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A COMPARATIVE STUDY OF THE IN-VITRO EFFECTS OF THE ANTIOXIDANT POTENTIAL AND ANTIMICROBIAL ACTIVITIES OF THREE DIFFERENT MEDICINAL PLANTS OF SOUTHERN AFRICA

George Saramma[1]* and Sajini Souda[2]

[1]Department of Biomedical Sciences, Faculty of Medicine, University of Botswana, Gaborone, Botswana.
[2]Department of Pathology, Faculty of Medicine, University of Botswana, Gaborone, Botswana.

***Corresponding Author: George Saramma**
Department of Biomedical Sciences, Faculty of Medicine, University of Botswana, Gaborone, Botswana.

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ABSTRACT

The present study was conducted to evaluate the *in-vitro* effect of the antioxidant potential and antimicrobial effects of the methanol extract of three different medicinal plants of Southern Africa. The plants used in this study were *Ocimum gratissimum*, *Momordica balsamina* and *Hypoxis hemerocallidea*, they were named as methanol extracts of MEOG, MEMB and MEHHC respectively. The total antioxidant status was evaluated using DPPH, TBA and ABTS assays and the Folin-Ciocalteu reagent method was used to determine total phenolic content. Phytochemical screening was conducted to detect bioactive constituents. Antimicrobial activity was determined with diffusion assays, minimum inhibitory concentration and minimum bactericidal concentration assays. The results show that MEHHC exhibited the maximum antioxidant activity and ability to scavenge DPPH radicals. A relatively high TPC was observed and there was a strong association between antioxidative activities and phenolic compounds (R =0.871), suggesting that the antioxidant potential was due to the phenolic content. The MEHHC exhibited maximum anti-fungal activity against *C. albicans* and variable antibacterial activity against the Gram positive and Gram negative bacteria. There was more pronounced activity against the tested Gram positive bacteria, particularly *S. aureus* (including the MRSA ATCC 430043 strain) whereas *E. coli*, *S. typhimurium* and *K. pneumoniae* displayed the lowest susceptibility. MEHHC showed the maximum antioxidant potential followed by MEOG and MEMB. Antimicrobial activity was also greater for MEHHC followed by MEMB and in lesser amounts for MEOG.

KEYWORDS: Antioxidants, antimicrobial activity, total phenolic content, phytochemicals, traditional medicine, antibiotics.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Plants are one of the most important sources of medicines for treating illnesses since the beginning of human civilization. Herbal drug therapy is regarded as an important alternate, leading the researchers to focus and evaluate the traditionally recommended medicinal plants for their efficacy in various disease conditions.[1] In recent times, folk medicine has taken an important place, especially in developing countries, where health services are limited.[2] The indiscriminate use of commercially available antibiotics for the treatment of infectious diseases developed multiple drug resistance in microorganisms, putting a new challenge before the drug industries for identification of new efficient antimicrobial compounds.[3] The use of plant extracts and phyto-products is gaining attention due to their availability, cost effectiveness, proven nature of specificity, biodegradability, low toxicity, and minimum residual toxicity in the ecosystem.[4] Most of phytochemicals from plant extracts have been identified to exhibit antioxidant activities. These phytochemicals protect us from adverse effects of oxygen free radicals by stabilizing free electrons from excited state to ground state.[5] Thus many of the biological functions, such as anti-mutagenicity, anti-carcinogenicity, and anti-aging, among others, originate from this property. Some of the better-known phytochemicals include: Isoflavones, Anthocyanins, and Carotenoids such as lycopene, beta-carotenes.[6]

Ocimum species are known for their medicinal properties and are used extensively in Ayurvedic preparations.[7] *O. gratissimum* commonly known as wild basil belongs to Lamiaceae family, highly branched shrub of about 1-2 meters height, with ribbed stem and laborious branches.

Its leaves are simple, oppositely arranged, 3-5 centimeters long, 1.2 centimeters wide.[8] The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, diseases of the eye, skin diseases, pneumonia, cough, fever and conjunctivitis.[9]

In folk medicine, the African potato had been used for centuries to treat many ailments such as arthritis, diabetes mellitus, high blood pressure, and cancer. Hypoxis is a family of plants that are extensively used for medicinal purposes in the Southern African region.[10] Taxonomically, *Hypoxis hemerocallidea* belongs to the hypoxidaceae (Star lily family) and the plant has recently drawn attention of researchers because of its beneficial medicinal effects.[11] The tuberous part of the *H. hemerocallidea* is the one that is believed to possess bioactive compounds.[12] Conceivably the production of immune modulating phytochemicals such as antioxidants and phytosterols in *Hypoxis* species including *H. hemerocallidea* may trigger innate immune system pathways that inadvertently have an antimicrobial effect on microbial pathogens during an infection.[13]

Momordica balsamina, African pumpkin (Cucurbitaceae), is a tendril-bearing, wild climber containing wide spectrum of medicinal and nutritional values and has been used as a traditional folk medicine in many countries.[14] Traditional medicinal use of this plant is widespread and diverse. The leaves, fruits, seeds, and bark of the plant are used by traditional healers. The leaves are also important source of nutrients having 17 amino acids with adequate mineral composition like potassium, magnesium, phosphorus, calcium, sodium, zinc, manganese and iron.[15] Recent studies on all *the above plants* proved to be a useful medication for people living with Human Immuno deficiency Virus (HIV), and Acquired Immuno-Deficiency Syndrome (AIDs).[16,17,18]

The aim of this study was to investigate for the first time the antioxidant and the antimicrobial properties of the methanol extracts of the above mentioned plants and to explore the relationship between these activities and the phenolic content present in them. To compare the anti-microbial activities of these plant extracts and establish their efficacy in controlling microbial diseases and to check whether the antioxidant system is facilitating the antimicrobial activities.

METERIALS AND METHODS

Collection and identification of plants

All the plants were collected locally from Botswana, and all of them are seasonal. For *O.gratissimum* and *M. balsamina* the aerial parts were used and for *H. hemerocallidea* the underground corms were used. The identification of the plants were done by Dr. Muzila at the University of Botswana Herbarium (UCBA). The voucher specimens were submitted in the herbarium and voucher Numbers were (2006/G, A01), (G2016/, A02), (G2016/, A01), respectively.

Preparation of the extract: Methanol extract

For the African potato the corms were cut into small pieces, dried in the shade, coarsely powered and soaked in 70% methanol for three days at room temperature. For *O.gratissimum* and *M. balsamina* the aerial parts are used. The plant was cut into small pieces and dried in the shade, then coarsely powered and soaked in 70% +30% methanol and distilled water for three days at room temperature. The extracts were filtered and made it solvent free by using a Buchi type rotary evaporator (65^{o}C) and dried completely in the vacuum. The yields were 7.8%, 7.3 and 7.6(W/W) respectively**.** The extracts obtained were used to carry out the experiments as MEOG (Methanol extract of *O.gratissimum),* MEMB (Methanol extract of *M. balsamina)* and MEHHC (Methanol extract of *Hypoxis hemerocallidea* corm) respectively.

Chemicals

All the chemicals used were analytical grade and bought from Sigma-Aldrich Chemical Company, (St. Louis, MO) USA. Reagents DPPH (2,2-diphenyl-l-picrylhydrazyl) reagent [Molecular formula C18H12N5O6 molecular weight 394 g/mol] was purchased from Fluka Chemicals (Steinheim, Germany), Ascorbic acid and anhydrous sodium carbonate all analytically pure were purchased from Unilab (South Africa). Gallic acid (AR) was obtained from Sigma Chemicals (Steinheim, Germany). The solvents used for the extraction process were also of analytical grade.

Phytochemical screening

The phytochemical tests were carried out in duplicates and are briefly described here.[19]

1. *Flavonoids*

The extract (1 mL) was added to a concentrated sulphuric acid (0.2 mL) and 0.5 g of Mg. A pink or red coloration that disappear on standing (3 min.) indicated the presence of flavonoids. OR, Lead acetate solution (10 %) drops were added to the extract (1 mL). Formation of a yellow precipitate showed the presence of flavonoids.

2. *Tannins*

The extract (1 mL) was added to 2 mL of water followed by drops of dilute ferric chloride solution (0.1 %). A green to blue-green (cathechic tannins) or a blue-black (gallic tannins) coloration were positive indicators.

3. *Saponins*

The extract (1 mL) was shaken vigorously with distilled water. A stable persistent froth for 20 min. was a positive indicator.

4. *Coumarins*

NaOH (2 mL, 10 %) was added to 1 mL of extract and formation of yellow color indicates the presence of coumarins.

5. *Terpenoids*
The extract (2 mL) was added to acetic anhydride (2 mL) and concentrated $H_2S\ O_4$ drops. Formation of blue, green rings indicated the presence of terpenoids.

6. *Fatty acids*
The extract (0.5 mL) was mixed with 5 mL of ether. The solution was allowed to evaporate on filter paper. The appearance of transparence on dried filter paper indicated the presence of fatty acids.

7. *Phenols*
Ferric chloride test, an extract (1 mL) was treated with drops of ferric chloride (5%) and observed for the formation of deep blue or black colour.

8. *Amino acids and Proteins*
The extract (1 mL) was treated with drops of ninhydrin solution (1 % ninhydrin solution) and placed in a boiling water bath for 2 minutes. The formation of purple colour was a positive test.

9. *Quinones*
An extract (1 mL) was treated with conc. HCl drops and observed for the formation of yellow precipitate or coloration.

10. *Oxalate*
The extracts (2 mL) were treated with a few drops of glacial acetic acid. A greenish black coloration indicates presence of oxalates.

Antioxidant status

TLC - Semi Quantitative DPPH Assay
0.2 % DPPH solution in methanol was prepared and kept in the fridge for further use. The grid space was marked with 1.0 cm^2 space on an aluminum based TLC sheet (Merck silica gel 60F254) and a stock solution of all the extracts together with the standard were prepared in methanol. A series of dilutions of the stock together with the standard were prepared ranging from 400 µL to 0.01µL for the last dilution. The grid on the TLC sheet was labeled with extract on the horizontal axis and amount of extract on the vertical axis. The extracts of different concentrations and the standards were plotted on the TLC sheets and the spots allowed to dry for at least 2 hours. Care was taken to keep the volume of the extracts spotted the same so that all the spots were of the same size for a fair comparison. The sheet was sprayed with 0.2% DPPH solution and the appearance of yellow spots against the white background showed the antioxidant activity. Photographs of the TLC were taken after 2 hours, 6 hours, and 24 hours and this could be used for further references because the DPPH gets faded with time. This procedure was adapted and revised from the methods which were previously used by Juma and Majinda (2004).[20]

2-Azobis-3-ethyl benzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity
ABTS radical scavenging activity was determined by the method described by Pellegrini *et al.*,[21] The ABTS radical cations are produced when ABTS (7 mM) reacts with potassium persulfate (2.45 mM) when incubated at room temperature in the dark for 16 hrs. The solution thus obtained was further diluted with phosphate buffer saline (PBS) to give an absorbance of 1.000. Different concentrations of the test sample in 50 µl were added to 950 µl of ABTS working solution to give a final volume of 1 mL. The absorbance was recorded immediately at 734 nm. Gallic acid was used as reference standard. Inhibiting concentrations of extracts were tested at 2.5, 5, 10, 25, 50 and 100µg/mL. Reference standard (gallic acid) was tested at 1, 2, 4, 8 and 16µg/mL. The percent inhibition was calculated from the following equation:

% inhibition = [(Absorbance of control – Absorbance of test sample) /Absorbance Control]x 100.

DPPH Assay - Spectrophotometric Method
The free radical scavenging activity was measured using DPPH method modified by Yeboah and Majinda (2009).[22] Solutions of 500 µM DPPH (i.e. 0.02 % or 0.2 mg/mL) in methanol (AR) was prepared. Also different concentrations of each of the plant extracts and standards were prepared (ascorbic acid and gallic acid) ranging from 0.001-0.05 mg/mL in methanol. Each extract or standard solution (2 mL) was added to an equal volume of the DPPH solution, making a total reaction volume of 4 mL. A control reaction mixture was prepared consisting of 2 mL methanol without extract and an equal volume of DPPH solution. The test tubes were tightly closed, vigorously shaken and placed in a dark cupboard for 30 minutes. The absorbance of each solution was measured at 517 nm, and methanol was used as the blank for baseline correction, after 2 h and finally after 24 h. The percentage inhibition of DPPH, I % was calculated using the following formula:

$$I\% = \frac{\text{(Absorbance control- Absorbance sample) x 100}}{\text{Absorbance control}}$$

From the inhibition curves, (I % versus sample concentration in µg/mL), the concentration of extract or standard required to inhibit DPPH radical activity by 50% (IC_{50}) was determined from non-linear regression equations that best fitted the curves. The experiment was carried out in triplicate and the IC_{50} values reported as the average of three trails in µg/ mL + the standard deviation.

Thiobarbituric acid (TBA) Assay
The method followed was described by Rezaeizadeh *et al.* (2011). [23] Briefly, 2mL of the extracts (1 mg/mL) were added to 1 mL aqueous trichloroacetic acid (20 %) and thiobarbituric acid (0.67%, 2 mL). After boiling for 10 min. the mixture was cooled and centrifuged at 3,000 rpm for 30 min. Absorbance of the supernatant was

recorded at 532 nm. The antioxidant activities were calculated by percentage of inhibition as follows:

% Inhibition = 100-[(A1-A0) ×100], where A0 is the absorbance of the control and A1 is the absorbance of the sample extracts.
Measurements were done in triplicates.

Total Phenolic Content Determination
The total phenolic content (TPC) of the extracts were determined using the Folin-Ciocalteu reagent method as described by Yeboah and Majinda (2009).[22] Five different concentrations of the standard, gallic acid and the extracts in methanol were prepared ranging from 0.01 to 0.05 mg/mL. 5 mL of 90% aqueous methanol and 0.5 mL Folin-Ciocalteu reagent were added to 0.5 mL of each of the standard solutions and to 0.5 mL of each extract solution (1 mg/mL) in screw cap test tubes. After 3 min, 1 mL of 2% Na2CO3 was then added to each test-tube and the mixture was vigorously shaken for 2 minutes and left to stand for 2 hours at room temperature. The absorbance of the supernatant solution was determined at 725 nm using 90% aqueous methanol as a solvent blank. A gallic acid standard curve was prepared and the equation derived by linear regression (y =36.84 x+0.1069) was used to determine the TPC of each extract in mg of gallic acid equivalents/g of extract (mg GAE/g). The experiment was performed in triplicate and TPC was reported as the average value of 3 trials ± the standard deviation.

Antimicrobial Activity
Microbial cultures
ATCC strains of microorganisms for testing the antimicrobial activity were obtained from Department of Microbiology, School of Allied Medical Sciences, University of Botswana, Botswana. The microorganisms included Gram positive cocci : *Staphylococcus aureus (ATCC 25923),* Methicillin resistant *Staphylococcus aureus (ATCC 43300), Staphylococcus aureus* from a patient sample, *Staphylococcus epidermidis (ATCC 12228) and Streptococcus agalactiae (ATCC 27956),* Gram positive bacilli: *Listeria monocytogenes,* Gram negative bacilli: *Escherichia coli (ATCC 10536), Klebsiella pneumonia (ATCC700803), Proteus mirabilis (ATCC 25933), Pseudomonas aeruginosa(ATCC 27853), Salmonella typhimurium(ATCC 43300) and a fungus: Candida albicans(ATCC 90028).* All organisms were tested for purity and maintained in nutrient agar plates (OXOID).[24]

Antimicrobial Susceptibility Testing
The methanol extract of all three plants, *Ocimum gratissimum, Momordica balsamina and Hypoxis hemerocallidea* were dissolved in 10% dimethyl sulfoxide (DMSO) to give a final concentration of 2gm/10 mL. Antimicrobial susceptibility testing to the different microorganisms were carried out by well diffusion on Mueller Hinton agar plates (MHA) (MAST) with some modification.[25] Using the base of a glass Pasteur pipette, sterilized by the hot flame from the Bunsen burner, 6 mm diameter wells were made in the MHA agar plate for carrying out the well diffusion method. Lawn cultures of each of the bacterial suspensions in Tryptone Soya broth (OXOID), adjusted to 0.5 McFarland standard turbidity (equivalent to a bacterial suspension of 1.5 X 10^8 colony forming units per ml (CFU/mlL) were made on the MHA plates. 100 microliters (µl) of the extract which gives a concentration of 20mg, was added to each well. DMSO (100µl) was also added to the wells in each plate as the control. Ampicillin (10µg) (Mast Diagnostics) discs were used as the positive control for each of the organism, meropenem (10µg) (Mast Diagnostics) for *Staphylococcus aureus* (ATCC 430043), ceftazidime (30µg) (Mast Diagnostics) for *Pseudomonas aeruginosa* and fluconazole (25µg) (Mast Diagnostics) for C.*albicans*. Co-trimoxazole (CTX 25µg) (Mast Diagnostics) was used for *Klebsiella pneumonia*.[24]

The plates were incubated at 37◦ C in ambient air for 24 hours and zones of inhibition measured using a vernier calipers. The tests were done in duplicates.

Minimum Inhibitory Concentration
Minimal inhibitory concentration (MIC) was also determined for each of the microorganisms to the extracts by a micro well dilution method with some modifications.[25] 96 -well plates were used for the assay. The 96 well plates were first prepared by adding 300µl of the 1gm/10ml of the extracts dissolved in 10% dimethylsulfoxide (DMSO) to the first well.150 µl of Tryptone Soya broth (TSB) was then added to all other wells.150 µl of the extract from the first well was transferred to the next well making the dilution of the extract 1:2 and 150 µl from the second well transferred to the third well and so on till a dilution of 1:512 was reached. 3 µl of bacterial suspension was added to all the wells. The last two wells contain positive control which is the bacterial suspension (3 µl in TSB) and negative control which is the TSB (150 µl) alone.[24]

The plates were covered and incubated at 37° C for 24 hours and then examined for visual inhibition of growth in the wells indicated by turbidity.

The MIC was considered the lowest concentration of the sample that prevented visible growth.

The minimum bactericidal concentrations (MBC) were also determined by sub-culturing the suspension from all the wells on to a MHA plate and incubated at 37◦ C in ambient air for 24 hours. The MBC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms.[26] The tests were done in duplicate.

The dilution assays are more reliable than diffusion assays due to many factors.[27] The MBC values are more reliable than the MIC values which depends only on the

visual observation of turbidity.[28] MIC values <1mg/ mL expressed by crude plant extracts are regarded as indicators of good antimicrobial activity with potential physiological relevance *in vivo*.[29]

Statistical analysis

All data were expressed as the mean ±S.E. mean of n=5. Analysis of variance was performed by one way ANOVA and the significant difference between the means were determined by the Holm-Sidak method. The *p* value ≤0.05 was regarded as significant. In all these cases, Statistical Software Stata 13.1 was used to analyze the data.

RESULTS

Phytochemical screening

Bioactive ingredient such as tannins, flavonoids, saponins, steroids, phenols and terpenoids were detected in the plant extracts (Table 1). Fatty acids were seen only in MEHHC. Amino acids and proteins were not detected in any of the extracts.

Table 1: Phytochemical screening of MEOG MEMB and MEHHC.

Test	MEOG	MEMB	MEHHC
Flavanoids	+	+	+
Tannins	+	+	+
Saponins	+	-	-
Coumarins	-	+	+
Terpenoids	-	-	+
Fatty acids	-	-	+
Phenols	+	+	+
Amino acids	-	-	-
α proteins	-	-	-
Quinones	+	-	-
Oxalate	-	-	-

MEOG: Methanol extract of *O.gratissimum*, MEMB: Methanol extract of *M. balsamina*
MEHHC: Methanol extract of *H. hemerocallidea*, GA-Gallic acid, AA Ascorbic acid,

Antioxidant Status

TLC - Semi Quantitative DPPH

The free radical, DPPH in the extracts gets reduced in the presence of an antioxidant molecule, giving rise to a colorless methanol solution. Figure-1 illustrates the decrease in the concentration of DPPH radical due to scavenging ability of hydro alcoholic extract of plant, vitamin C and gallic acid. The intensity of the antioxidant potential is dependent on the basis of the decolourisation of the extracts with DPPH. It was observed that MEOG showed the highest in these three extracts followed by MEOG and MEMB.

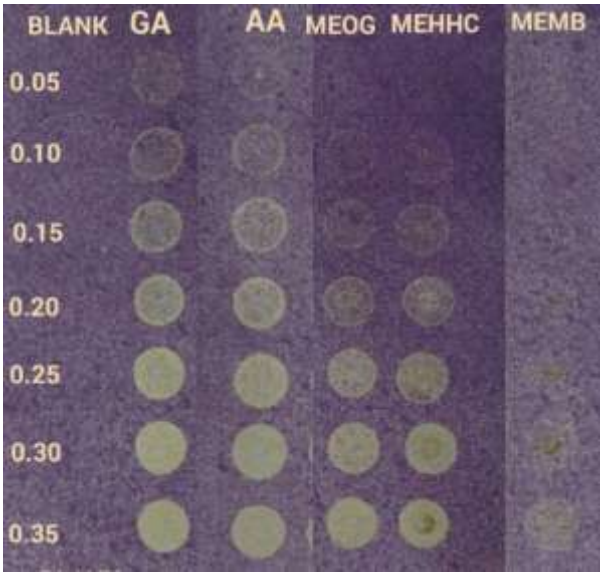

Fig 1: Semi quantitative TLC-DPPH radical scavenging activity of MEOG MEMB and MEHHC.
MEOG: Methanol extract of *O.gratissimum*, MEMB: Methanol extract of *M. balsamina.*
MEHHC: Methanol extract of *H. hemerocallidea*, GA-Gallic acid, AA Ascorbic acid.

ABTS Radical Scavenging Activity

This method is used for the screening of antioxidant activity and is reported as a decolourisation assay applicable to both lipophilic and hydrophilic antioxidants, the influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation are taken into account when determining the antioxidant activity.

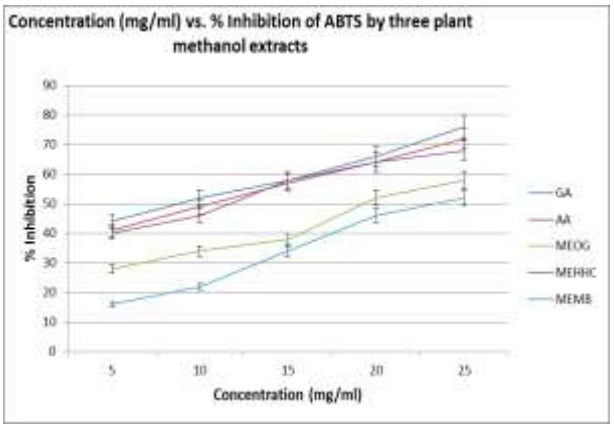

Fig 2: % inhibition of ABTS for MEOG, MEMB and MEHHC.
MEOG: Methanol extract of *O.gratissimum*, MEMB: Methanol extract of *M. balsamina.*
MEHHC: Methanol extract of *H. hemerocallidea*, GA-Gallic acid, AA Ascorbic acid.

DPPH ASSAY Spectrophotometric Method

DPPH is a free radical, stable at room temperature, and it produces a purple colour solution in methanol and is reduced in the presence of an antioxidant molecule, giving rise to uncoloured methanol solutions. This

method has been used extensively to predict the antioxidant activities due to its relatively short time required for reducing the purple colour. Fig: 3 show the radical scavenging activity of the extracts with DPPH and the results illustrates that the radical scavenging activity of the extract is increasing with the concentration. The positive DPPH test suggests that the samples posses' free radical scavenging activities.

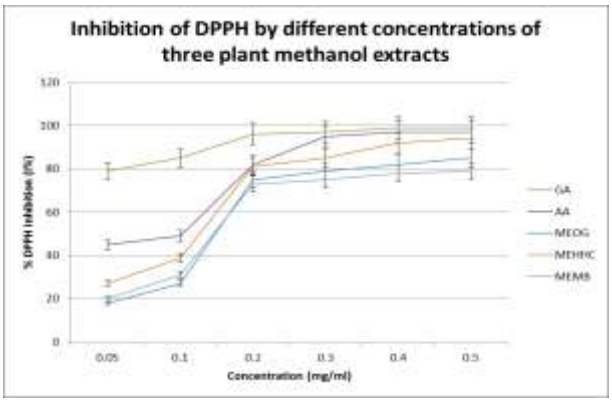

Fig 3: % inhibition in DPPH assay for MEOG, MEMB and MEHHC.
MEOG: Methanol extract of *O.gratissimum,* MEMB: Methanol extract of *M. balsamina.*
MEHHC: Methanol extract of *H. hemerocallidea,* GA-Gallic acid, AA Ascorbic acid.

Thiobarbituric acid (TBA) assay
Malondialdehyde assay is the most generally used test in the appreciation of the role of oxidative stress in disease. Malondialdehyde is one of the several products formed during the radical induced decomposition of polyunsaturated fatty acids. The methanol exhibits a good range of activity in % inhibition at a dose of 1 mg/mL. The activity of the methanol extract is more than that of VIT C but lower than that of BHT, Butylate hydroxyanisole which is a synthetic antioxidant. The comparison of the three extracts was presented in Table 2 clearly indicate that these extracts possess antioxidant potential, MEHHC showed the maximum followed by MEMB and MEOG.

Table 2: % Inhibition of TBA assay.

No	Extracts and Standards	% Inhibition at 30 Min	% Inhibition at 1 Hour
1	MEOG	52.11 ±1.21	56.92 ±.06
2	MEMB	56.88 ±.08	58.73 ±.04
3	MEMHC	70.56±.05	76.53±.09
4	BHT	95.66±.87	96.22±.05
5	AA	23.45±.12	25.06±.43

MEOG: Methanol extract of *O.gratissimum,* MEMB: Methanol extract of *M. balsamina*
MEHHC: Methanol extract of *H. hemerocallidea,* BHT-Butylate hydroxyanisole, AA Ascorbic acid

Total Phenolic Content
Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. Many natural antioxidants are phenolic compounds so the determination of total phenolic content could give useful information that could be correlated with antioxidant capacity of the sample. In the extracts used, TPC was calculated from the linear regression equation of the standard curve y =36.84 x+0.1069 as illustrated in Fig 4. From this equation the equivalent concentration of gallic acid 254.21± 0.43 mg mL was determined for each extract and converted to mg of gallic acid equivalents/g of dry extract (mg GAE/g). The total phenolic content of the extracts showed Table 3 indicated that MEHHC showed the maximum followed by MEOG and MEMB showed the least.

Table 3: Total phenolic content.

	Total Phenolic content(mg GAE/100g)		
Concentration (mg/ml)	MEOG	MEHHC	MEMB
0.1	0.71	27.66	2.84
0.2	10.64	54.96	17.02
0.3	18.09	60.28	26.24
0.4	31.91	76.60	38.30
0.5	54.96	79.79	49.29

MEOG: Methanol extract of *O.gratissimum,* MEMB: Methanol extract of *M. balsamina*
MEHHC: Methanol extract of *H. hemerocallidea,* GAE-Gallic acid.

Antimicrobial activity
The activities of methanol extracts of all the three plants against the different microorganisms were examined and their potency were qualitatively and quantitatively assessed by the zone inhibition, MIC values and MBC values. (Table: 3 & Table: 4).

In the well diffusion assay, methanol extract of *H.hemerocallidea* has comparatively greater antimicrobial activity towards the Gram positive organisms, with pronounced activity against Staphylococcus sp. *L.monocytogenes* is also more susceptible to this extract. All three extracts seems to have considerable activity against *S.agalactiae.* The antimicrobicidal activities of the three extracts against Gram negative organisms are comparatively lower than that for the Gram positive organism, although *P. mirabilis* is highly susceptible to all three extracts. *H.hemerocallidea* and *M.balsamina* extracts also has considerable antifungal activity against *C.albicans. O.gratissimum* has minimal antifungal activity.

Table 4: The antimicrobial activities of MEOG, MEMB and MEHHC.

Well diffusion (100ul = 20mg) Zone of inhibition(mm)				
Organisms	**MEOG**	**MEMB**	**MEHHC**	**Control (ampicillin-10ug)**
Gram positive Organisms				
Staphylococcus aureus (ATCC 25923)	12.5 ± 0.5	14	21	33
Staphylococcus aureus(Methicillin resistant) (ATCC 43300)	14	11	23	29[a]
Staphylococcus aureus. (patient sample)	14	15	17	26
Staphylococcus epidermidis (ATCC 12228)	12	15	18	28
*Streptococcus agalactiae (*ATCC 27956)	16	18	17	34
Listeria monocytogenes	13	12	18	37
Gram negative Organisms				
Escherichia coli (ATCC 10536)	11	12.5±0.5	10	19
Klebsiella pneumonia (ATCC 700803)	13	10	13	23[b]
Proteus mirabilis (ATCC 12228)	22	20	20	30
Pseudomonas aeruginosa (ATCC 27853)	14	13	15	27[c]
Salmonella typhimurium (ATCC 14028)	15	13.5±0.5	14	24
Fungus				
Candida albicans (ATCC 90028)	11	15 ± 1	17	25[d]

[a]*Meropenam (10ug),* [b]*CTX (25ug),* [c]*Ceftazidime (30ug),* [d]*Fluconazole (25ug).*

The MIC of the extracts ranged between *0.117 -7.5* mg/mL for MEOG, 0.234 – 1.875 mg/mL for MEMB and 0.234 - 0.938 mg/mL for MEHHC.The MBC of the extracts ranged between *0.117 -15* mg/mL for MEOG, 0.234 – 1.875 mg/mL for MEMB and 0.234 – 1.875 mg/mL for MEHHC. MBC of less than 1 mg/mL for MEOG is recorded only for *S.agalactiae* (0.117mg/mL) and highest for staphylococcal species and the Gram negative *S.typhimurium* (15mg/mL). All other organism has an MBC greater than or equal to 1.875mg/mL. MEMB showed a MBC of less than 1 mg/mL for *S. aureus* (ATCC 25923), *S.agalactiae, L. monocytogenes, P. mirabilis, S.typhimurium.* Higher MBC of 1.875 mg/mL is noted for all other Gram positive and Gram negative organisms. In the case of MEHHC, except for Gram negative *E.coli, K. pneumonia* and the fungus *C.albicans* all the other organisms had a MBC of < 1mg/mL.

Table 5: The MIC and MBC of MEOG, MEMB and MEHHC.

	MBC (mg/ml)	**MIC (mg/ml)**	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**	**MIC (mg/ml)**
	MEOG		**MEMB**		**MEHHC**	
Gram positive Organisms						
Staphylococcus aureus (ATCC 25923)	7.5	0.938	0.938	0.234	0.469	0.234
Staphylococcus aureus(Methicillin resistant) (ATCC 43300)	15	7.5	1.875	0.469	0.938	0.938
Staphylococcus aureus (patient sample)	3.75	1.875	1.875	0.938	0.938	0.938
Staphylococcus epidermidis (ATCC 12228)	1.875	1.875	1.875	0.938	0.938	0.938
*Streptococcus agalactiae (*ATCC 27956)	0.117	0.117	0.234	0.234	0.469	0.234
Listeria monocytogenes	3.75	0.938	0.469	0.234	0.234	0.234
Gram negative Organisms						
Escherichia coli (ATCC 10536)	3.75	1.875	1.875	1.875	1.875	0.469
Klebsiella pneumonia (ATCC 700803)	3.75	0.469	1.875	0.469	1.875	0.469
Proteus mirabilis (ATCC 12228)	3.75	0.938	0.938	0.234	0.938	0.469
Pseudomonas aeruginosa (ATCC 27853)	3.75	0.469	1.875	0.234	0.469	0.469
Salmonella typhimurium (ATCC 14028)	15	3.75	0.938	0.469	0.938	0.938
Fungus						
Candida albicans (ATCC 90028)	3.75	0.938	1.875	0.938	1.875	0.938

DISCUSSION

Medicinal plants have been used as therapeutic agents for centuries because of the presence of secondary metabolites, referred to as 'phytochemicals', that have been shown to be effective in combating or preventing disease due to their antioxidant ability. [30] In recent years, there has been growing scientific evidence supporting the fact that many of the observed pharmacological activities of plant extracts such as anticancer, anti-diabetic and antiviral are all directly linked to their antioxidant activity. The study on the

medicinal plants is essential to promote the proper use of herbal medicine in order to determine their potential as a source for the new drugs.[31] In many developing countries, a large proportion of the population relies on traditional practitioners and their medicinal plants in order to meet health care needs. Plant sources have been widely investigated for the search of new and innovative antibacterial agents to be used for the treatment of infectious diseases. Medicinal plants are an enormous source of alternative antimicrobial therapy, particularly in the face of emerging resistance against orthodox antimicrobial drugs. From time immemorial, many plants have been used by man as source of treatment of various disease conditions particularly at the community level. These plants are referred to as alternative or complimentary medicines.[32]

Antioxidant Status

Plants synthesize many complex compounds referred to as secondary metabolites that have no immediate obvious growth or metabolic functions but serve survivability and fecundity functions for the plant itself.[33] One important class of such secondary metabolites is the phenol class. Phenolic compounds have been shown to possess remarkable antioxidant properties, due to their redox properties, which enables them to act as free radical scavengers and inhibitors of lipid peroxidation.[34] The total antioxidant status test with DPPH, ABTS and TBA assays revealed that all the above extract showed antioxidant potential in a dose dependent manner. When comparing its antioxidant potential, MEHHC showed the maximum followed by MEOG and MEMB. Antioxidants such as dietary flavonoids work by scavenging free radicals, chelation of metal ions, and decomposition of peroxides such as lipid peroxides.[35] They are thought to play important role in the prevention of various human disorders as free radical scavengers. Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Phenolic compounds possess hydroxyl and carboxyl groups, able to bind particularly iron and copper.[36]

Antimicrobial activity

The phytochemicals present in the plant extracts like phenols, flavonoids and tannins have been reported to complex with proteins and polysaccharides leading to inactivation of microbial adhesion enzymes, cell envelope, and the transport protein accounting for its antimicrobial activity. The phenolic component can cause leakage of intracellular ATP and potassium ions leading to bacterial cell death. The flavonoids complexes with bacterial cell wall and disrupt the membrane integrity. Tannins have been reported to inhibit microbial adhesion enzymes and cell envelope proteins. Tannins also have been shown to have antiviral activity by inhibiting viral reverse transcriptase.[29]

The MEHHC seems to be having more potent antimicrobicidal activity compared to MEMB and MEOG. This may be due to the presence of more phytochemicals and higher concentration of these phytochemicals in the MEHHC. As reported in this study, the total phenolic content and the antioxidant potential of MEHHC was greater than MEOG and MEMB.

The dilution assays are more reliable than diffusion assays due to many factors.[36] The MBC values are more reliable than the MIC values which depends only on the visual observation of turbidity.[37] MIC values <1mg/ mL expressed by crude plant extracts are regarded as indicators of 'good' antimicrobial activity with potential physiological relevance *in vivo*.[27]

All extracts had more activity against the Gram positive organisms than the Gram Negative organisms. This is attributed to the difference in cell wall structure of the organisms which determines the binding of active component to the cell wall, entry of these components inside the bacterial cell and final disruption of the metabolism inside the cell and death of the microorganism. The cell wall of Gram negative bacteria has an outer membrane of lipopolysaccharides (LPS) which is hydrophilic and absent in Gram positive bacteria. This layer is also anionic due to acidic proteins, carboxyl and phosphate groups connected to the LPS.[38] The MEHHC in this study had high phenol content and phenols are hydrophobic. Thus the hydrophilic outer membrane of the Gram negative bacteria would be an inhibitory barrier for efficient penetration of hydrophobic phenolic phytochemicals, enabling the Gram negative bacteria to be less susceptible to the extracts.[38]

The presence of rooperol, a phytochemical present in *H. hemerocallidea* has been attributed to its greater activity of this extract against *S.aureus.* It has strong affinity for phosphatidylglycerol. The postulated mechanism of action involves binding of this component, rooperol, specifically to phosphatidyl glycerol which is abundant on the cell membrane of *S.aureus*, causing leakage and subsequently inhibition of bacterial growth.[39]

A ribosome inhibiting protein (RIPs) called balsamin and a Cucurbitane-type triterpenoids compounds found as active components in the extracts of *M.balsamina*, has a DNase like activity and inhibitory activity of the efflux pump system of Gram-positive bacteria, respectively. This also contributes to its antimicrobicidal activity.[40,41,42]

The methanol extract of *H. hemerocallidea* and *M.balsamina* showed considerable antifungal activity against *Candida albicans* species though at a higher concentration [MIC - 0.938mg/ml and MBC-1.875mg/ml]. Momordica plants produce a number of proteins and peptides that are indicative of antifungal activity, including trypsin inhibitors, lectins, ribosome-inactivating proteins and ribonucleases.[43]

CONCLUSION

The present study suggested that all the tree extracts used possess a considerable amount of antioxidant as secondary metabolites; it could be used as the potential source of natural antioxidants and thus could be useful as therapeutic agents as it showed a high reducing and radical scavenging activity. In the antimicrobial studies all the three extracts had more activity against the Gram positive organisms than the Gram Negative organisms. Further works should include the separation and characterization of active components which shows the antioxidant potential and antimicrobial activity.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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REFERENCES

1. World Health Organization. Legal Status of Traditional Medicines and Complementary/Alternative Medicine: A Worldwide Review, 2009. WHO Publishing www.who.int/topics/traditional medicine.
2. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan. 1993.
3. Hostettmann K, Marston A. Twenty years of research into medicinal plants: Results and perspectives. Phytochem Rev, 2002; 1: 275–285.
4. Ogbo, E. M. Oyibo, A. E. Effects of three plants extracts (*Ocimum gratissimum*, *Acalypha wilkesiana* and *Acalypha macrostachya*) on post-harvest pathogen of *Persea Americana*. J Med Plants Res., 2008; 2(11): 311-314.
5. Buricova L Reblova Z, Zech. Medicinal plants as possible sources of antioxidants. Czech.293 J. Food Science, 2008; 26: 132-138.
6. Jyothi A, Venkatesh K, Chakrapani P, Roja R. Phytochemical and pharmacological potential of Annona cherimola - A review. Int J Phytomed, 2011; 3: 439-47.
7. Vieira, R. F., J. E. Simon. Chemical characterization of basil (Ocimum spp.) found in the markets and used in traditional medicine in Brazil. Economic Botany, 2000; 54(2): 207-216.
8. Chaturvedi, P., George, S., John, A..Preventive and protective effects of wild Basil in ethanol –induced liver toxicity in rats. Bri J Biomed Sci., 2007; 64(1): 10-12.
9. Joshi, R. K. Chemical composition, in vitro antimicrobial and antioxidant activities of the essential oils of Ocimum gratissimum, O. sanctum and their major constituents. Ind J Pharm Sci., 2013; 75(4): 457.
10. Van Wyk B-E, Van Outshoorn B, Gericke N. *Medicinal Plants of South Africa*, 2nd edn. Briza Publications: Pretoria, 2002; 156–157.
11. B.-E. Van Wyk, "The potential of South African plants in the development of new medicinal products," South African J Botany, 2011; 77(4): 812–829.
12. G.J. Boukes, M. VandeVenter, and V.Oosthuizen. Quantitative and qualitative analysis of sterols/sterolins and hypoxoside contents of three*Hypoxis* (African potato) spp., African J. Biotech, 2008; 7(11): 1624–1629.
13. Boukes G, J., Van de Venter M. In vitro modulation of the innate immune response and phagocytosis by three *Hypoxis* spp. and their phytosterols. South African J. Botany, 2016; 102: 120-126.
14. Thakur, Gulab S.; Bag, Manoranjan; Sanodiya, Bhagwan S.; Bhadauriya, Pratiksha; Debnath, Mousumi; Prasad, G. B.K.S.; Bisen, P. S. *Momordica balsamina*: A Medicinal and Neutraceutical Plant for Health Care Management. Cur Pharma Biotech, 2009; 10(7): 667-682.
15. Puri, Munish "Momordica balsamina: A Medicinal and Neutraceutical Plant for Health care Management". Comments: Biotechnological Potential of M. balsamina Revealed. Cur Pharma Biotech, 2010; 11(2): 229-229.
16. YS Bot, LO Mgbojikwe, C Nwosu, A Abimiku, J Dadik, D Damshak Screening of the fruit pulp extract of *Momordica balsamina* for anti HIV property. African J. Biotech, 2007; 6(1).
17. Elujoba, A.A. Studies on the antidiarrhoea activity of Ocimum gratissimum. University of Ile Ife Press, 2000.
18. Morris K. South Africa tests traditional medicines. Lancet nfect Dis., 2002; 2: 319.
19. Mazimba, O., Wale, K., Kwape, T. E., Mihigo, S. O., & Bokolo, M. K. Cinnamomum verum: Ethylacetate and methanol extracts antioxidant and antimicrobial activity. J Med Plants Studies, 2015; 3(3): 28-32.
20. Juma, B.F., Majinda. R.R.T. Erythrinaline alkaloids from the flowers and pods of *Erythrina lyistemon* and their DPPH radical scavenging properties. Phytochemistry, 2004; 65: 1397-1404.
21. Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine, 1999; 26(9): 1231-1237.
22. Yeboah, E. M., & Majinda, R. R. Radical scavenging activity and total phenolic content of extracts of the root bark of Osyris lanceolata. Natural product communications, 2009; 4(1): 89-9.

23. Rezaeizadeh A, Zuki ABZ, Abdollahi M, Goh YM, Noordin MM, Hamid M Determination of antioxidant activity in methanolic and chloroformic extracts of *Momordica charantia*. Afri J Biotechnol, 2011; 10(24): 4932-4940.
24. Souda, S. et al., An Evaluation of the Antioxidant Status and Antimicrobial Activity of the Methanol Extract of Ocimum Gratissimum.IRA-Inter J. Applied Sciences, 2016; 5(1): 40-56. doi:http://dx.doi.org/10.21013/jas.v5.n1.
25. Marie B. Coyle, Manual of Antimicrobial Susceptibility Testing, 2005.
26. Nakamura, C. V., Ueda-Nakamura, T., Bando, E., Melo, A. F. N., Cortez, D. A. G., & Dias Filho, B. P. Antibacterial activity of Ocimum gratissimum L. essential oil. *Memórias do Instituto Oswaldo Cruz*, 1999; 94(5): 675-678.
27. Rios, J.L., Recio, M.C. & Villar, A., Screening methods for natural products with antimicrobial activity: A review of the literature. *Journal of Ethnopharmacology*, 1988; 23(2–3): 127–149.
28. Junaid, S. A., Olabode, A. O., Onwuliri, F. C., Okwori, A. E. J. and Agina, S.E., The antimicrobial properties of Ocimum gratissimum extracts on some selected bacterial gastrointestinal isolates. *African Journal of Biotechnology*, 2006; 5(22): 2315–2321. Available at: http://www.academicjournals.org/AJB.
29. Mann, A. Phytochemical constituents and antimicrobial and grain protectant activities of clove basil (Ocimum gratissimum L.) grown in Nigeria. *International Journal of Plant Research*, 2012; 2(1): 51-58.
30. Ogunniran, K. O. Antibacterial effects of extracts of Ocimum gratissimum and piper guineense on Escherichia coli and Staphylococcus aureus. *African Journal of Food Science*, 2009; 3(3): 77-81.
31. Sofowora, E. A. *Medicinal plants and Medicine in Africa* 2nd eds. John Wiley and Sons, 1993; 6.
32. Kunle O F, Egharevba H O & Ahmadu P O, Standardization of herbal medicines - a review. *International Journal of Biodiversity and Conservation*, 4(2012): 101–112.
33. Mshana, N.R., et, al. Traditional medicine and pharmacopoeia contribution to the revision of Ethnobotanical and floristic studies in Ghana, Scientific, *Technical and Research Commission of the Organization of African Unity*, 2000.
34. Dufresne, C.J., Farnsworth, E.R., A review of the latest findings on health promoting properties in tea. Journal of Nutritional Biochemistry, 2001; 12: 404–421.
35. Joshi, R. K. Chemical composition, in vitro antimicrobial and antioxidant activities of the essential oils of Ocimum gratissimum, O. sanctum and their major constituents. *Indian journal of pharmaceutical sciences*, 2013; 75(4): 457.
36. Gandhiraja N, Sriram MV, Kavitha C, Sasikumar C, Rajeswari R. Phytochemical screening and antimicrobial activity of the plant extracts of *Mimosa pudica* L against the selected microbes. Ethnobotanical leaflets, 2009; 13: 618-24.
37. Gajalakshmi S, Vijayalakshmi S, Rajeswari V. Phytochemical and pharmacological properties of Annona muricata: A review. Int J Pharm Pharmaceu Sci, 2012; 4(2): 3-6.
38. Mannathoko, N, et, al., An *in-vitro* Analysis of the Antioxidant and Antimicrobial Properties of the Methanol Extract of *Hypoxis hemerocallideacorm* (MEHHC) from Botswana. *Microbiology Research Journal International*, 2017; 19(6): 1-12.
39. Laporta O, Perez-Fons L, Mallavia R, Caturla N, Micol V. Isolation, characterization and antioxidant capacity assessment of the bioactive compounds derived from Hypoxis rooperi corm extract (African potato). *Food Chemistry*, 2007; 101: 1425-1437.
40. Ajji, P.K., Walder, K. & Puri, M., Functional Analysis of a Type-I Ribosome Inactivating Protein Balsamin from Momordica balsamina with Anti-Microbial and DNase Activity. *Plant Foods for Human Nutrition*, 71(3): 265–271. Available at: http://dx.doi.org/10.1007/s11130-016-0555-4.
41. Kaur, I. et al., Balsamin, a novel ribosome-inactivating protein from the seeds of Balsam apple Momordica balsamina. *Amino Acids*, 2012; 43(2): 973–981.
42. Ramalhete, C. et al., Cucurbitane-type triterpenoids from the African plant Momordica balsamina. *Journal of Natural Products*, 2009; 72(11): 2009–2013.
43. Burger, Y. et al., Variation in antifungal activity in extracts from Momordica plants. *Israel Journal of Plant Sciences*, 2010; 8(1): 1–7. Available at: http://www.sciencefromisrael.com/openurl.asp?genre=article&id=doi:10.1560/IJPS.58.1.1.