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Research Article

IN VITRO ANTIMICROBIAL ACTIVITY OF SELECTED MEDICINAL PLANTS IN LOSHO, NAROK COUNTY KENYA

Chalo Mutiso Duncan [1]*, Lukhoba Catherine [1], Dossaji Saiffudin [1], Nguta Joseph [2]
[1]School of Biological Sciences, University of Nairobi, Box 30197-00100, Nairobi, Kenya
[2]College of Agriculture and Veterinary Sciences, University of Nairobi Box 30197-00100, Nairobi, Kenya
*Corresponding Author Email: dunmutiso6@gmail.com

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ABSTRACT

In Kenya, microbial infections are a major cause of morbidity. Antibiotic effectiveness is threatened by increasing resistance of pathogenic microbes against most available drugs as new pathogens continue to emerge. Currently, herbal remedies offer hope considering they are readily and cheaply available. Antimicrobial activity of crude extracts of four selected plants namely *Schrebera alata* (Hochst.)Welw. (Oleaceae), *Ormocarpum kirkii* (Taub.) Engl. (Papilionoideae), *Helichrysum forskahlii* (J.F. Gmel.) Hilliard & B.L. Burttv (Asteraceae) and *Cussonia holstii* Harms ex Engl. (Araliaceae) that are medicinally used by herbalists from Losho, Narok County Kenya for treatment of ear, nose and throat infections (ENT), gastrointestinal disorders and skin ailments was done. Qualitative antimicrobial susceptibility test against five microorganisms, methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans* was investigated using Agar diffusion methods to generate inhibition zones and data accrued analyzed using Analysis of variance (ANOVA). Minimum inhibitory concentrations (MICs) were determined by broth micro dilution method. It was observed that the organic crude extracts of *H. forskahlii* had the highest inhibition zone diameters against MRSA of 19.5 and 18.5 mm in agar well and agar disk diffusion respectively. Moreover, organic extracts of *H. forskahlii* revealed the highest antifungal inhibition zone value equal to 8.5 mm in agar well diffusion. MIC values varied with plant samples from 15.625 to 250 mg/ml. The study has shown that *H. forskahlii* and *O.kirkii* possess promising antimicrobial activity against microbes of health importance and could lead to the isolation of new and effective antimicrobial compounds

KEY WORDS: Medicinal plants; Antimicrobial activity; Medicinal plants; Agar diffusion methods; Narok County; Kenya.

INTRODUCTION

Microbial infections remain a threat to millions of lives of those individuals and currently there is an increased problem of antibiotic resistance due to microbial persistence [1] . E.g *Pseudomonas aeruginosa* is a significant nosocomial Gram-negative bacterium commonly found in soil and ground water and the increasing use of fluoroquinolones has led to increasing resistance [2]. Though some strains of *E.coli* are harmless, some cause bloody diarrhea while others like O157:H7 strain may also cause severe anemia or kidney failure, which can lead to death [3]. These bacteria under stress often transfer multiple drug-resistance plasmids to other species hence important reservoir of transferable antibiotic resistance [4]. MRSA which causes pneumonia, life-threatening bloodstream infections and surgical site infections has evolved from a controllable nuisance into a serious public health concern during the past decades [5]. *B. cereus* exhibit resistance of their endospores to various stress and their long-term survival under unfavorable conditions and hence widely distributed in nature [6].*Candida albicans* causes most life-threatening yeast infection particular in immunocompromised individuals and its resistance is attributed its capacity to grow in a diversity of morphological forms [7].

Bacterial infections are prevalent due to various factors such as the HIV/AIDS pandemic, poor hygiene, overcrowding and resistance to convectional antimicrobials and without urgent coordinated action, the world is heading towards a post-antibiotic era [8].The rapid rise in microbial resistance to synthetic drugs has urged formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents [9 10]. Traditional medicine has been recognized as a part of primary health care program in many African countries including Kenya and [11] estimates that up to 80% of the population in some developing countries uses it as a compliment to the conventional medicine.

In this present study, four plants of different families were selected to access their antimicrobial activity. Some of the plants are known for their use as traditional medicine to cure candidiasis toothache and diarrheal diseases [12 13].

MATERIAL AND METHODS

Collection of the plant materials

Four plants selected based on their ethno pharmacological usage were collected from Losho, Narok County, Kenya with the help of herbalists. The plants and parts were; barks of *Schrebera alata,* aerial parts of *Ormocarpum kirkii*, bark of *Cussonia holstii* and the whole plant of *Helichrysum forskahlii.* The plant parts were thoroughly washed with running tap water and air dried at room temperature for six weeks, after which they were chopped into small pieces and ground into powder.Identification of the voucher specimens were confirmed by a taxonomist at the School of Biological Sciences, University of Nairobi and voucher specimens deposited at the University of Nairobi Herbarium (NAI).Table 1 shows the list of four plants used in this study

Preparation of crude extracts

The ground materials were extracted by cold maceration method. Fifty grams of ground plant material were extracted with distilled water (500 ml) to obtain aqueous extracts and Dichloromethane-

Methanol mixture (1:1) for 48 hours to obtain organic extracts. The aqueous extracts were filtered and the filtrate kept in a deep freezer then lyophilized (freeze dried) resulting to a dry powder. Organic extracts were filtered and concentrated using a rotary evaporator under reduced pressure at 40°C to obtain crude extract. These were then stored in airtight containers at 4°C awaiting bioassays and phytochemical screening [14].

Preparation of test strains

Sub cultures of methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 1385, *Pseudomonas aeruginosa* ATCC 27823 were obtained from KEMRI, Centre for Microbiology Research (CMR) while *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC10231 were from the department of Public health, Pharmacology and Toxicology, University of Nairobi. The microbial suspensions were standardized according to the Clinical and Laboratory Standards Institute procedures [15 16]. Bacteria were grown in Muller–Hinton agar for 18hrs and fungus in Sabouraud agar for 48hrs to obtain freshly growing strains. Then the microbial suspensions were standardized with sterile saline to turbidity equivalent to 0.5 McFarland (approximately 1.5×10^8 CFU/ml for bacteria and1.5×10^6 CFU/ml for *Candida* sp.) and stored at 4°C until used during antimicrobial test.

Antimicrobial susceptibility testing

Agar diffusion methods according to [14] were used to evaluate antimicrobial activities of the crude extracts. Approximately 20 ml of sterile Muller-Hinton Agar and Sabouraud Dextrose Agar was poured into sterile Petri plates and allowed to set. An inoculum suspension was swabbed uniformly to solidified 20 ml Mueller-Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for fungi and the inoculum was allowed to dry for 5 min. Three concentrations (400, 200, 100 mg/ml) of each test extract were prepared for susceptibility testing using 1% DMSO for organic extracts and distilled water for aqueous extracts.

Agar well diffusion

Holes of 10 mm in diameter were made in the seeded agar using sterile cork borer.100 µl of the test extracts was introduced into the wells using microtiter-pipette and allow to stand on the bench for 1h for proper diffuse into agar and thereafter incubated at 24hrs at 37° C. Microbial growth was determined by measuring the diameter of zone of inhibition in millimetres (mm). For each microbial strain, controls were maintained where pure solvents were used instead of the crude extracts [17]. The experiment was performed in triplicates under sterile condition and the mean values presented.

Disk Diffusion

Commercially prepared sterile discs of 6 mm in diameter were impregnated with 100 µl of each crude extract dried and placed aseptically onto plates inoculated with 1ml overnight growth test microorganism. Bacterial cultures and fungal culture were incubated at 24hrs at 37° C. Chloramphenicol 30µg/ml (for bacteria) and Amphotericin B 30 µg/ml (for fungi) were used as standards, while discs with diluting solvents only were used as negative controls. Each extract was tested in triplicate under sterile conditions. Microbial growth was determined by measuring the diameter zone of inhibition in millimeters[14].

Determination of Minimum Inhibitory Concentration (MIC)

Broth micro dilution method was used to determine minimum inhibitory concentration for the active crude extracts against the test microorganisms. The procedures was done as recommended [14 15] 0.5 mL of 24 h culture of test organisms (10^7 CFU/mL) adjusted to McFarland turbidity standard 0.5 McFarland (approximately 1.5×10^8 CFU/ml for bacteria and1.5×10^6 CFU/ml for *Candida* sp. were incubated in serial dilution 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.625mg/ml. Incubation was done for 24hrs at 37^0 C. The least concentration of the plant extract that did not permit any visible growth of the inoculated test microorganism in broth culture as indicated by lack of turbidity was regarded as visual MIC in each case. Tubes inoculated with microbes alone and media alone served as control. All the experiments were done in triplicates and results were recorded [18].

Data analysis

Statistical analysis of antimicrobial activity was done using SPSS to compare the various extracts from various plants to find out whether the plants exhibited growth inhibition of the various bacteria and fungi differently. Once differences were identified further ANOVA using Dunnett test was done to compare the treatments with the positive controls (Amphotericin B and Chloramphenicol) to find out whether any treatment had any bioactivity comparable to the positive control. MS Excel 2007 was used to determine mean inhibition zones while MS Word 2007 was used to draw tables

RESULTS

Antimicrobial activity

In both agar well diffusion and disk diffusion, organic extracts of *H. forskahlii* had the highest antimicrobial activity with inhibition zone diameter of 19.5 and 18.5 mm respectively compared to the rest of the tested extracts (Table 2 and 3). Moreover, its activity was noted in all the tested microorganisms. Also in both methodologies, it was observed that growth of MRSA was inhibited by all organic extracts of *S. alata, C. holstii, O. kirkii* and *H. forskahlii*. Furthermore, only water extracts of *H. forskahlii* showed antibacterial activity against MRSA. It was also noted that organic extracts of *S. alata, C.holstii* and *H. forskahlii* had antifungal activity. However, organic extract of *H. forskahlii* revealed the highest antifungal activity with inhibition zone value equal to 8.5 mm.

Minimum inhibitory concentration (MIC) of plant extracts

Results of minimum inhibitory concentration (MIC) of plant extracts against the test microorganisms were as shown in Table 4. MIC values varied with plant samples from 15.625 to 250 mg/ml. The MIC values of the test extracts also varied against different test pathogens. The results obtained from these assay revealed that MRSA was the most sensitive bacteria at lower concentrations with highest number of MIC values of 15.625mg/ml.

DISCUSSION

The presence of antibacterial substances in the higher plants is well established [19].The results by both agar diffusion methods were found not to differ significantly from each other ($P>0.05$) and this was in agreement with [17].More so, the principle of the agar well diffusion is the same as that of the agar disk diffusion method [20]. Interestingly, MIC values of less than 100mg/ml were observed in broth micro dilutions but not in antimicrobial susceptibility test(AST), and this could be due to micro dilution method providing a potentially useful technique for determining MICs increased sensitivity for small quantities of extracts which is important if the antimicrobial is scarce as is the case for many natural products and dilution testing methods may be quantitative (MIC), in addition to qualitative (susceptible, intermediate and resistant) whereas disk methods are only qualitative method [20 21 22].

From this study, it is clear that the dichloromethane: methanol (1:1) solvent extracts of all the plants tested were more potent than their

corresponding aqueous extracts against all the tested microbes. This observation is of particular interest, given that traditionally, the preparation of herbal remedy is often with water. This might have resulted from the lack of solubility of the active constituents in aqueous solution. [23] indicated that aqueous extraction could be ineffective because water soluble compounds might interrupt the antimicrobial effect. In addition, antimicrobial phytochemicals are soluble in moderate polar solvents. [24] who reported that the inactivity of water extracts may have been because they (extracts) were not prepared according to the traditional methods, which in some cases involved boiling for several hours.

The tested plant extracts were more active against gram positive bacteria compared to gram negative. The most sensitive bacterium was MRSA which was inhibited by the organic crude extracts of all the selected plants. Generally, Gram negative bacteria are more resistant than Gram positive bacteria [25 26] .The higher sensitivity of Gram-positive bacteria (MRSA) could be due to the exposure of the outer peptidoglycan layer [27] while Gram-negative bacteria bear an extra outer membrane (OM) which includes the asymmetric distribution of the lipids with phospholipids and lipopolysaccharide (LPS) located in the inner and outer leaflets, respectively can act as additional barrier which hinders the movement of foreign substance into the cell [28].Among the tested plant extracts only organic extract from *H.forskhalii* at 400mg/ml against MRSA had similar activity as the positive control. This antimicrobial activity of *H.forskhalii* is in agreement with [13 29]. However, [13] showed the essential oil from *Helichrysum forskahlii* presents activity against *E.coli* (MIC between 0.2 and 0.8mg/ml) which varied with study MIC value (62.5 mg/ml) against the same bacterium strain.

Table 1: Plant species from Losho, Narok County based on traditional reputation for their use as antimicrobial agents

Plant species &Voucher specimen number)	Vernacular name	Habit	Part used	Treatment preparation	Disease(s) treated
Schrebera alata (Hochst.)Welw (Oleaceae) DMC2014/001	Enchenienkashe	Tree	Bark	Boiling	stomach pains, blood in stool and diarrhoea, relieves pain from pregnancy.
Ormocarpum kirkii (Taub.) Engl (Leguminosae) DMC2014/002	Alaimurunyai	Shrub	Aerial part	Decoction, crushing	Cuts on swellings or in blisters and stomach problems
Cussonia holstii Harms ex Engl (Araliaceae) DMC2014/003	Oloiwurur	Tree	Bark	Decoction	Gonorrhea, antiflammatory, wound healing
Helichrysum forskahlii (J.F. Gmel.) Hilliard & B.L. Burttv (Asteraceae) DMC2014/004	Eleleishua-enkop	Shrub	Whole plant	Decoction	toothache, headache, stomachache

Table 2: Growth inhibition of crude extracts against microbes in agar well diffusion in mg/ml

Table 2.		MRSA			*P.a*			*C.a*			*B.c*			*E.coli*		
Plant		100	200	400	100	200	400	100	200	400	100	200	400	100	200	400
S.alata	Or	5.0	8.0	8.5	-	-	6.0	-	-	5.0	-	-	-	-	-	-
	Aq	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C.holstii	Or	7.5	6.5	10.5	-	-	-	-	-	6.0	-	-	-	-	-	-
	Aq	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O. kirkii	Or	15.0	15.5	16.0	-	-	-	-	-	-	-	-	-	-	-	-
	Aq	-	-	-	-	-	10.0	-	-	-	-	-	-	-	-	-
H.forskahlii	Or	15.5	15.5	18.5	-	-	10.0	-	9.0	8.5	-	4.5	5.0	-	3.5	6.0
	Aq	8.5	9.0	9.0	-	-	5.0	-	-	-	-	-	-	-	-	-
Positive control		Chloramphenicol 23.7			Chloramphenicol 20.7			Amphotericin B 16.0			Chloramphenicol 19.3			Chloramphenicol 22.0		

Table 3: Growth inhibition of crude extracts against microbes in agar disk diffusion in mg/ml:

Table 3.		MRSA			*P.a*			*C.a*			*B.c*			*E.coli*		
Plant		100	200	400	100	200	400	100	200	400	100	200	400	100	200	400
S.alata	Or	3.0	6.5	7.5	-	-	6.0	-	-	5.0	-	-	-	-	-	-
	Aq	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C.holstii	Or	7.0	7.7	9.0	-	-	-	-	-	6.0	-	-	-	-	-	-
	Aq	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O. kirkii	Or	15.0	11.5	15.0	-	-	-	-	-	-	-	-	-	-	-	-
	Aq	-	-	-	-	-	10.0	-	-	-	-	-	-	-	-	-
H.forskahlii	Or	15.0	13.5	19.5	-	-	8.0	-	8.0	7.0	-	6.0	7.0	-	4.5	6.0
	Aq	4.5	5.0	10.0	-	-	6.0	-	-	-	-	-	-	-	-	-
Positive control		Chloramphenicol 23.7			Chloramphenicol 20.7			Amphotericin B 16.0			Chloramphenicol 19.3			Chloramphenicol 22.0		

Table 4: Minimum inhibitory concentration (MIC) in mg/ml

Plant	Solvent	*E.coli*	*P.aeruginosa*	MRSA	*B.cereus*	*C.albicans*
O. kirkii	Organic	-	-	31.25	-	-
	Aqueous	-	250	-	-	-
S. alata	Organic	-	15.625	15.625		62.5
	Aqueous	-	-		-	-
C. holstii	Organic	-	-	15.625	-	62.5
	Aqueous	-	-	-	-	-
H. forskahlii	Organic	62.5	31.25	15.625	31.25	31.25
	Aqueous	-	-	15.625	-	-

Key: Or-Organic extracts; Aq-Aqeous extracts; *S.alata-Schrebera alata*; *C.holstii-Cussonia holstii*; *O.kirkii- Ormocarpum kirkii*; *H.forskahlii- Helichyrsum forskahlii*; MRSA- methicillin resistant *Staphylococcus aureus*; *B.c-Bacillus cereus; E.coli- Escherichia coli; P.a-Pseudomonas aeruginosa and C.a-Candida albicans*

Antibacterial and antifungal activity of *Schrebera alata* was reported for the first time in this area of study though in Samburu , studies show that the root and bark of this plant is pounded or chewed as treatment for candidiasis and toothache [12]. Sensitivity of *S. alata* to MRSA, *P. aeruginosa and C. albicans* showed activity similar to another species of the same genus *S. swietenioides Roxb* which was found to exhibit potent inhibitory activity [30]. Among the *C. holstii* extracts tested for antimicrobial activity, only the organic extracts were active against MRSA and *C. albicans* in both agar well and disc diffusion methods. This study is in agreement with previous findings [31] that *C.holstii* is used for traditional management of ear, nose and throat (ENT) diseases in central Kenya. More so, Cussonia species are used in African traditional medicine mainly against pain, inflammation, gastro-intestinal problems, malaria and sexually transmitted diseases [32]. Sensitivity of the plant against the tested microbes was in line with activity of some other species of the same genus [32 33]. Aqueous extracts of *O. kirkii* were active against only gram negative bacteria, *P. aeruginosa* whereas organic extracts of *O. Kirkii* were only active against MRSA. MIC value (31.25mg/ml) was revealed by organic extracts against MRSA was in line with [34]. Lack of antibacterial activity showed against *B.cereus* from this study was not in agreement with [34] earlier report on MeOH and n-Hexane extracts of the same species exhibited antibacterial activity against this microorganism. However activity against *P.aeruginosa* and MRSA was in line with species of the same genus *Omorcarpum trichocarpum* which showed antibacterial activity [35].

CONCLUSION AND RECOMMENDATION

Bioactivity of organic and aqueous extracts of *S. alata, O. kirkii, C. holstii* and *H. forskahlii* on bacterial and fungal strains is noteworthy. From this study *H. forskahlii* is a better source of antimicrobial agents and can be of interest in the development of new chemotherapeutic drugs hence recommending phytochemical screening of active secondary metabolites. Further evaluation for cytoxicity can be carried out of the most active crude extracts. Nature has demonstrated to be a good source of lead compounds for treatment of microbial infections.

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