Cytotoxic, antimicrobial, antioxidant, antilipoxygenase activities and phenolic composition of *Ozoroa* and *Searsia* species (Anacardiaceae) used in South African traditional medicine for treating diarrhoea

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Abstract

The safety and effectiveness of many of the medicinal plants used in traditional medicine by rural people with little or no access to allopathic drugs is yet to be evaluated. With this in mind, *Ozoroa* and *Searsia* (previously known as *Rhus*) species traditionally used in South Africa to treat microbial infections and gastrointestinal disorders were selected for *in vitro* evaluation of biological activities and cytotoxicity.

Phenolic-enriched leaf extracts were prepared using mixture of 1% HCl acidified 70% acetone and n-hexane. The crude extract was further fractionated with solvent of different polarities. Crude extracts and fractions were tested against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, *Enterococcus faecalis*, *Aspergillus fumigatus*, *Candida albicans* and *Cryptococcus neoformans*. In addition, the antioxidant potentials were determined by DPPH, ABTS, hydroxyl radical scavenging and linoleic acid peroxidation inhibition. The cytotoxic activity of the crude extracts was assayed against Vero cells.

The crude extract and the various fractions had good biological activities. The most noteworthy activity is the growth inhibition of the hexane and dichloromethane fractions with MIC values as low as from 19 µg/ml. The ethyl acetate and butanol fractions had moderate to low antimicrobial activities with MICs ranging from 39 to 2500 µg/ml. The polar fractions were more active against the fungal pathogens compared with the non-polar fraction. In the DPPH antioxidant assays, the active compounds were concentrated in the polar fractions (IC_{50} of the crude extract ranged between 0.90 and 15.82 µg/ml). The ethyl acetate fraction was the most active (IC_{50} ranging between 0.84 and 7.92 µg/ml). Although the water fraction was the most polar, the antioxidant activities were low due to the transfer of the active components into the ethyl acetate and butanol fractions. The

crude extracts also had good linoleic acid peroxidation inhibition (LC_{50} ranging between 13.99 and 40.45 µg/ml).

Crude extracts and fractions of the *Ozoroa mucronata*, *O. paniculosa, Searsia leptodictya*, *S. pendulina*, and *S pentheri* species tested in this study had good activities relating to diarrhoea mechanisms of pharmacological relevance. However, use of phenolic-enriched crude extracts from these plants for diarrhoeal treatment or any other diseases need to be applied with caution as most of the plant extracts were reasonably toxic against Vero cell line. A next step in the possible application of these extracts to treat diarrhoea would be to identify the bioactive and toxic compounds.
Keywords: *Ozoroa*; *Searsia (Rhus)*; diarrhoea; antimicrobial; antioxidant; cytotoxicity

1. Introduction

The use of medicinal plants to alleviate diarrhoeal symptoms is a common practice in South African Traditional Medicine (McGaw *et al*., 2000). More than 80% of the rural populations and urban settlements still rely on traditional medicine for healthcare delivery. Aside from being the backbone of ethnomedicine, plant phytochemicals have also been a major source of drugs or drug templates used in modern medicine. In an outbreak of diarrhoea, decoctions, concoctions or infusions of the medicinal plants are taken three or four times a day, until the symptoms are resolved, though the dosage is not controlled in most cases (Arnold and Gulumian, 1984).

Diarrhoeal symptoms are a consequence of altered intestinal motility and fluid accumulation in the gastrointestinal tract (GIT). These conditions are usually caused by microbial infections, intestinal parasitic burdens, intestinal inflammation, stress (physical and oxidative), or manifestation of other forms of diseases such as ulcerative colitis (Westbrook *et al*., 2010), Crohn‘s disease (Lomax *et al*., 2006), diabetes (Forgacs and Patel, 2011) and malaria (Gessler *et al*., 1995). Chronic diarrhoea is also a serious challenge in immune-suppressed people, as in cases of human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) where patients are susceptible to opportunistic infections including diarrhoeal pathogens (Dworkin *et al*., 1985). One of the consequences of diarrhoea in HIV/AIDS patients is the reduction of the absorption and bioavailability of anti-retroviral drugs. South Africa has the largest HIV infection rate in the world and many patients depend on medicinal plants to get relief from diarrhoea and intestinal disorders. In infants, diarrhoea

may cause malnutrition, resulting in stunted growth, a defective immune system and increased susceptibility to other infections (Fleckenstein *et al*., 2010).

Economically, diarrhoea is one of the major health challenges to humans as it causes loss of productive life due to premature mortality, disability and increased health-care costs (Guerrant *et al.*, 2005). In animal production, diarrhoea symptoms are presumed to impose heavy productivity losses on affected farms, although the true effects in monetary terms cannot be easily appreciated. The apparent on-farm losses are reduction in productivity (milk, wool, egg, meat and meat quality), increased mortality and morbidity, weight loss and abortion (Chi *et al.*, 2002). Episodes of diarrhoeal diseases can also affect the export market and hurt consumers' confidence in the products (Yarnell, 2007). Oddly enough, diarrhoea can be considered physiologically beneficial to the GIT as it provides an important mechanism of flushing away harmful luminal substances (Valeur *et al.*, 2009). However, diarrhoea becomes pathological and fatal when the loss of fluids and electrolytes exceeds the body's ability to replace the losses.

The most common modern way of managing diarrhoeal symptoms is fluid replacement in combination with pharmacological therapy. Fluid and electrolyte replacement is used to replace fluid losses by administrating sugar-electrolyte solution, usually referred to as oral rehydration therapy (ORT), as supportive treatment (Thapar and Sanderson, 2004). However, ORT does not facilitate the re-absorption or lessen the secretion of fluid, and therefore cannot stop diarrhoea but only reduce adverse effects that may result in death. Symptomatic treatment with antimicrobial or supportive therapy with preparations, compounds and drugs possessing antispasmodic, antimotility, antioxidant, anti-secretory/pro-absorptive, antitoxin and anti-inflammatory properties, depending on the causative agents, can be used in alleviating and treating diarrhoea (Wynn and Fougere, 2007). In cases of established infectious pathogens, specific therapeutic intervention using antimicrobial agents targeting the causative microbes may be applied as standard therapy. These standard therapeutic options are insufficient because of limited available modalities with broad based activities against the large numbers of diarrhoeal mechanism and apparent side effects (Ryu *et al.*, 2004).

In aerobic living systems, oxidative species are generated endogenously in processes such as oxidative phosphorylation in mitochondria, liver mixed function oxidases, microbial phagocytosis, xanthine oxidase activity, transition metal catalysis, drugs and xenobiotic metabolism. These oxidative species create homeostatic imbalance which generate oxidative stress resulting in tissue injury and

cell death (Kumar and Kakkar, 2008). Oxidative damage in the human and animals plays an important causative role in the initiation and progression of diseases. These include inflammation, microbial infection, autoimmune pathology, and gastrointestinal disorders such as diarrhoea through cytotoxic by-products of intestinal mucosal membrane lipid peroxidation. To overcome the deleterious effects of oxidative stress, multiple defence systems commonly referred to as antioxidants are inherent in living organisms. The protective efficiency depends on the balance between oxidative species and bioavailability of antioxidants in the microenvironment of a cell (Mathew and Abraham, 2006). However, endogenously antioxidant mechanisms may not be enough to remove the oxidative species, therefore additional antioxidant from medicinal plant or food source are needed. Medicinal plant preparations are used for treating more than one disease and probably had high bioactivity against common aetiologies.

The plant kingdom provides a non-exhaustive source of natural phytochemicals with bioactivity efficient in different applications for treating health disorders and maintenance of good health. Many plant species from the *Ozoroa* and *Searsia* (syn. *Rhus*) genera in the Anacardiaceae family such as *Ozoroa insignis* (Liu and Abreu, 2006), *Ozoroa reticulata* (Maregesi *et al*., 2007), *Ozoroa mucronata* (Yamagiwa *et al*., 1987), *Ozoroa obovata* (Bandeira *et al*., 2001), *Ozoroa sphaerocarpa* (Sibandze *et al*., 2010), *Ozoroa paniculosa*, *Searsia lancea*, *Searsia incisa* (Van der Merwe *et al*., 2001; McGaw *et al*., 2008), *Searsia javanica* (Vareinshang and Yadav, 2004), *Searsia natalensis* (John *et al*., 1995), *Searsia gueinzii* (Elgorashi *et al*., 2003), *Searsia leptodictya* diels (Sebothoma, 2010), *Searsia rogersii* Schonland (Samie *et al*., 2010), and *Searsia pendulina* (Coates-Palgrave, 2002) are used by traditional medicine practitioners in South Africa and other African countries for treating intestinal disorders, including diarrhoea. The antidiarrhoeal activities of these plants may be due to synergistic or individual components acting on one or more mechanisms of diarrhoea. *Searsia pentheri* is reported to be used to cure epilepsy in Zululand of South Africa (Svenningsen *et al*., 2006). However, *S. javanica* is the only species studied for antidiarrhoeal properties (Vareinshang and Yadav, 2004), although the antimicrobial of many *Ozoroa* and *Searsia* species have been reported in the literature.

Phytochemical evaluations of several species from the family have led to isolation of many bioactive compounds such as anacardic acid from *O. mucronata* and *O. paniculosa*. This compound has been reported to possess anti-inflammatory properties by inhibition of LOX (Ha and Kubo, 2005)

and PG synthase inhibition (Kubo *et al*., 1987). Isolation of moronic acid as an antimicrobial principle from *O. mucronata* has also been reported (Hostettmann-Kalda and Nakanishi, 1979).

Alongside the medicinal beneficial constituents, plants also have the capacity to produce some potentially toxic compounds and therefore attention needs to be focused on the safety of plant preparations. However, a literature search on the biological and phytochemical evaluation of some of these species generated little information. The aims of this work were to scientifically validate and evaluate the pharmacological properties of *O. mucronata*, *O. paniculosa*, *S. leptodictya*, *S. pendulina*, and *S. pentheri* against some diarrhoeal aetiology. Therefore, the antimicrobial activities of leaf extracts against some microbes associated with diarrhoea; the antioxidant activity of the extract using various standard protocols, the phenolic composition of the extracts, anti-inflammation through the soybean 15-lipoxygenase enzyme inhibition and the cytotoxicity of the extracts using an *in vitro* Vero cell line model were evaluated.

2. Materials and Methods

2.1. Plant collection

The species investigated were selected based on traditional use and availability in the University Botanical Garden and leaves were investigated because it represents a sustainable resource. The leaves of *Ozoroa mucronata* (Bernh.) R.Fern & A.Fern (Ozm), *Ozoroa paniculosa* (Sond.) R.Fern. & A.Fern.(Ozp), *Searsia leptodictya* (Diels) T.S.Yi, A.J.Mill. & J.Wen (Sle), *Searsia pendulina* (Jacq.) Moffett, comb. nov. (Spd), and *Searsia pentheri* (Zahlbr.) Moffett (Spt) were collected from the University of Pretoria's Manie van der Schijff Botanical Garden. The plant material was identified and authenticated by Lorraine Middleton and Magda Nel at the University of Pretoria. The voucher specimen numbers for the herbarium specimens deposited at the H.G.W.J. Schweickerdt Herbarium (University of Pretoria) are PRU 68928, PRU 66851, PRU 70151, PRU 84141 and PRU 709769 for Ozm, Ozp, Sle, Spd, and Spt respectively.

2.2. Preparation of extracts

The polyphenolic-enriched leaf extracts of Ozm, Ozp, Sle, Spd and Spt were prepared by simultaneous extraction and fractionation of 20 g of plant material using a mixture 200 ml of 70% acetone acidified with 0.1% HCl (Naczk and Shahidi, 2004) and 200 ml of n-hexane for 12 h. The phenolic and glycosides enriched aqueous phase lower phase fraction was separated from the

chlorophyll and triterpenoid enriched n-hexane upper phase fraction. This was repeated twice and n-hexane fractions were combined. The combined aqueous fraction was fractionated using solvent of increasing polarity to obtain dichloromethane (DCM), ethyl acetate (ETOAc), butanol (BuOH) and residual water fractions. The crude extract and various fractions were concentrated under reduced pressure using rotary evaporator (Buchi). The organic fractions of n-hexane, DCM, ETOAc and BuOH were air dried at room temperature for 72 h while the crude extract and residual water fraction were freeze-dried. The dried crude extract and fractions were reconstituted in acetone and 70% acetone at a concentration of 10 mg/ml for the bioassays.

2.3. Antimicrobial assays

2.3.1. Minimum inhibitory concentration (MIC) determination against bacteria

The minimum inhibitory concentration (MIC) values for the crude extracts against *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212) were evaluated using the serial dilution assay with tetrazolium violet added as growth indicator (Eloff, 1998). Overnight cultures of the bacteria (*Staphylococcus aureus*, 2.6×10^{12} cfu/ml; *Enterococcus faecalis*, 1.5×10^{10} cfu/ml; *Pseudomonas aeruginosa*, 5.2×10^{13} cfu/ml and *Escherichia coli*, 3.0×10^{11} cfu/ml) were ten-fold diluted with sterilized Mueller-Hinton (MH) broth. The extracts (100 µl at 10 mg/ml) were serially diluted two-fold with sterile water in a sterile 96-well microtitre plate for each organism, and 100 µl of the bacterial culture was added to each well. The plates were sealed with transparent parafilm and incubated at 37^{o}C for 24 h. The MIC values were obtained by adding 40 µl of 0.2 mg/ml INT and inhibitory activity read after 30 min and 12 h. The screening was done in triplicate and repeated twice, and where there were variations, the experiment was repeated again. Gentamicin (concentration ranges from 25 µg/ml in the first well to 0.19 µg/ml in the last well) and 70% acetone solution were used as positive and negative control respectively.

2.3.3. Minimum inhibitory concentration (MIC) determination against fungi

Minimum inhibitory concentrations (MIC) against three pathogenic and opportunistic fungi were determined using the serial dilution assay (Eloff, 1998) and with some modifications (Masoko *et al*., 2005). The organisms tested were *Aspergillus fumigatus*, *Candida albicans*, and *Cryptococcus neoformans*. Overnight cultures of the fungi (*Candida albicans*, 2.5×10^{6} cfu/ml, *Aspergillus fumigatus*, 8.1×10^{6} cfu/ml and *Cryptococcus neoformans*, 2.6×10^{6} cfu/ml) were ten-fold diluted with sterilized

Sabouraud broth. The extracts (100 µl at 10 mg/ml) were prepared by two-fold serial dilution with sterile water in a sterile 96-well microtitre plate for each organism. Then, 100 µl of the fungal culture and 40 µl of 0.2 mg/ml INT were added to each well. The plates were sealed with transparent parafilm and incubated at 37^{o}C. The MICs were read after 24 h and 48 h respectively. The screening was done in triplicate and repeated twice, and where there were variations, the experiment was repeated again. Amphotericin B (concentration ranges from 50 µg/ml in the first well to 0.39 µg/ml in the last well) and 70% acetone solution were used as positive and negative controls respectively.

2.4. Antioxidative assays

2.4.1. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Radical scavenging activity of the extracts and fractions was estimated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams *et al*., 1995) with some modifications to a 96-well microtitre plate. A 40 µl of 0.3 - 500 µg/ml of crude extracts, fractions and controls (trolox and ascorbic acid) were added to 160 µl of a 25 mg/L solution of DPPH in a 96-well microtitre plate. After incubation in the dark for 30 min, the absorbance of each test sample was read at 516 nm using Versamax microtitre plate reader.

2.4.2. 2, 2‘-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical decolourization assay

The free radical decolourization assay using 2, 2‘-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) of the crude extract, fractions and controls was performed with modification to 96-well microtitre plate format (Re *et al*., 1999). ABTS free radical was generated in 75% methanol solution by mixing ABTS solution at 7 mM concentration and potassium persulfate at 2.45 mM, and placed in the dark for 12-16 h at room temperature. For the assay, the ABTS solution was diluted to absorbance value range of 0.70±0.02 at 734 nm and equilibrated at room temperature (A_o). A 40 µl aliquot of 0.3-500 µg/ml of crude extracts, fractions and controls (trolox and ascorbic acid) was added to 160 µl of ABTS and the absorbance read after 6 minutes of mixing (A_t). The blank was prepared the same way with dissolving solvent (A_b).

2.4.3. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activities of the test samples were measured by the salicylic acid method with some modifications (Smirnoff and Cumbes, 1989). The hydroxyl radical was

generated using the Fenton reaction by which 50 ml of $FeCl_3$ (8.0 mM), 80 ml of H_2O_2 and 50 ml of distilled water was allowed to stand for 1 h. The mixture was filtered to remove the debris. The presence of the hydroxyl radical was determined by mixing 120 µl of the hydroxyl radical solution with 66 µl of each extract followed by 14 µl of salicylic acid (20 mM). The mixture was incubated for 30 min at 37^{o}C and absorbance taken at 510 nm.

2.4.4. Linoleic acid peroxidation inhibitory assay

Lipid peroxidation inhibitory activity of the crude extracts against linoleic acid was determined as described by Kishida *et al*. (1993). Each reaction mixture contained 4.1 ml of 2.5% linoleic acid in ethanol and 10 ml of 0.2 M phosphate buffers (pH 7.4), and 1 ml of 0.0025 mg/ml $FeSO_4$ was added as catalyst. Different concentrations of the samples (0.01-500 mg/ml) were added to the reaction mixture in a centrifuge tube. The reaction mixture was incubated at 40^{o}C for 2 h. The reaction was terminated by adding 1 ml of 25% trichloroacetic acid and 1 ml of 0.67% thiobarbituric acid. The reaction mixture was heated at 95^{o}C for 30 min and then cooled for 15 min. The mixture was extracted with butanol and absorbance measured at 500 nm.

2.5. *In vitro* cytotoxicity assay

Cytotoxicity of the extract was determined by the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide] assay (Mosmann, 1983) using Vero African green monkey kidney cell lines. The cells were cultured in Minimal Essential Medium (MEM) Earle's Base, supplemented with 2 mM L-glutamine, 16.5 mM $NaHCO_3$, 0.1% gentamicin and 5% foetal calf serum. Confluent monolayer culture suspensions of the cells were seeded into 96-well tissue culture microtitre plates at a density of 2×10^3 cells per well and incubated for 24 h at 37^{o}C in a 5% CO_2 incubator. Extracts re-suspended in DMSO and diluted in MEM, positive control (berberine chloride, Sigma) and negative control were added to the cells and incubated for 5 days. The cells were observed using an inverted microscope to check for cytopathic effects caused by the extract. The cell proliferation and viability was examined by addition of 30 µl of a 5 mg/ml solution of MTT in PBS to each well and incubation for another 4 h at 37^{o}C. The medium was carefully removed from the wells without disturbing the MTT concentrate and washed twice with PBS. The liquid was aspirated from the cells and 50 µl of DMSO was added to each well to dissolve the crystallized MTT formazan. The amount of reduced MTT was measured as

absorbance at 570 nm using a Versamax microtitre plate reader. The results were expressed as a percentage of the control cells and IC_{50} values were calculated.

2.6. Phytochemical analysis

2.6 1. Determination of total phenolic constituents of the extracts

The total phenolic (TP) compositions of the crude extracts were evaluated using the Folin-Ciocalteau method with some modifications (Makkar, 2003). The extracts (50 µl) at a ratio of plant material: extractant of 1:1 were made up to 500 µl with distilled water. Subsequently, 250 µl of commercial Folin-Ciocalteau reagent diluted with distilled water (1:1) and 1250 µl of 20% sodium carbonate solution was added. Absorbance was recorded at 725 nm after incubation for 40 min at room temperature. The amount of polyphenols (expressed as mg gallic acid/g dry weight) was calculated from a linear equation of the standard curve (0.0019-0.25 mg/ml gallic acid) prepared at the same time and the equation used was absorbance = 4.9022 × TP (mg GAE), R^2=0.9804.

2.6.2. Determination of total tannin

The total tannin (TT) content of the extracts was determined using the polyvinylpyrrolidone (PVPP) binding method (Makkar, 2003). The tannin-bound mixtures were prepared by mixing 100 mg of PVPP, 1 ml of distilled water and 1 ml of phenolic-containing extracts in a centrifuge tube. The mixtures were vortexed and refrigerated at 4^{o}C for 15 min and then filtered. The filtrate (100 µl) was transferred to a test tube and the phenolic contents were evaluated as described in section 2.6.1. Non-tannin phenolic contents were determined from the standard curve of catechin expressed as catechin equivalent in mg/g dry material. The tannin content was calculated as the difference between the total phenolic and non-phenolic content of the extracts as the tannin constituents were bound and precipitated by PVPP.

2.6.3. Determination of proanthocyanidin

The proanthocyanidin (PA) content of the extracts was determined using butanol-HCl reagent (Makkar, 2003). The extract (500 µl) was dispensed into a test tube and made up to 10 ml with 70% acetone. A volume of 3 ml of butanol/HCl reagent (95:5 v:v) and 100 µl of 2% ferric ammonium sulphate in 2N HCl were added. The test tubes were loosely covered and heated in a boiling water

bath for 50 min. The absorbance was recorded at 550 nm after the tubes and contents were cooled to room temperature. Absorbance of the unheated mixture was used as blank.

2.6.4. Determination of condensed tannin

The condensed tannin (CT) content of the extracts was determined using vanillin/HCl reagent (Heimler *et al*., 2006). The extract (0.5 ml) was mixed with 3 ml of vanillin reagent containing 4% concentrated HCl and 0.5% of vanillin in methanol in a test tube. The mixture was allowed to stand at room temperature for 15 min and absorbance was recorded at 500 nm against methanol as blank. The amount of condensed tannin in the extracts was expressed as catechin equivalent (CE)/g dry plant material. The standard curve ranged from 0.0019 to 0.25 mg/ml and the equation used was absorbance= 0.1791 × CT (mg CE) + 0.0504, R^2=0.944).

2.6.5. Determination of hydrolysable tannin (gallotannin)

The hydrolysable tannin as gallotannin (GT) constituent of the extracts was determined with the potassium iodate assay (Vermerris and Nicholson, 2006). The extract (3 ml) was mixed with1 ml of saturated solution of potassium iodate and allowed to stand at room temperature for 40 min and absorbance was read at 550 nm. A standard curve was prepared using gallic acid under the same conditions as the extracts and results were expressed as gallic acid equivalent (GAE)/g dry plant material and the equation used was absorbance= 0.8264 × GT (mg GAE) + 0.0392, R^2=0.9155.

2.6.6. Determination of total flavonoids and flavonol

The total flavonoid (TF) content of the extracts was determined using aluminium chloride solution with some modifications (Abdel-Hameed, 2009). The extract (100 µl) was mixed with 100 µl of 20% $AlCl_3$, two drops of glacial acetic acid and 3 ml of methanol. The mixture was allowed to stand at room temperature for 40 min and absorbance was read at 415 nm. Blank samples were prepared with the extract without $AlCl_3$. Standard curve was prepared using quercetin (3.9-500 µg/ml) in methanol under the same condition. The amount of flavonoids was expressed as mg quercetin equivalent/g of dry plant material (Absorbance= 4.9747 × FT (mg quercetin), R^2=0.9846).

The flavonol content (FLL) of the extracts was determined with some modifications using aluminium chloride solution (Abdel-Hameed, 2009). The extract (1 ml) was mixed with 1 ml of 20 mg/ml of $AlCl_3$ and 3 ml of 50 mg/ml of sodium acetate. The mixture was allowed to stand at room

temperature for 2.5h and absorbance read at 440 nm. A standard curve was prepared using quercetin (0.0019-0.0312 mg/ml) in methanol under the same condition. The amount of flavonol was expressed as mg quercetin equivalent/g of dry plant material and the equation used was absorbance= 34.046 × FLL (mg quercetin), R^2=0.9853.

2.7. Lipoxygenase inhibition

Inhibition of 15-lipoxygenase (15-LOX) was carried out using soybean lipoxygenase type 1-B (Sigma South Africa) (Lyckander and Malterud, 1992). Measurements of increase in absorbance at 234 nm at intervals of 30 s for 5 min after enzyme addition were done in 0.2 M borate buffer (pH 9.00) with linoleic acid (134 µM) as substrate and an enzyme concentration of 200 U/mL, using test substance solutions in DMSO as blanks. All experiments were performed in triplicate. Lipoxygenase inhibitory activity was expressed as the percentage inhibition of lipoxygenase, calculated as (%) inhibition following the equation

(%) inhibition = ((C – TS)/C) × 100

where C is the change in absorbance of the assay without the plant extracts (control) and TS is the change in absorbance of the assay with the plant extracts (test sample).

2.8. Statistical Analysis

All values were expressed as mean ± SD. The sigmoidal dose-response (variable slope) best fit EC_{50} for each extracts and fractions was computed with the associated standard error. The analysis was performed using GraphPad Prism for Windows, version 5.03 (GraphPad Software, San Diego, CA, USA). Statistical analysis of the results was performed by using one way ANOVA, followed by Bonferroni multiple comparison post hoc tests and the significant difference was set at $P<0.05$.

3. Results and Discussion

3.1. Yield

The percentage yield of the phenolic-enriched extracts and fractions were determined as presented in Fig 1. The percentage yields of the crude extracts and fractions were expressed as the ratio of the dry weight of plant extract to the dry weight of the plant material used for the extraction process. The maximum yield of the crude extract was obtained from *S. leptodictya* (44.5±3.47%)

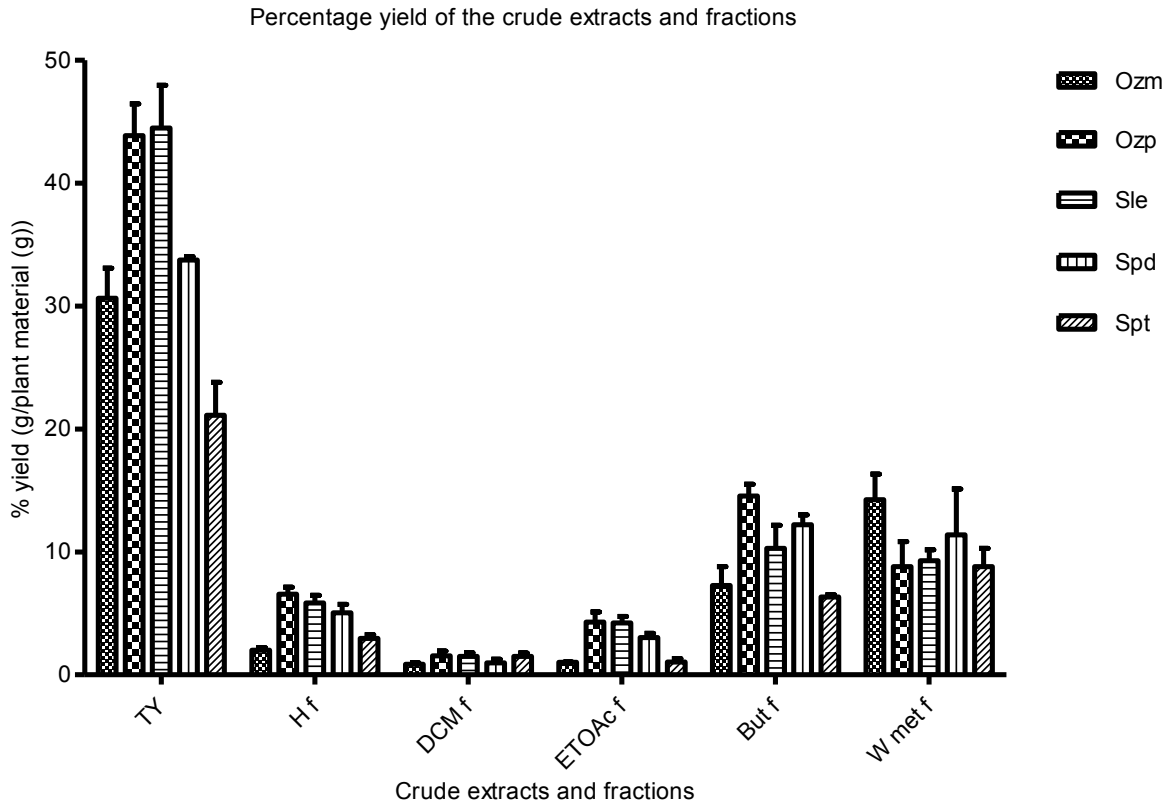

Fig 1: Percentage yield of the crude extracts and fractions.
Ozm (*Ozoroa mucronata*); Ozp (*O. paniculosa*); Sle (*Searsia leptodictya*); Spd (*S. pendulina*); Spt (*S. pentheri*), TY (total yield), H f (hexane fraction), DCM f (dichloromethane fraction), ETOAc f (ethyl acetate fraction), But f (butanol fraction), W met f (water fraction soluble in methanol).

followed by *O. paniculosa* (43.87±2.60%) while *S. pentheri* gave the lowest yield (21.13±2.67%). The acidified 70% acetone solution was an effective extractant of phenolic-enriched constituents considering the yields of the crude extracts. For the fractions, the yield varied according to the polarities of the different compounds present in the crude extracts.

3.2. Phenolic constituents

In this study, the total phenolic, total tannin, condensed tannin, hydrolysable tannin as gallotannin, flavonoids and flavonol constituents of the phenolic-enriched crude extracts are evaluated and the results presented as equivalents of various standard compounds in Fig. 2. The amount of total phenolics varied widely and ranged from 74.91±1.26 mg GAE/g dried plant material in *O. mucronata* to 370.89±4.80 mg GAE/g dried plant material in *O. paniculosa*. For the *Searsia* species, the highest amount of total phenolic content was obtained from *S. pendulina* (339.80±5.10 mg GAE/g dried plant material) followed by *S. leptodictya* (324.78±2.09 mg GAE/g dried plant material) and the

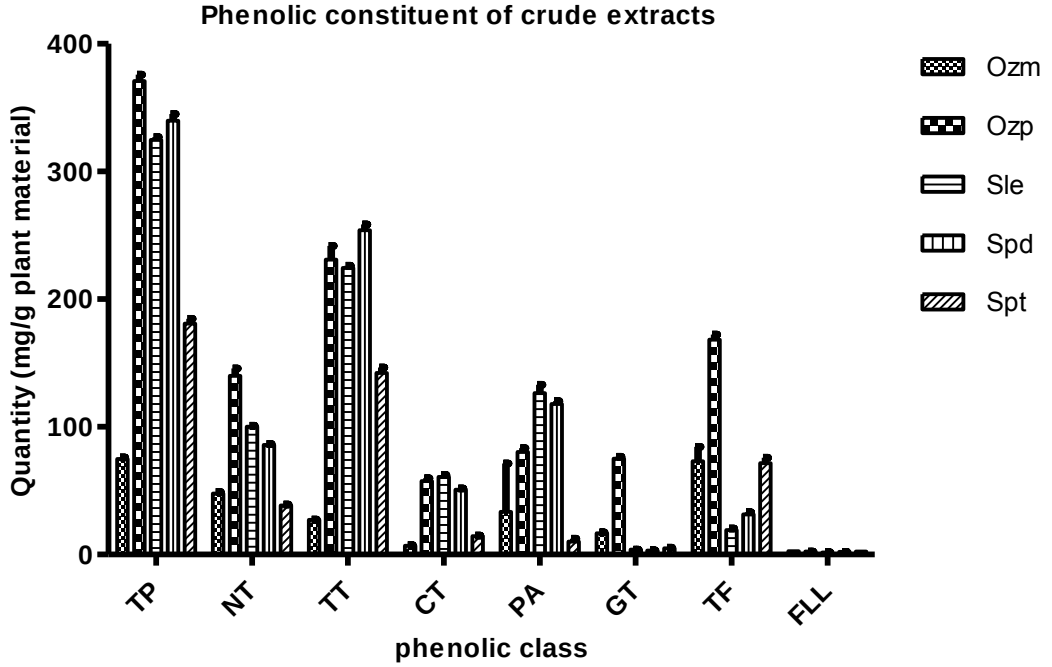

Fig 2: Phenolic constituent of the acidified 70% acetone leaf extracts of Ozoroa and Searsia species.
Ozm (*Ozoroa mucronata*); Ozp (*O. paniculosa*); Sle (*Searsia leptodictya*); Spd (*S. pendulina*); Spt (*S. pentheri*); TP (Total phenolic (mg GAE/g)), TT (Total tannin (mg GAE/g)), NT (Non-tannin (mg GAE/g)), CT (Condensed tannin (mg CE/g)), PA (proanthocyanidin (mg LE/g)), GT (gallotannin (mg GAE/g)), TF (total flavonoid (mg QE/g)), FLL (flavonol (mg QE/g)).

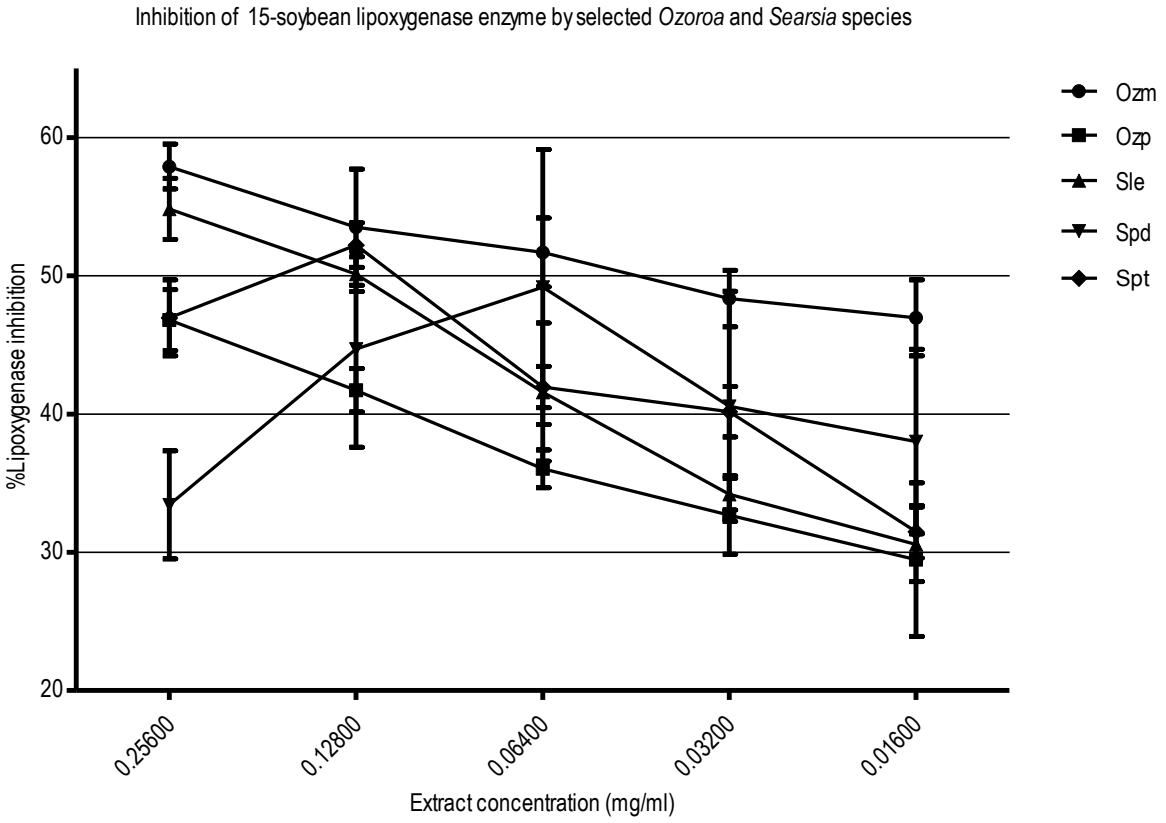

Fig. 3. Percentage inhibition of the soybean 15-lipoxygenase enzyme by the crude extracts.
N.B: Ozm (*Ozoroa mucronata*); Ozp (*O. paniculosa*); Sle (*Searsia leptodictya*); Spd (*S. pendulina*); Spt (*S. pentheri*)

least amount found in *S. pentheri* (180.86±3.68 mg GAE/g dried plant material). Phenolic compounds are important bioactive components of medicinal plant extracts exhibiting multifunctional pharmacological properties. These groups of phytochemicals are primarily natural antioxidants which act as reducing agents, metal chelators and single oxygen quenchers. Phenolic-enriched extracts correlate with a wide range of physiological and health benefits other than antioxidative activity through the modulation of multiple signalling pathways (Rodrigo *et al*., 2011). These features could be exploited for treatment of multi-causal diseases such as diarrhoea with one or more combinations of aetiologies including microbial infection, stress, allergy, inflammation, immune dysfunctions.

The extracts of *O. paniculosa* which had the highest total phenols, contained relatively low levels of total tannin with the highest amount obtained from *S. pendulina* (254.03±4.42 mg GAE/g dried plant material) followed by *O. paniculosa* (230.97±10.71 mg GAE/g dried plant material). Extracts of *O. mucronata*, whose total phenolic content was lowest, also ranked last on the total tannin content (27.15±0.18 mg GAE/g dried plant material). The tannin constituents are mostly of the proanthocyanidin type with the highest amount obtained from *S. leptodictya* (126.54±6.46 mg. LE/g plant material) followed by *S. pendulina* (117.83±2.24 mg. LE/g plant material) and the least amount was obtained from *S. pentheri* (10.08±2.41 mg. LE/g plant material). Interestingly, the *Ozoroa* species contained relatively high gallotannin (hydrolysable tannin) with the highest amount obtained from *O. paniculosa* (75.11±1.38 mg GAE/g dried plant material) followed by *O. mucronata* (16.78±0.61 mg GAE/g dried plant material) while *Searsia* species have low gallotannin and ranged from 3.24±0.16 mg GAE/g dried plant material in *S. pendulina* to 4.96±0.38 mg GAE/g dried plant material in *S. pentheri*.

The highest levels of total flavonoids were obtained in extracts of *O. paniculosa* (168.27±4.12 mg QE/g plant material) followed by *O. mucronata* (73.07±11.25 mg QE/g plant material). The extracts of *S. leptodictya and S. pendulina*, which had high total phenolic contents, contained relatively low levels of total flavonoid contents of 19.18±1.15 and 31.44±1.87 mg QE/g plant material respectively. The flavonol contents of all the extracts were low and ranged from 1.69±0.08 mg QE/g plant material in *S. leptodictya* to 2.36±0.09 mg QE/g plant material in *S. pentheri*.

3.3. Antimicrobial activities of the crude extracts and fractions

The crude extract and fractions of various polarities from the leaf extracts of *Ozoroa* and *Searsia* species tested in this study had a broad spectrum of microbial growth inhibition. For volatile oils minimum inhibitory concentrations of 0.28-1.27 mg/ml are considered as ~~-~~extremely high activity" (Aligiannis et al., 2001). For plant extracts there appear to be reasonable consensus that only MICs of less than 0.1 mg/ml are pharmacologically interesting (Eloff 2004, Rios and Recio, 2005). In this study where extracts could potentially be dosed to animals, the activity of the samples were classified as pharmacologically interesting if the MIC was less than 100 µg/ml, good if the MIC was between 100 and 200 µg/ml, moderate if the MIC was between 200 and 625 µg/ml, weak for MICs of 625-1000 µg/ml and inactive if the MIC was greater than 1000 µg/ml (Eloff, 2004; Kuete, 2010).The antibacterial activities of the test samples against ATCC strains of *E. coli, E. faecalis, P. aeruginosa and S. aureus* are summarized in Table 1. The hexane and DCM fractions of all the plant species had varying antibacterial activities with MIC values ranging between 19 and 1250 µg/ml. The polar fractions (ethyl acetate and butanol) also had moderate to good antibacterial activities with MIC values ranged between 39 and 625 µg/ml. However, some of the organisms were resistance to the crude extracts and residual water fractions with MICs ranging between 312 to above 2500 µg/ml.

Increasing new fungal infections and the emergence of resistant strains in humans and animals now pose a serious health problem worldwide. Opportunistic fungal infections have also become more important especially due to the immune deficiency induced by HIV/AIDS (Groll *et al*., 1996). The antifungal activities of the extracts and fractions are presented in Table 1 against clinical pathogenic strains of *Candida albicans*, *Aspergillus fumigatus*, and *Cryptoccoccus neoformans*. The antifungal activities of the five plant species were potentiated in the hexane and DCM fractions with MICs ranging between 39 and 625 µg/ml. The ethyl acetate and butanol fractions also had moderate to good antifungal activities with MICs ranging between 78 and 1250 µg/ml. The residual water fraction had poor to no activity against some fungal strains, especially *C. albicans*. In a related study, the non-polar extracts of *Combretum vendae* (A.E. van Wyk) (Combretaceae), *Khaya anthotheca* (Welm.) C. DC (Meliaceae) and *Ochna natalitia* (Meisn.) Walp. (Ochnaceae) have good activities against some selected microorganisms with MIC values ranged between 50 and 90 µg/ml (Suleiman *et al*., 2010).

Table 1: Minimum Inhibitory Concentration (MIC) of the *Ozoroa* and *Searsia* species expressed in µg/ml against some bacteria and fungi associated with GIT infectious diseases including diarrhoea

	Ozoroa mucronata							*Ozoroa paniculosa*							*Searsia leptodictya*						
	E. c	*E. f*	*P. a*	*S. a*	*C. a*	*C. n*	*A. f*	*E. c*	*E. f*	*P. a*	*S. a*	*C. a*	*C. n*	*A. f*	*E. c*	*E. f*	*P. a*	*S. a*	*C. a*	*C. n*	*A. f*
Cru	1250	625	312	2500	625	156	625	1250	1250	156	156	312	78	312	1250	625	625	312	312	156	625
Hex	78	39	156	19	312	312	39	78	39	39	19	156	312	78	39	39	39	19	39	156	78
DCM	39	19	39	19	156	156	156	39	19	39	19	78	39	78	39	19	39	19	39	39	39
ET	156	625	312	625	156	39	39	39	156	78	78	156	39	312	78	156	39	78	ND	ND	ND
B	312	1250	2500	1250	625	156	156	625	78	156	156	312	156	156	2500	625	625	312	312	312	156
W					625	2500	1250					625	1250	625					625	1250	312

	Searsia pendulina							*Searsia pentheri*						
	E. c	*E. f*	*P. a*	*S. a*	*C. a*	*C. n*	*A. f*	*E. c*	*E. f*	*P. a*	*S. a*	*C. a*	*C. n*	*A. f*
Cru	1250	625	625	312	625	312	625	2500	1250	156	312	2500	312	2500
Hex	39	156	78	78	39	156	78	39	39	19	19	625	1250	78
DCM	39	19	78	78	78	39	39	39	19	19	19	78	19	19
ET	78	312	19	39	156	39	156	156	156	78	156	312	39	625
B	1250	625	2500	625	625	156	78	2500	1250	2500	312	156	312	156
W					625	625	312					1250	1250	625
Gent	0.39	0.78	1.56	0.78	-	-	-							
Amp B	-	-	-	-	6.25	3.12	0.78							

N.B: Cru = crude extract, Hex = hexane fraction, DCM= dichloromethane fraction, ET = ethyl acetate fraction, B = butanol fraction, W = water fraction, *E. c* = *Escherichia coli*, *E. f* = *Enterococcus faecalis*, *P. a* = *Pseudomonas aeruginosa*, *S. a* = *Staphylococcus aureus, A. f* = *Aspergillus fumigatus*, *C. a* = *Candida albicans* and *C. n* = *Cryptococcus neoformans*, Gent = gentamicin, Amp B = amphotericin B.

The antimicrobial activity of extracts generally depends on the plant phytochemical composition, extracting solvent, effective solubility and miscibility of the active component in the test medium, the vulnerability of the test organisms, and the method used in evaluation. Antimicrobial components of the plants tested in this study are concentrated in the non-polar fractions. Many other studies have also reported the exceptional antimicrobial activities of non-polar extracts and fractions (Kotze and Eloff, 2002; Matu and Van Staden, 2003; Eloff *et al*., 2005a; Eloff *et al*., 2005b), suggesting that the antimicrobial agents are concentrated in these fractions. Thus, for infectious diarrhoea, plant decoctions, concoctions or infusions usually used in traditional medicine may be ineffective except as supportive therapy in the case of oxidative stress or inflammatory involvement. The use of alcoholic solutions which may extract a broad spectrum of compounds is recommended instead of water extracts.

In infectious diarrhoea, many bacteria, protozoa, viruses and parasites have been implicated as causative agents. These agents include *Vibrio cholera*, *Escherichia coli*, *Shigella dysenteriae*, *Bacillus cereus*, *Stapylococcus aureus*, *Clostridium difficile*, *Entamoeba histolytica, Salmonella typhi* and *Giardia lamblia* (Laohachai *et al*., 2003, Kelly, 2011). Some viruses such as rotavirus and adenovirus have also been implicated as causative agents of diarrhoeal diseases. The infectious mechanisms of the pathogenic strains of the enteric microbes include microbial attachment, localized effacement of the epithelial mucosa lining, production and elaboration of secretory enterotoxins, production of cell-destroying cytotoxins, and direct epithelial cell invasion. In this study, the emphasis was on *E. coli*, *S. aureus*, *E. faecalis* and *C. albicans* as diarrhoeal pathogens. Seven virulence groups of diarrhoeagenic *E. coli,* namely enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), diarrhoea-associated haemolytic *E. coli* (DHEC) and cytolethal distending toxin (CDT)-producing *E. coli* have been classified (Clarke, 2001). On a global scale EPEC, EHEC, ETEC and EIEC are the most important diarrhoeal agents, accounting for 4 - 8%, 0 - 1%, 12 - 20% and 0 - 2% respectively in terms of total episodes (Bhan, 2000). The virulence mechanisms of ETEC, EHEC, *S. aureus*, *E. faecalis* and some strains of *V. cholerae* include production of endotoxin, cytotoxins and ROS. The use of antimicrobial therapy may not be effective in the diarrhoea cases involving these organisms because the toxins, if already present in contaminated food or water, do not need the pathogens to exert activity. Therefore, non-antimicrobial therapy may be required in such cases.

The effective antimicrobial activities of the hexane and DCM fractions, and the moderate activities of the ethyl acetate and butanol fractions justify the application of the organic extracts of the plant species in treating infectious diarrhoea. The mechanism of action may be microbicidal or microbiostatic. However, targeting specific pathways such as anti-toxigenic, antioxidative, anti-inflammatory, antisecretory and pro-absorptive activities of the medicinal plant extracts provides novel approaches to combating diarrhoea.

3.4. Antioxidant

The concentration-dependent DPPH scavenging activity of the crude extracts and fractions of various polarities expressed as EC_{50} are presented in Table 2. The radical scavenging activities are potentiated in the crude extract, and polar fractions with the ethyl acetate and butanol fractions exhibiting activities comparable to the reference controls (ascorbic acid and trolox). The hexane and DCM fractions results revealed limited DPPH radical scavenging ability, indicating that the non-polar components present in the fractions are low radical scavengers. The mechanism of DPPH radical scavenging is through hydrogen transfer from the extract to DPPH, forming yellow coloured reduced adducts of DPPH-H. A high DPPH radical scavenging capacity is an indication of the hydrogen donating potential of the bioactive components of the extracts and these relate directly to the number of free phenolic hydroxyl groups and their position in the molecule.

The concentration-dependent DPPH scavenging activity of the crude extracts and fractions of various polarities expressed as EC_{50} are presented in Table 2. The mechanism of ABTS radical scavenging effect is by electron transfer from the extracts to blue-green $ABTS^{+}$ to form decolourized ABTS molecule. The crude extract, ethyl acetate and butanol fractions of all the *Ozoroa* and *Searsia* species had good radical scavenging capacity while the hexane and DCM fraction activities were weak. Apart from giving an insight into the mechanistic pathway of antioxidant activity of extracts, the ABTS and DPPH radical scavenging assay have no biological relevance.

The crude extracts and the polar fractions (ethyl acetate and butanol) had good concentration-dependent hydroxyl ion scavenging capacity. The results are calculated as percentage radical scavenged and from the plot best fit curve of log concentration against percentage inhibition, the EC_{50} were calculated and are presented in Table 2. Hydroxyl radicals are highly reactive species, which react non-selectively with bio-molecules such as DNA, proteins and membrane phospholipids

Table 2: Radical scavenging activities of the *Ozoroa* and *Searsia* species expressed as EC_{50} (µg/ml)

	Ozoroa mucronata			*Ozoroa paniculosa*			*Searsia leptodictya*			*Searsia pendulina*		
	DPPH	ABTS	OH	DPPH	ABTS	OH	DPPH	ABTS	OH	DPPH	ABTS	OH
Cru	15.82±4.07	15.93±2.13	44.29±4.20	0.90±0.05	0.99±0.06	33.02±6.5	1.98±0.10	5.43±0.73	43.88±8.57	1.14±0.11	1.94±0.22	83.46±10.45
Hex	132.13±5.0	43.48±4.21	175.56±57	15.65±2.34	191.47±11.0	35.90±3.0	63.55±2.70	129.17±20.2	129.69±39	126.77±8.68	213±18.32	130.45±57
DCM	63.48±4.0	17.55±2.29	45.77±0.98	5.34±0.31	161.60±4.16	11.03±2.8	11.48±1.35	113.80±11.07	27.76±0.9	15.49±0.73	158.38±15.5	
ET	7.92±1.64	10.74±0.46		0.84±0.28	1.60±0.61	33.07±0.5	0.99±0.05	4.63±0.56	41.17±7.90	1.20±0.03	2.19±0.24	39.02±0.09
B	-	68.85±23.76	82.24±0.97	8.12±0.78	7.74±4.58	17.17±3.9	42.65±0.28	23.43±7.73	64.23±19.55	39.60±3.22	20.39±2.0	74.51±15.46
W	-	288.17±7.05		663.47±29	172.67±29.4		491.17±19.4	278.86±16.2		513.90±17.3	87.85±8.60	

	Searsia pentheri		
	DPPH	ABTS	OH
Cru	1.76±0.15	4.25±0.22	74.69±2.87
Hex	37.35±4.58	126.03±5.78	73.93±4.93
DCM	1.93±0.22	149.40±3.54	19.02±2.7
ET	1.01±0.16	2.38±0.15	22.59±6.5
B	404.87±62.03	171.73±33.68	38.16±4.39
W	522.40±12.93	426±33.33	

N.B: Cru = crude extract, Hex = hexane fraction, DCM= dichloromethane fraction, ET = ethyl acetate fraction, B = butanol fraction, W = water fraction, DPPH = 2, 2-diphenyl-1-picrylhydrazyl, ABTS = 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid, OH = hydroxyl radical.

to cause tissue damage and dysfunction. The body immunology and other metabolic biochemical pathways generate oxidative molecules such as superoxide, hydrogen peroxide and nitric oxide as defence mechanisms or signalling agents. However, overproduction of the reactive species results in oxidative stress. Although, superoxide and hydrogen peroxide are weak oxidizing molecules, in the presence of transition metal catalysts like Fe^{3+} or Cu^{2+}, they generate hydroxyl radicals. Therefore, extracts or components with good hydroxyl ion scavenging ability can help with degenerative diseases in which this reactive species is an initiator, such as inflammatory diarrhoea.

Table 3: Cytotoxicity, linoleic acid peroxidation and lipoxygenase inhibition of acidified 70% acetone crude extracts

Plant species	Cytotoxicity (LC_{50} (µg/ml))	Linoleic acid peroxidation inhibition (LC_{50} (µg/ml))	Lipoxygenase enzyme inhibition (LL_{50} (µg/ml))
Ozoroa mucronata	741.91±44.20	13.95±2.23	32.02±12.00
Ozoroa paniculosa	16.58±1.85	25.2±8.10	286±94.40
Searsia leptodictya	25.09±2.40	40.45±13.38	122.67±27.86
Searsia pendulina	22.30±2.46	30.21±5.49	-
Searsia pentheri	50.62±4.33	25.53±6.20	97.61±21.71
Berberine chloride (positive control)	4.74±0.41		

Lipid peroxidation (LPO) is a chain reaction process of radical attack on the membrane and other phospholipid constituents of the cell. The thiobarbituric method determines the amount of peroxide in the reaction medium, as peroxide is the main product generated in lipid peroxidation. The dose-dependent inhibition of linoleic acid peroxidation processes of the crude extracts are presented in Table 3. The results expressed as LC_{50} range from 13.95±2.23 to 40.45±13.38 µg/ml. The inhibition of lipid peroxidation may be due to termination of the radical chain reaction after its propagation by scavenging highly toxic lipid peroxy radicals. Peroxidation of membrane phospholipids, including intestinal epithelial tissue, can distort the assembly of the membrane, causing changes in fluidity and permeability, alterations of ion transport and inhibition of metabolic processes (Nigam and Schewe, 2000). Many cytolytic compounds such as reactive aldehyde products, including malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE), 4-hydroxy-2-hexenal (4-HHE) and acrolein are produced as metabolites of lipid peroxidation (Esterbauer *et al*., 1991). These metabolites inflict injury and cause malfunctioning of the tissues. In addition, LPO generate more ROS, causing exacerbation of oxidative stress. Combined effects of LPO deleterious mechanisms in the GIT results in intestinal inflammation (Stojiljković *et al*., 2009), and consequently diarrhoea unless efficient inhibition mechanisms of the

processes are in place. The good LPO inhibitory activity of the phenolic-enriched extracts of the *Ozoroa* and *Searsia* species tested in this work indicate that the plant have potential as supportive therapy in diarrhoea episodes.

3.5. Inhibition of soybean 15-lipoxygenase

All the extracts had poor anti-inflammatory activity when evaluating percentages of lipoxygenase inhibition, except *O. mucronata*, which displayed moderate activity (IC_{50} value: 32.02 ± 12.00 µg/ml). The IC_{50} values of the *O. paniculosa* and *S. leptodictya* against the soybean 15-lipoxygenase enzyme were higher than 100 µg/ml compared to the positive control (quercetin) as presented in Table 3. The lipoxygenases have been implicated in a number of pathological states, and LOX products stimulate neutrophil migration, increase adhesion of leukocytes to endothelial cells, cause smooth muscle contraction, increase vascular permeability, and increase ion and mucus secretion (Eberhart and Dubois, 1995). Thus, inhibition of LT synthesis may be useful for the treatment of human and animal inflammatory conditions. Inhibition of soybean 15-lipoxygenase is generally regarded as predictive for inhibition of the mammalian enzyme (Gleason *et al*., 1995; Gundersen *et al*., 2003; Lapenna *et al*., 2003).

Compounds used as antioxidant therapy may also be effective against inflammation (Sala *et al*., 2003). The results suggest that the components of the extract responsible for the antioxidant activity are not necessarily those responsible for the anti-inflammatory activity. However, it is hypothesized that polar extracts exhibit poor LOX inhibition but good antiradical activity while non-polar extracts exhibit good LOX inhibition but poor antiradical activity. However, the ‗near absence' of 15-lipoxygenase inhibitory activity in this study does not imply that the extracts do not had any anti-inflammatory activity as activity may be observed at higher concentrations. It is important to note that inflammation involves a multiple cascade of events, involving not only the 15-lipoxygenase pathway, but also the 5- and 12-lipoxygenases, as well as cyclooxygenase-1, cyclooxygenase-2, cytochrome P_{450} and epoxygenase pathway. It is possible for the extracts to exhibit good activities against some of these other inflammatory pathways in addition to their good antioxidant properties.

3.6. CytotoxicityCytotoxicity of the phenolic-enriched crude extracts is presented as LC_{50} in Table 3.

The extracts had some level of toxicity, except for *Ozoroa mucronata* with an LC_{50} of 741.91 µg/ml and the most toxic was *O. paniculosa* (LC_{50}=16.58 µg/ml) followed by *Searsia pendulina* (LC_{50}=22.30

µg/ml). The LC_{50} values for *S. leptodictya* and *S. pentheri* were 25.09 and 50.62 µg/ml respectively. The toxicity threshold level for extracts is LC_{50} less than 20 µg/ml (Zirihi *et al*., 2005), therefore, the phenolic-enriched extracts of *O. paniculosa*, *S. pendulina*, and *S. leptodictya* need to be applied orally with caution unless the fraction(s) containing toxic component(s) is identified and removed. It is interesting to note that the toxicity levels of the two *Ozoroa* species differed so extensively, but these species may have quite different chemical compositions to account for this discrepancy.

4. Conclusion

Medicinal plant-derived phytochemical preparations exhibiting multiple bioactivities such as broad-based antimicrobial, antioxidative, radical scavenging and anti-inflammatory activity have potential for treating diseases with multi-factorial pathoaetiologies like diarrhoea. Crude extracts and fractions of the *Ozoroa* and *Searsia* species show high activity both as radical scavengers and lipoxygenase inhibitors, and it is possible that these activities may be involved in their putative medicinal properties. The cytotoxicity of the crude extracts are low compared with berberine chloride (Sigma) which was used as a positive control, indicating that the plants have some level of safety though their use needs to be monitored (dosage) to avoid exceeding the toxicity limits. However, in vivo studies are necessary to determine the therapeutic and toxic effects of these extracts in normal physiological situations as in vitro studies results are not usually a true, complete reflection of drug action.

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