were activated with LPS and not treated served as the negative control. The plates were incubated for 24 h at 37°C under 5% CO_2.

2.3.3.2. Determination of nitrite production

The amount of nitric oxide (NO) produced by treated cells in the wells was assessed using the Griess reagent method (Elisha et al., 2016). In brief, after the 24 h incubation, 100 µL of supernatant was transferred to new 96 well plates and an equal volume of Griess reagent was added in the dark. The plates were covered with aluminium foil and incubated for 15 min in the dark. The absorbance of the wells was determined by reading plates at 550 nm using a BioTek

Synergy™ HT Multi-Detection Microplate Reader. The amount of NO was determined using a calibration curve established with known concentrations of sodium nitrite. The percentage inhibition of NO production of extract was calculated using the expression: 100 – (Absorbance $_{extract}$ – Absorbance $_{blank}$)/ (Absorbance $_{Negative\ control}$ – Absorbance $_{blank}$) x 100.

2.3.3.3. Determination of cell viability of RAW 264.7 macrophages exposed to extracts

The mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan was used as an indicator of cell viability using the method reported by Mosmann (1983) to establish if the observed inhibition of NO production was not due to cell death. After incubation for 24 h with LPS and extract, the cell culture media was completely removed, cells were washed using PBS and 100 µL of fresh DMEM was added. To this media, a 30 µL aliquot of 5 mg/mL MTT in PBS was added to each well and the plates were incubated for 4 h at 37°C in 5% CO_2. After incubation, the medium was carefully aspirated, and DMSO was added to dissolve the formed formazan salt. The plates were then read at 570 nm using a plate reader. Cell viability was calculated as percentage viability with reference to the negative control which contained LPS-stimulated cells. Doxorubicin was used as the positive control.

% Cell viability = ($A_{sample} - A_{blank}$) / ($A_{control} - A_{blank}$) x 100.

2.4. Cytotoxicity of selected plants on Vero cells

The cytotoxicity of five plant extracts showing satisfactory 15-LOX inhibitory activity was assessed on Vero cells using the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxyanilide salt (XTT) reduction assay. The cells were maintained in cell culture flasks containing DMEM supplemented with 1% antibiotics (100 U/mL penicillin, 100 µg/mL

streptomycin and 250 µg/mL fungizone) and 10% heat inactivated FCS. Incubation was done under standard cell culture conditions at 37°C under 5% CO_2.

Vero cells (cell density of 10 000 cells/ well) were seeded in a 96 well microtitre plate and incubated for 24 h at 37°C under 5% CO_2 atmosphere. After initial incubation, 100 µL of extract was added to all the wells and the plates were re-incubated for 72 h. The extracts were tested in triplicate with concentrations ranging from 3.13 to 400 µg/mL. The experimental layout also included DMSO as negative control and actinomycin-D as a positive control.

Actinomycin-D was tested with final concentrations ranging from 0.05 – 3.91 x 10^{-4} µg/mL. A colour control well in which Vero cells were absent was also prepared using a similar experimental procedure. After incubation, 100 µL of XTT was added to the test and colour wells and the plates were again re-incubated for 2 h. The absorbance was measured at 490 nm at a reference wavelength of 690 nm using a multi-well plate reader. Percentage cell growth inhibition was calculated in reference to untreated control and the 50% inhibitory concentration (IC_{50}) was determined.

2.5. In vitro radical scavenging assays

2.5.1. DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay

Scavenging of the DPPH radical was measured as described by Malterud et al. (1993). Briefly, an aliquot of test compound dissolved in DMSO (0.05 mL) was mixed with a solution of DPPH in methanol (A_{517} = 1.0; 2.95 mL) and the UV absorbance at 517 nm was measured for 5 min. Percent radical scavenging was calculated as 100 * ((295/300)*A_{start} – A_{end})/ ((295/300)*A_{start}), where A_{start} is the absorbance before addition of test compound and A_{end} is the absorbance value after 5 min of reaction time. For samples with high absorption at 517 nm, appropriate corrections were made.

2.5.2. Nitric oxide scavenging assay

The nitric oxide scavenging capacity of the extracts was determined according to the method described by Mayur et al. (2010) with slight modifications. Twenty microliters of a stock solution with concentration of 10 mg/mL of extract or positive control (reconstituted in ethanol) was added to the top well of a 96 well plate from which serial dilutions were made.

Extracts and positive control (ascorbic acid) were tested to final concentrations of 3.91 to 500 µg/mL. For the negative control, 10% ethanol was added in the place of extracts. A 10 mM solution of sodium nitroprusside was prepared in distilled water, and 50 µL of this solution was added to all the wells. The plates were allowed to incubate for 90 min at room temperature before the addition of 100 µL Griess reagent. Control wells were also prepared for all extracts in the same manner as the test wells. However, distilled water was added in the place of Griess reagent. The absorbance of the plates were read at 546 nm using a 96 well plate reader and the percentage scavenging capacity was calculated as 100 x ($A_c - A_s$) / A_c, where A_c and A_s are absorbance of control and samples respectively.

2.6. Nuclear Magnetic Resonance (NMR) analysis

The acetone extracts of the plants were characterized by ^{1}H and in some instances ^{13}C NMR spectroscopy. All the spectra were recorded on a Bruker AVII-600 (Bruker, Rheinstetten, Germany) instrument and analysis was performed in deuterated methanol (CD_3OD) with tetramethylsilane (TMS) as an internal standard. ^{1}H NMR spectra were recorded at 600 MHz while ^{13}C NMR spectra were recorded at 150 MHz.

2.7. GC-MS analysis

One microlitre (1 µL) of each plant extract was injected in the splitless mode with the injector temperature set at 250°C unto a Schimadzu GC-MS (QP2010 SE) and analysed in the Electron impact Ionization (EI) mode on an Inert Cap 5MS/NP capillary column (30 m×0.25 mm×0.25 µm; GL Sciences, Tokyo, Japan). The oven temperature was programmed at 50°C for 2 min, and increased at 15°C min^{-1} to a final temperature of 300°C and held for 20 min. Helium was used as the carrier gas at a constant flow rate of 1.0 ml min^{-1}. The ion source was operated at 250°C with an interface temperature of 250°C, and Mass spectral recorded between 35–550 m/z at 70 eV with a scan speed of 2000. Compounds were tentatively identified based on comparison of their mass spectra data with those of published mass spectral libraries [NIST11 and Wiley (10th edition)].

2.8. Statistical analyses

Results were expressed as mean ± SD for percentage inhibition and IC_{50} values. All data were subjected to one way analysis of variance (ANOVA) using GraphPad Prism 4 software. Where a significant difference ($P < 0.05$) was observed, Tukey's multiple comparison test was used to separate the means.

3. Results and discussion

3.1. Studies on crude extracts

3.1.1. Yield of extract, total phenolics and flavonoid content

The extract yields from the bark of the 12 medicinal plants used in this study are presented in Table 1. This was expressed as the ratio of the dried crude extract obtained after extraction to the weight of the plant material used for the extraction process. Extract yield ranged from approximately 90 – 790 mg by mass with the highest percentage yield of 13.2% obtained from the stem bark extract of *F. saligna* and the lowest yield of 1.5% obtained from stem bark extract of *O. tenax.* In this study, the observed trend was that slightly higher extract yields were obtained when stem bark was used compared to root bark. The exceptions were however the crude extract yield from root bark of *L. schweinfurthii* with a 9% yield, 3.2% from stem bark of *Z. capense* and 1.5% from *O. tenax* respectively.

Phenolic phytochemicals have been classified as important secondary metabolites in plants and they have also been documented as beneficial compounds due to the role they play in various human diseases. The phenolic content of plant extracts tested in this study ranged from 710 mg/g in *F. saligna* (with the highest phenolic content) to 190 mg/g GAE in *S. spinosa* which had the lowest phenolic content. The total phenolic and flavonoid content present in the plant extracts were reported as equivalent in mg/g of the standard used.

Acetone extracts of *F. abutilifolia, C. abbreviata, B. mollis, O. tenax* and *T. prunioides* also showed high phenolic contents, greater than 500 mg/g GAE (Fig. 1). The high yield of *F. saligna* was commensurate with the phenolic content present. Nonetheless, the flavonoid contained in the extract was relatively low (20 mg/g QE).

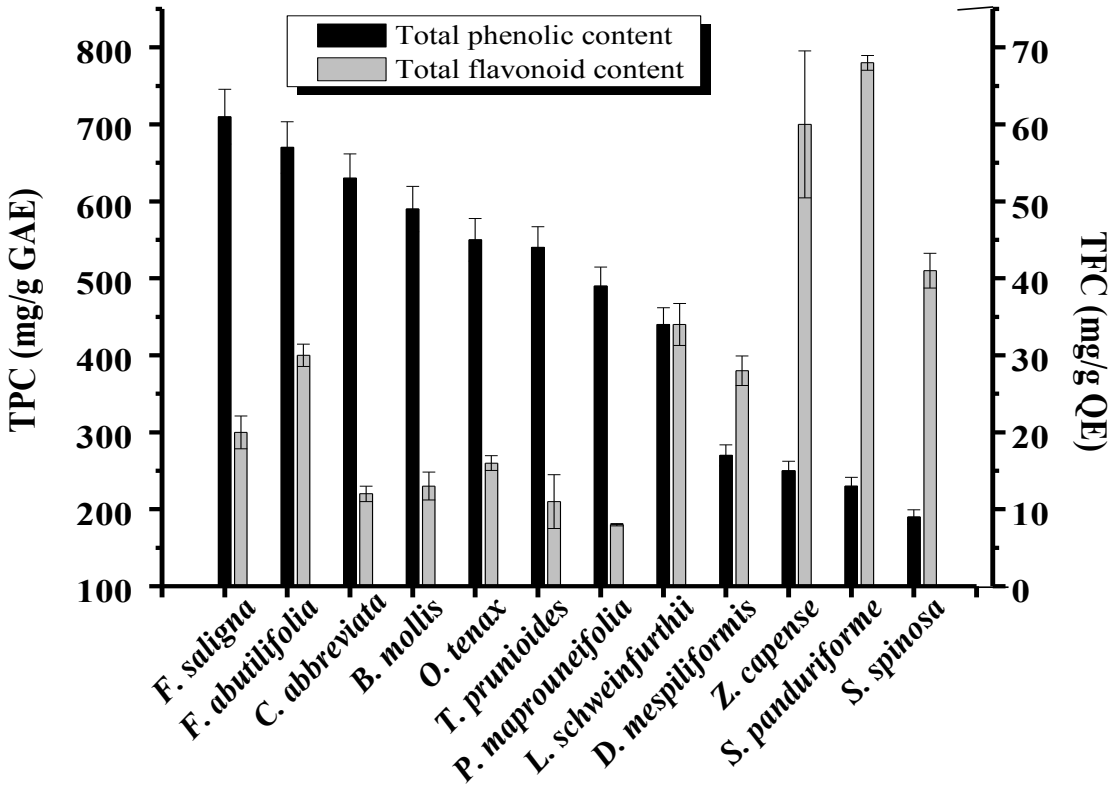

Fig. 1: Total phenolic and total flavonoid content of plant extracts.

It was observed that some of the extracts with higher phenolic contents subsequently had lower flavonoid content. A similar finding of an inverse relationship between the phenolic and flavonoid content in extracts was reported by Ahmed et al. (2014) and Abdillahi et al. (2010) in separate studies conducted on the phytochemical contents of some Anacardiaceae and *Podocarpus* species respectively.

The total flavonoid content was highest in the *S. panduriforme* extract (68.0 mg/g QE), followed by *Z. capense* (60 mg/g QE) and it was lowest in *P. maprouneifolia* (8.0 mg/g QE) (Fig. 1). Somewhat surprisingly, extracts from *S. panduriforme* which had the highest flavonoid content showed poor activity in both anti-inflammatory and anti-oxidant assays.

In accordance with our findings, root extracts from this plant have previously been shown to have no antimicrobial and anticandidal activity (More et al., 2008). A related species, *Solanum dulcamara*, was also reported to have no inhibitory activity against 5-LOX and showed weak activity against the COX-2 enzyme (Jäggi et al., 2004).

3.2. Anti-inflammatory activity of plant extracts

The potential of plant extracts to inhibit product formation by the peroxidative enzyme 15-LOX was monitored at 234 nm, and the findings are summarized in Table 2. Activity at four different test concentrations (166.7, 83.3, 41.7 and 20.8 µg/mL) were assessed, and it was observed that all extracts had inhibitory activity against 15-LOX, with best activity at the highest tested concentration. The extract from *L. schweinfurthii* showed the highest inhibitory activity against 15-LOX, with an IC_{50} value of 40 ± 3 µg/mL, compared to a value of 29 ± 1 µg/mL for the positive control quercetin.

This was followed by extracts from *F. abutilifolia*, *C. abbreviata* and *P. maprouneifolia*. IC_{50} values could not be established for these extracts, since at 234 nm the samples absorbed strongly at 83.3 and 166.7 µg/mL (above the upper limit of the spectrometer). The activity of the extracts were, however, assessed by determining the percentage inhibition at a test concentration of 41.7 µg/mL, at which they showed moderate activity. Extracts from *S. spinosa* and *S. panduriforme* showed the least inhibitory activity against the enzyme with IC_{50} value greater than 83.3 µg/mL.

The difference in 15-LOX activity of extracts could be attributed to the differences in their phytochemical profiles. The extract from *L. schweinfurthii* was observed to be rich in secondary metabolites which may act as 15- LOX inhibitors and as radical scavengers.

The methanol extract from the root of *L. schweinfurthii* has also been reported in a previous study as a good radical scavenger and inhibitor of acetylcholinesterase; the extract also contained phenols and to a lesser extent flavonoids (Adewusi and Steenkamp, 2011). The presence of these compounds in *L. schweinfurthii* extracts might be responsible for the observed anti-oxidant effect and inhibition of pro-inflammatory enzymes. Numerous scientific investigations (Johns et al., 1995; Maregesi et al., 2008, 2010; Gathirwa et al., 2008, 2011; Adewusi and Steenkamp, 2011; Okoth, 2014) have been carried out to validate some of the ethnobotanical uses of *L. schweinfurthii* in different parts of Africa; however, very little work has been done on the constituents of this plant (Wamyu, 2016; Yaouba et al., 2018). Compounds like quercetin, catechin, epicatechin and their methoxyl derivatives have been found to be present in a number of species belonging to the family Anacardiaceae to which the *Lannea* genus belongs (Ojewole et al., 2010; Schulze-Kaysers et al., 2015).

A close species of *F. abutilifolia* (*Ficus platypylla*) was studied for its analgesic properties and was found to be a good inhibitor of 5-LOX and COX-2 (Chindo et al., 2016). A glucoside, racemosic acid, was isolated from the bark extract of *Ficus racemosa.* This compound was reported to show inhibitory activity against COX-1 and 5-LOX enzymes (Li et al., 2004). With reference to the observed anti-inflammatory activity, some of the extracts showing noteworthy activity may be regarded as potential sources of new 15-LOX inhibitors. Recently, the role of 15-LOX in the promotion and progression of several life threating inflammatory diseases as well as cancer and obesity was reviewed (Sadeghian and Jabbari, 2016). It is therefore important to screen for new plant sources of inhibitors which might not only attenuate STI related inflammation but may have additional uses in management of other life threatening inflammatory disease and could be incorporated as part of chemotherapeutic agents.

Table 2: Anti-inflammatory, anti-oxidant and cytotoxic activity of South African medicinal plants used traditionally in the treatment of STIs and associated inflammation.

	IC_{50} values (µg/mL)					
Plant extract	15-LOX inhibition	XO inhibition	Inhibition of NO in RAW 264.7 cells	DPPH assay	NO scavenging	Cytotoxicity on Vero cells
B. mollis	56 ± 3 [c]	>83*	37.5 ± 0.6 [b, c]	14 ± 2 [b]	> 500	nd
C. abbreviata	**	46.8 ± 1.5 [b]	39.9 ± 0.3 [b,c]	17 ± 2 [b]	42.3 ± 3.4[a]	>100
D. mespiliformis	78 ± 5 [e]	142 ± 8 [e]	79.8 ± 2.7 [d]	25 ± 2 [c]	> 500	nd
F. saligna	53 ± 3 [c]	67 ± 3[c]	21.0 ± 0.7 [a]	13 ± 1 [b]	368.7 ± 3.6 [f]	nd
F. abutilifolia	**	74 ± 6 [c]	19.7± 0.2 [a]	6.4 ± 0.4 [a]	442.5 ± 2.2 [g]	>100
L. schweinfurthii	40 ± 3 [b]	71 ± 3 [c]	49.4 ± 8.0 [c]	7.6 ± 0.2 [a]	185.8 ± 2.0 [d]	>100
O. tenax	63 ± 2[d]	105 ± 5 [d]	35.3 ± 1.4 [b]	14 ± 1[b]	> 500	nd
P. maprouneifolia	**	110 ± 15 [d,e]	68.8 ± 4.5 [d]	13 ± 1 [b]	171.0 ± 3.2 [c]	84 ± 6.5
T. prunioides	55 ± 2 [c]	69 ± 2 [c]	44.9 ± 1.4 [c]	8.2 ± 0.3 [a]	313.3 ± 2.4 [e]	>100
S. panduriforme	> 83	>167	40.3 ± 6.2 [c]	> 167	> 500	nd
S. spinosa	> 83	>167	41.4 ± 7.3 [c]	> 167	> 500	nd
Z. capense	69 ± 5 [de]	125 ± 2 [e]	29.9 ± 1.9 [b]	> 167	> 500	nd
Quercetin	29 ±1 [a]	0.44 ± 0.02 [a]	30.0 ± 0.7 [b]	5.0 ± 0.3 [a]	__	__
Ascorbic acid	__	__	__	__	121.0 ± 2.6 [b]	__
Actinomycin D	__	__	__	__	__	0.03 ± 0.01
P value	***	***	***	***	***	
R^2	0.97	0.98	0.97	0.89	0.99	

--; not tested, *: not enough sample to test at 167µg/mL, **: IC_{50} value could not be determined due to strong absorptivity of extract at higher tested concentrations (less than 50% inhibition at 41.7 µg/ml), ***: Highly significant difference among the means ($P < 0.0001$), nd: Not determined. Means with different letters in the same column are significantly different at $P < 0.05$.

Several of the samples showing inhibitory activity against 15-LOX also had noteworthy activity against xanthine oxidase (Table 2). The extract of *C. abbreviata* was the most active, with an IC_{50} value of 46.8±1.5 µg/mL. In addition, extracts from *F. abutilifolia* (IC_{50} 74±6 µg/mL), *L. schweinfurthii* (IC_{50} 71±3 µg/mL), *T. prunioides* (IC_{50} 69±2 µg/ml) and *F. saligna* (IC_{50} 67±3 µg/mL) also showed good activity. The rest of the samples were less active, with IC_{50} values higher than 83 µg/mL. Quercetin, the positive control, had an IC_{50} value of 0.44±0.02 µg/mL, in accordance with previously reported data (Bräunlich et al., 2013).

For several of the plants investigated in the present study, no data on radical scavenging or 15-LOX inhibition appears to have been published previously. This is the case for *F. abutilifolia, O. tenax, P. maprouneifolia* and *S. panduriforme*. Similarly, no data for XO inhibition for any of the plants tested were found in the literature.

Previous work by Chauke et al., (2012) assessed radical scavenging activity of *B. mollis,* and an IC_{50} value of 130 µg/mL was reported for the acetone extract of the leaves. This differs somewhat from our result (14±2 µg/mL); which may be due to differences in the plant material (in our work, stem bark was extracted) or methodology employed. No data for 15-LOX inhibition have been found for this plant. For the methanol extract of roots of *C. abbreviata*, an IC_{50} value of 6.2±0.5 µg/mL was obtained for DPPH scavenging (Sobeh et al., 2018), a somewhat higher activity than our value for the stem bark, 17±2 mg/mL. Again, different plant parts might be the reason for this. In addition, inhibition of 15-LOX has not been previously reported for this plant.

Several groups have investigated *D. mespiliformis* for DPPH scavenging, but many of the results are not in formats that are comparable to our data. However, an IC_{50} value of 1.21 µg/mL was reported from an ethanol extract of roots and leaves (Tshikalange et al., 2016). This group

investigated the 15-LOX inhibitory activity of the extract, as well, finding an IC_{50} value of 188.1 μg/mL (Mamba et al., 2016), somewhat higher than the value obtained in our study (78±5 μg/mL).

The methanol extract of leaves of *F. saligna* and roots of *L. schweinfurthii* were reported to have IC_{50} values of 3.88 μg/mL (Makhafola et al., 2016) and 15±4 μg/mL (Adewusi and Steenkamp, 2011) in the DPPH scavenging assay. However, no data for 15-LOX inhibition have been found in published literature for these plants. Noteworthy DPPH scavenging activity and moderate 15-LOX inhibition was also observed with the extract of *F. saligna*, suggesting a positive correlation between the amount of phenolics and the overall performance of the extract in the radical scavenging bioassay.

For a decoction of *Strychnos spinosa* fruits, an activity of 112% compared to quercetin, the positive control, was reported (Kabine et al., 2015). Our IC_{50} values for *S. spinosa* acetone bark extract and quercetin give a ratio of less than 3%, which might mean that the radical scavenging activity is very different in the fruits and the bark. Inhibition measurements of 15-LOX by different leaf extracts were reported to give IC_{50} values of 143 to 164 μg/mL (Isa et al., 2014b). For *Terminalia prunioides*, DPPH scavenging was described as strong for an acetone extract of the leaves and moderate for the methanol extract (Masoko & Eloff 2007), but no quantitative data were given. Inhibition of 15-LOX has to our knowledge not been reported.

The DPPH scavenging and 15-LOX inhibitory properties of *Zanthoxylum capense* acetone leaf extracts were reported by Adebayo et al (2015). For DPPH scavenging, an IC_{50} value of 138.78±13.24 μg/mL was given. From a graphic display of 15-LOX inhibition, an IC_{50} value of approximately 18 μg/mL was estimated, with the positive control, quercetin, having an IC_{50} value

of 8.5 μg/mL. The activity ratio between extract and positive control appears to be in good agreement with our data.

In summary, all our data on xanthine oxidase inhibition and most of our data on 15-lipoxygenase inhibition are new. While DPPH scavenging has been reported for several of the plants, most investigations have employed other plant parts and/or other extractants, making comparison between our results and previous ones difficult. Therefore, we believe that our work represents new knowledge on these medicinally used plants.

In order to confirm the anti-inflammatory potential of extracts, their inhibitory effect on inducible nitric oxide (iNOS) produced by LPS stimulated RAW macrophage cells was assessed. The effect of plant extracts on the viability of the cells was also determined. Lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell wall, can activate macrophages to induce an inflammatory response by releasing different types of cytokines including iNOS-derived NO which can be easily converted to the cytotoxic oxidant peroxynitrite and superoxide anion (Poltorak et al., 1998). The overproduction of nitric oxide has been linked to several inflammatory conditions and usually, iNOS is expressed in cells only in response to certain inflammatory stimuli such as infections, bacterial products, cytokines and lipid mediators (Wu, 2004).

In this study, all tested extracts showed a moderate to high dose dependent suppression of NO production. The 50% inhibitory concentrations (IC_{50}) of tested plant extracts against NO production in LPS stimulated RAW macrophages are summarized in Table 2. At the highest tested concentration (100 μg/mL), several extracts inhibited NO production by approximately 90 %. With the exception of the extracts from *T. prunioides* (66.8%) and *D. mespiliformis* (68.1%), all other extracts tested were good inhibitors of iNOS- derived NO with inhibition of greater than 70%.

Ficus abutilifolia and *O. tenax* had IC_{50} values of approximately 20 µg/mL, better than the positive control quercetin.

The findings in this work revealed that the extracts from *T. prunioides* and *S. spinosa* were safest to RAW 264.7 cells at the highest tested concentration (100 µg/mL), with a cell viability of 100 and 97.3% respectively. Although extracts from *T. prunioides* only exhibited moderate NO suppression at the highest concentration, extracts from *S. spinosa* however still had good NO inhibitory activity (89.4%) at 100 µg/mL suggesting the observed NO suppression was not due to cytotoxicity of the extract. Previous studies by Isa et al. (2014a) reported acetone leaf extracts of *S. spinosa* as toxic to human U937 macrophages (IC_{50} 27.36 µg/mL), however, water and methanol leaf extracts from the same plant used in their studies were not cytotoxic.

It appeared that the *S. spinosa* extract showed selective anti-inflammatory activity by acting as a good, non-cytotoxic inhibitor of iNOS in RAW 264.7 macrophages, however, it did not have significant activity against 15-LOX and XO at the respective tested concentrations. In addition, the extract from this plant also had poor anti-oxidant activity. The observed poor activity might be due to the low phenolic and flavonoid content present in the extract (Fig. 1). Several anti-inflammatory phytochemicals such as triterpenoids and seco-iridoids have been isolated from the leaves and branches of *S. spinosa* (Hoet et al., 2007; Itoh et al., 2005; Miettinen et al., 2014). These bioactive compounds might have been present in small quantities which were insufficient to produce significant anti-inflammatory and anti-oxidant activity at the tested concentrations.

Other extracts such as *B. mollis, O. tenax, F. saligna* and *C. abbreviata* showed moderate cytotoxicity at 100 µg/mL, but were non-toxic to the cells at 50 µg/mL (cell viability >70%, NO inhibition activity >50%). Conversely, cells were only 4% viable when treated with 100 µg/mL of extract from *Z. capense* stem bark, a result very similar to that obtained for the positive control

(doxorubicin). This suggests that the extract is highly cytotoxic. Furthermore, at the lowest concentration where it could be regarded as safe to the cells, NO inhibition was not significant, which might discourage its continued use as an anti-inflammatory extract.

In a study by Adebayo et al. (2015), the acetone leaf extract of *Z. capense* was reported to possess very good anti-inflammatory activity and did not have significant effects on the viability of RAW 264.7 macrophages at the tested concentrations. The observed non-cytotoxic effect in their study could be due to the plant part used (leaves) as well as the tested concentrations which were low (25-3.12 µg/mL). Other reports of cytotoxic effect of extracts from *Z. capense* include moderate cytotoxicity to monocytic THP-1 cells (IC_{50} 45.7 µg/ml) and cytotoxic effect on human periodontal ligament fibroblasts and human gingival fibroblasts (Luo et al., 2011; Maja, 2009)

3.3. Cytotoxicity of selected plant extracts on Vero cells

Extracts of *C. abbreviata, F. abutilifolia, T. prunioides, L. schweinfurthii* and *P. maprouneifolia* were thus selected for further evaluation on Vero cells. All extracts, except *P. maprouneifolia,* were not cytotoxic (IC_{50} > 100 µg/mL) against Vero cells (Table 2). In accordance with our findings, the acetone extract of leaves and stem bark of *C. abbreviata* was reported as non-toxic to Vero cell lines with IC_{50} values of 294 and 170 µg/mL respectively (Mongalo et al., 2017) and also not toxic to peripheral blood mononuclear cells. No previous cytotoxicity report was found on plant parts or compounds from *F. abutilifolia* and *P. maprouneifolia*. Hence, this study provides the first cytotoxicity report on extracts from these plants. Previous studies by Adewusi et al. (2013) have, however, assessed the cytotoxicity of *L. schweinfurthii*. In their work, they reported that the ethanol extract of this plant was not cytotoxic to SH-SY5Ycells. Cytoprotective properties of extracts from this plant were reported in another study (Seoposengwe et al., 2013).

In the present study, some of extracts had mild to moderate cytotoxicity against RAW 264.7 macrophages cells at 100 µg/mL but were not toxic to Vero cells at the same concentration. The differences in cytotoxicity observed may be due to the differences in cell types, causing different sensitivity of the cells at the highest tested concentration of 100 µg/mL.

3.4. Radical scavenging activity

The DPPH assay conducted on the plant extracts (Table 2) revealed that most of the extracts tested were effective radical scavengers, some of them comparable to the positive control, quercetin. *Ficus abutilifolia* extract had the best scavenging property in the DPPH assay (IC_{50} = 6.4 ± 0.4 µg/mL), followed by extracts of *L. schweinfurthii* (IC_{50} = 7.6 ± 0.2 µg/mL) and *T. prunioides* (IC_{50} = 8.2 ± 0.3 µg/mL). Extracts of *Z. capense, S. panduriforme* and *S. spinosa* had poor DPPH scavenging properties with IC_{50} values of more than 167 µg/mL. It was observed that the extract of *Z. capense* showed selectivity; acting as an inhibitor of 15-LOX (IC_{50} 69 µg/mL) but not as a radical scavenger of DPPH and nitric oxide with IC_{50} > 500µg/mL in the nitric oxide assay.

Out of the twelve plants tested, six did not show significant nitric oxide scavenging activity at the tested concentrations (IC_{50} > 500 µg/mL; Table 2). The most noteworthy activity was observed with extracts of *C. abbreviata* with an IC_{50} of 42.3 ± 3.4 µg/mL, which represented statistically higher ($P < 0.05$) scavenging activity compared to that of ascorbic acid (IC_{50} = 121.0 ± 2.6 µg/mL). Extracts of *P. maprouneifolia* and *L. schweinfurthii* also showed good nitric oxide inhibition potential. The samples which were active in the xanthine oxidase inhibition assay also showed activity as radical scavengers and 15-lipoxygenase inhibitors. From linear regression calculations, a correlation between the DPPH scavenging and xanthine oxidase inhibitory activity of most of the extracts was observed (R^2 = 0.52). There was also a correlation between inhibitory activities of the extracts in the xanthine oxidase and 15-LOX assays (R^2 = 0.46), while the correlation

between DPPH scavenging and 15-lipoxygenase was poorer (R^2 = 0.18)**.** The low correlation between DPPH scavenging and 15-lipoxygenase inhibition is in accordance with previously reported results (Malterud et al., 1993).

3.5. NMR studies and GC-MS analysis of crude extracts

From the ^{1}H and ^{13}C NMR spectroscopic analysis (spectra shown in supplementary information Fig. S1- S12), it appeared that the active extracts contained a mixture of compounds such as lipids, aromatic glycosides, terpenoids, proanthocyanidins and some sugar moieties with interesting patterns in the aromatic and aliphatic regions. Some of the active extracts such as *L. schweinfurthii* and *F. abutilifolia* showed major signals of proanthocyanidins. Proanthocyanidins are a subgroup of flavonoids, and their role as free radical scavengers, as well as inhibitors of lipoxygenase enzymes, has been reported (Ariga, 2004; Bagchi et al., 2014; Bräunlich et al., 2013; Rigo et al., 2000).

Investigations of a related species *Lannea velutina*, reported extracts from this plant to be effective as 15-LOX inhibitors and radical scavengers, with proanthocyanidins, catechin and epicatechin as the major constituents from methanol root bark fractions (Maiga et al., 2007). It was also observed from the NMR spectra of the *F. abutilifolia* extract, (which also showed good activity against 15-LOX), that terpenoids as well as proanthocyanidins appeared to be present. The biological activity of this plant has only been reported a few times (Danmalam et al., 2012; Taiwo et al., 2016), however, several other species which belong to the genus *Ficus* have been studied in relation to their ethnobotanical use in the treatment of inflammation.

To obtain a detailed profile of compounds and secondary metabolites present in all the tested extracts in this study, a GC-MS analysis was conducted, from which a total of 67 known

compounds and 2 unknown compounds were identified. The results from the in depth study is summarized in Table S1 of the supplementary information and the peak number corresponds to those shown in the representative chromatogram of the active, moderately active and inactive extracts (Fig. 2).

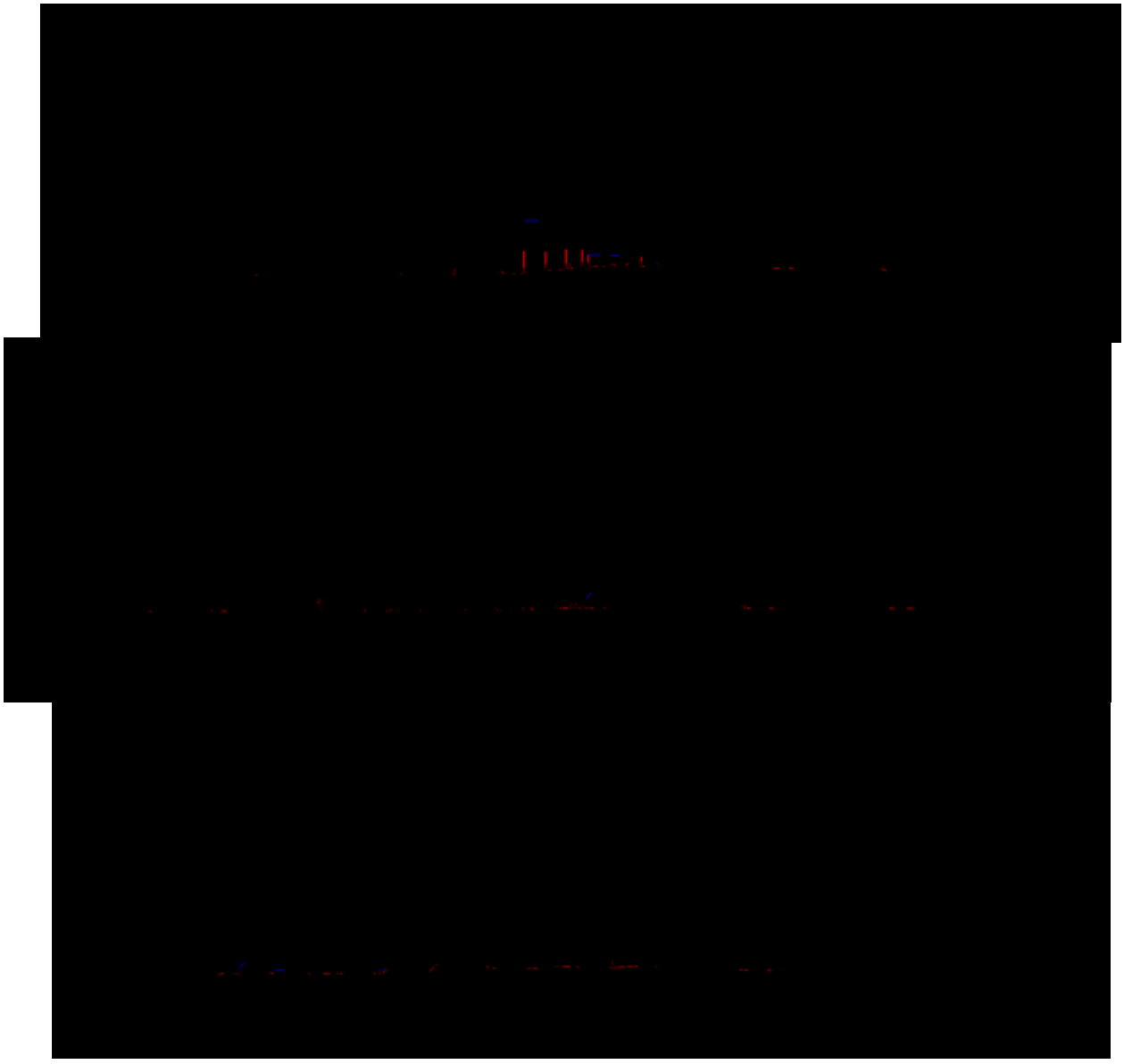

Fig. 2: Representative Total Ion Chromatographs (TIC) of crude extracts from *Lannea schweinfurthii* (A*), Bridelia mollis* (B) and *Strychnos spinosa* (C), most active, moderately active and least active plants respectively. Peak numbers and identity of compounds are as described on Table S1 of supplementary material.

Grouping of these compounds showed the presence of 13 major classes of secondary metabolites with percentage occurrence in each extract represented in Table 3. The difference in the proportion of chemical constituents as well as the presence or absence of metabolites may be attributed to the fact that the extracts belong to plants from different families. Compounds such as Phenol 3-pentadecyl, Methyl commate D, polygalitol, Benzoic acid and Octadecanoic acid-dihydroxypropyl ester were found to be present in highest proportion in *L. schweinfurthii, F. abutilifolia, F. saligna, P. maprouneifolia* and *C. abbreviata* respectively which are very active extracts (Tables S1). Some of these compounds are already known for their anti-inflammatory and various other biological activity (Adnan et al., 2019; Feresin et al., 2003; Venkata et al., 2012). It appears that the observed biological activity in some of the extracts may be due to a synergistic effect of different compounds present in the samples.

Table 3: Proportion (%) of secondary metabolites identified in acetone extract of 12 medicinal plants using GC-MS.

Class of compound	***B. mollis***	***C. abbreviata***	***D. mespiliformis***	***F. abutilifolia***	***F. saligna***	***L. schweinfurthii***	***O. tenax***	***P. maprouneifolia***	***S. spinosa***	***S. Panduriforme***	***T. prunioides***	***Z. capense***
Acids	14.3	9.1	-	-	16.7	5.9	10.0	16.7	25.0	-	15.8	9.1
Acid esters	28.6	27.3	16.7	22.2	16.7	11.8	40.0	33.3	50.0	16.7	10.5	36.4
Alcohols	14.3	-	16.7	11.1	8.3	23.5	20.0	16.7	12.5	-	15.8	-
Alkaloids	-	-	-	-	-	-	-	-	-	-	5.3	27.3
Aldehydes	-	-	-	-	-	5.9	-	-	-	-	-	-
Amides	4.8	-	-	-	-	-	-	-	-	-	5.3	-
Furan	-	-	-	-	8.3	-	-	-	-	-	-	-
Hydrocarbon	-	-	-	-	-	-	-	-	-	16.7	10.5	-
Ketones	4.8	-	-	-	8.3	-	-	33.3	-	-	-	9.1
Phenolics	4.8	18.2	-	11.1	-	29.4	10.0	-	-	33.3	15.8	9.1
Steroids	9.5	27.3	16.7	11.1	8.3	5.9	10.0	-	-	33.3	5.3	-
Sugars	9.5	9.1	-	-	33.3	-	-	-	12.5	-	10.5	-
Terpenoids	9.5	9.1	50	44.4	-	5.9	10.0	-	-	-	5.3	9.1
Unknown	-	-	-	-	-	11.8	-	-	-	-	-	-

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4. Conclusion

The anti-inflammatory potential of some South African medicinal plants used in treatment of STIs was confirmed by the results obtained in this study. Most of the plant extracts tested showed moderate to significant inhibitory activity against 15-LOX, XO and NO release, all of which have been reported to play significant roles in the aetiology of several inflammatory conditions.

Particularly, extracts of *C. abbreviata, F. abutilifolia, T. prunioides, L. schweinfurthii, P. maprouneifolia* and *F. saligna* had noteworthy activity in both anti-inflammatory and anti-oxidant assays. However, acetone extracts from *S. panduriforme* and *S. spinosa* recorded poor activity in all the assays. The acetone extract from *S. spinosa* was a selective and non-cytotoxic inhibitor of iNOS in RAW 264.7 macrophages, but did not have significant activity against 15-LOX and XO at the tested concentrations. The acetone extract from *Z. capense* also showed selectivity, acting as a moderate inhibitor of 15-LOX and XO, but not as a radical scavenger.

With regard to cytotoxicity analyses, extracts from *Z. capense* had the highest cytotoxic effect on RAW 264.7 macrophages followed by the acetone extract from *P. maprouneifolia* and *D. mespiliformis* suggesting that their NO inhibitory activity at higher concentrations could be due to the toxic effect of the extracts on the cells. In addition, only the extract from *P. maprouneifolia* had moderate cytotoxic activity on Vero cells. None of the other extracts exhibited strong cytotoxic effects on cells compared to doxorubicin used as the positive control. They could also be considered non-cytotoxic at their respective IC_{50} values in both the anti-inflammatory and anti-oxidant assays.

The registered activity of most of the tested extracts may be due to a synergistic effect of different classes of compounds present in them. The observed activity supports the administration

of these plants by traditional healers in treatment of STIs and the associated inflammation. Studies on anti-inflammatory potential of these plants may serve as a lead towards their incorporation in the effective treatment of STI-related inflammation. Similarly, controlling progression of the inflammatory response could serve as an additional benefit in reducing the risk of HIV acquisition by STI patients. This is based on the fact that the presence of STIs in patients has been linked to elevated levels of inflammatory mediators.

As a follow-up to this study, plants showing good activity and moderate cytotoxicity are now being subjected to bioassay-guided fractionation, isolation of bioactive compounds and determination of mode of action. For several of these plants, the active constituents are thus far unknown.

Conflict of interest

The authors declare no conflict of interest.

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References

Abdillahi, H.S., Finnie, J.F., Van Staden, J., 2010. Anti-inflammatory, anti-oxidant, anti-tyrosinase and phenolic contents of four *Podocarpus* species used in traditional medicine in South Africa. Journal of Ethnopharmacology 136, 496–503.

Adebayo, S.A., Dzoyem, J.P., Shai, L.J., Eloff, J.N., 2015. The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. BMC Complementary and Alternative Medicine 15, 159. DOI: 10.1186/s12906-015-0669-5.

Adewusi, E.A., Fouche, G., Steenkamp, V., 2013. Effect of four medicinal plants on Amyloid- β induced neurotoxicty in SH-SY5Y cells. African Journal of Traditional, Complementary and Alternative Medicine 10, 6–11.

Adewusi, E.A., Steenkamp, V., 2011. *In vitro* screening for acetylcholinesterase inhibition and anti-oxidant activity of medicinal plants from southern Africa. Asian Pacific Journal of Tropical Medicine 4, 829–835.

Adnan, M., Chy, M. N. U., Kamal, A. M., Barlow, J. W., Faruque, M. O., Yang, X., Uddin, S. B., 2019. Evaluation of anti-nociceptive and anti-inflammatory activities of the methanol extract of *Holigarna caustica* (Dennst.) Oken leaves. Journal of Ethnopharmacology 236: 401–411.

Ahmed, A.S., Mcgaw, L.J., Moodley, N., Naidoo, V., Eloff, J.N., 2014. Cytotoxic, antimicrobial, anti-oxidant, antilipoxygenase activities and phenolic composition of *Ozoroa* and *Searsia* species (Anacardiaceae) used in South African traditional medicine for treating diarrhoea. South African Journal of Botany 95, 9–18.

Allott, E.H., Olshan, A.F., Carey, L.A., Moorman, P.G., Troester, M.A., 2014. Non-steroidal anti-inflammatory drug use , hormone receptor status , and breast cancer-specific mortality in the Carolina Breast Cancer Study. Breast Cancer Research and Treatment 147, 415–421.

Ariga, T., 2004. The antioxidative function , preventive action on disease and utilization of proanthocyanidins. BioFactors 21, 197–201.

Bagchi, D., Swaroop, A., Preuss, H.G., Bagchi, M., 2014. Fundamental and molecular mechanisms of mutagenesis free radical scavenging , anti-oxidant and cancer chemoprevention by grape seed proanthocyanidin : An overview. Mutation Research 768, 69–73.

Bräunlich, M., Rune, S., Wangensteen, H., Brede, C., Malterud, K.E., Barsett, H., 2013. Extracts, anthocyanins and procyanidins from *Aronia melanocarpa* as radical scavengers and enzyme inhibitors. Nutrients 5, 663–678.

Buwa, L. V., Van Staden, J., 2006. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. Journal of Ethnopharmacology 103, 139–142.

Château, A., Seifert, H.S., 2016. *Neisseria gonorrhoeae* survives within and modulates apoptosis and inflammatory cytokine production of human macrophages. Cell Microbiology 18, 546–560.

Chauke, M.A, Shai, L., Mogale, M., Tshisikhawe, M., Mokgotho, M., 2015. Medicinal plant use of villagers in the Mopani district, Limpopo province, South Africa. African Journal of Traditional, Complementary and Alternative Medicine 12, 9-26.

Chauke, A.M., Shai, L.J., Mphahlele, P.M., Mogale, M.A. 2012. Radical scavenging of selected medicinal plants from Limpopo province of South Africa. African Journal of Traditional, Complementary and Alternative Medicine 9, 426-430.

Chindo, B.A., Schröder, H., Koeberle, A., Werz, O., Becker, A., 2016. Analgesic potential of standardized methanol stem bark extract of *Ficus platyphylla* in mice : Mechanisms of action. Journal of Ethnopharmacology 184, 101–106.

Dobrian, A.D., Lieb, D.C., Cole, B.K., Taylor-fishwick, D.A., Chakrabarti, S.K., Nadler, J.L., 2011. Functional and pathological roles of the 12- and 15-lipoxygenases. Progress in Lipid Research 50, 115–131.

Danmalam, U.H., Allahmagani, P.K., Ilyas, N., Abdurahman, E.M., Yaro, A.H., Magaji, M.G., 2012. Phytochemical and anticonvulsant studies on the aqueous ethanol extract of the root-bark of *Ficus abutilifolia* (Miq.) Miq. (Family: Moraceae). Journal of Applied Pharmaceutical Science 2, 234-237.

Dzoyem, J.P., Donfack, A.R.N., Tane, P., McGaw, L.J., Eloff, J.N., 2016. Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 macrophages and 15-LOX activity by anthraquinones from *Pentas schimperi*. Planta Medica 82, 1246-1251.

Dzoyem, J. P., Eloff, J. N. (2015). Anti-inflammatory, anticholinesterase and antioxidant activity of leaf extracts of twelve plants used traditionally to alleviate pain and inflammation in South Africa. Journal of Ethnopharmacology, 160, 194-201.

Eldeen, I.M.S., Elgorashi, E.E., Van Staden, J., 2005. Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. Journal of Ethnopharmacology 102, 457–464.

Elisha, I.L., Dzoyem, J.P., McGaw, L.J., Botha, F.S., Eloff, J.N., 2016. The anti-arthritic, anti-inflammatory, anti-oxidant activity and relationships with total phenolics and total flavonoids of nine South African plants used traditionally to treat arthritis. BMC Complementary and Alternative Medicine 16, 307. DOI: 10.1186/s12906-016-1301-z

Eloff, J.N., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology 60, 1-8

Feresin, G. E., Tapia, A., Sortino, M., Zacchino, S., de Arias, A. R., Inchausti, A., ...Schmeda-Hirschmann, G., 2003. Bioactive alkyl phenols and embelin from *Oxalis erythrorhiza*. Journal of ethnopharmacology 88(2-3), 241-247.

Fernandes, L., Rensburg, C.E.J. van, Hoosen, A.A., Steenkamp, V., 2008. *In vitro* activity of medicinal plants of the Venda region, South Africa, against *Trichomonas vaginalis*. South African Journal of Epidemiology and Infection 23, 26–28.

Gathirwa, J.W., Rukunga, G.M., Mwitari, P.G., Mwikwabe, N.M., Kimani, C.W., Muthaura, C.N., Kiboi, D.M., Nyangacha, R.M., Omar, S.A., 2011. Traditional herbal antimalarial therapy in Kilifi district, Kenya. Journal of Ethnopharmacology 134, 434-442.

Gathirwa, J.W., Rukunga, G.M., Njagi, E.N.M., Omar, S.A., Mwitari, P.G., Guantai, A.N., Tolo, F.M., Kimani, C.W., Muthaura, C.N., Kirira, P.G., Ndunda, T.N., Amalemba, G., Mungai, G.M., Ndiege, I.O., 2008. The *in vitro* anti-plasmodial and *in vivo* anti-malarial efficacy of combinations of some medicinal plants used traditionally for treatment of malaria by the Meru community in Kenya. Journal of Ethnopharmacology 115, 223-231.

Ho, G.T.T., Bräunlich, M., Austarheim, I., Wangensteen, H., Malterud, K.E., Slimestad, R., Barsett, H., 2014. Immunomodulating activity of *Aronia melanocarpa* polyphenols. International Journal of Molecular Science 15, 11626-11636.

Hoet, S., Pieters, L., Muccioli, G.G., Habib-Jiwan, J.-L., Opperdoes, F.R., Leclercq, J.Q., 2007. Antitrypanosomal activity of triterpenoids and sterols from the leaves of *Strychnos spinosa* and related compounds. Journal of Natural Products 70, 1360–1363.

Isa, A.I., Awouafack, M.D., Dzoyem, J.P., Aliyu, M., Magaji, R.A., Ayo, J.O., Eloff, J.N., 2014a. Some *Strychnos spinosa* (Loganiaceae) leaf extracts and fractions have good antimicrobial activities and low cytotoxicities. BMC Complementary and Alternative Medicine 14, 456-464.

Isa, A.I., Adebayo, S.A., Mohammed, A., Ayo, J.O., Suleiman, M.M., Saleh, M.I.A., Sadau, Y., Eloff, J.N., 2014b. *In-vitro* lipoxygenase inhibitory activity and total flavonoid of *Strychnos spinosa* leaf extracts and fractions. Nigerian Journal of Pharmacuetical Sciences 13, 35-43.

Itoh, A., Oya, N., Kawaguchi, E., Nishio, S., Tanaka, Y., Kawachi, E., Akita, T., Nishi, T., Tanahashi, T., 2005. Secoiridoid Glucosides from *Strychnos spinosa*. Journal of Natural Products 68, 1434–1436.

Iwalewa, E., McGaw, L., Naidoo, V., Eloff, J., 2007. Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. African Journal of Biotechnology 6, 2868–2885.

Jacob, J., Prakash Kumar, B., 2015. Dual COX / LOX inhibition : screening and evaluation of effect of medicinal plants of Kerala as anti-inflammatory agents. Journal of Pharmacognosy and Phytochemistry 3, 62–66.

Jäggi, R., Würgler, U., Grandjean, F., Weiser, M., 2004. Dual inhibition of 5-lipoxygenase / cyclooxygenase by a reconstituted homeopathic remedy ; possible explanation for clinical efficacy and favourable gastrointestinal tolerability. Inflammation Reseaarch 53, 150–157.

Jeengar, M.K., Shrivastava, S., Nair, K., Singareddy, S.R., Putcha, U.K., Talluri, M.V.N.K., Naidu, V.G.M., Sistla, R., 2014. Improvement of bioavailability and anti-inflammatory potential of curcumin in combination with Emu oil. Inflammation 37, 2139–2155.

Johns, T., Faubert, G.M., Kokwaro, J.O., Mahunnah, R.L.A., Kimanani, E.K., 1995. Anti-giardial activity of gastrointestinal remedies of the Luo of East Africa. Journal of Ethnopharmacology 46, 17-23.

Kabine, O., Samba, B.M., Fatoumata, B., Namagan, K., Luopou, H.N., Mamadou, B.A., 2015. Anti-oxidative activity of fruit extracts of some medicinal plants used against chronic diseases (diabetes, hypertension) in Kankan, Guinea. Journal of Plant Sciences 3, 1-5.

Kambizi, L., Afolayan, A.J., 2008. Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhoea*. African Journal of Biotechnology 7, 012–015.

Li, R.W., Leach, D.N., Myers, S.P., Lin, G.D., Leach, G.J., Waterman, P.G., 2004. A new anti-inflammatory glucoside from *Ficus racemosa* L. Planta Medica 70, 421–426.

Luo, X., Pires, D., Aínsa, J.A., Gracia, B., Mulhovo, S., Duarte, A., Anes, E., Ferreira, M.U., 2011. Antimycobacterial evaluation and preliminary phytochemical investigation of selected medicinal plants traditionally used in Mozambique. Journal of Ethnopharmacology 137, 114–120.

Lyckander, I.M., Malterud, K.E., 1992. Lipophilic flavonoids from *Orthosiphon spicatus* as inhibitors of 15-lipoxygenase. Acta Pharmaceutica Nordica 4, 159-166.

Maiga, A., Malterud, K.E., Mathisen, G.H., Paulsen, R.E., Thomas-Oates, J., Bergström, E., Reubsaet, L., Diallo, D., Paulsen, B.S., 2007. Cell protective antioxidants from the root bark of *Lannea velutina* A . Rich ., a Malian medicinal plant. Jornal of Medicinal Plants Research 1, 66–79.

Maja, M.P., 2009. *In vitro* cytotoxicity and antibacterial activity of selected South African medicinal plants used in the treatment of periodontitis. M. Sc. Thesis, University of Pretoria, South Africa.

Makhafola, T.J., Elgorashi, E.E., Mcgaw, L.J., Verschaeve, L., Eloff, J.N., 2016. The correlation between antimutagenic activity and total phenolic content of extracts of 31 plant species with high antioxidant activity. BMC Complementary and Alternative Medicine 16, 490. DOI:10.1186/s12906-016-1437-x

Malterud, K.E., Farbrot, T.L., Huse, A.E., Sund, R.B., 1993. Anti-oxidant and radical scavenging effects of anthraquinones and anthrones. Pharmacology 47 (suppl 1), 77–85.

Mamba, P., Adebayo, S.A., Tshikalange, T.E., 2016. Anti-microbial, anti-inflamatory and HIV-1 reverse transcriptase activity of selected South African plants used to treat sexually transmitted diseases. International Journal of Pharmacognosy and Phytochemical Research 8, 1870-1876.

Maregesi, S., Miert, S. Van, Pannecouque, C., Haddad, M.H.F., Hermans, N., Wright, C.W., Vlietinck, A.J., Apers, S., Pieters, L., 2010. Screening of Tanzanian medicinal plants against *Plasmodium falciparum* and human immunodeficiency virus. Planta Medica 76, 195–201.

Maregesi, S.M., Pieters, L., Ngassapa, O.D., Apers, S., Vingerhoets, R., Cos, P., Vanden Berghe, D., Vlietinck, A.J., 2008. Screening of some Tanzanian plants from Bunda district or antibacterial, antifungal and antiviral activities. Journal of Ethnopharmacology 119, 58-66.

Masoko, P., Eloff, J.N., 2007. Screening of twenty-four South African *Combretum* and six *Terminalia* species (Combretaceae) for antioxidant activities. African Journal of Traditional, Complementary and Alternative Medicine 4, 231-239.

Masson, L., Arnold, K.B., Little, F., Mlisana, K., Lewis, D.A., Mkhize, N., Gamieldien, H., Ngcapu, S., Johnson, L., Lauffenburger, D.A., Karim, Q.A., Karim, S.S.A., Passmore, J.S., 2015a. Inflammatory cytokine biomarkers to identify women with asymptomatic sexually transmitted infections and bacterial vaginosis who are at high risk of HIV infection. Sexually Transmitted Infection 0, 1–8.

Masson, L., Passmore, J.A.S., Liebenberg, L.J., Werner, L., Baxter, C., Arnold, K.B., Williamson, C., Little, F., Mansoor, L.E., Naranbhai, V., Lauffenburger, D.A., Ronacher, K., Walzl, G., Garrett, N.J., Williams, B.L., Couto-Rodriguez, M., Hornig, M., Lipkin, W.I., Grobler, A., Abdool Karim, Q., Abdool Karim, S.S., 2015b. Genital inflammation and the risk of HIV acquisition in women. Clinical Infectious Diseases 61, 260–269.

Mayur, B., Sandesh, S., Shruti, S., Sung-yum, S., 2010. Anti-oxidant and α-glucosidase inhibitory properties of *Carpesium abrotanoides* L . Journal of Medicinal Plants Research 4, 1547–1553.

Miettinen, K., Dong, L., Navrot, N., Schneider, T., Burlat, V., Ilc, T., Verpoorte, R., Pollier, J., Woittiez, L., Krol, S. Van Der, Martinoia, E., Bouwmeester, H., Goossens, A., Memelink, J., 2014. The seco-iridoid pathway from *Catharanthus roseus*. Nature Communications 5, 3606. DOI: 10.1038/ncomms4606.

Mitchell, C., Prabhu, M., 2013. Pelvic inflammatory disease: current concepts in pathogenesis, diagnosis and treatment. Infectious Disease Clinics of North America 27, 1–21.

Mlisana, K., Naicker, N., Werner, L., Roberts, L., Van Loggerenberg, F., Baxter, C., Passmore, J.A.S., Grobler, A.C., Sturm, A.W., Williamson, C., Ronacher, K., Walzl, G., Abdool Karim, S.S., 2012. Symptomatic vaginal discharge is a poor predictor of sexually transmitted infections and genital tract inflammation in high-risk women in South Africa. Journal of Infectious Diseases 206, 6–14.

Mongalo, N.I., 2013. Antibacterial activities of selected medicinal plants used to treat sexually transmitted infections in Blouberg Area, Limpopo Province. PhD Disseratation, University of Zululand, KwaDlangezwa, South Africa.

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.

More, G., Tshikalange, T.E., Lall, N., Botha, F., Meyer, J.J.M., 2008. Antimicrobial activity of medicinal plants against oral microorganisms. Journal of Ethnopharmacology 119, 473–477.

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

Mu, M.M., Chakravortty, D., Sugiyama, T., Koide, N., Takahashi, K., Mori, I., Yoshida, T., Yokochi, T., 2001. The inhibitory activity of quercetin on lipopolysaccharide-induced nitric

oxide production in RAW 264.7 macrophage cells. Journal of Endotoxin Research 7, 431-438.

Mulaudzi, R.B., Ndhlala, A.R., Kulkarni, M.G., Finnie, J.F., Van Staden, J., 2011. Antimicrobial properties and phenolic contents of medicinal plants used by the Venda people for conditions related to venereal diseases. Journal of Ethnopharmacology 135, 330–337.

Mulaudzi, R.B., Ndhlala, A.R., Kulkarni, M.G., Finnie, J.F., Van Staden, J., 2013. Anti-inflammatory and mutagenic evaluation of medicinal plants used by Venda people against venereal and related diseases. Journal of Ethnopharmacology 146, 173–179.

Mulaudzi, R.B., Ndhlala, A.R., Van Staden, J., 2015. Ethnopharmacological evaluation of a traditional herbal remedy used to treat gonorrhoea in Limpopo province, South Africa. South African Journla of Botany 97, 117–122.

Naidoo, D., Van Vuuren, S.F., Van Zyl, R.L., De Wet, H., 2013. Plants traditionally used individually and in combination to treat sexually transmitted infections in northern Maputaland, South Africa: Antimicrobial activity and cytotoxicity. Journal of Ethnopharmacology 149, 656–667.

Nair, J.J., Mulaudzi, R.B., Chukwujekwu, J.C., Van Heerden, F.R., Van Staden, J., 2013. Antigonococcal activity of Ximenia caffra Sond. (Olacaceae) and identification of the active principle. South African Journal of Botany 86, 111–115.

Niu, X., Wang, H., Fu, Z.F., 2011. Role of chemokines in rabies pathogenesis and protection. Advances in Virus Research 79, 73–89.

Nguyen, T.N., Rusten, A., Bugge, M.S., Malterud, K.E., Diallo, D., Paulsen, B.S., Wangensteen, H., 2016. Flavonoids, gallotannins and ellagitannins in *Syzygium guineense* and the traditional use among Malian healers. Journal of Ethnopharmacology 192, 450-458.

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

Ojewole, J.A.O., Mawoza, T., Chiwororo, W.D.H., Owira, P.M.O., 2010. *Sclerocarya birrea* (A. Rich) Hochst. ["Marula"] (Anacardiaceae): A Review of its phytochemistry, pharmacology and toxicology and its ethnomedicinal uses. Phytotherapy Research 24, 633–639.

Okoth, A.D., 2014. Phytochemistry and bioactive natural products from *Lannea alata, Lannea rivae, Lannea schimperi* and *Lannea schweinfurthii* (Anacardiaceae). PhD Dissertation, University of KwaZulu-Natal, South Africa.

Poltorak, A., He, X., Smirnova, I., Liu, M., Huffel, C. Van, Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M., Ricciardi-Castagnoli, P., Layton, B., Beutler, B., 1998. Defective LPS signaling in C3H / HeJ and C57BL / 10ScCr Mice : Mutations in Tlr4 gene. Science 282, 2085–2089.

Rigo, A., Vianello, F., Clementi, G., 2000. Contribution of proanthocyanidins to the peroxy radical scavenging capacity of some Italian red wines. Journal of Food Chemistry 48, 1996–2002.

Sadeghian, H., Jabbari, A., 2016. 15-Lipoxygenase inhibitors: a patent review. Expert Opinion on Therapeutic Patents 26, 65–88.

Samie, A., Tambani, T., Harshfield, E., Green, E., Ramalivhana, J.N., Bessong, P.O., 2010. Antifungal activities of selected venda medicinal plants against candida albicans, Candida krusei and cryptococcus neoformans isolated from South African AIDS patients. African Journal of Biotechnology 9, 2965–2976.

Schulze-Kaysers, N., Feuereisen, M.M., Schieber, A., 2015. Phenolic compounds in edible species of the Anacardiaceae family – a review. RSC Advance 5, 73301–73314.

Seoposengwe, K., Van Tonder, J.J., Steenkamp, V., 2013. *In vitro* neuroprotective potential of four medicinal plants against rotenone-induced toxicity in SH-SY5Y neuroblastoma cells. BMC Complementary and Alternative Medicine 13, 1–11.

Shai, L.J., Chauke, M., Magano, S.R., Mogale, M., Eloff, J.N., 2013. Antibacterial activity of sixteen plant species from Phalaborwa , Limpopo Province , South Africa. Journal of Medicinal Plant Research 7, 1899–1905.

Shisana, O., Rhele, T., Simbayi, L.C.C., Zuma, K., Jooste, S., Zungu, N., Labadarios, D., Onoya, D., 2012. South African national HIV prevalence, incidence and behaviour survey, 2012. HSRC Press, Cape Town, South Africa.

Sobeh, M., Mahmoud, M.F., Abdelfattah, M.A.O., Cheng, H., El-Shazly, A.M., Wink, M., 2018. A proanthocyanidin-rich extract from *Cassia abbreviata* exhibits antioxidant and hepatoprotective activities in *vivo*. Journal of Ethnopharmacology 213, 38-47.

Sutcliffe, S., Zenilman, J.M., Ghanem, K.G., Jadack, R.A., Sokoll, L.J., Elliott, D.J., Nelson, W.G., De Marzo, A.M., Cole, S.R., Isaacs, W.B., Platz, E.A., 2006. Sexually transmitted infections and prostatic inflammation/cell damage as measured by serum prostate specific antigen concentration. Journal of Urology 175, 1937–1942.

Taiwo, F.O., Fidelis, A.A., Oyedeji, O., 2016. Antibacterial activity and phytochemical profile of leaf extracts of *Ficus abutilifolia*. British Journal of Pharmaceutical Research 11(6), 1-10.

Tlakula, P.K., 2016. Ethnobotanical Survey and Conservation of Medicinal Plants used by Local People of Thulamela Municipality, Limpopo Province. M.Sc Thesis, University of Pretoria, South Africa.

Tshikalange, T.E., Meyer, J.J.M., Lall, N., Munoz, E., Sancho, R., Van de Venter, M., Oosthuizen, V., Munoz, E., Sancho, R., Venter, M. Van De, Oosthuizen, V., 2008. *In vitro* anti-HIV-1 properties of ethnobotanically selected South African plants used in the treatment of sexually transmitted diseases. J. Ethnopharmacol. 119, 478–481.

Tshikalange, T.E., Mamba, P., Adebayo, S.A., 2016. Antimicrobial, anti-oxidant and cytotoxicity studies of medicinal plants used in the treatment of sexually transmitted diseases. International Journal of Pharmacognosy and Phytochemical Research 8, 1891-1895.

Venkata, R. B., Samuel, L.A., Pardha, S. M., Narashimha, R. B., Naga, V. K. A., Sudhakar, M. and Radhakrishnan, T.M., 2012. Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. Asian Journal of Pharmaceutical and Clinical Research 5 (2): 99-106.

Viljoen, A., Mncwangi, N., Vermaak, I., 2012. Anti-inflammatory iridoids of botanical origin. Current Medicinal Chemistry 19, 2104–2127.

Wamyu, K.R., 2016. Antibacterial, antifungal and phytochemical screening of the plant species *Lannea schweinfurthii* (Engl.) Engl. M. Sc. Thesis, Kenyatta University, Nairobi, Kenya.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd Edition. Livingstone Ltd., Edinburgh UK.

World Health Organization, 2016. , Sexually transmitted infections (STIs). WHO Fact sheet no.110. World Health Organization. http://www.who.int/mediacentre/factsheets/fs110/en/ , accessed February 13, 2018.

Wu, C.C., 2004. Nitric oxide and inflammation. Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents 3(3), 217–222.

Yaouba, S., Koch, A., Guantai, E.M., Derese, S., Irungu, B., Heydenreich, M., Yenesew, A., 2018. Alkenyl cyclohexanone derivatives from *Lannea rivae* and *Lannea schweinfurthii*. Phytochemistry Letter 23, 141-148.