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In Vitro Screening of Ten Selected Traditionally Used Medicinal Plants in Vihiga County, Kenya for Antibacterial and Antifungal Activity

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Abstract: *The current role of plants in infection control in Vihiga County, Kenya involves the management of minor oral, skin or wound infections as well as gastrointestinal and sexually transmitted infections, and provision of a supportive role in opportunistic infections in HIV/AIDS. The study aimed to explore the potential anti-bacterial and antifungal activity of ten selected plants with a view to providing scientific basis for their continued ethno-medicinal use. The in vitro antibacterial and antifungal activity of plant extracts (water, methanol and chloroform), 2500 µg/well, against standardized inoculum of S. aureus and C. albicans, was evaluated using the agar well diffusion method. For the most active extracts, the minimum inhibitory concentration was determined as well as the active fractions through bioautographic agar overlay procedure. Of the 30 extracts tested, 70 % had activity against C. albicans and 90 % against S. aureus. Extracts of F. africana, B. axillaris, C. mimosoides, P. peruviana, L. trifolia and A. sessiliflora were the most active (MIC 1.56 - 50 mg/ml). The exhibited activity corroborates the folkloric use and may inform the continued traditional use of the herbal remedies or use of isolated constituents as possible lead compounds in the development of newer antimicrobial agents.*

Keywords: *ethno-medicinal evaluation, in vitro activity, antifungal activity, antibacterial activity*

1. INTRODUCTION

The emergence of fungal and bacterial opportunistic infections as well as the challenges in their management has led to the continuous need for exploration, discovery and development of innovative antifungal and antibacterial drug molecules including those from plant sources. Ethno-pharmacological information on plants is often of importance while searching for plants of potential medicinal value or new ways of using an already known plant. It has been estimated that 74% of the pharmacologically active plant derived components were discovered after ethno-medical evaluation of the respective plants [1-3]. Several plants in Kenya have similarly been evaluated and found to possess antimicrobial activity [4].

The current role of plants in infection control in Vihiga County, Kenya involves the management of minor oral, gastrointestinal and sexually transmitted infections, skin or wound infections and provision of a supportive role in opportunistic infections in HIV/AIDS. Some of the commonly used plants in this regard were selected for this study. The whole plant or their parts are traditionally used individually or in combination prepared in diverse ways. The whole plant may be boiled into a decoction that is taken orally or applied topically. The root and stem infusions are similarly used. The dried whole plant, after burning into ash, is applied to the affected areas, as is the fine powder of the pounded dried whole plant. Chewing of the fresh leaves and flowers of some of these plants, for example, *Spilanthes mauritiana* (Rich. ex Pers.) DC reportedly gives quick relief from mouth infections [5].

With the continued need to evaluate the local medicinal plants varieties, the study aimed to explore any potential antibacterial and antifungal activity of ten selected plants with a view to providing scientific basis for their continued traditional use in treating of microbial infections. The *in vitro* antibacterial and antifungal activity of the plants crude extracts against bacteria and fungi of medicinal importance was thus evaluated.

2. MATERIALS AND METHODS

A. Plant Material Collection and Identification

Using the ethno-medicinal approach, plant species frequently used traditionally for the treatment of infectious diseases were selected. The whole plant or aerial parts as traditionally used were collected from Vihiga County in western Kenya and were identified and voucher specimens deposited at the School of Biological Sciences (formerly Department of Botany) Herbarium, University of Nairobi. The voucher specimens are preserved under voucher number series BA/01/2004 to BA/10/2004. The study plants are: *Sida tenuicarpa* Vollesen (Malvaceae), *Spilanthes mauritiana* (Rich. ex Pers.) DC. (Asteraceae), *Fuerstia africana* T.C.E.Fries (Labiatae), *Chamaecrista mimosoides* (L.) Greene (Caesalpiniaceae), *Alectra sessiliflora* (Vahl.) Kuntze (Scrophulariaceae), *Blumea axillaris* (Lam.) DC.(Asteraceae), *Cucumis anguria* L. var. *longaculeatus* J.H.Kirkbride (Cucurbitaceae), *Hibiscus fuscus* Garcke (Malvaceae), *Physalis peruviana* L.(Solanaceae) and *Lantana trifolia* L. (Verbenaceae).

B. Plant Material Preparation

The collected plant materials were spread out and dried at room temperature away from direct light for about 10 days. The dried plant materials were then ground to a fine powder using a commercial mill. About 100 g of the powdered material was then subjected to a cold maceration process with chloroform (500 ml) for 24 h with stirring. Fresh batches of the powdered material were similarly each extracted with methanol and distilled water, respectively. The macerate from each extraction was filtered through a Whatman no. 1 filter paper (Whatman International Ltd, England). The chloroform and methanol filtrate was concentrated each to dryness on a Heidolph VV 2000 rotary evaporator at 50 ^{0}C. The water extracts were freeze dried on a Heto FD 1.0, 110 model freeze drier. All dried extracts were stored at 4 ^{0}C until use.

C. Microorganism Strains and Inoculums Preparation

Eight microorganisms; five bacteria, *Staphylococcus aureus* (NC07447), *Escherichia coli* (ATCC 25922) *Bacillus pumilus* (local strain), *Pseudomonas aeruginosa* (local strain) and *Shigella dysenterae* (local strain) and three fungi, *Aspergillus niger* (NCPF 2275), *Candida albicans* (NCPF 3179) and *Cryptococcus neoformans* (local strain) were used. The reference micro organisms with NC prefix were obtained from the United Kingdom National Culture Collections while those with ATCC number from the American Culture Collections courtesy of the National Quality Control Laboratory, Ministry of Health, Kenya. The local strains were clinical isolates obtained from Kenyatta National Hospital Laboratory Department. Tryptone Soya (TS) Agar and Sabouraud's dextrose (SD) agar both obtained from Oxoid Ltd, England were used as the nutrient medium for the bacteria and fungi, respectively. The test microorganisms were grown on their respective nutrient medium at 37 °C for bacteria and at 30 ^{0}C for fungi and then maintained on nutrient agar slants stored at 4 °C. Standardized microbial suspension (10^6-10^7 colony forming units (c.f.u)/ml) from one-day-old subcultures were used in screening for activity [6]. The zone diameter was read using a Wezu electronic digital calliper (Wezu Messzenge GmbH, Germany) to the nearest 0.01 mm.

D. Microbiology Procedures

All glassware used in the microbiology procedures were dry heat sterilized in a Memmert universal oven(Memmert GmbH, Germany) while the nutrient media and water were sterilized using a portable autoclave(Dixons, U.K). The bacteriological loop and cork-borer were sterilized over open bunsen burner flame before use. All aseptic and microbiological procedures were carried out under a bioflow laminar flow equipment (Vermeulen, L.J., Belgium) while a Freezer-1 incubator (Analis, Belgium) was used to incubate all inoculated plates.

E. Antimicrobial Activity Testing

Antibacterial and Antifungal Activity Testing

The *in vitro* antibacterial and antifungal activity of the plant extracts, at fixed concentrations of 2500 µg/ well, against *S. aureus* and *C. albicans* standard inoculums, was done by the disk diffusion method [7]. The molten nutrient agar prepared as per the manufacturer's instructions was inoculated uniformly with the standardised test organism's suspension before allowing setting. Extracts were applied in reservoirs formed by cutting out the set agar with a cork borer to form 6.80 mm diameter and 3.00 mm depth cylindrical wells in the agar layer. Using a fixed volume micropipette, the wells

were each filled with 50 µl of the solutions of the extracts (50 mg/ml, 2500 µg/well). A similar volume of sterile distilled water or 1 % dimethylsulfoxide (DMSO) were used as negative controls while standard drug concentrations, chloramphenicol 1.0 mg/ml (50 µg/well) for bacteria and nystatin 1.0 mg/ml (50 µg/well) for fungi were the positive controls. A diffusion period of 1 h at refrigerator temperature was allowed before incubation at the standardised conditions of temperature and time (37 ^{0}C for bacteria and 30 ^{0}C for fungi for 8 h). The zone of inhibition diameter observed after incubation, was taken as the quantitative parameter. All determinations were in triplicate and results are given as the means and standard error of the means (s.e) of the zone diameters.

Minimum Inhibitory Concentration Determination

The minimum inhibitory concentrations (MICs) for the most active extracts against the test microorganisms were determined in 24-well micro-titre plates by serial two-fold dilution of extracts by a modification of the broth micro-dilution method as described by the National Committee for Clinical Laboratory Standards [8]. For each test micro-organism, 50 µl of test extracts concentrations of 1.56 mg/ml to 50 mg/ml were each added in the respective wells. Two wells were used as controls with the respective solvent as negative control whilst the standard drugs were also used as positive controls. After adding 200 µl nutrient media each uniformly inoculated with 20 µl of the microbial suspension to each well containing extract, the micro-plate was sealed and incubated at the optimum conditions for each microorganism.

After incubation period, 20 µl of a 0.2 mg/ml solution of 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each row and the plate was returned to the incubator for an extra one hour to ensure adequate colour development. Inhibition of growth was indicated by a yellowish colour in well. The MIC of the extract was defined as the lowest concentrations of the extract that visually showed the yellow coloration compared with purple colour in negative control wells.

Bioautographic Agar Assay

The bioautographic agar overlay procedure as described by Nostro *et al*. was used to determine the active fractions of the chloroform and methanol extracts that had shown marked antimicrobial activity at screening [9]. Extracts were reconstituted to 10 mg/ml in the mobile phase and 50 µl of each extract spotted on two preparatory (20 cm x 20 cm) thin layer chromatography (TLC) plates. The two TLC plates were developed in an optimized chromatographic system consisting of silica gel 60 F_{254}, 0.75 mm as stationary phase and chloroform-methanol (19:1, v/v) as mobile phase. One plate was visualized by the relevant reagents (ultra violet, UV, light at 254 nm, Iodine vapour and Vanillin reagent) and served as the reference chromatogram whilst the other was uniformly and evenly covered with the standardized inoculum suspension of the test microbes in molten agar. After media solidification, the test TLC plate was incubated overnight at the optimum conditions for the microorganism. After incubation the plates were sprayed evenly with MTT solution for visualization of zones.

3. RESULTS

A. Antibacterial and Antifungal Activity Screening of Extracts

Thirty chloroform, methanol and water extracts from whole or aerial parts of the selected ten plant species belonging to 8 families were screened against *S. aureus* and *C.albicans*, known pathogenic bacteria and fungi, respectively. Table 1 shows the zones of growth inhibition diameters and are presented as the arithmetic average (mean value ± s.e) of the triplicate determinations for each extract. Overall, diameters equal to or greater than 7.00 mm were considered susceptible to extracts tested and a diameter of ≥ 14.0 mm high activity [10]. Of the extracts tested, 90 % inhibited bacterial growth (diameters, 9.25-30.00 mm). Only the *C. mimosoides* water extract (2500 µg/well), (diameter, 30.00 mm), had greater activity than the positive control, standard antibiotic, tested at 50 µg/well. The descending order of antibacterial activity was found to be: *C. mimosoides* (water), *L. trifolia* (chloroform and methanol), *A. sessiliflora* (methanol), *B. axillaris* (methanol), *F. africana* (chloroform), *C. mimosoides* (chloroform and methanol), *F. africana* (water) and *P. peruviana* (chloroform) for the most active extracts. Of the 30 extracts tested, 21 (70 %) had activity against *C. albicans* (diameters, 10.00 - 13.88 mm).The exhibited antifungal activity is noted to be much less than that of the reference antifungal agent, nystatin (21.33 mm) at the tested concentrations. All extracts with antifungal activity in addition possessed antibacterial activity.

Further screening of the most active extracts against a wider range of pathogenic bacteria and fungi showed varied activity (Table 2). The methanol extracts of *B. axillaris* and *A. sessiliflora* gave appreciable antibacterial and antifungal activity across the expanded list of microorganisms. The narrowest activity was by *S. mauritiana* and *A. sessiliflora* chloroform extracts. The most resistant bacteria to the extracts' activity were *E.coli*, *P aeruginosa* and *S. dysenteriae*. All the extracts with activity against *C. albicans* a yeast, also exhibited activity against the filamentous fungi *A. niger* except *P. peruviana* and *S. mauritiana* chloroform extracts. It was however noted activity was least against C. *albicans* compared to *A. niger* and the standard antifungal.

B. Minimum Inhibitory Concentrations

The MIC of the most active extracts against the test microbes (Table 3) lay between 1.56 and 50 mg/ml. In addition to *C. mimosoides* water extract, other extracts that showed good antibacterial activity (1.56 mg/ml) include the methanol and chloroform extracts of *L. trifolia.* Notable antibacterial activity as shown by low MIC values, include *C. mimosoides*(water and chloroform), *A. sessiliflora* (methanol), *B. axillaris* (methanol), *F.africana*(chloroform) *P. peruviana* (chloroform and methanol) and *S. mauritiana* (methanol) extracts, in this order. The lowest MIC against the test fungi was 3.13 mg/ml with the descending order of the top 10 antifungal activities as *F. africana* (chloroform), *S. mauritiana* (methanol), *L. trifolia* (methanol), *B. axillaris* (methanol), *C. mimosoides* (methanol), *L. trifolia* (chloroform), *B. axillaris* (chloroform), *C. mimosoides* (chloroform) and *A. sessiliflora* (chloroform and water) extracts.

Table1. *Inhibitory effect of plant extracts on bacterial and fungal pathogens*

Plant	Extract solvent	Inhibition zone, mm, (±s.e)	
		Staphlococcus aureus	*Candida albicans*
Alectra sessiliflora	Chloroform	11.78 (±0.76)	11.50 (±1.21)
	Methanol	17.46 (±0.18)	10.90 (±0.09)
	Water	13.68 (±0.30)	11.46 (±0.55)
Blumea axillaris	Chloroform	10.84 (±0.17)	11.83 (±0.30)
	Methanol	16.41 (±0.31)	13.09 (±1.07)
	Water	11.67 (±0.18)	11.20(±0.13)
Chamaecrista mimosoides	Chloroform	15.32 (±1.34)	11.61 (±0.09)
	Methanol	14.50 (±0.52)	12.78 (1.13)
	Water	30.00(±1.46)	NI
Cucumis aculeatus	Chloroform	13.35 (±1.05)	10.10 (±0.14)
	Methanol	9.72 (±0.21)	NI
	Water	NI	NI
Fuerstia africana	Chloroform	15.88 (±0.54)	13.86 (±0.18)
	Methanol	13.11 (±0.24)	11.10 (±1.05)
	Water	13.93 (±0.32)	NI
Hibiscus fuscus	Chloroform	10.46 (±0.13)	10.40 (±0.65)
	Methanol	11.42 (±0.26)	10.82 (±0.27)
	Water	NI	NI
Lantana trifolia	Chloroform	20.97 (±0.88)	12.29 (±0.83)
	Methanol	20.59 (±0.92)	13.37 (±0.74)
Physalis peruviana	Water	13.23 (±1.04)	10.47 (±1.04)
	Chloroform	13.78 (±0.79)	11.36 (±0.72)
	Methanol	13.66 (±0.31)	10.83 (±1.04)
	Water	12.78 (±0.61)	10.68 (±0.91)
Sida tenuicarpa	Chloroform	11.29 (±1.12)	NI
	Methanol	9.62 (±0.84)	NI
	Water	NI	NI
Spilanthes mauritiana	Chloroform	9.33 (±0.24)	10.00 (±0.22)
	Methanol	12.47 (±0.54)	13.40 (±0.61)
	Water	9.25 (±0.82)	NI
Chloramphenicol		21.17 (±0.11)	-
Nystatin		-	21.33 ((±0.17)

NI-no inhibition; well size: 6.80 mm; extract concentration, 2500 µg/well; chloramphenicol 50 µg/well; nystatin 50 µg/well.

Table2. *Antibacterial and antifungal effect of active extracts against selected bacteria and fungi*

Extract	**Solvent**	**Bacteria**					**Fungi**	
		Staphylococcus aureus	*Pseudomonas aeruginosa*	*Escherichia coli*	*Shigella dysenteriae*	*Bacillus pumilus*	*Candida albicans*	*Aspergillus niger*
Alectra sessiliflora	Chloroform	++	+	_	_	+++	+	+
Alectra sessiliflora	Methanol	++++	+++	+++	++	++++	+	++
Blumea axillaris	Chloroform	++	+	++	++	+++	++	+++
Blumea axillaris	Methanol	++++	++	+++	++	++++	++	++++
Chamaecrista mimosoides	Chloroform	++++	++	+++	+++	++	++	++
Chamaecrista mimosoides	Methanol	++++	+	++	_	+	++	++
Chamaecrista mimosoides	Water	++++	+++	+++	++	++++	_	_
Fuerstia Africana	Chloroform	++++	++	+	++	++++	+++	+++
Lantana trifolia	Chloroform	++++	+++	+++	++	+++	++	++
Lantana trifolia	Methanol	++++	++	+++	++	+	++	++
Physalis peruviana	Chloroform	++++	++	+	+	++	+	_
Physalis peruviana	Methanol	++++	++	+	+	+	+	_
Spilanthes mauritiana	Chloroform	+	_	_	_	++	+	_
Spilanthes mauritiana	Methanol	++	++	+++	++	+++	+++	+++

(-) -no inhibition; +: 9-10.5 mm; ++: 10.6-12.5 mm; +++: 12.6-13.5 mm and ++++: more than 13.6 mm zone of inhibition diameter

Table3. *Minimum inhibitory concentrations of active extracts*

Plant (extract)	Minimum inhibitory concentration (mg/ml)	
	Staphylococcus aureus	*Candida albicans*
Alectra sessiliflora (chloroform)	12.5	12.5
Alectra sessiliflora (methanol)	3.13	25.0
Blumea axillaris (chloroform)	12.5	12.5
Blumea axillaris (methanol)	3.13	3.13
Chamaecrista mimosoides (chloroform)	6.25	6.25
Chamaecrista mimosoides (methanol)	6.25	6.25
Chamaecrista mimosoides (water)	1.56	-
Fuerstia Africana (chloroform)	6.25	3.13
Lantana trifolia (chloroform)	1.56	6.25
Lantana trifolia (methanol)	1.56	3.13
Physalis peruviana (chloroform)	6.25	12.5
Physalis peruviana (methanol)	6.25	25.0
Spilanthes mauritiana (chloroform)	50.0	25.0
Spilanthes mauritiana (methanol)	6.25	3.13
Chloramphenicol	0.25	-
Nystatin	-	0.12
(-) no inhibition		

A. Bioautographic Assay

To get an indication of the bioactive compounds in each of the active extracts, *F. africana*, *B. axillaris*, *C. mimosoides*, *P. peruviana*, *L. trifolia* and *A. sessiliflora*, the chloroform and methanol extracts' bioautoassay was performed. The water extracts were not included in this assessment because most of the extracts due to their polar nature did not separate appreciably on the TLC systems employed. The retention factor, R_f of the respective inhibition spots, as visualized by MTT spray reagent was calculated as shown in Table 4.

With the exception of *F. africana* methanol extract which showed no appreciable inhibition, the rest of extracts showed varied inhibition. A total of 89 spots were visualized with an average of 7 spots per extract. Of the total spots, 52 spots (58.4 %) showed inhibition on bio-autograph, a further indication of presence of bioactive constituents in the extracts. Twenty five spots had antifungal activity while 38 spots had antibacterial activity including 27 with exclusive antibacterial activity.

Table4. *Bioautoassay results showing R_f on TLC and inhibition spots against test microorganisms*

TLC and inhibition spots R_f		
Extract	***Staphylococcus aureus***	***Candida albicans***
Alectra sessiliflora[a]	0.06, 0.13, 0.18, 0.24, **0.31**, 0.43, 0.56, 0.66, **0.73**, 0.81	**0.06**, 0.13, 0.18, 0.24, 0.31, 0.43, 0.56, 0.66, 0.73, 0.81
Alectra sessiliflora[b]	0.03, **0.06**, **0.09**, 0.20, **0.31**, 0.39, 0.50, 0.66, 0.75, 0.81	0.03, 0.06, **0.09**, 0.20, 0.31, 0.39, 0.50, 0.66, 0.75, 0.81
Blumea axillaris [a]	0.18, **0.36**, 0.51, **0.68**	0.18, 0.36, **0.51**, 0.68
Blumea axillaris [b]	0.04, 0.23, 0.41, **0.60**, 0.73, 0.82	**0.04**, 0.23, 0.41, 0.60, **0.73**, 0.82
Chamaecrista mimosoides [a]	0.10, 0.15, **0.38**, **0.59**, **0.65**, 0.89	**0.10**, 0.15, 0.38, 0.59, **0.65**, 0.89
Chamaecrista mimosoides [b]	**0.10**, **0.18**, 0.35, 0.41, 0.79	**0.10**, 0.18, 0.35, 0.41, 0.79
Fuerstia africana [a]	0.06, 0.15, **0.38**, 0.47, **0.59**, **0.65**	0.06, 0.15, 0.38, **0.47**, 0.59, **0.65**
Lantana trifolia [a]	**0.05**, **0.18**, **0.36**, 0.64, 0.77	0.05, 0.18, **0.36**, 0.64, 0.77
Lantana trifolia [b]	0.06, **0.15**, **0.29**, 0.42, **0.63**, 0.79, 0.87	0.06, 0.15, 0.29, 0.42, 0.63 ,**0.79**, 0.87
Physalis peruviana [a]	**0.10**, **0.15**, 0.38, 0.59, 0.65, 0.81	**0.10**, 0.15, 0.38, 0.59, **0.65**, 0.81
Physalis peruviana [b]	**0.05**, **0.13**, **0.17**, 0.36, 0.58, **0.64**, 0.83	0.05, **0.13**, 0.17, 0.36, **0.58**, 0.64, **0.83**
Spilanthes mauritiana [a]	**0.04**, 0.06, 0.12, 0.56, **0.65**, **0.74**, 0.82	**0.04**, 0.06, 0.12, **0.56**, 0.65, **0.74**, 0.82
Spilanthes mauritiana[b]	**0.08**, **0.12**, 0.18, 0.26, 0.44, 0.58, 0.64, 0.71, **0.77**, **0.83**	0.08, 0.12, 0.18, 0.26, 0.44, **0.58**, 0.64, 0.71, **0.77**, 0.83

Stationary phase: Silica gel 60 F_{254}, 0.75 mm. Mobile phase: chloroform-methanol (19:1, v/v)

[a]:chloroform extract, [b]:methanol extract. Bold values- inhibition spots ; bold & underlined- dual activity

4. DISCUSSION

The ethno-medicinal approach employed in this study to select plants for investigation was found to be useful in gathering and harnessing important information regarding the targeted plants and the conditions managed by the herbal remedies. The use of the microbiological screening procedures was found to be effective and relatively rapid in the preliminary activity search in the complex plant matrix without necessarily having to carry out clean up procedures before screening or knowing the chemical nature or structure of the bioactive constituents. Another advantage of this technique is the possibility of screening for activity against an array of microorganisms at the same time. The agar well diffusion method has also been reported to give better results compared to disk diffusion method as the free hydroxyl groups in paper disks may interfere with the diffusion of cationic polar molecules [11]. The choice of *S. aureus* and *C. albicans*, microorganisms of medical importance, as the test microorganisms gives a direct clinical application of the results. The use of MTT spray reagent facilitated accuracy of activity detection. The purple color is due to formation of a formazan dye (purple), being a clear indication of live microorganisms´ dehydrogenase reduction activity on the tetrazolium salt (yellowish). The yellow colour in contrast is indicative of death of the microorganism which implies the presence of a localized bioactive compound(s) at that specific R_f. This characteristic explains why formazan dyes are in addition commonly used in cell proliferation and toxicity assays [12].

The present study has clearly demonstrated good antibacterial and antifungal activity in *A. sessiliflora*, *B. axillaris*, *C. mimosoides F. africana*, *L. trifolia* and *S. mauritiana* extracts. The solvent of extraction has also been shown to contribute to activity as exemplified by C. *mimosoides* in which the water extract that had the highest antibacterial activity whilst its methanol and chloroform extracts exhibited relatively less activity. It is therefore not surprising that *C. mimosoides* is a common ingredient in traditional cough remedies that are aqueous in nature [13]. The observation of activity in water and to an extent methanol extracts is indicative of probable water soluble antimicrobial constituents. Some known water soluble antibacterial compounds from plant extracts include hydrolysable tannins and saponins. The presence of activity in the polar extract is of importance in traditional herbal medicine as traditionally, macerates or hot water decoctions are used in the preparation of some of the herbal medicines that would probably be rich in water soluble bioactive constituents. The polar portions of *A. sessiliflora* extracts, that showed high *in vitro* antibacterial and antifungal activity has since yielded three bioactive compounds, p-coumaric acid, 3,4-dihydroxybenzoic acid and luteolin[14].

Some inhibition on bioautoassay was seen as broad zones as opposed to distinct spots that could be due to activity overlap of several chemically related compounds that were not adequately separated on chromatographic system used giving apparent similar R_f values. Some of the commonly encountered antimicrobial phytochemicals belong to varied specific chemical classes such as phenolics and polyphenols, quinones, flavones, flavonoids and flavanols, tannins, coumarins, terpenoids and essential oils, alkaloids, lectins and polypeptides, polyamines, glucosides and thiosulfinates [15]. Careful choice of solvent systems as guided by bioautographs is useful when attempting to extract and isolate the respective phytochemicals[16].

To an extent, the investigated plants exhibited *in vitro* antibacterial and antifungal activity against the tested microorganisms to corroborate their folkloric use.The findings will add onto earlier reports of ethno-pharmacological as well as bioactivity information on some of these traditionally used medicinal plants.

5. CONCLUSIONS

Most of the screened plant extracts have some level of activity with F. *africana, B. axillaris, C. mimosoides*, *P. peruviana*, *L. trifolia* and *A. sessiliflora* extracts exhibiting appreciable combined antibacterial and antifungal activity. It is further presumed that the active phyto-constituents in the plants might be ideal lead molecules for the development of new generation antimicrobial agents. Reported results obtained may inform the continued traditional use of the plants as herbal remedies or use of any future isolated constituents as possible lead compounds in the development of new antimicrobial agents. Further scientific work on extracts is however recommended especially toxicity studies and use of combinational and computational techniques in synthesis of new leads that may lead to more useful compounds to tackle the current or any future emerging microbial infections.

ABBREVIATIONS

DMSO- dimethylsulfoxide

MICs- Minimum inhibitory concentrations

MTT- 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide

R_f - retention factor,

S.E- standard error of the mean

TLC- thin layer chromatography

UV- ultra violet

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