

Evaluation of Anti-Microbial, Anti-Inflammatory and Anti-Oxidative Properties *Artemisia afra*, *Gunnera perpensa* and *Eucomis autumnalis*

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Abstract

Antimicrobial, anti-inflammatory and free radical scavenging activities of crude root extract fractions from *Artemisia afra*, *Gunnera perpensa* and *Eucomis autumnalis* were determined. Minimum inhibitory concentration was determined by using micro dilution method. In order to assess antioxidant scavenging capacity of plant extracts and fractions, 2, 2-di (4-tert-octylphenyl)-1-picrylhydrazyl and 2, 2'-azinobis (3-ethylbenzothiazoline)-6-sulfonic was used as substrate. Anti-inflammatory activity of the plant extracts against 15-soybean lipoxygenase enzyme was evaluated by measuring change in absorbance at 234 nm using linoleic acid as substrate. The highest activity was obtained from methanol fraction of *Gunnera perpensa* with EC₅₀ value of 1.069 µg/ml against 2,2-di (4-tert-octylphenyl)-1-picrylhydrazyl. *Eucomis autumnalis* crude and acetone fraction displayed DPPH[•] free radical scavenging activity of EC₅₀ of 2.891 µg/ml and 2.41 µg/ml respectively. *Artemisia afra* crude fraction and fractions of acetone and methanol displayed activity (EC₅₀ for DPPH[•] radical, 2.113 µg/ml with crude 4.393 µg/ml with acetone fraction, 4.715 µg/ml, with methanol fraction and with ABTS^{•+} radical cation, 6.447 µg/ml and 6.208 µg/ml from crude and methanol fraction respectively). The antioxidant properties of the extracts increased with the polarity of the fractions. *Gunnera perpensa* crude extract and fractions displayed antimicrobial properties with the methanol fraction being the most active with an EC₅₀ of 80 µg/ml, against *Pseudomonas aeruginosa* and EC₅₀ of 160 µg/ml against *Candida albicans*. *Artemisia afra* acetone and methanol fractions displayed inhibitory activities of 20 µg/ml against *Escherichia coli* and good-moderate activity ranging 160-320 µg/ml for the crude extract. *Eucomis autumnalis* had activities ranging 160-320 µg/ml by the crude extract and fractions against the organisms tested except for crude extract activity against *Escherichia coli* of 630 µg/ml. The activities validate claims by the traditional healers use for cure offering possible alternative as dietary supplements to the management of inflammation related conditions.

Keywords: Minimum inhibitory concentrations (MIC); Reactive oxygen species (ROS); Reactive nitrogen species (RNS)

Abbreviations: ABTS^{•+}: 2,2'-Azinobis(3-ethylBenzothiazoline)-6-Sulfonic acid radical; DPPH[•]: 2,2-Di(4-tert-octylPhenyl)-1-PicrylHydrazyl radical; 15-LOX: 15-Soybean Lipoxygenase

Introduction

Infectious disease is an illness resulting from the invasion of the host species by a pathogenic microbial agent, and outcome of the disease depends on the degree of success of the invading pathogen and immune system of the host [1]. They are considered a major threat to human health, because of the unavailability of vaccines or limited chemotherapy even in the developed parts of the world, although developing countries are carrying the major part of the burden Sub Saharan African countries, including South Africa are mostly affected by respiratory infections, diarrhea, HIV/AIDS, tuberculosis and malaria [2,3] The continued resurfacing of antibiotic-resistant infections drives research to produce better drugs to combat the more resistant pathogens [4]. Auto immune diseases such as lupus are becoming a major concern in both the developed and third world countries [5]. Investigating plants that could be included in affected people's diet would assist in managing such diseases.

Fighting infections with natural products will be more advantageous and affordable in the southern African context over the conventional drugs to most patients since they are readily available in the environment in which they live and some are cultivated to ensure product quality and safety [6]. Antimicrobials of plant origin have enormous therapeutic potential in the treatment of infectious diseases [7]. They are effective as well as have the advantage of mitigating many of the side effects that are often associated with synthetic antimicrobials

[8]. The beneficial medicinal effects of plant phytochemicals typically result from the synergism of secondary products present in the plant [9].

Infection is usually accompanied by microbial invasion followed by the occurrence of oxidative stress and serious inflammation [7]. Activation and proliferation of pro-inflammatory cytokines in respiratory epithelial cells and macrophages are down regulated by supplying and maintaining sufficient levels of exogenous and endogenous antioxidants [10].

Phytochemicals from medicinal plants have provided unlimited opportunities for new drug discoveries because of their inherent chemical diversity, which has prompted a continuous search for plant sources with medicinal value [11,12]. *Artemisia afra* Jacq. ExWilld. (Asteraceae), *Gunnera perpensa* L. (Gunneraceae) and *Eucomis autumnalis* (Mill) (Hyacinthaceae) Chitt are medicinal plants mainly used in the Mabandla village of UMzimkhulu Local Municipality,

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Kwa-Zulu Natal, South Africa for the treatment of infectious and some inflammatory mediated diseases (Table 1). Therefore, it is of interest to observe if these plant extracts can affect these mechanisms of pathogenic changes that lead to diseases, if over-expressed. This study focuses on the antimicrobial, antioxidant and anti-inflammatory properties of selected plant extracts, namely their ability to directly scavenge free radicals; their effects on soya bean derived 15 LOX inhibitory activities and inhibition of microbial growth (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia Coli*, *Pseudomonas aeruginosa* *Aspergillus fumigatus*, and *Candida albicans*).

Material and Methods

Plant selection and collection

Artemisia afra, *Gunnera perpensa* and *Eucomis autumnalis* (Table 1) from Mabandla village of UMzimkhulu Local Municipality, KwaZulu-Natal, and South Africa were used for the study. They were identified and collected by the aid of the head of traditional healers from the village (Mr. Sanoyi Paulos Dlamini, Traditional healer, KwaZulu-Natal 2009). Authentication of plants was carried out at South Africa National Biodiversity Institute, Pretoria and their voucher specimens were deposited in the Pretoria National Herbarium.

Plant treatment

Plant root and bulb materials were washed in water, air dried at room temperature for three weeks and ground to powder using a Lasec Polymix PX-MFC 90D crusher. The dried pulverized material was stored in glass containers in a cool dry place. The extraction was carried out by shaking the powder (200 g) in a ratio of 1 g to 10 ml solvent for 6 h on a Remi R S-12R shaker (Rajendra Electrical India Ltd, India) at 200 rpm [13]. Excess solvent was recovered on a rotary vapor until the

extract was concentrated. The concentrated slurry was dried at room temperature in the fume hood. The crude plant extract was stored in the fridge at 4°C. Fractions of different polarities were then produced by solvent/ solvent extraction from the crude extracts as shown in the protocol in Figure 1. Percentage yields for the crude, and the different fractions were reported in Table 2.

Quantitative evaluation of the biological activities of the plant extracts

Determination of antimicrobial activity: The following microorganisms were used as test organisms in the screening: two Gram-positive reference strains (*Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212)), and two Gram-negative reference strains (*Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli*

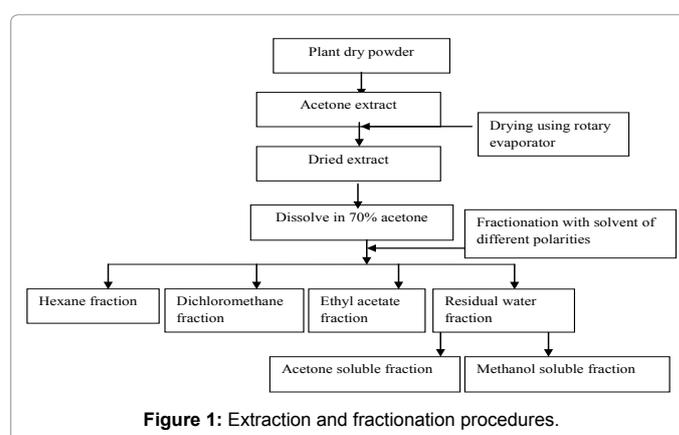


Figure 1: Extraction and fractionation procedures.

Botanical name and specimen Voucher numbers	South African name	Biological activities and isolated compounds from previous studies	Traditional medicinal use
<i>Artemisia afra</i> : 3166-4001	Umhhlonyane (Xhosa), Mhloniyane (isiZulu), Lanyana (Sesotho), Lengana (Tswana), African wormwood (English), and Wilde also (Afrikaans). Van Staden et al., (2009)	Among the volatile secondary metabolites, there are 83 monoterpenoids, 8 and 50 sesquiterpenes and under the non-volatile, 2 sesquiterpens, 7 glaucolides, 6 guaianolides, 4 triterpenes, 6 long chain alkanes, 5 coumarins, 1 organic acid, 1 glycoside, 11 flavonoids (Watt and Breyer- Branwijk, (1962); Van Staden et al., 2009) Liu et al, 2008).	Rhizomes and roots are used for, colds, influenza, stomach and respiratory ailments, rheumatism and wounds (Somova et al (2001)
<i>Gunnera perpensa</i> : 2537-1	Ugobho (isiZulu)	2-methyl-6-(3-methyl-2-butenyl) benzo-1,4-quinone and -hydrxxy-8-methyl-2,2-dimethyl-2H-benzopyran from <i>Gunnera perpensa</i> . Respective MIC range = 70 µg/ml and 75 µg/ml of isolate (Drewes et al., 2005)	Roots extracts are used to aid pot-partum expulsion, relief of dysmenorrhea, psoriasis and cure for female infertility (Kaidoo, T. L., (1997) Van Wyk et al, (1997))
<i>Eucomis autumnalis</i> : 3790-4001	Umathinga (isiZulu) Khapumpu (Sesotho)	3,7-dihydroxy-1methyl-6H-dibenzo[b,d.]pyran-6-one (autumnariol) 3,4,7-trihydroxy-1-methyl-6H-dibenzo[b,d]pyran-6-one (autumnariol) 5,7-dihydroxy-8methoxy-3-(4'-methoxybenzyl)-4-chromanone(4'-O-methyl-3,9-dihdropunctatin) 5,7-dihydroxy-6-methoxy-3-(4'-hydrxxybenzyl)-4-chromanone(3,9-dihydrocucomnalin or 3,9-dhydroautumnalin) 3,5,7-trihydroxy-3-(4'-methoxybenzyl)-4-chromanone(eucomol) (E)-5,7-dihydroxy-6-methoxy-3-(4'-hydroxybenzylidene)-4-chromanone(autumnalin) (Z)-5,7-dihydroxy-6-methoxy-3-(4'-hydroxybenzylidene)-4-chromanone(autumnalin) 5,7-dihydroxy -8-methoxy-3-(4'-methoxybenzylidene)4-chromanone(4'-O-methylpunctatin) (23S)-17,23-epoxy-3β,29-dihydroxy-27-norlanost-8-ene-15,24-dione(eucosterol) (23S)-17,23-epoxy-3β,16β,29-trihydroxy-27-norlanost-8-ene-15,24-dione(16β-hydroxyeucosterol) 3β-hydroxy-4β-hydroximethyl-4α,14α-dimethy-15-oxo-24-nor-5α-chola-8,16-dien-23-oic acid R(-)-2-(4'-hydroxybenzyl)-malic acid (eucomic acid) (Taylor and van Staden, (2001); Stafford et al., (2004).)	Bulbs are used for a variety of ailments including urinary diseases, stomach ache and fevers. (Jager et al, (1996); Taylor and van Staden, (2001); Stafford et al., (2004).

Table 1: Ethno pharmacological information of the plant use in this study.

Plant species	C	H	DCM	ET	AC	Met	W
<i>Artemisia afra</i>	2.73	0.55	12.08	1.09	57.69	1.09	
<i>Gunnera perpensa</i>	6.45	1.70	0.39	0.46	4.03	40.23	38.20
<i>Eucomis autumnalis</i>	2.66	0.19	4.71	0.56	0.56	56.12	

C= crude extract, H= hexane fraction, DCM= dichloromethane fraction, ET = ethyl acetate fraction, AC = acetone fraction and Met= methanol fraction, W= water fraction

Table 2: The % yield of the crude and fractions of the extracts.

(ATCC 25922)). In addition, two pathogenic clinical isolate of fungi (*Candida albicans* and *Cryptococcus neoformans* (supplied by the Phytomedicine laboratory, University of Pretoria, South Africa) were also employed as test organisms. Stock cultures were maintained at 4°C on slopes of nutrient agar. The cultures were diluted to achieve optical densities corresponding to 2.0×10^6 colony forming units (CFU/ml) for bacterial and 2.0×10^5 spore/ml for fungal strains.

Antimicrobial assay

Inoculate of the bacteria were made from 24 h Mueller–Hinton broth (Sigma) cultures and standardized using 0.5 McFarland standards comparable to a bacterial suspension of 1.0×10^8 cell/ml. Crude extracts and fractions of various polarities were reconstituted in 70% acetone (10 mg/ml). Gentamicin and 70% acetone were used as the positive and negative controls respectively. Minimum inhibitory concentration (MIC) values of the samples and controls were determined using a micro-well dilution method over three independent experiments [13]. The sterile 96-well microtitre plates were prepared with 100 µl of Millipore filtrated water per well and a two-fold serial dilution was prepared from 100 µl crude extracts or fractions in 70% acetone (10 mg/ml) added to the first well. An aliquot (100 µl) of the standardized inoculum was added to wells and the plates were incubated overnight at 37°C. The final concentration in wells ranged from 2.5 to 0.019 mg/ml. Bacterial growth was determined by adding 40 µl p-iodonitrotetrazolium (INT) violet (Sigma, UK) 2 mg/ml in water. The extracts were then incubated for 30 min and bacterial growth was determined by INT formazan production.

DPPH radical scavenging assay: The DPPH scavenging activity of the extracts was measured from the bleaching of the of purple DPPH solution in methanol [14]. Twenty microliter extract or reference standards in methanol (500-1.9 µg/ml) were added to 180 µl of 6.5×10^{-5} M DPPH methanol solution in 96-well microtitre plates. The plates were incubated at room temperature in the dark for 30 min after which the optical density was recorded at 517nm using a microtitre plate reader. For the positive control, 160 µl solution of DPPH in methanol was mixed with 40 µl methanol. Trolox and ascorbic acid were used as positive control. The assay was carried out in triplicate wells on each plate and with three independent experiments for 30 min. Percentage inhibition of free radical DPPH was calculated thus: Percentage inhibition (%IP) = $[(A_{t=0 \text{ min}} - A_{t=30 \text{ min}})/A_{t=0}] \times 100$. The EC₅₀ value (µg/ml) (concentration of extract required to inhibit DPPH radical formation by 50%) was determined via extrapolation from best fit non-linear analysis, using the Graph Pad Prism software (California, USA).

ABTS⁺ scavenging assay: The scavenging activity against ABTS cation radical was assayed to determine the Trolox Equivalent Antioxidant Capacity value (TEAC) using a reported method [15] The blue-green ABTS⁺ was produced by reacting 7 mM ABTS and 2.45 mM potassium persulfate in water and the solution was diluted with methanol to a final absorbance of 0.7 ± 0.02 at 734 nm. Forty microliters of antioxidants solution were added to 160 µl ABTS⁺ solution, mixed vigorously, and measured promptly at the absorbance 734 nm against

a blank solution of 40 µl solvent and 160 µl ABTS⁺. For the positive control, 160 µl solution of ABTS⁺ in methanol was mixed with 40 µl methanol. Trolox and ascorbic acid were used as positive control. The assay was carried out in triplicate wells on each plate and with three independent experiments. Percentage inhibition of free radical ABTS⁺ was calculated thus: Percentage inhibition (%IP) = $[(A_{t=0 \text{ min}} - A_{t=30 \text{ min}})/A_{t=0}] \times 100$. The EC₅₀ value (µg/ml) (concentration of extract required to inhibit ABTS⁺ radical formation by 50%) was determined via extrapolation from best fit non-linear analysis, using the Graph Pad Prism software (California, USA).

In vitro lipoxygenase inhibition assay: The soybean derived 15- lipoxygenase type I-B (15-LOX) was evaluated as described by Malterud and Rydland (2000) [16]. The reaction mixture contained 20 mM borate HCl buffer (pH 8.8), test compound, linoleic acid solution (40 µM), and soybean lipoxygenase (131.000 U/mL). For inhibition experiments, 15-LOX was incubated with inhibitor for 5 min in a 10 mm path length cuvette at room temperature. The reaction was then started by the addition of 10 µl of linoleic acid solution (substrate). The control was run in DMSO the solvent used to dissolve the inhibitor. After 5 min, the absorbance was read at 234 nm as a measure of the conjugated diene produced. For the determination of IC₅₀, the inhibitor was varied at the constant substrate concentration of 80 µM linoleic acid. The % inhibition of 15-LOX activity by extract was calculated from the absorbance values at 234 nm at the end of 5 min: Inhibition (%) = $(A_c - A_s/A_c) \times 100$ where A_c and A_s were absorbance of the control (without test sample) and the sample, respectively. All measurements are an average of triplicate measurements and expressed as the 50% inhibition concentration value (IC₅₀) from the control without inhibitor. The enzyme solution was kept on ice, and controls were measured at intervals throughout the experimental periods to ensure that the enzyme activity was constant.

Results and Discussion

The antioxidant scavenging capacity of plant extracts and fractions were determined using the DPPH[•] and ABTS[•] free radical scavenging assays and the results were expressed as EC₅₀ as presented in Table 2 and antimicrobial assays in Figures 2-4. The results for the 15-LOX activities are summarized in Table 3. The percentage yield (Table 2) of fractions showed that for *Gunnera perpensa* and *Eucomis autumnalis* more than 50% of total extract for each plant was in the polar solvents of methanol and water.

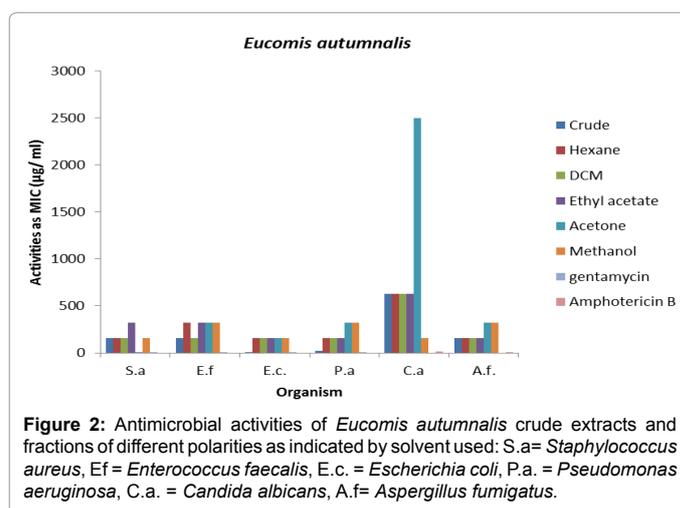


Figure 2: Antimicrobial activities of *Eucomis autumnalis* crude extracts and fractions of different polarities as indicated by solvent used: S.a= *Staphylococcus aureus*, E.f = *Enterococcus faecalis*, E.c. = *Escherichia coli*, P.a = *Pseudomonas aeruginosa*, C.a. = *Candida albicans*, A.f= *Aspergillus fumigatus*.

Antimicrobial assays

Gunnera perpensa crude extract and fractions displayed high activities (Figure 4) against the organisms tested using the micro plate

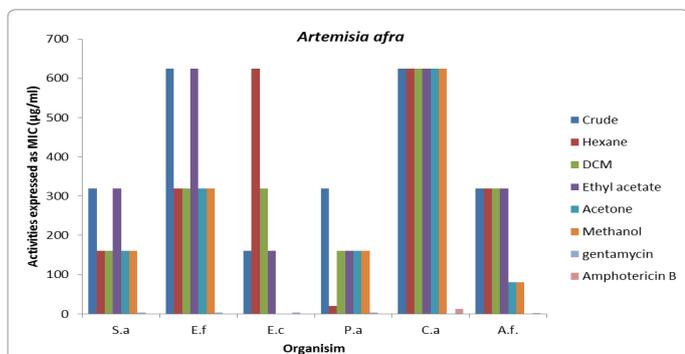


Figure 3: Antimicrobial activities of *Artemisia afra* crude extracts and fractions of different polarities as indicated by solvent used: S.a= *Staphylococcus aureus*, E.f= *Enterococcus faecalis*, E.c. = *Escherichia coli*, P.a. = *Pseudomonas aeruginosa*, C.a. = *Candida albicans*, A.f= *Aspergillus fumigatus*.

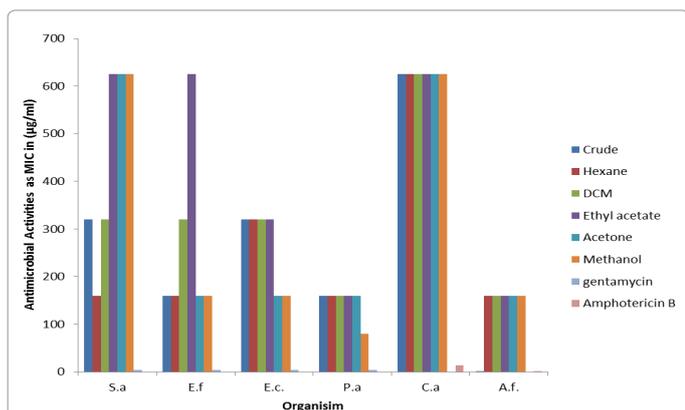


Figure 4: Antimicrobial activities of *Gunnera perpensa* crude extracts and fractions of different polarities as indicated by solvent used: S.a= *Staphylococcus aureus*, E.f= *Enterococcus faecalis*, E.c. = *Escherichia coli*, P.a. = *Pseudomonas aeruginosa*, C.a. = *Candida albicans*, A.f= *Aspergillus fumigatus*.

plant species	EC ₅₀
G. Perpensa	81.18
E. autumnalis	42.76
A. afra	21.84

Table 3: Soya bean based 15 LOX inhibitory activity expressed as EC₅₀ values for the crude in 25 µg/ml.

Sample	A. afra				G. perpensa				E. autumnalis			
	DPPH		ABTS		DPPH		ABTS		DPPH		ABTS	
	EC ₅₀	R ²										
Cr	2.113	0.5043	6.447	0.9697	1.069	0.346	32.49	0.9302	2.891	0.7544	12.18	0.8771
H	29.5	0.4448	96.66	0.8541	46.88	0.5588	646.5	0.4257	21.67	0.5432	929.40	0.6797
DCM	36.03	0.8452	89.08	0.9166	48.71	0.8813	164.9	0.6992	87.71	0.9615	130.00	0.726
ET	199.3	0.6795	68.57	0.9308	57.67	0.7889	11.39	0.8678	95.32	0.8442	24.44	0.9709
Ac	4.393	0.9288	32.95	0.8637	2.795	0.2938	261.5	0.4348	2.461	0.7564	24.44	0.9709
Met	4.715	0.8696	6.028	0.8994	2.795	0.2938	292.9	0.4577	6.621	0.7286	15.17	0.9446

Cr=crude extract, H=hexane fraction, DCM=dichloromethane fraction, ET=ethyl acetate fraction, Ac=acetone fraction, Met=methanol fraction, Positive standards: trolox = 0.04 ± 0.001, ascorbic acid=0.04 ± 0.001

Table 4: Free radical scavenging activity of the crude extract and fractions of varied polarities (µg/ml).

dilution method. Ethno medicine is one method of discovering new drugs which may offer a solution to cure for disease caused by pathogens which have resistance to known drugs [17]. In this investigation, *Gunnera perpensa* extracts and fractions displayed good broad based antimicrobial activities against the organisms tested using the micro plate dilution method. The most active fraction was the methanol fraction (80 µg/ml) against *Pseudomonas aeruginosa*. *Gunnera perpensa* fractions (hexane, DCM, ethyl acetate, acetone and methanol) have good activity of 160 µg/ml against *Candida albicans*. The antimicrobial activities of water, ethyl acetate and ethanol fractions of the root extract of *Gunnera perpensa* against some bacteria and fungi has been reported before for *Bacillus subtilis*, 12.5 mg/ml by Buwa and Van Staden [18]. Crude water extract of *Gunnera perpensa* have antimicrobial activity against *Escherichia coli* (0.78 mg/ml); *Klebsiella pneumoniae* (0.78 mg/ml); and *Candida albicans* (25 mg/ml) while the corresponding ethyl acetate and ethanol fractions were less active (Buwa and van Staden. However, the reported values are not considered to be significantly high by the phytomedicine program. This explains the use of the plant by South African Traditional healers as treatment against venereal diseases [19]. Drewes et al., isolated 1,4 -benzoquinone derivatives, which were identified as 2, methyl-6-(3-methyl-2-butenyl) benzo-1,4- quinone (MIC of 18 µg / ml against *Bacillus cereus*) and 3-hydroxy-2-5-(3-methyl-2-butenyl)benzo-1,4- quione (MIC of 37 µg / ml against *Candida albicans* and 75 µg / ml and against *Cryptococcus neoformans*) the active antimicrobial components against the bacteria and fungus [19]. Although this characterization is not exhaustive, it explains why *Gunnera perpensa* is so active against the organisms tested (Table 4). South African traditional healers use *Gunnera perpensa* aqueous decoction to inducing labor, facilitating the expulsion of placenta and relief of dysmenorrhea [20]. *Gunnera perpensa* also displayed antinociceptive and anti-inflammatory activity [21].

Artemisia afra acetone and methanol fractions have good inhibitory activities (20 µg/ml) against *E. coli* and good-moderate activity ranging 160-320 µg/ml for the crude extract and all fractions against the organisms tested (Figure 3) against *Aspergillus fumigatus* and *Staphylococcus aureus*. *Artemisia afra* is used in traditional medicine in the treatment of a variety of diseases ranging from respiratory infections to dysmenorrhea, diabetes and malaria [22,23]. The plant rich in terpenes such as artemisia alcohol, camphene, camphor and artemisia ketone [23] and displayed a variety of biological activities (using disc diffusion method) against bacteria such as *Staphylococcus aureus* (MIC 2.0 mg/ml), *Mycobacterium smegmatis* (MIC 1.9 mg/ml) fungi such as *Candida albicans* (% minimum inhibitory percentage of 0.25) and protozoa such as *P.falciparum* (IC₅₀ of 4,4 µg/ml) tested in the investigations [23]. This explains why the percentage yield for the polar fraction from *Artemisia afra* (Table 2) was lower compared to the other two plants. *Artemisia afra* is used to treat various inflammatory related diseases as displayed in Table 4.

Eucomis autumnalis displayed good to moderate (160-320 µg/ml) activity from the crude extract and fractions against the organisms tested except for the crude extract against *E. coli* in which the activity of 630 µg/ml, which was low. The various antimicrobial activities demonstrate the validity of the healing capacities of the plant that the traditional healers of the community claim it possesses. Invasion of the body by organisms such as *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa* and other such pathogens can result in chronic inflammation [7].

Antioxidant and 15-LOX activity

The highest activity is obtained from methanol fraction of *Gunnera perpensa* with EC₅₀ value of 1.069 mg/ml against DPPH[•], EC₅₀. The lowest activity was displayed by hexane fraction from *Eucomis autumnalis* (929.4 µg/ml). *Gunnera perpensa* crude and fractions were more active with DPPH[•] (57.67 mg/L) than with ABTS^{•+} (11.39 µg/ml) except for the ethyl acetate fraction. *Eucomis autumnalis* crude and acetone fraction displayed high DPPH[•] free radical scavenging activity of EC₅₀ of 2.891 µg/ml and 2.41 µg/ml respectively. ABTS^{•+} free radical activity for the plant sample was generally lower for all samples except for the ethyl acetate moderate activity of 24.44 µg/ml. *Artemisia afra* crude extract and methanol fraction DPPH[•] and ABTS^{•+} free radical scavenging activity as displayed by the curves on Figures 2 to 4 and the EC₅₀ values. High activity is also displayed by the acetone fraction DPPH[•] anti-radical activity *Gunnera perpensa* had the highest amount of yield of extract of 6.2% and 38% of the *Gunnera perpensa* extract was water soluble. Acetone and methanol extracts were also much higher for *Gunnera perpensa* and *Eucomis autumnalis* extracts. The minimum inhibitory concentrations of the methanol crude extracts and fractions were presented in Table 4.

Artemisia afra forms part of southern African indigenous medicines with a range of applications including treatment of conditions associated with chronic inflammation such as rheumatism fever, diabetes, asthma, malaria and wounds [23,24]. *A. afra* volatile oils have been reported to demonstrate antioxidant activity when sprayed by DPPH[•] during a TLC screening method of evaluation and during non-enzymatic lipid peroxidation in liposomes [24]. In this investigation, high anti-oxidant activities were displayed (Table 5). Simelane et al. 2010 have reported DPPH and ABTS scavenging activity IC₅₀ of 1.6 µg/ml for DPPH and 0. µg/ml for ABTS values which are comparable (EC₅₀ of 1-2.8 µg/ml) to those obtained in this study.

Several reports on the biological activities of *Artemisia afra* have been made before including the isolation and characterization of volatile and nonvolatile metabolites [25]. The fact that *Artemisia afra* crude fraction and fractions of acetone and methanol displayed high activity (EC₅₀ for DPPH[•] radical, 2.113 µg/ml with crude 4.393 µg/ml with acetone fraction, 4.715 µg/ml, with methanol fraction and EC₅₀ with ABTS^{•+} radical cation, 6.447 µg/ml and 6.208 µg/ml from crude and methanol fraction respectively). The soya bean 15 Lox inhibitory activity was relatively low with an EC₅₀ of 21.8 in 25 µg/ml of crude compared to that of *Eucomis autumnalis* and *Gunnera perpensa* (Figure 3).

Eucomis autumnalis crude fraction displayed EC₅₀ with ABTS^{•+} radical cation, 12.89 µg/ml and 6.21 µg/ml against DPPH (Table 4). *Eucomis autumnalis* is used to treat various inflammation related diseases ranging from post-operative recovery, healing fractures to treating syphilis [26]. *Eucomis autumnalis* bulbs displayed a 58% 15-LOX inhibition activity during this study (Table 3), which was moderate. In previous reports on *E. autumnalis* anti-inflammatory activity, its bulbs inhibited prostaglandin synthesis 90% relative to

indomethacin (0.5 µg/ml) at 65% and also displayed selective COX-2 activity [26,27]. *Gunnera perpensa* acetone, methanol fraction and crude extract displayed high activity of DPPH free radical scavenging of 2.795 µg/ml (Table 4) illustrating ant oxidant capability. In previous research, *Gunnera perpensa* methanol extracts demonstrated strong ABTS^{•+} (78.45) and DPPH[•] (78%) scavenging at a concentration of 50 µg/ml [21]. *Gunnera perpensa* EC₅₀=81.18 µg/ml exhibited some soya bean 15-LOX inhibitory activity. *Gunnera perpensa* methanol and aqueous extracts has been demonstrated to possess analgesic and anti-inflammatory activity before [21]. In previous research, *Eucomis autumnalis* also inhibited totally prostaglandin synthesis and displayed preferential COX-2 inhibitory activity [6] and these pharmacological properties make the plant suitable for treating inflammation and would be ideal as dietary supplements to sufferers of autoimmune diseases and other inflammation related conditions.

Conclusions

The plants in this investigation exhibited good anti-microbial activity on the organisms tested. *Gunnera perpensa* and *Eucomis autumnalis* methanol fractions exhibited high free radical activity against both ABTS^{•+} and DPPH[•] radicals displaying some nutritional value. Biological activities of fractions of different polarities (from hexane to methanol) were highlighted in this study are compared to reports that have been made before of the same plants but different fractions from previous studies. These plants are used for treating the various disorders related to inflammation by the traditional Zulu practitioners (Table 1). The activities also validate claims by the traditional healers' use of plants for cure. These results were produced from *in vitro* assays using artificial radicals ABTS, DPPH, microbes, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli*, *Candida albicans* and *Aspergillus fumigatus* therefore the plant extracts and fractions activity *in vivo* still need to be carried out in order to fully validate their activities as antioxidants in the body. The soya bean derived 15-LOX enzyme inhibitory activities were used only to serve as a guideline to demonstrate inhibitory ability. *In vivo* experiments using human derived 15-LOX still need to be carried out to provide a better picture of the inhibitory activities of the crude extracts reported here.

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