applied sciences

MDPI

Article

Chemical Constituents and Biological Activities of Essential Oils of *Hydnora africana* Thumb Used to Treat Associated Infections and Diseases in South Africa

Olubunmi Abosede Wintola and Anthony Jide Afolayan *

Medicinal Plants and Economic Development (MPED) Research Centre, Department of Botany, University of Fort Hare, Alice 5700, South Africa; olubunmiwintola@yahoo.com

* Correspondence: aafolayan@ufh.ac.za; Tel.: +27-822-022-167; Fax: +27-866-282-295

Academic Editor: Hidenori Otsuka

Received: 21 September 2016; Accepted: 1 December 2016; Published: 27 April 2017

Abstract: *Hydnora africana* (*HA*) Thumb is a member of the genus *Hydnora.* The roots are used in the treatment of infectious diseases in South Africa folk medicine. Though the root extracts are used to treat various human diseases including inflamed throat, there is a dearth of scientific data on the biological activities of essential oil isolated from this plant in the literature. Therefore, the present study was conducted to determine the chemical components and certain biological activities of the essential oil using standard bioassay methods. The plant essential oil exhibited a moderate free radical scavenging activity that was dependent on the radical species. Similarly, the essential oil was active against the growth of all thirteen opportunistic bacteria apart from *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. The essential oil was also active against *Aspergillus niger* among all the nine fungi selected. In addition, the species is typified by substantial amounts of classes of compounds including; carboxylic acids (30.68%), terpenes (10.70%), alkyl aldehydes (4.86%) and esters (0.82%), identified as antioxidant and antimicrobial agents. The essential oil of *H. africana* could be said to have pharmacological properties, and these agents in the essential oil of *H. africana* could justify the folklore usage of this plant in the treatment of infections and related diseases.

Keywords: *Hydnora africana*; chemical composition; biological activities; infections; GC-MS

1. Introduction

Medicinal plants have contributed immensely to health care in Africa. These plants are easily accessible and cheap sources of therapeutic agents [1]. Essential oils from medicinal plants contain certain constituents that play a pharmacological role in the prevention and treatment of chronic diseases such as cancer, cardiovascular disease, diarrhoea diseases and infections [2].

Infection is the invasion of the body tissues by disease-causing agents like bacteria, virus and fungi. The host tissue's reaction to these organisms as well as the toxins produced by the organisms is attributed to inflammation in patients with HIV or AIDS, cancer, solid organ transplantation, immune deficiencies, chronic inflammatory gastrointestinal, liver, respiratory, urinary tract and other infections [3]. The bacteria associated with common infections like wounds, gastrointestinal and urinary tract infections include *Klebsiella pneumonia*, *Streptococcus pyogenes* and *Serratia mercescens* [4,5]. Essential oils are known to soothe infections by acting as antimicrobial agents, or by reducing and neutralising reactive oxygen species (ROS) generated during the pathophysiology of disease infections [6].

Medicinal plants play important roles as a source of antimicrobial and antioxidant. One of these plants is the genus *Hydnora africana* (HA) Thumb (Hydronaceae (Piperales)) (Figure 1A,B). The plant is

also known as Jackal food, Ubnklunga (Xhosa) or Umavumbuka (Zulu) [7]. It comprises 15 species in South Africa. This plant, similar to about ten species of parasitic flowering plant, lives a greater part of its life cycle underground and is unable to create chlorophyll or perform photosynthesis. In traditional medicine, the root of *Hydnora* species, tuber, fruits, leaves and fruit pulp (such as potato) is used for the treatment of infectious diseases such as dysentery, diarrhoea, amenorrhoea, bladder and kidney complaints. Other ailments include swollen glands and inflamed throat [7]. Although progress has been made in validating the chemical components and biological activities of the root parts of the plant, the chemical composition, antioxidant and antimicrobial properties of HA essential oil remain obscure.

Therefore, this study aimed at identifying the chemical constituents of *H. africana* essential oil using gas chromatography interfaced with mass spectrometry (GC-MS). We also aimed to assess its antioxidant, antibacterial and antifungal potential, and to relate our findings to its possible use in folk medicine.

(**A**) (**B**)

Figure 1. *Hydnora africana* plant parts; Source http://www.botany.org/Parasitic_Plants/. (**A**) The flower of *Hydnora africana*; (**B**) The root of *Hydnora africana* used.

2. Materials and Methods

2.1. Plant Collection and Preparation

The roots of *H. Africana* as shown in Figure 1B were collected in December 2013 at the Ntselamanzi area location in Nkonkobe Municipality, Eastern Cape Province, South Africa. This lies at the latitude and longitude as described by Masika and Afolayan [8]. The samples were collected and the specimen of the voucher (Win 2014/1) was kept in the Giffen's herbarium, University of Fort Hare, Alice, South Africa. The plant materials were washed using tap water and then stored in the refrigerator at 4 °C until it was time for essential oil extraction.

2.2. Extraction of the Essential Oil

The essential oil was isolated from *H. Africana* roots by hydro-distillation following the procedure from European Pharmacopoeia [9]. Here, 250 g of the dry roots were subjected to hydro-distillation for 3 h in an all-glass Clevenger (model and manufacturer name). Through the supply of heat to the heating mantle (50 °C), the essential oil was extracted with 4 litres of water in 3 h until no more essential oil was coming out. The average percentage yield was 0.3%. Appropriate 10% DMSO vehicles in between 80 were utilized to dissolve the essential oil for more in vitro bioassay activities.

2.3. Chemicals and Reagents Used

The chemicals used consist of 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), butylated hydroxyl toluene (BHT), vanillin, potassium persulphate, rutin, sodium nitroprusside ($Na_2[Fe(CN)_5NO]2H_2O$), glacial acetic acid (CH_3COOH), sulfanilic acid, gallic acid, tannic acid, ferric chloride ($FeCl_2$), aluminum chloride ($AlCl_3$), potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid (TCA), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), hydrochloric acid (HCL), sodium dihydrogen phosphate (NaH_2PO_4), disodium

hydrogen phosphate (Na_2HPO_4), Mueller-Hinton agar (MHA), dimethyl sulfoxide (DMSO), ascorbic acid, ciprofloxacin and amoxicillin, 2-thiobarbituric acid (TBA), potassium acetate (CH_3CO_2K), Mueller-Hinton dextrose broth (MDB), diclofenac sodium and sodium chloride (NaCl), These chemicals were bought from Merck and Sigma-Aldrich, Gauteng, South Africa. Every chemical utilised in this study was of analytical grade.

2.4. Antioxidant Assay

The antioxidative capacities of the essential oil of *H. africana* were assessed in terms of DPPH, nitric oxide, hydrogen peroxide and ABTS inhibitory assays.

2.4.1. 1,1-diphenyl-2-picrylhydrazyl DPPH Radical Scavenging Activity Assay

The method of Liyana-Pathiranan and Shahidi [10] was employed to determine DPPH free radical scavenging activity. A solution of 0.135 mM 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in methanol was briefly prepared. Briefly, 1.0 mL of this solution was mixed with 1.0 mL of the HA oil and standard drugs (BHT and gallic acid) (0.025 to 0.5 mg/mL). The reaction mixture was then vortexed and put in the dark at room temperature for a period of 30 min. The absorbance of the mixture was measured using a spectrophotometer at 517 nm. All tests and analysis were run in triplicates. The plant extract's scavenging ability was then calculated using the following equation:

$$\text{DPPH Scavenging activity (\%)} = [(\text{Abs control} - \text{Abs sample})/(\text{Abs control})] \times 100$$

where Abs control is the absorbance of DPPH + methanol and Abs sample is the absorbance of DPPH radical + sample (sample or standard).

2.4.2. 2,2′-Aazino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) Radical Scavenging Assay

The method described by Oyedemi et al. [11] was adopted for the determination of ABTS scavenging activity. The stock solution of 7 mM ABTS solution and 2.4 mM potassium persulfate solution was prepared. The working solution was prepared by mixing the two stock solutions in equal proportions and then keeping it in the dark room for 12 h. The solution was then diluted by mixing 1 mL $ABTS^+$ solution with 60 mL of methanol to get an absorbance of 0.708 ± 0.001 units at 734 nm with the use of the spectrophotometer. The plant's essential oil (1 mL) and the controls were allowed to react with 1 mL of the $ABTS^+$ solution and the absorbance was taken at 734 nm after 7 min using a spectrophotometer. The $ABTS^+$ scavenging capacity of the essential oil was then compared with that of the standards. The percentage inhibition was then calculated as follows:

$$\text{Inhibition \%} = [(\text{A blank} - \text{A sample})/\text{A blank}] \times 100 \quad (1)$$

where A blank is the absorbance of ABTS radical + methanol used as control and the A sample is the absorbance of ABTS radical + sample extract/standard.

All the tests were carried out in triplicate. The activity was expressed as 50% inhibitory concentration (IC_{50}). The lower the IC_{50} value, the higher the antioxidant activity.

2.4.3. Nitric Oxide Scavenging Activity Assay

The nitric oxide radical scavenging activity was determined by the modified method described by Oyedemi et al. [11]. A volume of 2 mL of 10 mM of sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4) mixed with 0.5 mL of plant extracts and standards, separately, to make different concentrations from 0.025 to 0.5 mg/mL. The mixture was incubated at 25 °C for a period of 150 min after which 0.5 mL of incubated solution was mixed with 0.5 mL of Griess reagent (1.0 mL of sulfanilic acid reagent (0.33% prepared in 20% glacial acetic) acid at room temperature for 5 min with 1 mL of naphthylenediamine dichloride (0.1% w/v). After incubating the mixture was for

30 min and taking absorbance at 540 nm, the amount of nitric oxide radicals inhibited by the essential oil was calculated by the use of the equation below:

$$\text{NO radical scavenging activity (\%)} = [(\text{Abs control} - \text{Abs sample})/(\text{Abs control})] \times 100$$

where Abs control is the absorbance of NO radical + methanol and the Abs sample is the absorbance of NO radical + extract or standard.

2.4.4. Hydrogen Peroxide (H_2O_2) Scavenging Activity Assay

The H_2O_2 inhibition activities of the extracts were assessed by the method of Oyedemi et al. [11]. A solution of hydrogen peroxide (4 mM) was prepared in phosphate buffer (0.1 M; pH 7.4) and incubated for 10 min after which 1 mL of the essential oil (0.025 to 0.5 mg/mL) was added to a 0.6 mL of hydrogen peroxide solution (4 mM). After 10 min of incubation, the absorbance of the reacting mixture was measured spectrophotometrically at 230 nm, against a blank solution containing phosphate buffer solution without hydrogen peroxide. BHT and Gallic acid (0.025 to 0.5 mg/mL) were used as positive control. Below is the formula used to calculate percentage scavenging of hydrogen peroxide of samples:

$$H_2O_2 \text{ inhibition capacity (\%)} = [1 - (\text{Absorbance of sample}/\text{Absorbance of blank})] \times 100 \quad (2)$$

2.5. Antimicrobial Activity

2.5.1. Microorganisms and Media

The bacteria and fungi employed in this study were selected mainly on the basis of their significance as opportunistic pathogens of humans with diarrheal diseases. The bacterial strains include *Shigella flexneri* KZN, *Proteus vulgaris*, *Klebsiella pneumonia* ATCC 4352, *Staphylococcus aureus*, *Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* ATCC 19582 and *Serratia mercescens* ATCC 9986. The fungi strains of the American Type Culture Collection (ATCC) include *Triphophytonrubrum* ATCC 28188, *Trichophyton tonsurans* ATCC 28942, *Trichophyton mucoides* ATCC 201382, *Microsporum canis* ATCC 36299, *Microsporum gypseum* ATCC 24102, *Aspergillus niger* ATCC 16888, *Aspergillus fumigatus* ATTC 204305, *Penicillium chrysogenum* ATCC 10106 and *Penicillium aurantiogriesum* ATCC 16025. The bacteria test organisms were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, South Africa and fungi test organisms were purchased from Davies Diagnostics (Pty) Ltd., Gauteng, South Africa, respectively.

The Mueller–Hinton dextrose agar (MHA), Mueller-Hinton dextrose broth (MHB), potato dextrose agar (PDA) and Sabouraud dextrose broth (SDB) were prepared according to the instruction from the manufacturer. The nutrient agar was suspended in demineralized water, heated and stirred until it completely dissolved and then sterilised by autoclaving at 121 °C for 15 min. The fungi and bacteria were maintained at 4 °C on MHA and PDA. Scraped cell mass in 0.85% salt solution was diluted and adjusted to 0.5 McFarland standards and authenticated by spectrophotometric reading at 580 nm assays [12] to prepare the inoculums for the assays.

Finally, the cell suspensions were diluted 1:100 in nutrient broth to present an estimated inoculum of 10^4 CFU·mL^{-1} in comparison with McFarland standard for use in the assays [12].

2.5.2. Antibacterial and Antifungal Susceptibility Test

The agar well diffusion technique as described by Prabuseenivasan et al. [2] was modified and used to test for the antibacterial and antifungal activity. Briefly, 100 µL of 0.5 McFarland solutions of bacterial or fungal strain cultures in 0.85% sterile distilled water (SDW) was placed over the surface of an agar plate and spread with a sterile inoculation loop. In each agar plate, three wells were cut with a cooled, flamed cork borer of 5 mm diameter, while a sterile needle was used to remove the agar plugs. A 50 µL of the amoxicillin (0.0125 mg/mL) or nystatin (0.03 mg/mL) were added to the first well for

antibacterial and antifungal susceptibility test respectively to serve as a positive control. In the second well, 50 µL of the corresponding extract solvent were added while in the third, 50 µL of the acetone extract/aqueous extract/essential oil (50 mg/mL) were added. The essential oil had been prepared by dissolving in 10% DMSO with Tween 80 (0.5% v/v for easy diffusion) and sterilised by filtration through a 0.45 µm membrane filter [2]. Preliminary studies confirmed that the carrier vehicle did not inhibit the growth of bacteria. Each test was done in triplicate. The culture plates were incubated at 37 °C, and the results were examined after 24 h and 72 h for antibacterial and antifungal susceptibility test, respectively. The clear zone around each of the wells was measured in mm, showing the plant fractions' activity against the tested organisms.

2.5.3. Minimum Inhibitory Concentration (MIC) Assay

The broth microdilution method using 96 well microtiter plates was used to verify the minimum inhibitory concentration (MIC) of the plant extracts which indicated antibacterial or antifungal activity [12]. After adding 120 µL of SDW into every well of the first (A) and the last (H) rows and into every well of the last column, 120 µL of Nutrient broth (NB) was put in all the wells of the second row (B) after which a 150 µL of NB was put in the wells left in the first column and 100 µL in the remaining wells from the second column towards the right. There was then the addition of fifty microliters of the essential oil (20 mg/mL) into the third well of the first column as well as the addition of 50 µL of the positive (amoxicillin or nystatin) and negative control (SDW) independently into the wells left in the first column. A serial dilution was carried out in two folds. The contents in all the wells of the first column (beginning from the third row) were mixed and 100 µL was transferred into the second well of the same row. This process was replicated up to the 11th well after which the 100 µL was discarded. Consequently, the dilution of the fractions of the plant and the control in the wells brought about a range of concentration from 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, 0.039, 0.019, 0.0098 and 0.005 mg/mL.

Subsequently, there was the inoculation of 20 µL of 0.5 McFarland bacterial or fungal suspensions into the wells apart from the ones which had SDW. The test organism's growth was measured through the determination of the absorbance at 620 nm with an automatic ELISA microplate reader (Synergy Mx, BioTek®, Winooski, VT, USA) prior to, and following incubation. The plates were incubated at 37 °C for 24 h and 72 h for bacteria and fungi, respectively. The MIC 50 was defined and recorded as the concentration of the test antibacterial and antifungal agent that revealed 50% inhibition of bacteria and fungi growth, respectively.

2.6. Chemical Composition Evaluation

2.6.1. Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analyses were performed on Agilent 5977A MSD and 7890B GC System, Chemetrix (Pty) Ltd., Midrand, South Africa; Agilent Technologies, Deutschland, Germany with a Zebron-5MS column (ZB-5MS 30 m × 0.25 mm × 0.25 um) (5%-phenylmethylpolysiloxane). The column and temperature conditions employed include GC grade helium at a flow rate of 2 mL/min and splitless 1 mL injection was also employed. The injector, source and oven temperatures were set at 280 °C, 280 °C and 70 °C, respectively. The ramp settings were; 15 °C/min to 120 °C, then 10 °C/min to 180 °C, then 20 °C/min to 270 °C and held for 3 min.

2.6.2. Identification of Components

The identification of the chemical components of the essential oil was influenced by their GC retention times, percentage composition (area %) and retention indices. The interpretation and identification of their mass spectra were confirmed by mass spectral incorporated library. The identification was further confirmed by search using the National Institute of Standards and Technology (NIST) database (NIST/EPA/NIH mass spectral library 2014) with those of published data [13].

Empirical searches were conducted using the PubChem Project (https://pubchem.ncbi.nlm.nih.gov/) and Drug Bank (www.drugbank.ca/) to identify the known pharmacological properties associated with these compounds.

2.6.3. Statistical Analysis

All experiments were carried out in triplicates and the results were expressed as Mean ± SD. Where appropriate, the data were subjected to one-way analysis of variance (ANOVA) employing the Minitab program (version 12 for windows). $p < 0.05$ were considered significant.

3. Results and Discussion

3.1. Antioxidant Activities

In this study, the DPPH, ABTS, nitric oxide and hydrogen peroxide scavenging activities have been used as in vitro models to evaluate the antioxidant capacity of the essential oil of *Hydnora africana*, in comparison with the standards BHT and Gallic acid. Figure 2 illustrates the DPPH radical scavenging activity of the essential oil of *Hydnora africana* compared with BHT and Gallic acid. We observed weak DPPH inhibitory activities of the essential oil of *Hydnora africana*, compared with the reference compounds used. Previous studies have indicated that essential oils from different sources possess varying capacities to scavenge DPPH radicals, probably due to the differences in their composition. Factors like stereo-selectivity of the radicals or the solubility of the oil in the different testing systems have been reported to affect the capacity of essential oils in quenching different radicals [14]. Our result was similar to that of Negue et al. [15] who also found weak DPPH radical scavenging activity using the essential oil of *P. brazzeana* (RSA = 14% for 1 g/L, SC50 = 1.5 mg/mL and SC50 = 3.23 mg/mL). In contrast, Ndoye et al. [16] showed that the essential oil of *Allium sativum* was a more effective DPPH radical scavenger (SC50 = 7.67 mg/mL). It was observed that increasing the concentration of the oil or standards was not accompanied by any significant increases in their DPPH radical scavenging activities. It appears that the lowest concentrations tested (0.025 mg/mL) was sufficient, in each case, to produce the maximum antioxidant capacity of the standards. It is possible, therefore, that much higher doses of the oil than those tested in this study may produce higher DPPH radical scavenging activities. The IC_{50} values could not be determined precisely for both standards as they were lesser than the lowest concentration tested (0.025 mg/mL). Similarly, IC_{50} for the essential oil was greater than the highest concentration tested (0.5 mg/mL) and could not be precisely determined. The results observed in this study indicated that the *Hydnora africana* essential oil hasa low effectiveness in donating a hydrogen proton to the lone pair electron for the radical.

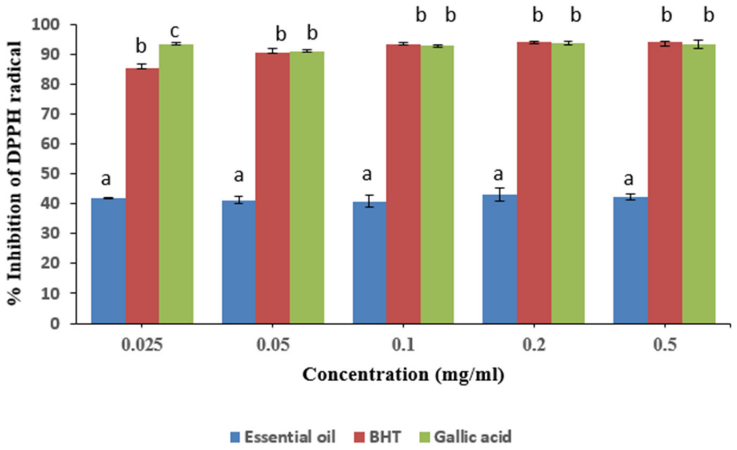

Figure 2. DPPH radical scavenging activity of the essential oil of *H. africana*. Bar graphs with different letters within the same concentration are significantly different ($p < 0.05$).

The ABTS radical scavenging activity of *H. africana* essential oil is illustrated in Figure 3. The ABTS radical scavenging activity of the essential oil was also significantly lower ($p > 0.05$) when compared to those of BHT and Gallic acid at all the concentrations investigated (Figure 3). The % inhibition of ABTS by the essential oil was found to be concentration- dependent. The IC_{50} value for the ABTS radical scavenging activity of the essential oil was 0.55 mg/mL, while those of the standards could not be determined precisely as they were lower than the lowest concentration tested. This result here is similar to the work by Mamadalievaa et al. [17] who found that three *Uzbek Scutellaria* species also exhibited weak ABTS radical scavenging activity.

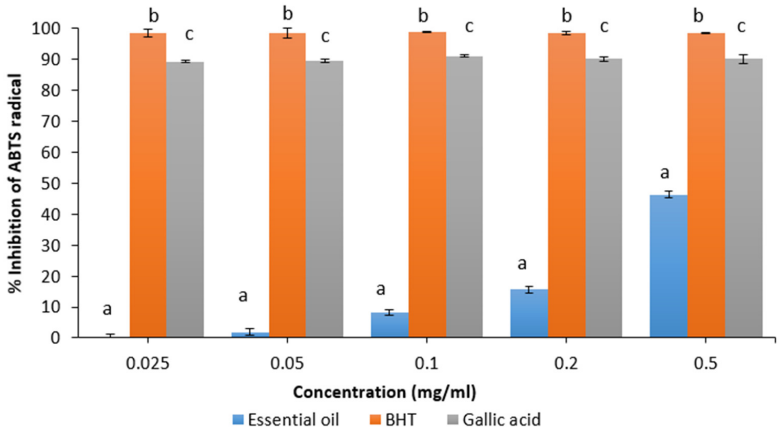

Figure 3. ABTS radical scavenging activity of the essential oil of *H. africana*. Bar graphs with different letters within the same concentration are significantly different ($p < 0.05$).

The nitric oxide radical scavenging activities of the essential oil and the reference compounds are presented in Figure 4. At all the concentrations tested, the essential oil produced a significantly higher ($p < 0.05$) nitric oxide radical scavenging activity, when compared with both BHT and Gallic acid. However, the capacities of all the tested compounds to scavenge nitric oxide were largely concentration-dependent. Nitric oxide, in itself, can alter cell structure and function by modifying different signalling pathways. Its toxicity usually results when it combines with the superoxide anion to form peroxynitrite, which is a very strong oxidant [18]. The ability of the essential oil of *H. africana* to effectively scavenge nitric oxide radicals in this study suggests it could be useful against oxidative conditions involving this radical.

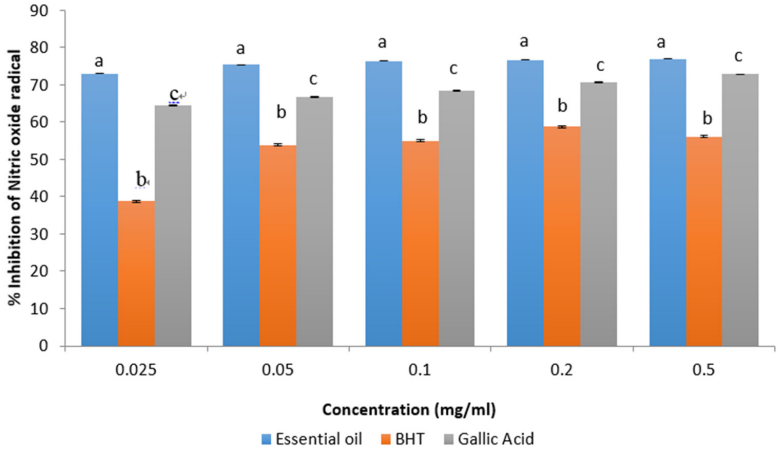

Figure 4. Nitric oxide radical scavenging activity of the essential oil of *H. africana*. Bar graphs with different letters within the same concentration are significantly different ($p < 0.05$).

The scavenging activities of the essential oil and the standards against hydrogen peroxide are depicted in Figure 5. For both the oil and the standards, the amount of hydrogen peroxide remaining in the reaction mixtures, decreased as their concentrations were increased. There was no significant difference observed between the oil and BHT at nearly all the concentrations ($p < 0.05$) except at 0.05 mg/mL where BHT showed a significantly higher activity than the oil ($p > 0.05$). At all the concentrations tested (0.025 to 0.5 mg/mL), the essential oil showed lesser hydrogen peroxide antioxidant activity as compared to the standards (BHT and gallic acid), only able to reduce hydrogen peroxide by less than 20%, while gallic acid had the highest activity, reducing the H_2O_2 by over 80%.

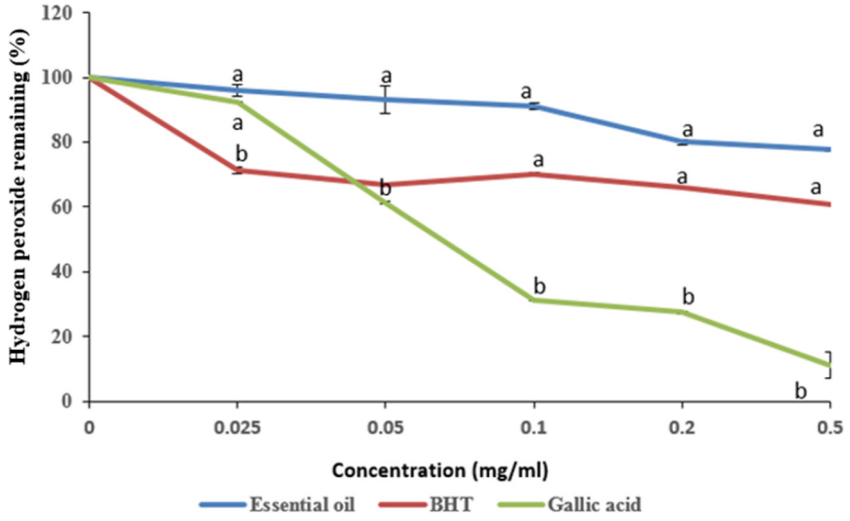

Figure 5. Hydrogen peroxide scavenging activity of the essential oil of *H. africana*. Lines with different letters within the same concentration are significantly different ($p < 0.05$).

3.2. Antimicrobial Analysis

The essential oil was tested against thirteen opportunistic bacteria. The results showed that the plant was active against the growth of all organisms except *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. The essential oil of *H. africana* showed activity against growth of some pathogenic bacteria that cause infections associated with diarrhoeal diseases (Tables 1 and 2). The antibacterial activity could also be attributed to the presence of some chemical constituents in the essential oil fraction of *H. africana*. For example, Terpinen-4-ol, both in aqueous solutions and vapour form, acts as an antimicrobial agent against bacteria of Legionella genus [19]. β-cymene possesses antibacterial and antifungal activities [20]. Napthalene has antibacterial and antifungal properties against various human pathogens [21]. Diphenyl ether possesses bactericidal activity against gram-positive and gram negative bacteria [22]. (−)-Spathulenol, an oxygenated sesquiterpene, has antifungal, antibacterial, antiviral and cytotoxic activities [23].The zones of inhibition varied from 10 to 25 mm (Table 1). The HA essential oil showed a significant growth inhibitory effect against the ten organisms ($p < 0.05$) having IC_{50} values ranging from 5 to 0.005 mg/mL (Table 2). The lowest MIC was observed with *Enterococcus faecalis* ($p < 0.05$) (Table 2). Of the selected and tested nine fungal isolates, the plant species inhibited only the growth of *Aspergillus niger* whose MIC activity was significantly similar to the control drug ($p < 0.05$) (Tables 3 and 4).

Table 1. Inhibition zone diameters caused by the essential oil (50 mg/mL) in the tested opportunistic bacteria.

Organism	Essential Oil	Positive Control (Amoxicillin)
Salmonella typhimurium −	NA	36 ± 1.1^{a}
Enterococcus faecalis −	17 ± 0.2^{a}	34 ± 1.2^{b}
Escherichia coli −	15 ± 1.3^{a}	35 ± 0.2^{b}
Pseudomonas aeruginosa −	NA	NA
Bacillus cereus +	10 ± 1.6^{a}	32 ± 1.2^{b}
Shigella sonnei −	15 ± 0.3^{a}	35 ± 2.2^{b}
Streptococcus pyogens +	22 ± 0.2^{a}	35 ± 2.1^{b}
Bacillus subtilis +	22 ± 0.2^{a}	40 ± 0.2^{b}
Shigella flexneri −	19 ± 0.2^{a}	40 ± 1.1^{b}
Klebpneumoniae −	23 ± 2.1^{a}	33 ± 2.2^{b}
Staphylococcus aureus +	25 ± 3.1^{a}	37 ± 0.2^{b}
Proteus vulgaris −	NA	38 ± 1.1^{a}
Serretiamercenscens −	25 ± 3.1^{a}	35 ± 2.2^{b}

The bacteria are denoted thus by: St (*Salmonella typhimurium*), Ef (*Enterococcus faecalis*), Ec (*Escherichia coli*), Pa (*Pseudomonas aeruginosa*), Bc (*Bacillus cereus*), Ss (*Shigella sonnei*), Sp (*Streptococcus pyogens*), Bs (*Bacillus subtilis*), Sf (*Shigella flexneri*), Kp (*Kleb pneumoniae*), Sa (*Staphylococcus aureus*), Pv (*Proteus vulgaris*) and Sm (*Serratia marcescens*). Data expressed as means ± SD; $n = 3$; values along a row with different subscripts are significantly different ($p \leq 0.05$).

Table 2. Minimum inhibitory concentrations (MIC) of the *H. africana* essential oils against the tested opportunistic bacteria.

Solvent	Inhibition Zone Diameter (mm)												
	St	**Ef**	**Ec**	**Pa**	**Bc**	**Ss**	**Sp**	**Bs**	**Sf**	**Kp**	**Sa**	**Pv**	**Sm**
Essential oil	NA	2.5^{a}	$>5^{a}$	NA	$>5^{a}$	$>5^{a}$	0.02^{a}	0.02^{a}	0.02^{a}	2.5^{a}	0.02^{a}	NA	0.02^{a}
Positive control	0.01	0.01^{b}	0.01^{b}	NA	0.01^{b}	0.01^{b}	$<0.01^{b}$	0.01^{b}	0.01^{b}	$<0.01^{b}$	0.01^{b}	0.01^{b}	0.01^{b}

The bacteria are denoted thus by: St (*Salmonella typhimurium*), Ef (*Enterococcus faecalis*), Ec (*Escherichia coli*), Pa (*Pseudomonas aeruginosa*), Bc (*Bacillus cereus*), Ss (*Shigella sonnei*), Sp (*Streptococcus pyogens*), Bs (*Bacillus subtilis*), Sf (*Shigella flexneri*), Kp (*Kleb pneumoniae*), Sa (*Staphylococcus aureus*), Pv (*Proteus vulgaris*) and Sm (*Serratia marcescens*). Data expressed as means ± SD; $n = 3$; values with the different superscript in the same column are significantly different ($p \leq 0.05$). NA indicates not active.

Table 3. Inhibition zone diameters caused by the essential oil (50 mg/mL) in the tested opportunistic fungi.

Organism	Essential Oil	Positive Control (Nystatin)
Aspergillus fumigates	NA	30 ± 1.1
Aspergillus niger	20 ± 1.2^{a}	20 ± 1.2^{a}
Microsporium gypsum	NA	25 ± 0.2
Triphophytonrubrum	NA	25 ± 0.2
Triphophytontonsurans	NA	30 ± 1.2
Triphophytonmucoides	NA	25 ± 2.2
Penicillium aurantrognereum	NA	30 ± 2.1
Penicillium chrysogenum	NA	25 ± 0.2
Microsporum canis	NA	35 ± 1.1

The fungi are denoted thus by: Af (*Aspergillus fumigatus*), An (*Aspergillus niger*), Mg (*Microsporium gypsum*), Tr (*Triphophyton rubrum*), Tt (*Triphophyton tonsurans*), Tm (*Triphophyton mucoides*), Pa (*Penicillium aurantrognereum*), Pc (*Penicillium chrysogenum*) and Mc (*Microsporum canis*). Data expressed as means ± SD; $n = 3$; values along a row with different subscripts are significantly different ($p \leq 0.05$). NA indicates not active.

Table 4. Minimum inhibitory concentrations (MIC) of the *H. africana* essential oils against the tested opportunistic fungi.

Solvent	Inhibition Zone Diameter (mm)								
	Af	**An**	**Mg**	**Tr**	**Tt**	**Tm**	**Pa**	**Pc**	**Mc**
Essential oil	NA	2.5 [a]	NA	NA	NA	NA	NA	NA	NA
Positive control	0.01	2.5 [a]	0.02	2.5	<0.01	0.02	0.01	0.02	0.01

The fungi are denoted thus by: Af (*Aspergillus fumigatus*), An (*Aspergillus niger*), Mg (*Microsporium gypsum*), Tr (*Triphophyton rubrum*), Tt (*Triphophyton tonsurans*), Tm (*Triphophyton mucoides*), Pa (*Penicillium aurantrognereum*), Pc (*Penicillium chrysogenum*) and Mc (*Microsporum canis*). Data expressed as means ± SD; $n = 3$; values along a row with differentsubscripts are significantly different ($p \leq 0.05$). NA indicates not active.

Although compounds in the oil are known to have antimicrobial activity independently, they could be acting independently or in combination to potentiate the plants' potentials [24]. Therefore, it is crucial to isolate and elucidate the bioactive compounds and determine their pharmacological properties. This should facilitate the identification of novel antimicrobial agents.

GC-MS analysis of the essential oil fraction revealed fraction revealed the presence of 67 compounds in different chemical classes including terpenoids, aldehydes, ketones, fatty acid esters and carboxylic acids (Table 5). The details of certain compounds could, however, not be confirmed in the NIST database. Among identified compounds, carboxylic acids, dominated by *n*-Hexadecanoic acid and *cis*-9-hexadecanoic acid, constituted the largest group of compounds (30.68% of total oil composition). The higher content of carboxylic acids in the essential oil, compared to other groups of compounds, is an unusual finding, as most other essential oils reportedly contain terpenes and other hydrocarbons. The results of this study, however, bear a similarity to those observed for the composition of essential oils from *Tetrapleura tetraptera* Udourioh and Etkudoh, [25], who also reported a high content of carboxylic acids in the oil.

Terpenoids identified in the oil made up 10.70% of the total oil composition. They included Beta-Myrcene, *o*-limonene, Terpien-4-ol, pulegone, Beta-bisabolene, (−)-spathulenol, *cis*-lanceol, alloaromadendrene, Longifolene, Phenanthrene, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-1, 1,4b,7-tetramethyl-, [4aS-(4a.alpha.,4b.beta., 7.alpha.,8a.alpha.)]- and Bicyclo [2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1*S*)-Bicyclo. Aldehydes (4.86%); Ketones (2.85%) and fatty acid esters (1.82%) were also among compounds whose identity was confirmed from the library. The low percentage of terpenoids and relatively high content of carboxylic acids in the oils extracted from *Hydnora africana* may suggest that the oil may not be considered as a true essential oil [25]. This may be the reason for the weak antioxidant effects shown by the oil against DPPH, ABTS and nitric oxide. However, the potential applications of the oil in the cosmetic industries, due to the presence of some terpenoids may be very useful.

Table 5. Chemical composition of the essential oils of the root part of *H. africana*.

#	RT	Name	Area (%)	Class of Compound	Molecular Formula	Molecular Weight
1	3.585	2-Heptanone	1.07	Ketone	$C_7H_{14}O$	114
2	3.674	Heptanal	0.97	Aldehyde	$C_7H_{14}O$	114
3	3.714	2H-Isoindole-1,3(1H,3H)-dione, 5-amino-2-(2-methoxyphenyl)-	0.52	n/a	n/a	n/a
4	4.215	Benzaldehyde	0.45	Aldehyde	C_7H_6O	106
5	4.351	2-Hepten-2-one, 6-methyl-	0.21	n/a	n/a	n/a
6	4.393	Beta-Myrcene	0.64	Monoterpene	$C_{10}H_{16}$	136
7	4.487	Octanal	0.27	Aldehyde	$C_8H_{16}O$	128
8	4.675	Benzene, 1-methoxy-3-methyl	0.47	n/a	$C_8H_{10}O$	122
9	4.725	*p*-Cymene	0.58	Alkylbenzene	$C_{10}H_{14}$	134
10	4.765	*o*-Limonene	3.05	Monoterpene	$C_{10}H_{16}$	136
11	5.008	Octyl chloroformate	0.37	n/a	$C_9H_{17}ClO_2$	193
12	5.199	8-Nonen-2-one	1.31	Ketone	$C_9H_{16}O$	140
13	5.250	2-Nonanol	0.28	Alcohol	$C_9H_{20}O$	144
14	5.308	Nonanal	0.84	Aldehyde	$C_9H_{18}O$	142
15	5.527	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, *trans*-	0.18	n/a	$C_{10}H_{18}O$	154
16	5.617	Cyclopentane, 1,3-bis (methylene)-	6.38	n/a	C_7H_{10}	94
17	5.756	Bicyclo [2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1S)-	0.42	Monoterpenoid	$C_{10}H_{16}O$	152
18	5.808	Nona-3,5-dien-2-one	0.47	Ketone	$C_9H_{14}O$	138
19	5.916	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1. alpha, 2. beta, 5. alpha.)-(.+/−.)-(Menthacamphor)	0.30	n/a	$C_{10}H_{20}O$	156
20	5.976	Terpinen-4-ol	0.22	Monoterpenoid	$C_{10}H_{18}O$	154
21	6.012	Sorbic acid vinyl ester	0.63	n/a	$C_8H_{10}O_2$	
22	6.089	Naphthalene	0.81	n/a	$C_{10}H_8$	128
23	6.184	2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-	0.28	n/a	$C_{10}H_{14}O$	150
24	6.452	Pulegone	0.16	Monterpenoid	$C_{10}H_{16}O$	152
25	6.520	2-Decanal, (*E*) −	0.17	Aldehyde		
26	6.588	3,5-Dimethoxytoluene	0.23	n/a	$C_9H_{12}O_2$	152
27	6.696	Phenol, 4-ethyl-2-methoxy-	0.50	n/a	$C_9H_{12}O_2$	152
28	6.735	Phenol, *p*-tert-butyl	0.38	n/a	$C_{10}H_{14}O$	150
29	6.927	2,4-Decadienal, (*E*,*E*) −	0.17	Aldehyde	$C_{10}H_{16}O$	152
30	6.959	Benzene, 4-ethyl-1,2-dimethoxy-	0.26	n/a	$C_{10}H_{14}O_2$	166
31	7.243	Cyclotetradecane	0.24	Cycloalkane	$C_{14}H_{28}$	196
32	7.302	6-Bromomethyl-5-methyl-bicyclo[3.1.0]hexan-2-one	0.51	n/a	n/a	n/a
33	7.362	Butanoic acid, butyl ester	0.20	Fatty acid ester	$C_8H_{16}O_2$	144
34	7.610	Diphenyl ether	0.77	n/a	$C_{12}H_{10}O$	170
35	7.833	5,9-Undecadien-2-one, 6,10-dimethyl-,(*Z*)-(Nerylacetone)	0.37	n/a	$C_{13}H_{22}O$	194
36	8.008	2-Undecanone, 6,10-dimethyl-	0.18	n/a	$C_{13}H_{26}O$	198
37	8.142	Methyl (*Z*)-dec-2-en-4,6-diynoate	0.24	n/a	$C_{11}H_{12}O_2$	176
38	8.244	Beta-Bisabolene	1.49	Sesquiterpene	$C_{15}H_{24}$	204
39	8.682	1,7-Dimethyl-4-oxa-tricyclo[5.2.1. 0(2,6)]decane-3,5,8-trione	0.52	n/a	n/a	n/a
40	8.787	(−)-Spathulenol	0.87	Sesquiterpenoid	$C_{15}H_{24}O$	220

Table 5. *Cont.*

#	RT	Name	Area (%)	Class of Compound	Molecular Formula	Molecular Weight
41	8.848	Lanceol, *cis*	0.68	Sesquiterpenoid	$C_{15}H_{24}O$	220
42	8.898	3-Pyridinecarboxylic acid, 1,6-dihydro-6-oxo-	2.29	Carboxylic acid	$C_6H_5NO_3$	139
43	8.965	Alloaromadendrene	0.43	Sesquiterpene	$C_{15}H_{24}$	204
44	9.054	Longifolene	0.23	Sesquiterpene	$C_{15}H_{24}$	204
45	9.098	3-Cyclohexene-1-acetaldehyde, alpha.,4-dimethyl-(Carvomenthenal)	1.99	Aldehyde	$C_{10}H_{16}O$	152
46	9.225	3-Methyl-2-butenoic acid, 2-methyloct-5-yn-4-yl ester	1.12	Fatty acid ester	$C_{14}H_{22}O_2$	222
47	9.283	Bicyclo[3.1.1]heptan-3-one, 2-(but-3-enyl)-6,6-dimethyl-	0.45	n/a	n/a	n/a
48	9.325	Triisopropylphosphite	0.96		$C_9H_{21}O_3P$	208
49	9.360	2-Cyclohexen-1-one, 3,6-dimethyl-6-(1-methylethyl)-	0.75	n/a	n/a	n/a
50	9.472	3-Methylbut-2-enoic acid, 3,5-dime	2.23	n/a	n/a	n/a
51	9.567	*s*-Indacene, 1,2,3,5,6,7-hexahydro-1,1,4,8-tetramethyl-	0.27	n/a	n/a	n/a
52	9.714	4-[3-(4-Nitrophenoxy)propyl]-1H-imidazole	3.55	n/a	n/a	n/a
53	9.818	Ethyltetramethylcyclopentadiene	2.84	n/a	n/a	n/a
54	9.902	Dodecanoic acid, 10-undecen-1-yl ester	0.37	n/a	n/a	n/a
55	9.971	Neoisolongifolene, 8,9-epoxy-	2.86	n/a	n/a	n/a
56	10.048	3-Metthoxybenzyl alcohol	1.02	n/a	n/a	n/a
57	10.268	1,2-Benzenedicarboxylic acid, butyl octyl ester	0.50	Fatty acid ester	$C_{20}H_{30}O_4$	335
58	10.305	4,4′-Bis (tetrahydrothiopyran)	1.02	n/a	n/a	n/a
59	10.365	3-Fluorobenzoic acid, 3,5-dimethyl cyclo hexyl ester	0.34	n/a	n/a	n/a
60	10.501	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-	0.30	n/a	$C_{18}H_{30}O$	262
61	10.571	Hexanoic acid, 2-tetradecyl ester	0.49	n/a	n/a	n/a
62	10.745	Dibutyl phthalate	2.78	n/a	$C_{16}H_{22}O_4$	278
63	10.877	*Cis*-9-hexadecenoic acid	4.09	Carboxylic acid	$C_{16}H_{30}O_2$	254
64	11.355	1,5,9-Decatriene, 2,3,5,8-tetramethyl-	10.52	n/a	$C_{14}H_{24}$	192
65	11.396	*n*-Hexadecanoic acid	24.30	Carboxylic acid	$C_{16}H_{32}O_2$	256
66	11.421	7-Oxabicyclo[4.1.0]heptane, 1,5-dimethyl-	3.18	n/a	n/a	n/a
67	12.218	Phenanthrene, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-1,1,4b,7-tetramethyl-, [4aS-(4a.alpha., 4b.beta.,7.alpha.,8a.alpha.)]-	2.51	Diterpene	$C_{20}H_{32}$	273
		Total	100.05			

n/a = compound details not found in the NIST database. Please note the names in red are synonyms for the ones in black. They could be used instead. The name, nature, retention time (RT), molecular weight, molecular formula and percentage composition (%) of the identified compounds in essential oil.

4. Conclusions

In this study, we conclude that the essential oil of *H. africana* possess antioxidant and antibacterial activities and could be attributed to the presence of bioactive compounds observed in the study. However, the oils exhibited poor antifungal activity which could be due to the weak effect of its component in eradicating opportunistic fungi. These results have partially justified the folklore usage of this plant in the treatment of infections and some diarrhoeal diseases.

Acknowledgments: The authors greatly appreciate the National Research Foundation (NRF) (Grant No. T304) and Govan Mbeki Research and Development Centre (GMRDC) of the University of Fort Hare, South Africa (Grant No. C275) for financing this study.

Author Contributions: O.A.W. conceived the study, participated in the design, prepared the extracts and carried out the study. A.J.A. participated in the design of the study, coordinated and helped to revise the manuscript. Both authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Shaba, E.Y.; Mann, A.; Yisa, J. Antimicrobial and cytotoxic activities and GC-MS analysis of phytocomponents of methanolic extract of *Curculigo pilosa* (Schum and Thonn) Engl. (Hypoxidaceae) rhizomes. *Br. J. Pharm. Res.* **2014**, *4*, 1552–1567. [CrossRef]
2. Prabuseenivasan, S.; Jayakumar, M.; Ignacimuthu, S. In Vitro antibacterial activity of some plant essential oils. *BMC Complement. Altern. Med.* **2006**, *6*, 39. [CrossRef] [PubMed]
3. Casqueiro, J.; Casqueiro, J.; Alves, C. Infections in patients with diabetes mellitus: A review of pathogenesis. *Indian J. Endocrinol. Metab.* **2012**, *16*, S27–S36. [PubMed]
4. Longdoh, N.A.; Assob, J.C.N.; Nsagha, S.D.; Nde, P.F.; Kamga, H.L.F.; Nkume, A.F.; Emmanuel, T.K. Uropathogens from diabetic patients with asymptomatic bacteriuria and urinary tract infections. *West Lond. Med. J.* **2013**, *5*, 7–14.
5. Al-Salihi, S.H.; Jumaah, I.A.M. Bacterial infection of diabetic foot ulcer. *Diyala J.* **2013**, *9*, 15–21.
6. Sharma, N.; Samarakoon, K.W.; Gyawali, R.; Park, Y.; Lee, S.; Jong Oh, S.; Lee, T.H.; Jeong, D.K. Evaluation of the antioxidant, anti-inflammatory and anticancer activities of Euphorbia hirta ethanolic extract. *Molecules* **2014**, *19*, 14567–14581. [CrossRef] [PubMed]
7. Nickrent, D.L.; Blarer, A.; Qiu, Y.L.; Soltis, D.E.; Soltis, P.S.; Zanis, M.J. Molecular data place *Hydnoraceae* with Aristolochiaceae. *Am. J. Bot.* **2002**, *89*, 1809–1817. [CrossRef] [PubMed]
8. Masika, P.J.; Afolayan, A.J. An ethnobotanical study of plants used for the treatment of livestock diseases in the Eastern Cape Province, South Africa. *Pharm. Biol.* **2003**, *41*, 16–21. [CrossRef]
9. Council of Europe. *European Pharmacopoeia Commission European Directorate for the Quality of Medicines & Healthcare*, 4th ed.; European Pharmacopoeia; Council of Europe: Strasbourg, France, 2002.
10. Liyana-Pathiranan, C.M.; Shahidi, F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *J. Agric. Food Chem.* **2005**, *53*, 2433–2440. [CrossRef] [PubMed]
11. Oyedemi, S.O.; Bradley, G.; Afolayan, A.J. In vitro and in vivo antioxidant activities of aqueous extract of *Strychonos henningsii* Gilg. *Afr. J. Pharm. Pharacol.* **2010**, *4*, 70–78.
12. Otang, W.M.; Grierson, D.S.; Ndip, R.N. Antifungal activity of *Arctotis arctotoides* (L.f.) O. Hoffm. and *Gasteria bicolor* Haw. against opportunistic fungi associated with human immuno deficiency virus/acquired immunodeficiency syndrome. *Pharmacogn. Mag.* **2012**, *8*, 135–140. [PubMed]
13. Shahat, A.A.; Ibrahim, A.Y.; Hendawy, S.F.; Omer, E.A.; Hammouda, F.M.; Abdel-Rahman, F.H.; Saleh, M.A. Chemical composition, antimicrobial and antioxidant activities of essential oils from organically cultivated fennel cultivars. *Molecules* **2011**, *16*, 1366–1377. [CrossRef] [PubMed]
14. Guerrini, A.; Sacchetti, G.; Rossi, D.; Paganetto, G.; Muzzoli, M.; Andreotti, E. Bioactivities of *Piper aduncum* and *Piper obliquum* essential oils from Eastern Ecuador. *Environ. Toxicol. Pharmacol.* **2009**, *27*, 39–48. [CrossRef] [PubMed]

15. Nyegue, M.A.; Ndoyé, F.; AmvamZollo, H.; Etoa, F.X.; Agnaniet, H.; Menut, C. Chemical and biological evaluation of essential oil of *Pentadiplandra brazzeana* (Baill) roots from Cameroon. *Adv. Phytother. Res.* **2002**, *43*, 91–107.
16. Ndoye Foe, F.M.; Tchinang, T.F.K.; Nyegue, A.M.; Abdou, J.; Yaya, A.J.G.; Alembert, T.T.; Essame, J.O.; Etoa, F.X. Chemical composition, in vitro antioxidant and anti-inflammatory properties of essential oils of four dietary and medicinal plants from Cameroon. *BMC Complement. Altern. Med.* **2016**, *16*, 117. [CrossRef] [PubMed]
17. Mamadalievaa, N.Z.; Sharopovb, F.; Satyalc, P.; Azimovaa, S.S.; Winkb, M. Composition of the essential oils of three Uzbek *Scutellaria* species (Lamiaceae) and their antioxidant activities. *Nat. Prod. Res.* **2016**, *30*, 1–5. [CrossRef] [PubMed]
18. Pacher, P.; Beckman, J.S.; Liaudet, L. Nitric oxide and peroxynitrite: In health and disease. *Physiol. Rev.* **2007**, *87*, 315–424. [CrossRef] [PubMed]
19. Mondello, F.; De Bernardis, F.; Girolamo, A.; Cassone, A.; Salvatore, G. In vivo activity of terpinen-4-ol, the main bioactive component of *Melaleuca alternifolia* Cheel (tea tree) oil against azole-susceptible and resistant human pathogenic Candida species. *BMC Infect. Dis.* **2006**, *6*, 158. [CrossRef] [PubMed]
20. Zengin, H.; Baysal, A.H. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. *Molecules* **2014**, *19*, 17773–17798. [CrossRef] [PubMed]
21. Salae, A.; Karalai, C.; Ponglimanont, C.; Kanjana-Opas, A.; Yuenyongsawad, S. Naphthalene derivatives from *Diospyros wallichii*. *Can. J. Chem.* **2010**, *88*, 922–927. [CrossRef]
22. Tang, X.; Xiea, M.; Sunb, Y.X.; Liu, J.H.; Zhong, Z.C.; Wang, Y.L. Synthesis and antibacterial activity of brominated 2′(4′)-nitro-3-hydroxy diphenyl ethers. *Chin. Chem. Lett.* **2009**, *20*, 435–438. [CrossRef]
23. Perez-Hernandez, N.; Ponce-Monter, H.; Ortiz, M.I.; Cariño-Cortes, R.; Joseph-Nathan, P. Structure-activity relationships of *Aromandendrene* in uterus-relaxant activity. *Z. Naturforsch. C* **2009**, *64*, 840–846. [CrossRef] [PubMed]
24. Ashafa, A.O.T.; Grierson, D.S.; Afolayan, A.J. In vitro antioxidant activity of extracts from the leaves of *Felicia muricata* Thunb. an underutilized medicinal plant in the Eastern Cape Province, South Africa. *Afr. J. Tradit. Complement. Altern. Med.* **2010**, *7*, 315–321. [CrossRef]
25. Udourioh, G.A.; Etokudoh, M.F. Essential oils and fatty acids composition of dry fruits of *Tetrapleura tetraptera*. *J. Appl. Sci. Environ. Manag.* **2014**, *18*, 419–424.