Antibacterial and anti-inflammatory activity of plant species used in traditional poultry ethnomedicine in Zimbabwe: a first step to developing alternatives to antibiotic poultry feed additives

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Abstract

Ethnopharmacological relevance: Global interest in phytogenic feed additives as alternatives to antibiotics in feed has been spurred by the banning of antibiotic growth promoters by several countries. Suitable plant extracts for development of phytogenic feed additives should have therapeutic value and should also be safe. The aim of this study was to evaluate the antibacterial, antioxidant and anti-lipoxygenase activities as well as cytotoxicity of selected plant species used in poultry ethnomedicine in Zimbabwe.

Methodology: Antibacterial activity was determined against three ATCC strains (*Staphylococcus aureus, Escherichia coli, Salmonella* Enteritidis) and two clinical strains isolated from chickens (*Escherichia coli* and *Salmonella* Gallinarum) using a two-fold serial microdilution assay. Qualitative antibacterial bioautography was also carried out using the ATCC strains. Antioxidant activities of crude acetone and methanol extracts were determined using free radical scavenging assays whilst anti-lipoxygenase activity was evaluated using a ferrous oxidation-xylenol orange (FOX) assay. Cytotoxicity was evaluated using a tetrazolium-based colorimetric assay (MTT assay) on Vero monkey kidney cells.

Results: *Erythrina abyssinica* had the best antibacterial activity against both ATCC strains and clinical strains with minimum inhibitory concentrations (MIC) values ranging from 0.02 – 0.156 mg/ml. *Aloe greatheadii, Adenia gummifera* (leaves), *Senna singueana*, *Aloe chabaudii* had moderate activity against the poultry pathogens. Bioautography showed that all ten plant species have antibacterial activity against the tested microorganisms with *E. abyssinica* and *S. singueana* having prominent bands of inhibition against both Gram-negative and Gram-positive bacteria. The acetone extract of *S. singueana* and the methanol extract of *Euphorbia matabelensis* had the most powerful antioxidant activities with mean IC_{50} values of 1.43 µg/ml and 1.31 µg/ml respectively in the ABTS assay which were comparable with those of the positive controls (ascorbic acid and trolox). *Bobgunnia madagascariensis, A. chabaudii*, *E. abyssinica* and *Tridactyle bicaudata* extracts had reasonable antioxidant activity. The *S. singueana* extract had the most potent anti-lipoxygenase activity with a mean IC_{50} value of 1.72 µg/ml. The cytotoxicity results showed that only the acetone extracts of *A. greatheadii* and *S. singueana* were relatively safe at concentrations that were active against the tested microorganisms (selective index >1). Regarding anti-lipoxygenase activity, extracts of *B. madagascariensis*, *S. singueana, T. bicaudata* and *E. matabelensis* were more active than toxic (selective index >5) indicating anti-inflammatory potential.

Conclusions: This study showed that *S. singueana* had a cocktail of therapeutic activity and supports further investigation of this plant species for development phytogenic poultry feed additives. Other plant species with noteworthy biological activities include *B. madagascariensis, E. abyssinica, A. greatheadii, T. bicaudata* and *E. matabelensis*.

Keywords: antimicrobial resistance, antibiotic growth promoters, cytotoxicity, anti-inflammatory

Abbreviations: ABTS, 2,2-azino-bis (3-ethylben-zothiazoline-6 sulfonic acid; AMR, antimicrobial resistance; ATCC, American Type Culture Collection; BEA, benzene/ethanol/ammonium hydroxide; CCC, carvacrol + cinnamaldehyde+capsicum oleoresin; CEF, chloroform/ethyl acetate/formic acid; DPPH, 1, 1 diphenyl-2-picryl hydrazyl; EMW, ethyl acetate/methanol/water; FOX, ferrous oxidation-xylenol orange; IC_{50}, 50% inhibitory concentration; INT, p-iodonitrotetrazolium violet; LC_{50}, 50% lethal concentration; MIC, minimum inhibitory concentration; MH, Mueller Hinton; MTT, methyl thiazolyl tetrazolium; NO, nitric oxide; PBS, phosphate-buffered saline; PFA, poultry feed additive;

PUFA, poly-unsaturated fatty acid; SI, selectivity index; TLC, think layer chromatography; UV, ultraviolet

1. Introduction

Plant based products have emerged as a viable alternative to in-feed antibiotics. In response to the scourge of antimicrobial resistance (AMR) the European Union banned use of in feed antibiotics in 2006 (Castanon, 2007). In Asia, South Korea and Thailand have prohibited the addition of antibiotics at sub-therapeutic levels in animal feed (Lee *et al.*, 2014; Nhung *et al.*, 2016). Australia, New Zealand, Brazil, Mexico and Vietnam have also ratified and enforced partial bans (Harbarth *et al.*, 2015). The bans on antibiotic feed additives are justified since antibiotic growth promoters have had significant effects on the natural selection and promulgation of antimicrobial resistance globally through the food chain and release to the environment. However, prohibiting antibiotic growth promoters in poultry production may not be enough. There has been an increase in therapeutic use of antibiotics in some countries which have banned antibiotic growth promoters and antimicrobial resistance in major zoonotic bacteria did not decrease (Jensen and Hayes, 2014). Alternatives to in-feed antibiotics should therefore be availed in order to perform the same role of maintaining health and improving feed efficiency in poultry production.

In addition to probiotics, prebiotics, antimicrobial peptides, organic acids, hyperimmune antibodies and bacteriophages, phytogenic feed additives are one of the available alternatives to antibiotic feed additives. Phytogenic feed additives are currently the focus of extensive research as they are regarded as a viable, cheaper and safer alternative to antibiotic growth promoters. Phytogenic feed additives are plant derived compounds added to animal diets in order to improve animal performance through enhanced digestibility, nutrient absorption and elimination of disease causing microbes in the animal gut (Alloui *et al*., 2014). The phytochemicals or secondary metabolites which can be used as feed additives are an extremely large group of compounds which can be categorised into major groups of chemicals such as essential oils, acids, alkaloids, tannins, saponins, steroids and so forth (Hashemi and Davoodi, 2011). Phytobiotics contain a complex blend of diverse secondary metabolites with multiple bioactive compounds which may have different modes of antimicrobial action making it much more difficult for microorganisms to develop resistance against them (Suresh *et al.*, 2018).

All the products which have emerged as alternatives to in-feed antibiotics have direct or indirect antimicrobial activity (Suresh *et al.*, 2018). Antimicrobial activity is therefore a salient attribute of suitable herbal or botanical products that can be used as phytogenic feed additives as they should be able to mimic the action of antibiotic feed additives. The mechanism of action of antibiotic feed additives is not clearly understood. Researchers have postulated a number of mechanisms of growth promotion, most of which are based on antimicrobial effects on gut microbiota. The mechanisms include inhibition of subclinical infections, reduction of growth-depressing microbial metabolites i.e. ammonia and bile degradation products; reduced microbial use of nutrients and enhanced uptake of nutrients because of histologically thinner guts in antibiotic-treated animals (Brüssow, 2015).

Antioxidant activity is an important feature of phytogenic feed additives because antioxidants play a protective role in animal health (Alloui *et al*., 2014). Antioxidants promote animal health by scavenging reactive oxygen species *in vivo*, thereby preventing cellular damage. The first 100% botanical zootechnical additive to be officially approved in Europe for use as a growth promoter in broiler production is a phytonutrient formulation, CCC (carvacrol + cinnamaldehyde+capsicum oleoresin) which was approved in 2015 (Suresh *et al.*, 2018). Carvacrol is a monoterpenoid phenol with high antioxidant activity both *in vitro* and *in vivo* which is mainly found in pepperwort (*Lepidium flavum*), oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*) and wild bergamot (Alagawany *et al*., 2015). The presence of phytoconstituents with strong antioxidant capacity in the CCC formulation underlies the importance of this attribute in phytogenic feed additives.

Plants with anti-inflammatory properties are also potential candidates for development of phytogenic feed additives. Inflammation is coordinated by mediators released by activated macrophages which include nitric oxide (NO), leukotrienes, lipoxins and prostaglandins (Bosc *et al*., 2005). Nitric oxide (NO) is a short-lived free radical produced by the nitric oxide synthase from arginine. Cycloxygenases and 15-lipoxygenase are major enzymes responsible for synthesis and release of inflammatory mediators (prostangladins and leukotrienes) from poly-unsaturated fatty acids (PUFAs) respectively (Adamu and Adebayo, 2020). Plant derived products should be able to inhibit these key enzymes involved in synthesizing pro-inflammatory mediators if they are to be successfully used as anti-inflammatory agents.

Plant species used for development of phytogenic poultry feed additives should preferably have multiple biological activities. Above all application of phytogenic feed additives to livestock

must be safe to the animal, the consumer of the animal product and the environment (Windisch *et al.*, 2007). In the current study the antibacterial, cytotoxicity, antioxidant and anti-lipoxygenase activities of selected plant species used in poultry ethnomedicine in Zimbabwe were determined (Jambwa *et al.*, 2021). The aim of the study was to evaluate the therapeutic value of these plant species and assess their suitability for development of phytogenic poultry feed additives.

2. Methods

2.1. Plant collection, authentication, drying and storage

Ten plant species were collected from Bindura, Chipinge and Murehwa districts in Zimbabwe. The plant species were identified by Mr. Chapano and Mr Mapaura, botanists from the National Herbarium in Harare. They were also authenticated by Ms. Magda Nel, a botanist from the Department of Plant and Soil Science, University of Pretoria. Voucher specimens were deposited in the H.G.W.J. Schweickerdt Herbarium (PRU) at the University of Pretoria. The plant species and plant parts evaluated in this study are shown in **Table 1**. The plant materials were dried in a well-ventilated room at room temperature, and ground into a fine powder using a mill.

Table 1: Plant species and plant parts evaluated in the study

Botanical name	Common name	Place of collection	Plant part	Voucher number
Bobgunnia madagascariensis (Desv) J. H. Kirkbr. & Wiersema	Snake bean tree	Murehwa	Pods	PRU 0125425
Adenia gummifera (Harv.) Harms var. *gummifera*	Snake climber	Chipinge	Leaves Twigs Stem	PRU 0125433
Senna singeueana (Del.) Lock	Winter-flowering Senna	Chipinge	Leaves	PRU 0125450
Aloe chabaudii Shönland var *chabaudii*	Dwala aloe	Murehwa	Leaves	PRU 0125445
Aloe greatheadii Shönland var *greatheadii*	Greathead's spotted leaf aloe	Chipinge	Leaves	PRU 0125443
Agave sisalana Perrine	Sisal	Chipinge	Leaves	PRU 0125424
Albizia gummifera (*J.F.* Gmel.) C.A.Sm.	Smooth-barked flat-crown	Bindura	Pods	PRU 126149
Erythrina abyssinica Lam.	Luck bean tree	Murehwa	Bark	PRU 0125441
Euphorbia matabelensis Pax	Three forked eurphobia	Bindura	Bark	PRU 126150
Tridactyle bicaudata (Lindl.) Schltr. subsp. *bicaudata*	Wild orchid	Chipinge	Whole plant	PRU 0125422

2.2. Extraction of plant material

Ground material was extracted with acetone and methanol (Minema, South Africa) by adding 3g of material and 30 ml of solvent in 50 ml polyester centrifuge tubes and leaving them for

24 h at room temperature. The tubes were centrifuged at 4000 x g for 10 min (EBA 20, Labotec; South Africa) and the supernatant was filtered into pre-weighed honey jars through Whatman No 1 filter paper (Merck, United States). The process was repeated twice on the plant residues of each sample with the same volume of acetone in order to maximise extraction. The extracts were concentrated by drying under a stream of cold air and the dried extracts were weighed. Yield was determined by dividing mass extracted by initial mass.

2.3. TLC phytochemical profiling

This was done by spotting 10 µl of crude acetone extracts redissolved in acetone (10 mg/ml) on aluminium-backed silica gel plates (10 x 20cm, aluminium-backed, Merck, silica gel 60 F254) which were developed in three solvents with differing polarities, namely BEA (benzene/ethanol/ammonium hydroxide (90:10:1)-non polar solvent system, CEF (chloroform/ethyl acetate/formic acid (5:4:1)-intermediate polar solvent system, EMW (ethyl acetate/methanol/water (40:5.4:4)-polar solvent system (Kotzé and Eloff, 2002). The separated phytochemicals were visualized under visible and UV light (Lamp - CAMAG T-L900/U, Switzerland) at wavelengths of 254 nm and 365 nm, and visible bands were marked using a pencil. The TLC plates were then sprayed with vanillin-sulphuric acid reagent (freshly prepared, 0.1 g vanillin (Sigma-Aldrich, South Africa), 28 ml methanol, 1 ml sulphuric acid) and heated to 110°C until optimal colour development in oven (Masoko *et al*., 2005). Phytochemical profiling was also carried out on the crude methanol extracts using the same procedure in CEF and EMW developing solvents after re-dissolving them in methanol.

2.4. Antibacterial assay by TLC bioautography

The compounds in the crude extract were separated as described above but TLC plates were sprayed with bacterial cultures. After development, the TLC plates were dried overnight in a stream of cold air and sprayed with an actively growing concentrated suspension of American Type Culture Collection (ATCC) strains of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213) or *Salmonella enterica* subsp. *enterica* var. Enteritidis *(S.* Enteritidis ATCC 13076) in Mueller Hinton (MH) broth. The plates were dried and incubated overnight at 37°C in a closed sterile, humidified plastic container to allow growth of the bacteria on the plates. After incubation, the plates were sprayed with a sterile solution (2 mg/ml) of p-iodonitrotetrazolium violet (INT) (Sigma-Aldrich, United States) salt and incubated for a further 1 hour. Clear zones on chromatograms after incubation indicated inhibition of growth

as the INT is metabolized to a coloured formazan product by actively growing cells (Begue and Kline, 1972). The retention factors of the bands of inhibition were calculated.

2.5. Quantitative antibacterial assay by minimum inhibitory concentration assay

The antibacterial activity of the samples were determined by measuring minimum inhibitory concentration (MIC) values using a serial two-fold dilution method (Eloff, 1998). The following test organisms were used *S. aureus* (ATCC 29213), *S.* Enteritidis (ATCC 13076), *E. coli* (ATCC 25922) and clinical strains (*E. coli* and *Salmonella* Gallinarum)

The five bacterial isolates screened were prepared by inoculating single colonies from agar plates into 10 mL of sterile Mueller Hinton (MH) broth (Merck, South Africa). Cultures were incubated at 37 °C in an MRC orbital shaker incubator (150 rpm, United Scientific, South Africa) for 18 to 20 h before the experiment. Following incubation, bacterial cultures were diluted in MH broth and absorbance was measured at a wavelength of 560 nm (Epoch microplate reader: BioTek, Analytical & Diagnostic Products CC, Johannesburg, South Africa). Absorbance was adjusted to match a McFarland standard No 1 (corresponding to approximately 3×10^8 colony forming units (cfu)/mL).

Sterile water (100 µl) was added to all wells of 96-well microtitre plates (Lasec, South Africa). In the first row, 100 µl of extracts were added in triplicate, and then serially diluted two-fold to the last well after which 100 µl were then discarded with a micropipette. Gentamicin (Virbac, South Africa) was included as a positive control antibiotic and a sterility control with only water was included. This was followed by adding 100 µl of bacterial suspension to each well except for the sterility control. Microtitre plates were sealed with parafilm and incubated at 37˚C (IncoTherm, Labotec). After 24 h of incubation, 40 µl of a 0.2 mg/ml solution of iodonitrotetrazolium chloride (INT) (Sigma-Aldrich, United States) was added to each well and the plates were further incubated for at least half an hour to allow sufficient colour development. INT is a dehydrogenase activity detecting reagent converted to an intensely coloured red-purple formazan in the presence of metabolically active micro-organisms. Inhibition of growth is indicated by a clear solution or a definite decrease in colour development. This value was taken as the MIC of the extract. The assay was repeated once with each assay conducted in triplicate.

2.6. Cytotoxicity evaluation

Cytotoxicity evaluation was done on extracts which had moderate or significant antibacterial activity. The cytotoxicity of the plant extracts was determined using an *in vitro* tetrazolium-based colorimetric (MTT) assay by Mosmann (1983) using African green monkey kidney (Vero) cells purchased from the American Type Culture Collection (ATCC CCL-81™, Manassas, VA, USA). These cells were seeded at a density of (10 000 cells/per well) in 96-well microtitre plates. Plates were then incubated at 37°C in a 5% CO_2 incubator (Hera Cell 150, ThermoScientific Germany) in a humidified environment for 24 h to allow cell attachment.

After incubation the medium was removed by aspiration and replaced with fresh Minimal Essential Medium (MEM), (PAN Biotech, Biocom Africa, Johannesburg South Africa), supplemented with 0.1% gentamicin (Virbac, Johannesburg, South Africa) together with 5% foetal calf serum (Separation Scientific, South Africa). The extracts (100 µl) of varying concentrations were added to wells in which cells were seeded. The anticancer chemical (doxorubicin chloride, Pfizer Laboratories, Johannesburg, South Africa) was used as a positive control in this assay. A corresponding blank control with equivalent concentrations of fresh medium was also included, and plates were further incubated for 48 h in a 5% CO_2 incubator. Following incubation, medium in each well was aspirated from the cells, which were washed with about 150 µl phosphate-buffered saline (PBS) (Whitehead Scientific, Johannesburg, South Africa) and fresh medium (200 µl) was then added to each well. A 30 µl aliquot of MTT (Inqaba Biotec, Pretoria, South Africa), (5 mg/ml in PBS) was added to each well and the plates were incubated for 4 h at 37°C. The medium was completely aspirated from the wells and 50 µl DMSO was added to each well to dissolve the formazan crystals. Absorbance at 570 nmwas measured on a BioTek Synergy microtitre plate reader (Analytical & Diagnostic Products CC, Johannesburg, South Africa). Cell growth inhibition was expressed in terms of LC_{50} values for each extract. Selectivity index (SI) values were also calculated by dividing the LC_{50} by the MIC obtained from antibacterial assays for each extract. The cytotoxicity assay was repeated three times with four replicates in each assay.

2.7. Qualitative antioxidant activity

Thin layer chromatography plates (10 x 20cm, aluminium-backed, Merck, silica gel 60 F254) were spotted with 10 µl of the methanol extracts (re-suspended to 10 mg/ml) and dried before being developed in two mobile phase systems as described above but were not sprayed with

vanillin. The DPPH free radical test was performed by spraying the TLC plates with DPPH (0.2% w/v) in methanol to show antioxidant activity of the plant extracts. A change of colour from the purple background of the DPPH radical to yellow indicated presence of antioxidants (Bors *et al*., 1992).

2.8. Quantitative determination of antioxidant activity of the plant extract

2.8.1. *DPPH (1, 1 diphenyl-2-picryl hydrazyl) free radical assay*

The antioxidant activities of extracts were measured using the stable radical (DPPH.) in terms of radical scavenging ability (Brand-Williams et al., 1995) with minor modifications. Methanol solutions (40 µl) of extracts and positive controls (Trolox and ascorbic acid, Sigma-Aldrich, Johannesburg, South Africa) at various concentrations (1.56 -100 µg/ml) were prepared by serial dilution in a 96 well-microtitre plate (Lasec, South Africa). One hundred and sixty microliters (160) µl of DPPH (Sigma-Aldrich, Johannesburg, South Africa) in methanol adjusted to have an absorbance between 0.9 and 1.0 were added and plates were incubated at room temperature (25 °C) in the dark for 30 min. Absorbance was measured against a blank with a microtitre plate reader (Epoch microplate reader, BioTek, Analytical & Diagnostic Products CC, Johannesburg, South Africa) at 516 nm. The DPPH scavenging effect was determined using the following formula:

$$\text{DPPH Scavenging Effect (\%)} = [(A1\text{-}A2/A1)]\times 100$$

Where: A1 is the absorbance of the control reaction and A2 is the absorbance in the presence of the sample.

2.8.2. *ABTS (2,2-azino-bis (3-ethylben-zothiazoline-6 sulfonic acid) free-radical-scavenging assay*

Free radical-scavenging activity of extracts was determined using the ABTS cation decolourization method of Re et al. (1999) as a measure of hydrogen donating capacity with some modifications. ABTS (Sigma-Aldrich, Johannesburg, South Africa) radical solution (7 µM) was prepared by dissolving first 76.81 mg of ABTS in 20 ml of methanol and then 26.49 mg of potassium persulphate ($K_2S_2O_4$) in 20 ml methanol. The two solutions were mixed together and kept at room temperature in the dark for 12 h. Before running the assay, the ABTS radical solution was diluted with methanol to an absorbance between 0.7 and 0.8 at 734 nm. The extracts were serially diluted (40 µl) (1.56 -100 µg/ml) in 96 well-microtitre plate and 160

µl of ABTS radical solution was added to each well. The absorbance was taken exactly after 6 min of reaction at 734 nm (Epoch microplate reader, BioTek, Analytical & Diagnostic Products CC, Johannesburg, South Africa) and blank absorbance were prepared using respective samples without the ABTS radical. The scavenging effect was calculated using the formula:

$$\text{ABTS Scavenging Effect (\%)} = [(A1\text{-}A2/A1)]\times 100$$

The IC_{50} values were calculated from a graph plotted as inhibition percentage against concentration. A Trolox standard curve was prepared by plotting percentage inhibition of the ABTS+ radical against concentration of Trolox. Data from the test samples were similarly analysed.

2.9. Evaluation of the anti-lipoxygenase (15-LOX) activity of the extracts

Lipoxygenase inhibitory activity was determined according to (Taraporewala *et al.*, 1990) and (Lyckander and Malterud, 1996). Lipoxygenase inhibition was determined using a spectrophotometer based on formation of the complex Fe3+/xylenol orange (Pinto *et al*., 2007). In short, 20 µl of Tris-HCl buffer (pH 7.4) was added to all wells of 96-well microplates. Subsequently, 20 µl of extracts (1 mg/ml) were added to the first row of the microplates, followed by a two-fold serial dilution. The extracts were added both to the sample and the sample blank wells. Quercetin (Sigma-Aldrich, Johannesburg, South Africa) was included as the positive control, and Tris-HCl buffer was the negative control. After serial dilution, 40 µl of lipoxygenase enzyme (Sigma Aldrich, Germany) was added to each well and the plates were incubated for 5 min at room temperature. After incubation, 40 µl of linoleic acid (Darmstadt and Schuchardt, Germany) at a final concentration of 140 µM prepared in Tris-HCl buffer (50 mM, pH 7.4) was added to each well (except for the blanks). Plates were incubated for 20 min at 25°C in the dark. After the incubation period 100 µl of freshly prepared ferrous oxidation−xylenol orange (FOX) reagent (Searle Company, England) [sulfuric acid (30 mM), xylenol orange (100 µM), iron (II) sulphate (Merck, Germany), (100 µM) in methanol/water (9:1)] was added. The plates were incubated for 30 min at 25°C in the dark, after which 40 µl of linoleic acid was added to the blanks. The absorbance was measured at 560 nm using a microplate reader (Epoch, BioTek, Analytical & Diagnostic Products CC, Johannesburg, South Africa). The selectivity index (SI) values were also calculated by dividing cytotoxicity LD_{50} values by the IC_{50} values of the anti-lipoxygenase activity ($SI=LD_{50}/IC_{50}$) (Adamu and Adebayo, 2020).

2.10. Data analysis

Data was presented as mean ± standard deviation (SD) of the determinations. Statistical analyses for antioxidant and anti-lipoxygenase data were performed using the Tukey– Kramer multiple comparison post-hoc test, following one way ANOVA. A P-value of <0.05 was considered to be statistically significant. The results were computed using IBM SPSS Statistics.

3. Results

3.1. Plant extract yield

S. singueana had the highest yield (14.38%) followed by *E. matabelensis* (8.75%) whilst *Agave sisalana* (1.00%) had the lowest yield. The yields of the crude acetone extracts of the ten plant species are shown in Table 2.

3.2. TLC Phytochemical profiles

The best mobile phase system was BEA (90:10:1) as it achieved the best separation of compounds (Fig 1a). Terpenoids (purple or bluish purple bands) were the most common group of phytochemicals in the crude extracts. The phytochemical profiles of all the acetone extracts showed the presence of terpenoids which produce a bluish purple colour on spraying with vanillin (Taganna *et al*., 2011). Of the 12 crude extracts the *E. abyssinica* (EA) had the most diverse phytochemicals. The *E. abyssinica* BEA profile produced orange bands, brown bands, pink bands and bluish purple bands after spraying with vanillin-sulfuric acid (Fig 1a). The profiles of *A. gummifera* (AG_l, AG_t, AG_s) extracts showed that the different plant parts (leaves, twigs and stem) had similar compounds (Fig 1a). However leaves had more compounds followed by twigs with the stem extract having the least number of compounds. The *S. singueana* (SS) profile showed prominent bright red zones. The BEA (Fig 1a) and EMW (Fig 3a) solvent system were not able to separate the bright red compounds into distinct bands. They were separated by the CEF (Fig 2a) mobile phase system although the separation was not very clear.

3.3. Antibacterial bioautography

Bioautography showed that all the extracts had antibacterial activity against *S. aureus*, *E. coli* and S. Enteritidis. Bands of bacterial inhibition for all three microorganisms were observed on

the TLC plates developed using the three mobile phase systems (BEA, CEF and EMW) on all the profiles of the 12 extracts (Fig 1b, 1c, 1d, 2b, 2c, 2d, 3b, 3c, 3d). *Adenia gummifera* (leaves) and *E. abyssinica* profiles developed in BEA showed prominent bands of inhibition against *S. aureus* (Fig 1b). The active compound of *A. gummifera* (leaves) had a retention factor of 0.09 whilst one of the active compounds of *E. abyssinica* had a retention factor of 0.19 (Fig 1b). The other active compounds of *E. abyssinica* did not separate clearly with the BEA solvent system. The BEA profiles of *A. gummifera* (leaves), *A. greatheadii* and and *E. abyssinica* showed prominent bands of inhibition against *E. coli* (Fig 1c). The active compounds of *A. gummifera*(l) and *A. greatheadii* had retention factors of 0.06 and 0.13 respectively. Three active compounds with R_f values of 0.06, 013 and 0.17 were observed on the *E. abyssinica* profile. The TLC developed in BEA also indicated that *A. gummifera* (twigs) and *A. gummifera* (stem) had the same active compound against both *S. aureus* and *E. coli* with a retention factor of 0.25 (Fig 1b and Fig 1c). The BEA profiles of *A. gummifera* (leaves, twigs, stem), *A. chabaudii* and *E. abyssinica* had clear zones of inhibition against *S.* Enteritidis (Fig 1d). Two compounds active against *S.* Enteritidis with R_f values of 0.06 and 0.1 were observed in all three plant parts of *A. gummifera*. A large zone of inhibition against *S.* Enteritidis which did not move from the point of origin was observed on the *A. chabaudii* profile. Clear bands of bacterial inhibition were also observed on the *E. abyssinica* profile.

The *S. singueana* and *E. abyssinica* profiles developed using CEF had large clear zones of bacterial inhibition against *S. aureus* which did not separate into distinct bands of active compounds (Fig 2b). The TLC plates developed in CEF also showed that *S. singeuana* and *E. abyssinica* profiles had prominent zones of bacterial inhibition against *E. coli* with the *S. singueana* active compounds showing better separation (R_f values 0.13, 0.21, 0.36, 0.47) whilst the *E abyssinica* active compounds did not separate into distinct bands (Fig 2c). Bioautograhy of all the bacterial species also showed that the EMW solvent system could not separate the active compounds of the plant extracts into distinct bands. Only the EMW profile of *A. chabaudii* had one clear active compound against *S.* Enteritidis (Fig 3d).

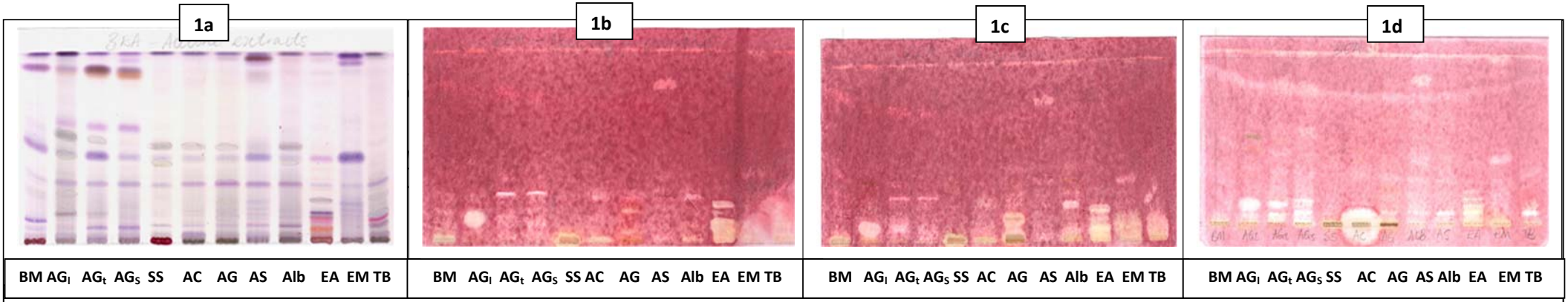

Fig 1a: Chromatogram developed in BEA (benzene: ethanol: ammonium hydroxide (90:10:1) of 12 acetone plant extracts sprayed with vanillin**. Fig 1b:** Bioautography of *S.aureus* (ATCC strain)-TLC plate developed with BEA (90:10:1). **Fig 1c:** Bioautography of *E. coli* (ATCC strain) -TLC plate developed with BEA (90:10:1). **Fig 1d:** Bioautography *S.* Enteritidis (ATCC strain)-TLC plate developed with BEA (90:10:1). White bands indicate compounds that inhibit bacteria. BM=*Bobgunnia madagascariensis*, AG $_l$= *Adenia gummifera leaves*, AG$_t$ = *Adenia gummifera twigs*, AG$_s$ = *Adenia gummifera stem*, SS =*Senna singueana*, AC=*Aloe chabaudii*, AG= *Aloe greatheadii* , AS= *Agave sisalana*, Alb =*Albizia gummifera*, EA =*Erythrina abyssinica*, EM =*Euphorbia matabelensis*, TB = *Tridactyle bicaudata*

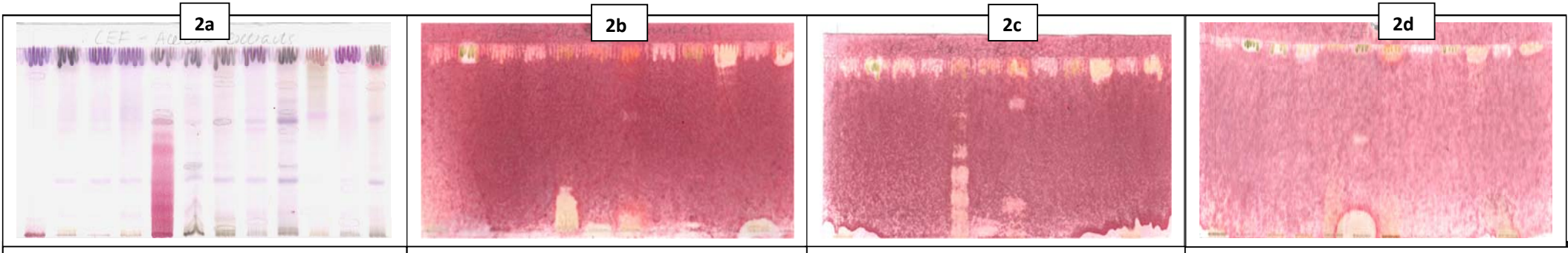

Fig 2a: Chromatogram developed in CEF (chloroform: ethyl acetate : formic acid (5:4:1) of 12 acetone plant extracts sprayed with vanillin**. Fig 2b:** Biaoutography of *S.aureus* (ATCC strain)-TLC plate developed with CEF (5:4:1). **Fig 2c:** Bioautography of *E. coli* (ATCC strain) -TLC plate developed with CEF (5:4:1). **Fig:2d:** Biaoutography of *S.* Enteritidis *(*ATCC strain) -TLC plate developed with CEF (5:41). White bands indicate compounds that inhibit bacteria. BM=*Bobgunia madagascariensis*, AG $_l$= *Adenia gummifera leaves*, AG$_t$ = *Adenia gummifera twigs*, AG$_s$ = *Adenia gummifera stem*, SS =*Senna sengueana*, AC=*Aloe chabaudii*, AG= *Aloe greatheadii* , AS= *Agave sisalana*, Alb =*Albizia gummifera*, EA =*Erythrina abyssinica*, EM =*Euphobia matabelensis*, TB = *Tridactyle bicaudata*

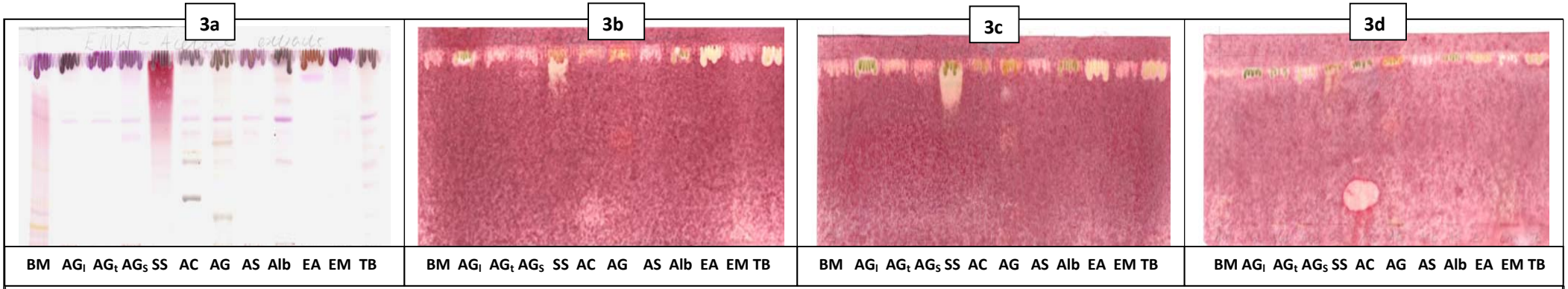

Fig 3a: Chromatogram developed in EMW (ethyl acetate :methanol: water (40:5.4:4) of 12 acetone plant extracts sprayed with vanillin**. Fig 3b:** Bioautography of *S. aureus* (ATCC strain)-TLC plate developed with EMW (40:5.4:4). White bands indicate . **Fig 3c:** Bioautography of *E. coli* (ATCC strain) -TLC plate developed with EMW (40:5.4:4) compounds that inhibit bacteria. **Fig 3d:** Bioautography of *S.*Enteritidis (ATCC strain)-TLC plate developed with EMW (40:5.4:4). BM=*Bobgunnia madagascariensis*, AG $_l$= *Adenia gummifera leaves*, AG$_t$ = *Adenia gummifera twigs*, AG$_s$ = *Adenia gummifera stem*, SS =*Senna singueana*, AC=*Aloe chabaudii*, AG= *Aloe greatheadii* , AS= *Agave sisalana*, Alb =*Albizia gummifera*, EA =*Erythrina abyssinica*, EM =*Euphorbia matabelensis*, TB = *Tridactyle bicaudata*

3.4. Qualitative antioxidant activity

Of the 12 methanol extracts only the extracts of *A. greatheadii*, *A. sisalana* and *A. gummifera* did not have clear bands of antioxidant activity. Antioxidant compounds of the plant species except for *T. bicaudata* did not elute in the CEF mobile phase system but remained on the point of origin (Fig. 4b). The *S. singueana* and *E. matabelensis* extracts had prominent zones of antioxidant activity in both CEF and EMW profiles (Fig 4b and 5b). The antioxidant compounds of these two plant species did not separate in the two mobile phase systems. However, some of the compounds migrated from the origin in the EMW mobile system. The phytochemical and antioxidant profiles of *S. singueana* shows that compounds which turned red on spraying with vanillin were responsible for antioxidant activity of the extract. The antioxidant compounds of *E. matabelensis* did not produce a colour with the vanillin spray. The *A. chabaudii* extract produced a large band with antioxidant activity ($R_f = 0.2$) which was separated by the EMW mobile phase system (Fig 5b). This compound produced a bluish band with vanillin spray. The EMW profiles of *E. abyssinica* and *T. bicaudata* also showed antioxidant compounds.

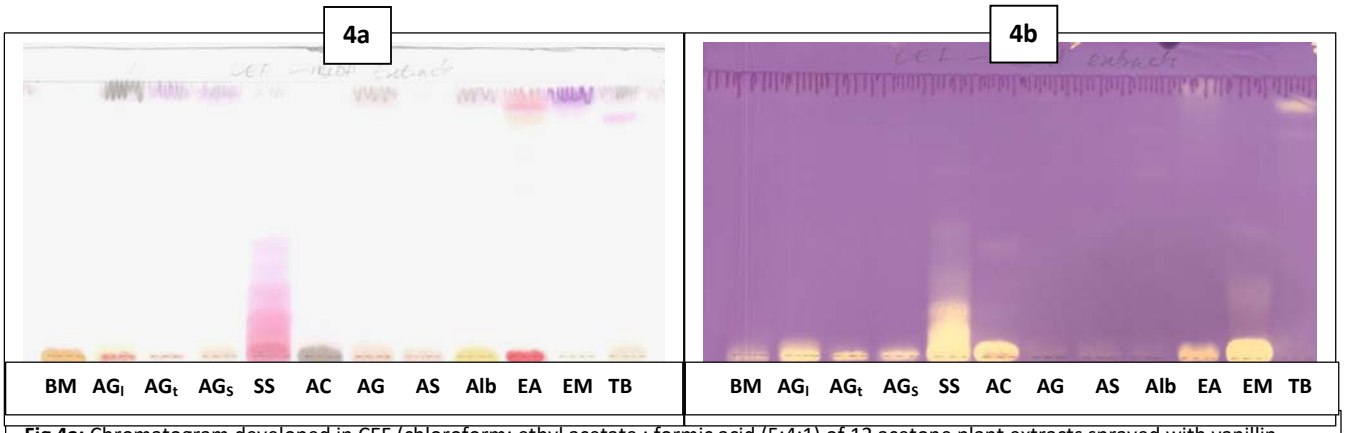

Fig 4a: Chromatogram developed in CEF (chloroform: ethyl acetate : formic acid (5:4:1) of 12 acetone plant extracts sprayed with vanillin**.**
4b: Antioxidant Bioautography -TLC plate developed with CEF and sprayed with DPPH. Yellowish bands indicate compounds antioxidant activivity. BM=*Bobgunnia madagascariensis*, AG_l= *Adenia gummifera leaves*, AG_t = *Adenia gummifera twigs*, AG_s = *Adenia gummifera stem*, SS =*Senna singueana*, AC=*Aloe chabaudii*, AG= *Aloe greatheadii* , AS= *Agave sisalana*, Alb =*Albizia gummifera*, EA =*Erythrina abyssinica*, EM =*Euphorbia matabelensis*. TB = *Tridactyle bicaudata*

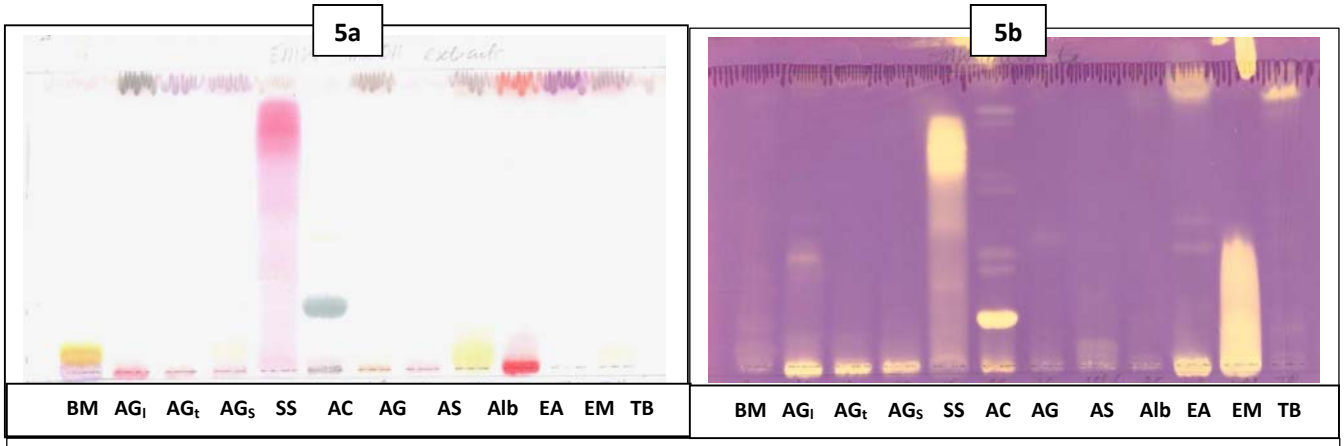

Fig 5a: Chromatogram developed in EMW (ethyl acetate :methanol: water (40:5.4:4) of 12 methanol plant extracts sprayed with vanillin**.**
Fig 5b: Antioxidant Bioautography-TLC plate developed with EMW (40:5.4:4) and sprayed with DPPH. Yellowish bands indicate compounds with antiodantnt activity. BM=*Bobgunnia madagascariensis*, AG_l= *Adenia gummifera leaves*, AG_t = *Adenia gummifera twigs*, AG_s = *Adenia gummifera stem*, SS =*Senna singueana*, AC=*Aloe chabaudii*, AG= *Aloe greatheadii* , AS= *Agave sisalana*, Alb =*Albizia gummifera*, EA =*Erythrina abyssinica*, EM =*Euphorbia matabelensis*, TB = *Tridactyle bicaudata*

3.5. Antibacterial activity (MIC values and total activity (TA))

The extracts had antibacterial activity ranging from significant to weak against the ATCC strains and clinical strains. Minimum Inhibitory Concentrations (MIC) results of the crude acetone are presented in Table 2 and Table 3. The *E. abyssinica* extract had significant antibacterial activity (mean MIC ≤ 100 µg/ml) against all the tested bacterial strains except for *E. coli* (both ATCC and clinical strains). *A. chabaudii*, *A. greatheadii* and *S. singueana* extracts had moderate activity (100<MIC≤625 µg/ml) against all the tested ATCC and clinical strains.

Extracts of *A. gummifera* (leaves) had moderate activity against all the tested ATCC and clinical strains except for the *S.* Enteritidis ATCC strain which had an MIC of 938 µg/ml which is considered to be weak. Moderate activity against two of the ATCC strains (*S. aureus* and *S.* Enteritidis) and one clinical strain (*E. coli*) was recorded with the *A. sisalana* extract. The other plant species *B. madagascariensis*, *A. gummifera* (twigs and stem*), E. matabelensis* and *T. bicaudata* had weak activity against the ATCC strains. However moderate activity was recorded with A. *gummifera* (twigs and stem*)* and *E. matabelensis* extracts against all the two clinical strains whilst the *T. bicaudata* was moderately active against one of the clinical strains. The *Albizia gummifera* extract had weak activity against all of the tested bacterial strains. The total antibacterial activity of each plant extract, obtained by dividing the quantity of material extracted from 1 gram in milligrams (mg) of each plant extract by the MIC value, is shown in Tables 2 and 3. *E. abyssinica*, *S. singueana*, *E. matabelensis* and *A. greatheadii* had mean total antibacterial activities of 812.38, 491.51, 121.33 and 96.16 ml/g respectively which were the highest amongst the plant extracts.

Table 2: Plant extract yield, minimum inhibitory concentration (MIC) and total activity of acetone plant extracts against ATCC strains.

		Staphylococcus aureus		***Escherichia coli***		***Salmonella* Enteritidis**	
Plant species	**%Yield**	**MIC (µg/ml)**	**TA (ml/g)**	**MIC (µg/ml)**	**TA (ml/g)**	**MIC (µg/ml)**	**TA (ml/g)**
Bobgunnia madagascariensis	2.50	>2.5	ND	1250±0.00	20.00	469±170	53.30
Adenia gummifera-leaves	3.00	625±0.00	48	625±0.00	48.00	938±342	32
Adenia gummifera-twigs	3.17	>2500	ND	>2500	ND	1875±685	15.11
Adenia gummifera-stem	2.83	>2500	ND	>2500	ND	>2500	ND
Senna singueana	14.38	313±0.00	459.27	625±0.00	230	169±77	849.75
Aloe chabaudii	3.00	313±0.00	95.85	625±0.00	48.00	625±0.00	48.00
Aloe greatheadii	1.25	117±43	106.84	117±43	106.84	117±43	106.84
Albizia gummifera	1.13	1250±0.00	9.00	>2.5	ND	1250±0.00	9.00
Agave sisalana	1.00	625±0.00	16.00	1250±0.00	8.00	625±0.00	16.00
Erythrina abyssinica	3.88	**23±8.00**	1660.71	117±43	331.20	**29±11.00**	1324.79
Euphorbia matabelensis	8.75	938±342	93.33	938±342	93.33	625±0.00	140.00
Tridactyle bicaudata	2.13	938±342	22.67	1875±685	11.33	625±0.00	34.00
Gentamicin	N/A	37±23.00	N/A	30±0.010	N/A	59±0.021	N/A

MIC values **≤ 100 µg/ml** indicate significant activity**, 100<MIC≤625 µg/ml** moderate activity and values **>625 µg/ml** indicate weak activity (Kuete, 2010). ND – Not determined. N/A – Not applicable. Values in bold indicate promising MIC lower than 100 µg/mL

Table 3: Plant extract yield, minimum inhibitory concentration (MIC) and total activity of acetone plant extracts against clinical strains.

	Escherichia coli		***Salmonella* Gallinarum**	
Plant species	**MIC (µg/ml)**	**TA (ml/g)**	**MIC (µg/ml)**	**TA (ml/g)**
Bobgunnia madagascariensis	469±170	53.30	625±0.00	40.00
Adenia gummifera-leaves	156±0.00	192.31	625±0.00	48.00
Adenia gummifera-twigs	313±0.00	90.52	469±0.00	60.41
Adenia gummifera-stem	625±0.00	50.67	938±342	33.78
Senna singueana	313±0.00	459.27	313±0.00	459.27
Aloe chabaudii	469±170	63.97	625±0.00	48.00
Aloe greatheadii	156±0.00	80.13	156±0.00	80.13
Albizia gummifera	938±342	12.00	>2500	ND
Agave sisalana	469±170	21.32	1250±0.00	8.00
Erythrina abyssinica	156±0.00	248.40	**78±0.00**	496.79
Euphorbia matabelensis	625±0.00	140.00	625±0.00	140.00
Tridactyle bicaudata	625±0.00	34.00	1250±0.0.00	17.00
Gentamicin	39±0.00	N/A	29,5±10.00	N/A

Values in bold indicate promising MIC lower than 100 µg/mL

3.6. Quantitative antioxidant activity

The antioxidant activity expressed as IC_{50} values for both DPPH and ABTS are shown in Figures 6 and 7. The mean IC_{50} values ranged from 4.43 to 195.26 µg/ml and from 1.31 to 76.94 µg/ml in the DPPH and ABTS assays respectively for both methanol and acetone extracts. Both assays showed that the acetone extract of *S. singueana* and the methanol extract of *E. matabelensis* had the strongest antioxidant capacity. The acetone extract of *S. singueana* was the most powerful with a mean IC_{50} value of 4.43µg/ml followed by the methanol extract of *E. matabelensis* which had an IC_{50} value of 4.52µg/ml in the DPPH assay. In the ABTS assay the methanol extract of *E. matabelensis* had the highest antioxidant capacity with a mean IC_{50} value of 1.31µg/ml followed by the acetone extract of *S. singueana* with an average IC_{50} value of 1.71µg/ml. The other extract which had notable antioxidant activity was the acetone extract of *B. madagascariensis* which had mean IC_{50} values of 7.61µg/ml in the DPPH assay

and 3.47µg/ml in the ABTS assay. The methanol and acetone extracts of *A. chabaudii*, *E. abyssinica* and *T. bicaudata* exhibited good antioxidant activities in the ABTS assay only.

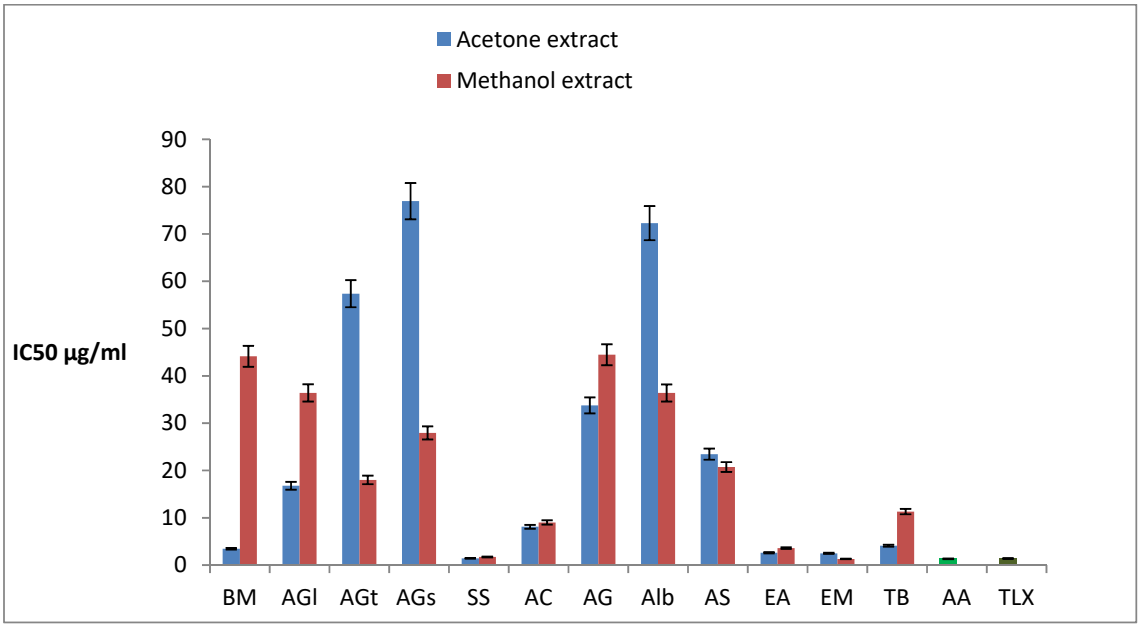

Fig 6: Antioxidant activity of methanol and acetone extracts of 10 plant species used poultry ethnomedicine in Zimbabwe - DPPH assay. BM=*Bobgunnia madagascariensis*, AG_l= *Adenia gummifera leaves*, AG_t = *Adenia gummifera twigs*, AG_s = *Adenia gummifera stem*, SS =*Senna singueana*, AC=*Aloe chabaudii*, AG= *Aloe greatheadii* , AS= *Agave sisalana*, Alb =*Albizia gummifera*, EA =*Erythrina abyssinica*, EM =*Euphorbia matabelensis*, TB = *Tridactyle bicaudata*

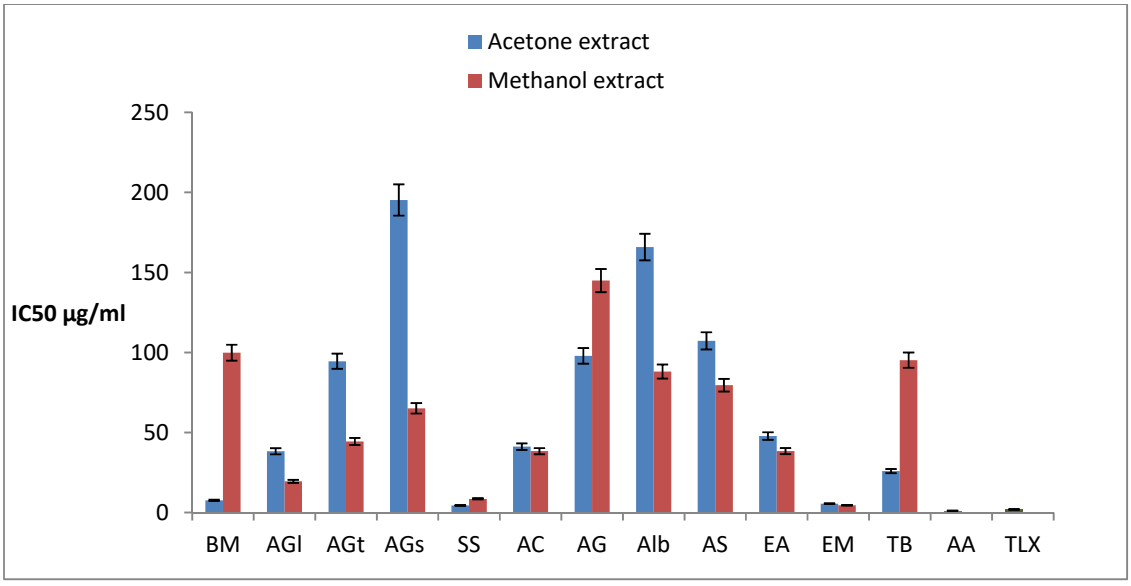

Fig 7: Antioxidant activity of methanol and acetone extracts of 10 plant species used poultry ethnomedicine in Zimbabwe - ABTS assay. BM=*Bobgunnia madagascariensis*, AG_l= *Adenia gummifera leaves*, AG_t = *Adenia gummifera twigs*, AG_s = *Adenia gummifera stem*, SS =*Senna singueana*, AC=*Aloe chabaudii*, AG= *Aloe greatheadii* , AS= *Agave sisalana*, Alb =*Albizia gummifera*, EA =*Eryhrina abyssinica*, EM =*Euphorbia matabelensis*, TB = *Tridactyle bicaudata*

3.7. Anti-lipoxygenase activity

The *S. singueana* extract had the best anti-lipoxygenase activity with an IC_{50} value of 1.72±0.28 µg/ml followed by the *B. madagascariensis* extract which had an IC_{50} value of 4.41±0.37 µg/ml (Figure 8). The *E. abyssinica* extract also had good anti-lipoxygenase activity.

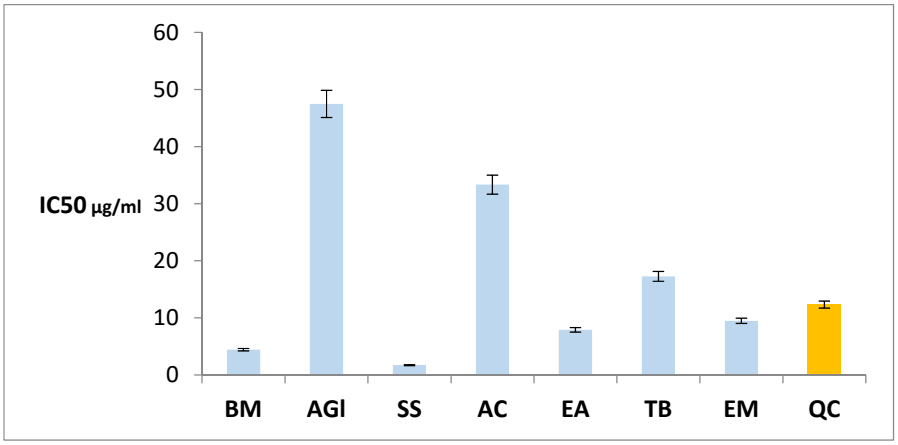

Fig 8: Anti-lipoxygenase activity of acetone extracts of 7 plant species used in poultry ethnomedicine in Zimbabwe. BM=*Bobgunnia madagascariensis*, AG_l= *Adenia gummifera leaves*, SS =*Senna singueana*, AC=*Aloe chabaudii*, AG= *Aloe greatheadii* , AS= *Agave sisalana*, Alb =*Albizia gummifera*, EA =*Erythrina abyssinica*, EM =*Euphorbia matabelensis*, TB = *Tridactyle bicaudata*

3.8. Cytotoxicity

According to the National Cancer Institute (United States), there are four group classifications for evaluation of cytotoxicity: Very active (**LC_{50} ≤ 20µg/mL**), moderately active (**LC_{50} >20 - 100 µg/mL**), weakly active (**LC_{50} >100 - 1000 µg/mL**), and inactive (**LC_{50} >1000 µg/mL**) (Nordin *et al.*, 2018; Zulaikha *et al.*, 2020). Thus, the results were interpreted using these four group classifications. Acetone extracts of *A. gummifera* (leaves), *Agave sisalana* and *E. abyssinica* had moderate cytotoxicity against Vero cells, whilst the extracts of *A. greatheadii*, *A. chabaudii*, *S. singueana*, *E. matabelensis* and *T. bicaudata* exhibited low toxicity (Table: 4). Only two extracts, *A. greatheadii* and *S. singueana* were more antibacterial than toxic (Selective index >1). However, regarding anti-lipoxygenase activity *B. madagascariensis*, *S. singueana*, *E. abyssinica*, *E. matabelensis* were more anti-inflammatory than toxic (Selective index >1), Table 5. The *B. madagascariensis* and *S. singueana* extracts in particular had good safety margins with selective indices of >10.

Table 4: The cytotoxicity (LC_{50} values) and selective indexes of the plant species with respect to antibacterial activity

		Test organisms and Selectivity index (SI) = LC_{50}/MIC				
Plant species	**LC_{50} (µg/ml)**	***S. aureus* (ATCC strain)**	***E. coli* (ATCC strain)**	***S.* Enteritidis (ATCC)**	***E. coli* (Clinical strain)**	***S.* Gallinarum (Clinical strain)**
Bobgunnia madagascariensis	304.3±14.7	ND	0.24	0.65	0.65	0.49
Adenia gummifera leaves	77.3±4.2	0.12	0.12	0.08	0.50	0.12
Senna singueana	509.6±40.3	**1.63**	0.82	**3.01**	**1.63**	**1.63**
Aloe chabaudii	300.2±19.0	0.96	0.48	0.48	0.64	0.48
Aloe greatheadii	183.7±10.3	**1.57**	**1.57**	**1.57**	**1.18**	**1.18**
Agave sisalana	90.8±9.3	0.15	0.07	0.15	0.19	0.07
Erythrina abyssinica	31.1±8.6	**1.35**	0.27	**1.07**	0.2	0.40
Euphorbia matabelensis	104.0±24.9	0.11	0.11	0.16	0.17	0.17
Tridactyle bicaudata	141.6±20.9	0.15	0.08	0.23	0.23	0.11
Doxorubicin	9.9±1.3	N/A	N/A	N/A	N/A	N/A

Values in bold indicate promising activity

Table 5: Cytotoxicity results (LC_{50} values) and selective index values of seven plant species regarding anti-lipoxygenase activity

Plant species	LC_{50} (µg/ml)	SI (15 – LOX)
B. madagascariensis	304.2±14.7	**68.9**
A. gummifera leaves	77.3±4.2	**1.63**
S. singueana	509.6±40.3	**296.9**
A. chabaudii	300.2±19.0	**9.0**
E. abyssinica	31.1±8.6	**3.9**
E. matabelensis	104.0±24.9	**11.0**
T. bicaudata	141.6±20.9	**8.2**
Doxorubicin	9.9±1.3	N/A

Values in bold indicate promising activity

9. Discussion

The yield is important in the determination of the total antibacterial activity of each plant extract. Total activity has been defined as a measure of the amount of material extracted from a plant in relation to the MIC of the extract, which gives an indication of the plant with the largest quantity of antibacterial compounds in it (Eloff, 2000). A higher yield and lower MIC gives a higher total antibacterial activity. Total activity can be an important source of feedback to rural farmers which has often been lacking, as the value indicates the volume to which bioactive compounds present in one gram of dried leaf material can be diluted and inhibit growth of the particular test organism (Eloff, 2000). This provides a fast basis for the use of evaluated plants for primary health care in rural communities before isolation of active compounds which is often a futile process. From the current study *E. abyssinica, S. singueana, E. matabelensis* and *A. greatheadii* had the highest total activities. *E. matabelensis* had a higher mean total activity despite its high MIC (weak antibacterial activity) against ATCC strains because of its higher yield.

The non-polar solvent system (BEA (90:10:1)) gave the most optimal separation of compounds during phytochemical profiling of the plant extracts. Similar studies have also achieved good separation of phytochemicals with BEA compared to CEF and EMW (Eloff *et al*., 2005; Mokoka *et al*., 2010; Masoko and Makgapeetja, 2015). Terpenoids were the most common class of compounds as they appeared to be present in all the 12 acetone extracts. This group of

phytochemicals is a major subdivision of secondary metabolites (Kabera, 2018). Purple or bluish purple coloration indicates the possible presence of terpenoids (Taganna *et al.*, 2011). *Erythrina abyssinica* had the most diverse range of phytochemicals. The presence of these diverse phytochemicals explains why antimicrobial bioautography showed that *E. abyssinica* had more active compounds against both Gram-positive and Gram-negative bacteria. The same compounds produced clear bands of inhibition against *E. coli*, *S. aureus* and *S.* Enteritidis.

The compounds in the *Senna singueana* acetone extract which were characterised by a bright red colour could only be separated by the CEF solvent which had intermediate polarity although the separation was not clearly defined. It is plausible that the *S. singueana* extract was rich in polyphenolic compounds with closely related structures hence they could not be clearly separated into distinct bands. The presence of polyphenolic compounds has been previously reported in *S. singueana* (Sobeh *et al.*, 2017). It is these polyphenols that were responsible for the prominent zones of bacterial inhibition that were observed on bioautography results. The TLC developed in CEF also produced the clear zones of inhibition shown on bioautography of *S. aureus* and *E. coli*. The bioautography of *S. aureus* showed that the antibacterial compounds of *S. singueana* were not separated into distinct bands and were close to the origin whilst the separation of the antibacterial compounds was better in the bioautography of *E. coli*. The fact that the antibacterial compounds were close to the origin suggests that some of the compounds are polar. This is also supported by the fact the TLC plates developed in EMW, a polar solvent system showed prominent bands of inhibition against *S. aureus* and *E. coli* close to the solvent front on the *S. singueana* profile.

Antibacterial bioautography also showed that the two *Aloe* species, *A. chabaudii* and *A. greatheadii* had prominent bands of inhibition against the Gram negative bacteria. The *A. chabaudii* profile showed the presence of a polar compound which was active against *S.* Enteritidis. The active compound did not move from the origin in the TLC developed in BEA and CEF suggesting that the compound was interacting more with the stationary phase comprised of silica, which is polar. The active compound also did not move a significant distance with a polar mobile phase (EMW) further giving credence to its polar nature. *A. greatheadii* had clear bands of inhibition against *E. coli* which were observed on the TLC plates developed in BEA and CEF. The bioautography profiles of parts of *Adenia gummifera* showed that the leaves had clear bands of inhibition compared to twigs and stem. This might be due to the fact that the phytochemical profile of the leaves showed that the leaves had more

compounds compared to the other parts. The active compounds of *A. gummifera* leaves were more pronounced against *S.* Enteritidis.

The MIC results showed that *E. abyssinica* was the most potent extract as it exhibited a broad spectrum of antibacterial activity with promising activity against all the tested microorganisms. Significant activity of methanol and dichloromethane extracts of *E. abyssinica* against both gram negative and gram positive bacteria has been previously reported (Marume *et al.*, 2018). The MIC results of the *E. abyssinica* correlated with bioautography profiles of the extract as they also showed prominent bands of inhibition against all the tested microorganisms. *Adenia gummifera* (leaves), *Senna singueana*, *Aloe chabaudii* and *Aloe greatheadii* extracts exhibited moderate antibacterial activity. In a previous study in which they used the disc diffusion assay Mbanga et al. (2010) reported the antibacterial activity of methanol and aqueous extracts of *A. chabaudii* against *S. aureus*, *E. coli* and *S.* Gallinarum.

Antioxidant bioautography showed that antioxidant compounds of most of the plant species were polar as they were not separated and did not move from the origin when eluted with CEF which is an intermediate polar solvent system. The compounds were retained by the silica which is polar. Similar studies have suggested that antioxidant compounds are polar in general (Masoko and Eloff, 2007; Mahlo *et al.*, 2013; Masoko and Makgapeetja, 2015). Studies have also shown that polar extracts do not have good antimicrobial activity (Eloff, 2019). This is despite the fact that most ethnobotanical surveys have documented that most phyto-remedies used traditionally to manage diseases caused by microorganisms are used in aqueous form. Their therapeutic value has been attributed to antioxidant activity because of the polar nature of antioxidant compounds. This seems to be the case with the plant species which were evaluated in this study as they are used in aqueous form by farmers in the non conventional treatment of poultry ailments in Zimbabwe (Jambwa *et al*., 2021).

The antioxidant bioautography results also showed that *S. singueana* and *E. matabelensis* had prominent zones of antioxidant activity. The antioxidant compounds of these two plant species are closely related as they could be separated into distinct bands by the two mobile phase systems. It can be deduced that the compounds which turned into a bright red colour during phytochemical profiling were responsible for antioxidant activity exhibited by the *S. singueana* extract. The deduction can be made by matching both the CEF and EMW phytochemical and antioxidant profiles of *S. singueana*. These were the same compounds that were responsible

for antibacterial activity of *S. singueana* as shown by the phytochemiacal profile and antibacterial bioautography of this plant species. The phytochemicals responsible for the antioxidant activity of the *E. matabelensis* extract did not change colour on spraying with vanillin. The antioxidant bioautography of *A. chabaudii* showed a large band of antioxidant activity which has a blue colour in the phytochemical profile.

In the quantitative antioxidant assays a lower IC_{50} value means that the plant extract was able to bleach the DPPH or ABTS at a low concentration which signifies strong antioxidant activity. The leaf methanol extract of *S. singueana* had a mean IC_{50} value of 8.59µg/ml which was lower than the previously reported IC_{50} value of 20.8 µg/ml which was obtained from the methanol extract of *S. singueana* bark (Sobeh *et al.*, 2017). The difference between the IC_{50} values of acetone extracts of *B. madagascariencis, A. gummifera*-leaves, *S. singueana, A. chabaudii, E. matabelensis, T. bicaudata* and those of positive controls (ascorbic acid and trolox) in the DPPH assays was not statistically significant ($p>0.05$). The same applies to the methanol extracts of *A. gummifera*-leaves, *S. singueana* and *E. matabelensis.* The extracts of *B. madagascariencis, A. gummifera*-leaves, *S. singueana, A. chabaudii, E. matabelensis, T. bicaudata* therefore exhibited good antioxidant activities in the DPPH assay. It is recommended to use more than one method when assessing the antioxidant capacity of plant extracts because different compounds are likely to act *in vivo* using different mechanisms (Katerere *et al.*, 2012). Therefore, no single method can fully evaluate the antioxidant status of plants. In the current study the ABTS assay was also used to evaluate antioxidant activity of the plant species. There was no significant difference between the mean IC_{50} values of acetone extracts of *B. madagascariensis, S. singueana, A. chabaudii, E. abyssinica, E. matabelensis, T. bicaudata* and those of positive controls (ascorbic acid and trolox) in the ABTS assay ($p>0.05$). The acetone extracts of these plant species therefore had good antioxidant activity. There was also no significant difference between the mean IC_{50} values of methanol extracts of *S. singueana*, *A. chabaudii, E. abyssinica, E. matabelensis, T. bicaudata* and those of positive controls in the ABTS assays ($p>0.05$). The acetone extract of *S. singueana* had better antioxidant than the methanol extract. The same applies to the pods of *B. madagascariensis* whose IC_{50} value of the acetone extract of *B. madagascariensis* was markedly lower than that of the methanol extract in both assays. The acetone extract of *B. madagascariensis* had notable antioxidant activity. This suggests that these two plant species (*S. singueana* and *B. madagascariensis*) have both polar and non-polar antioxidant compounds. Acetone is able to extract diverse compounds whereas methanol extracts mostly polar compounds. The methanol and acetone

extracts of *A. chabaudii*, *E. abyssinica* and *T. bicaudata* had good antioxidant activities in the ABTS assay. On the other hand, activity of these three plant species was not that good in the DPPH assay. It is possible to find different antioxidant activities among assays as each has different mechanism of action and different reaction conditions (Surveswaran *et al.*, 2007).

Antioxidant activity is an important attribute to consider in the search of plants that can be used in development of phytogenic feed additives that can replace antibiotic growth promoters. In addition to other health and technical benefits, phytogenics rich in antioxidants can also come in hand in conditions such as coccidiosis which is prevalent in poultry. Coccidiosis is associated with parasite-induced host cell destruction, which is associated with oxidative stress as well as lipid peroxidation. Antioxidants which are able to neutralise reactive oxygen species, play a protective role due to their ROS scavenging ability. A study by Naidoo et al. (2008) reported that *Tulbaghia violacea*, *Vitis vinifera* and *Artemisia afra* which are rich in antioxidants had beneficial effects similar to toltrazuril in the management of coccidiosis.

The selective index which takes into account the cytotoxicity (LC_{50}) and the bioactivity of the plant extract showed that most of the extracts were more toxic than antibacterial except for *A. greatheadii* and *S. singueana* which had selective indices of greater than 1. These two extracts which exhibited moderate activity against the poultry pathogens and were relatively safe may be good candidates for development of poultry phytogenic feed additives. The poultry gut is a complex and dynamic microbiome, with microbial cells being more numerous than host cells in a ratio of approximately 10:1 (Suresh *et al.*, 2018). Antibiotic feed additives are thought to have a bacteria-centric effect as it has been postulated that they act by inhibiting the growth of the normal gut microbiota thereby reducing maintenance costs and increasing nutrient availability to the host (Gaskin *et al*., 2002).

Senna singueana, which had a high total antibacterial activity and prominent bands of bacterial inhibition on bioautography is a potential candidate for the development of PFAs. The prospects of *S. singueana* as a good candidate for the development of phytogenic feed additives are further reinforced by its powerful antioxidant activity, potent anti-lipoxygenase activity and its low toxicity. It has also been proposed that antibiotic growth promoters function by permitting growth by means of an anti-inflammatory role (Niewold, 2007). Inhibition of gut inflammation saves energy and also prevents thickening of the intestinal wall. The *S. singueana* extract was able to significantly inhibit lipoxygenase, one of the key enzymes in the

coordination of inflammation. The mean IC_{50} value of the *S. singueana* acetone was significantly lower than that of the quercetin (positive control) ($p<0.05$). In addition, its safety regarding anti-lipoxygenase activity was good with a selective index of 296.9. It is widely held that biological efficacy is not owing to *in vitro* cytotoxicity when $SI \geq 10$ (Vonthron-Sénécheau *et al.*, 2003). The acetone extracts of *B. madagascariensis*, *E. abyssinica, E. matabelensis, T. bicaudata* also exhibited good anti-lipoxygenase as there was no statistically significant difference between their IC_{50} values and that of quercetin ($p>0.05$) showing anti-inflammatory potential. The selective index of the *B. madagascariensis* extract regarding anti-lipoxygenase activity was good as it was markedly greater than 10.

10. Conclusions

Potential candidates for development of botanical or herbal poultry feed additives should preferably have a cocktail of therapeutic activity. The study showed that *S. singueana* and *E. abyssinica* had multiple biological activities. The *S. singueana* acetone extract had moderate antibacterial activity, powerful antioxidant and potent anti-lipoxygenase activities whilst the *E. abyssinica* extract had significant antibacterial activity, good antioxidant and reasonable anti-lipoxygenase activity. However, the *E. abyssinica* had poor safety margins due to its moderate toxicity. Based on its anti-inflammatory potential, potent antioxidant activity and good safety margins *S. singueana* is a potential candidate for development of phytogenic feed additives. The other plant species which also had noteworthy therapeutic activity are *A. greatheadii* which had reasonable antibacterial activity and *B. madagascariensis, E. matabelensis and T. bicaudata* which had strong antioxidant activity and good anti-inflammatory activity. Future studies should investigate the possibility of isolating active compounds or partitioning fractions from these plant species that can be used on their own or blended to produce phytonutrient formulations with multiple biological activities which can be earmarked for use as phytogenic poultry feed additives.

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