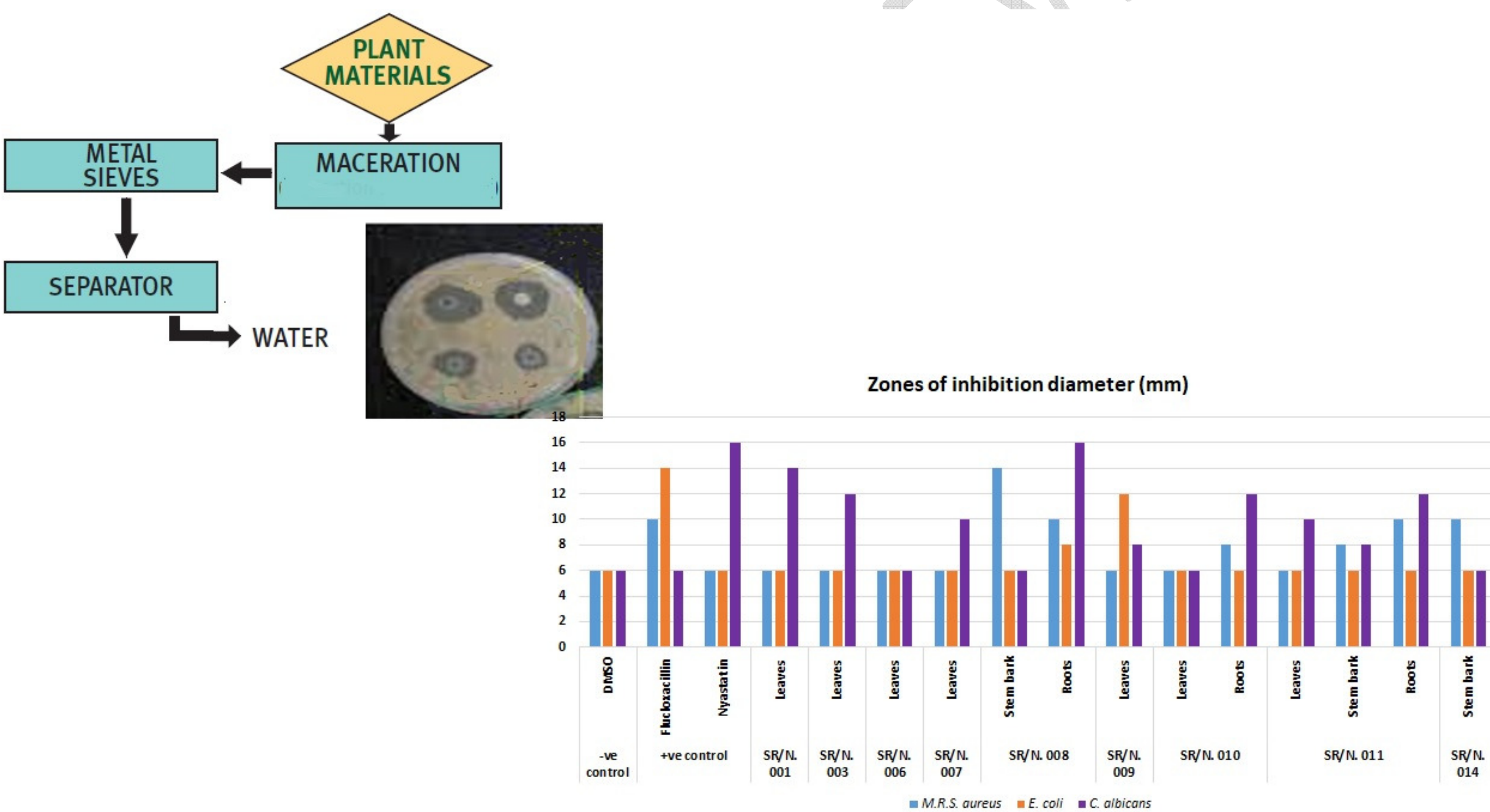
Original Research Article

Antimicrobial Assay of aqueous extracts of selected Ethno-pharmacologic alternatives used by the Maasai community of Narok, Kenya

Graphical Abstract



ABSTRACT

Aims: Antimicrobial resistance motivates the search for new antimicrobials. Besides Methicillin-Resistant *Staphylococcus aureus,* Carbapenem-Resistant *Klebsiella pneumoniae* strain has emerged worldwide over the last decade, posing a great challenge to healthcare. This paper reports a survey of Maasai ethno-pharmacy practices.

Study design: Key informant interviews utilizing e-questionnaires were used for data collection. Some 13 plants were found to be most commonly used for antibacterial and antifungal related ailments.

Methodology: Plants were identified, and the applicable parts taken as samples, dried, powdered then subjected to aqueous extraction. Using agar well diffusion method, the extracts were screened against gram positive, gram negative and fungal strains to establish antimicrobial activity.

Place and Duration of Study: The study was conducted at the School of Pharmacy & Health Sciences of the United States International University, Africa in Nairobi from January 2017 to December 2018.

Results: Out of the 24 different plant samples collected, 33% were leaves while 17%, 12.5% and 37.5% were fruits, stem bark and roots, respectively. The highest extract percentage yields were from the leaves of *Biden Pilosa* (5.11%)*, Psidium guajava* (4.65%) and *Tarchononthus comphoratus* (4.31%). While the minimum extracts yields were from *Solanum incum* roots (0.08%) and stem bark (0.09%). The extracts of *Toddalia asiatica* stem bark and roots; *Rhamnus staddo* roots; *Tarchonanthus camphoratus* stem bark and roots; and *Zanthroxyleum chelybeum* stem bark, all exhibited well defined inhibition diameters against *M.R.S. aureus* in the range 8mm to 14mm as compared to the standard drug (10mm). All these were extracts of non-leafy samples. The significant antimicrobial activity corresponded to presence of flavonoids and alkaloids as seen on TLC plates during phytochemical screening.

Conclusion: The results could be regarded as sufficient rationale for utilization of the plants identified as alternatives to antibiotics for management of antimicrobial infections.

Key words: Ethnopharmacology; Antimicrobials; Bioautography; Maasai

1. INTRODUCTION

Microbial infections remain a threat to millions of lives globally [1] as antimicrobial resistance (AMR) is reported to be on the increase against commonly used antibiotics [2, 3]. This rapid rise in AMR to synthetic and semi-synthetic drugs continues to inspire search for new antimicrobial agents. In the last two decades, β-lactamase producing *Enterobacteriaceae* (e.g. *Escherichia coli, Salmonella typhi* and *Klebsiella pneumoniae*) have been identified as the main gram-negative bacteria responsible for multidrug resistance [4, 5]. In addition to the methicillin resistant *Staphylococcus aureus* (MRSA), a carbapenem-resistant strain of *Klebsiella pneumoniae* has emerged worldwide over the last decade, posing a great challenge to healthcare [6, 7]. Multi-drug resistant (MDR) *Salmonella typhi* and *Shigella dysenteriae* strains have also notably worsened the AMR problem [8].

In this regard, focus has turned to research on the efficacy of natural plant secondary metabolites. We draw inspiration from the fact that most African communities have always used herbal remedies as a readily and cheaply available alternative to contemporary medicines. The gradual change from a nomadic to a more sedentary lifestyle for the Maasai of Narok, Kenya, has not really led to any dramatic loss of traditional plant knowledge. Medicinal plants continue to be used frequently for human ailments and for veterinary purposes.

The World Health Organizations estimates that more than 80% of the developing countries relies on traditional plant-based medicine for their primary healthcare needs. Furthermore, at least 25% of drugs in modern pharmacopoeia are derived from plants with many synthetic drugs also having been developed based on template compounds isolated from plants [9]. A major fraction of the plants in their natural habitat have not been investigated for phytochemical composition, antimicrobial and toxicity activities. Additionally, it is indicated that overdose by patients due to imprecise nature of diagnosis is a worldwide experience particularly with herbal remedies [10].

This paper reports an exploration of the cultural and ethnopharmacological practices of Maasai in the application of medicinal plants in infective conditions. After identification and documentation, the commonly utilized plants were collected, authenticated and extracted with water. The extracts were then screened against *Salmonella typhi, Shigella species, Klebsiella pneumoniae, Escherichia coli* and *Candida albicans* to mimic the traditional herbal in treating human and livestock microbial infections. Subsequent phytochemical assay to test for presence of saponins, tannins, alkaloids and flavonoids was rapidly performed with bioautography. Substantial information is thus presented revealing the place of medicinal plants utilized by the Maasai as alternative to antibiotics and their potency for development into conventional medicine therapies.

2. MATERIALS AND METHODS

2.1 About the Maasai people of Narok, Kenya

The Maasai populations of east Africa are mainly pastoralist for their socioeconomic well-being. Since time immemorial, these communities have used different wild plants as dietary and medicinal additives in beverages and in form of other food preparations. Basing on recent reports [11], the study was designed with investigations correlated to utilization by Maasai people. Plant identification was done with particular reference to the ingredients of popular preparations among the Maasai such as “almajani” (tea or herbal infusion); “motorí” (traditional soup); and “okiti” (psychoactive herbal tea). We also rationalize the cultural use of these Maasai food-medicines; and document their frequency of use through self-reports.

2.2 Apparatus, reagents and organism strains

Smart phones pre-installed with electronic questionnaire application; polyethene bags; cutter knives; Analar grade hexane, methanol, Dimethylsulphoxide all from Sigma-Aldrich Germany; MacConkey agar from Thermo-Scientific^{TM}, Oxoid^{TM} Mueller-Hinton agar, BD Difco^{TM} Sabouraud Dextrose Agar; Fisherbrand^{TM} Plastic Petri Dishes; Strains of standard organisms of *Methicillin Resistant Staphylococcus aureus* (ATTC N0. 2913), *Escherichia coli* (ATTC N0. 25922) and *Candida albicans* (ATTC N0.14053) cultivated in sterile BD ^{TM} Trypticase soy broth**.**

2.3 Ethnopharmacy practices survey

Traditional use of medicinal plants is prevalent amongst most Kenyan communities, the Maasai included. In this study, we surveyed systems and practices among the Maasais of Narok. A dual mode of study was adopted that involved direct collection of the medicinal plants based on published literature while at the same time interviewing the locals on their uses. Field surveys were conducted from the month of April to September and November to February to cover all the seasons of the year. Electronic Questionnaires on smart phones were used to conduct interviews.

Interviews were conducted during field visits, followed by examination of the specimens collected from their natural habitats by our Maasai community contacts. The respondents were chosen without bias in regard to gender or age. A standardized set of questions were pre-loaded to the E-Questionnaire used to inquire about each plant collected. This was by showing the locals collected plants and asking them questions about each plant regarding the traditional uses particularly related to bacterial and fungal infections. More specific information was recorded later by using structured interviews in which the e-questionnaire was completed to capture precise method of use and preparation of the folk medical remedies for each folk taxon quoted.

2.4 Key informant interviews

Because the Maasai people, based on locality, use different names to refer to the same species of plants [12], key informant interviews were conducted with 10 locals of mixed gender and age to verify the collected plants and their ethno-pharmacologic applications. This was through open-ended, semi-structured interviews to investigate the different utilization and relation to bacterial antifungal related ailments for linkage to the plant exploration as antibiotics alternatives. The identified key informants were adults comprising of 6 male and 4 females all residing within the community in the area where the plant specimens were collected.

2.5 Plant sampling, processing and extraction

Various parts of the identified plants were harvested as per ethno-pharmacological application. Portions of leaves, stem bark and roots were collected carefully, ensuring none destruction of the plant. The fresh plant parts were hygienically transported to the laboratory, and Voucher specimens prepared and deposited at the United States Internal University – Africa, School of Pharmacy & Health Sciences, Herbarium. The plant specimen were openly aerated at room temperature to dry completely before grinding into fine powder using a Willy mill. Digestion extraction method was then applied for extracting the active principles. In 500ml round bottom flasks, 100 g of the plant part powder was extracted with 250 ml of distilled water over a heating mantle at 45 °C to afford decoctions of the various plant parts. The aqueous extracts were then filtered through gauze cloth and then subjected to lyophilization obtaining powder samples ready for antimicrobial activity assays.

2.6 Antimicrobial sensitivity assays

To investigate the antibacterial and antifungal activity of plant samples, the lyophilized extracts were subjected to bioautographic evaluation against *M.R.S. aureus, E. coli* and *C. albicans* as also used by Dewanjee et al. [13]. Aliquots at a concentration of 100µg/mL were spotted on silica gel Kieselgel DGF254 TLC plates and eluted with Chloroform: Methanol (98:2) with drops of glacial acetic acid. After evaporation of the organic solvent the TLC plate was placed on sterile petri dish and flooded with 100mL of Muller Hinton agar seeded in 1% aqueous *M.R.S. aureus* suspension (10^8 cells mL^{-1}). Similarly, other two sets were prepared and flooded with *E. coli* and *C. albicans.* The TLC plates were then incubated at 37 ℃ for 48hours and 72hours for bacteria and fungi respectively. These were then flooded each with 50mL of microbial agar (10g) containing 0.05% of MTT (3-(4, 5-Dimethylthiazzol-2-yl)-2, 5-diphenyltetrazolium bromide). Cell growth inhibition indicating antibacterial and antifungal phytochemicals was observed as yellowish TLC spots on a purple background, allowing chromatographic retention factors to be established against the solvent front. The same was repeated with separate TLC plates, however, without flooding microorganism suspension but eluting in the usual way and observing under UV lamp to mark out the eluted spots. The retention factors established was then correlated to the TLC plates developed and flooded with microorganism suspensions.

2.7 Phytochemical screening

The categories of the active phytochemical principles in the active extracts was carried out by Thin Layer Chromatography. Qualitative chemical screening for identification of the various classes of constituents was done by spotting and developing the TLC plates with Chloroform: Methanol (98:2). The spots were then visualized after reactions with specific reagents as per methods of Haborne (1998) and under ultraviolent lamp.

3. RESULTS AND DISCUSSION

3.1 Ethnopharmacy practices

From the survey, we found 13 plants most regularly utilized for antibacterial and antifungal related ailments. All these were reported by two or more participants as being utilized by addition into beverages like traditional tea. Out of the 24 different parts combination utilizations, 33% applications in ethno-pharmacy was by plant leaves while 17%, 12.5% and 37.5% utilized fruits, stem bark and roots respectively (Table 1). The rationales for utilization of the plants identified as antimicrobial alternatives was deduced from the commonly mentioned ailments. These included skin infections, fever, sexually transmitted infections, stomach discomforts, tooth aches, oral pains, worm infestations, constipation, flu, back pain, breast pain, tonsils and constipation. All these could be highly attributable to ailments related to infections with bacteria or fungi.

3.2 Plant samples extractions

At the laboratory, the plant samples were extracted as total extracts by subjecting each to hot continuous extraction techniques. The obtained extracts were weighed, and the percentage yields calculated per plant part. This is appropriately tabulated in table 2.

Table 1: Ethno-pharmacologic practices utilizing plants identified

NO	BOTANICAL NAME	FAMILY	VERNACULAR NAME: Maasai	USE	PART USED	METHOD OF APPLICATION
1.	*Solanum incanum*	Solanaceae	Entulele/endulelei	Treats wound, skin infection, malaria	Fruits	- Fruit sap applied on swollen part to reduce pain - Fresh leaves soaked in warm water, applied for skin infection - Roots cut in small pieces then boiled for fever treatment - Stem soaked in water, treats stomach disturbance
2.	*Carissa edulis*	Apocynaceae	Olamuriaki Ochoka	Venereal disease, Stomach ache, Eye infection (animal)	Roots, stem sap	- Crushed roots are boiled and taken by women who are preparing to conceive to clean uterus infection. - Boiled and mixed with tea, soup, cream to treat kidney - Sap tapped from the stem and used to treat eye infection in livestock
3.	*Euclea divinorum*	Ebenaceae	olkinyei olkinye(e)	Stomach problem, chicken pox, tooth ache	Fruits, roots	- Leaves are mixed with beverages to treat chicken pox - Fresh roots chewed to treat tooth aches - Fruits treat stomach problems
4.	*Asystasia mysoriensis*	Acanthaceae	Olosida	Malaria, amoeba/typhoid	Leaves, roots	- Fresh leaves are boiled and patients inhale steam to relieve fever

						- Leaves crushed and soaked in water and taken orally to treat stomach upsets - Roots ground, soaked and taken to relieve pain from a lion bite
5.	*Warbugia salutaris (formerly ugandensis)*	Canellaceae	ol-sogunoi	Stomach disturbance, tooth ache, deworming, organic farming	Leaves, stem, roots	- Dried stem bark/roots are boiled together with soup to treat stomach disturbance - Fresh stem bark is boiled and mixed with soup/milk cream/goat fat and decoction is take by women after delivery - Stem used as tooth brush to stop tooth ache
6.	*Toddalia asiatica*	Rutaceae	Olebarmonyo,	Malaria, flu	Leaves, roots	- Leaves are boiled and taken for relieving fever - Steam from boiled leaves treat flu and fever - Roots soaked in cold water and taken for seven days by new mothers who have low milk production
7.	*Bidens pilosa L.*	Asteraceae	Black jack	Stomach upsets	Leaves	- Leaves are crushed, boiled in water for deworming and stomach upset
8	*Rhamnus staddo*	Rhamnaceae	Olkokokola	Back pain, STI's, headache, deworming	Roots, leaves	- Leaves are crushed, soaked in water and given to calves for deworming - Roots boiled and mixed with soup for sexually transmitted infections treatment and back pain

9	*Tarchonanthus camphoratus*	Asteraceae	Oleleshwa	Stomach ache, Breast pain,	Roots, leaves	- Root back boiled & mixed with soup to treat back pain, stomach problems, - Boiled roots also treat breast pain - Burnt leaves' smoke used to treat cows with breathing problem and can also be soaked in water and extract used as droplets
10	*Psiadia punctulata*	Asteraceae	Olabaai le partolu	Flu, joint ache, malaria, blood pressure, ulcers	Roots	- Fresh/dried roots are boiled, and extract filtered, then mixed with cream/ soup/animal fat and decoction is taken for joint pain
11	*Olinia rochetiana*	Penaeaceae	Orkirenyi	Stomach problem, Diarrhoea, tooth ache, chicken pox	Fruits, leaves, roots	- Fresh fruits taken to treat stomach disturbance and stop diarrhoea though in excess is poisonous - Fresh roots chewed treat tooth ache but extract is not swallowed
12	*Zanthroxyleum chelybeum*	Rutaceae	Oluisuti	Tonsils, tooth ache, relief constipation	Stem bark, leaves	- Fresh stem bark is chewed to relief tooth ache/mouth infection and tonsils - Crushed leaves are mixed with water and given to cow to relief constipation
13	*Psidium guajava*	Myrtaceae				- No clear information however infections

Table 2: Percentage yields for plant extracts

Plant species	Sample code	Part utilized	Extract weight (grams)	% yield
Psidium guajava	SR/N. 001	Leaves	4.65	4.65%
		Fruits	0.13	0.13%
		Stem bark	0.07	0.07%
		Roots	1.04	1.04%
Solanum incanum	SR/N. 002	Leaves	2.01	2.01%
		Fruits	1.09	1.09%
		Stem bark	0.09	0.09%
		Roots	0.08	0.08%
Ageratum convzoides	SR/N. 003	Leaves	2.04	2.04%
		Fruits	1.21	1.21%
		Stem bark	0.24	0.24%
		Roots	1.46	1.46%
Carissa edulis	SR/N. 004	Leaves	0.73	0.73%
		Fruits	0.57	0.57%
		Stem bark	1.02	1.02%
		Roots	0.91	0.91%
Euclea divinorum	SR/N. 005	Leaves	2.11	2.11%
		Fruits	0.48	0.48%
		Stem bark	0.11	0.11%
		Roots	1.09	1.09%
Asystasia mysoriensis	SR/N. 006	Leaves	1.22	1.22%
		Fruits	0.21	0.21%
		Stem bark	0.44	0.44%
		Roots	0.41	0.41%
Warbugia salutaris	SR/N. 007	Leaves	3.65	3.65%
		Fruits	0.96	0.96%
		Stem bark	1.21	1.21%
		Roots	1.09	1.09%
Toddalia asiatica	SR/N. 008	Leaves	0.34	0.34%
		Fruits	1.09	1.09%
		Stem bark	1.55	1.55%
		Roots	0.91	0.91%

Bidens pilosa L.	SR/N. 009	Leaves	5.11	5.11%
		Fruits	2.09	2.09%
		Stem bark	0.33	0.33%
		Roots	0.19	0.19%
Rhamnus staddo	SR/N. 010	Leaves	3.19	3.19%
		Fruits	0.35	0.35%
		Stem bark	0.11	0.11%
		Roots	2.98	2.98%
Tarchonanthus camphoratus	SR/N. 011	Leaves	4.31	4.31%
		Fruits	3.01	3.01%
		Stem bark	0.49	0.49%
		Roots	2.77	2.77%
Psiadia punctulate	SR/N. 012	Leaves	2.21	2.21%
		Fruits	0.77	0.77%
		Stem bark	2.11	2.11%
		Roots	3.09	3.09%
Olinia rochetiana	SR/N. 013	Leaves	0.32	0.32%
		Fruits	3.11	3.11%
		Stem bark	0.67	0.67%
		Roots	1.87	1.87%
Zanthroxyleum chelybeum	SR/N. 014	Leaves	3.21	3.21%
		Fruits	1.29	1.29%
		Stem bark	0.91	0.91%
		Roots	1.07	1.07%

The highest extract percentage yields were realized from the leaves of *Biden pilosa, Psidium guajava* and *Tarchononthus comphoratus*, with 5.11%, 4.65% and 4.31% respectively. While the minimum extracts yields were from *Solanum incum* as 0.08% and 0.09% for the roots and stem bark respectively. This was considered an indication that the phytochemicals in the *S. incum* were of a varied polarity range from that of water, the extraction solvent. The higher percentage yields in the leaves is typical or previous analysis of plants of the same family of asteraceae that showed similar trends for Percentage yields [14].

3.3 Antimicrobial activity screening

The ethno-pharmacologic practices indicated that the Maasai utilized the identified plants for both human ailments and treatments of animal diseases. The specific plants and respective parts that are applied most commonly for human ailments were the only ones screened in this study for antibacterial antifungal activity. Results indicated inhibitions by several samples. Extracts showed well defined inhibition zones in the assays respectively indicated in the Table 3 the inhibition zones. These are consistent with several other studies reporting plants that elicit medicinal activity against bacterial and fungal microorganisms [15].

The plant extract samples SR/N 008-S, SR/N 008-R, SR/N 010-R, SR/N 011-S, SR/N 011-R and SR/N 014-S, all illustrated well defined inhibition diameters on assaying with the gram positive bacteria M.R.S. aureus in the range 8mm to 14mm as compared to the standard drug that displayed an inhibition zone of 10mm. Incidentally these were noted to be either stem bark or roots of the plant. The diameter of inhibition were in correspondence to that identified to belong to flavonoids and alkaloids from the TLC plates developed in the phytochemical screening. This is also in agreement with past reports [16]. Activity against the gram-negative bacteria was only exhibited by SR/N 008-R and SR/N 009-L, illustrated by well-defined inhibition zones in the range 8mm to 12mm compared to 14mm for the standard.

Table 3: Bactericidal, fungicidal activity inhibition zones

Sample code	**Part utilized**	**Inhibition spots band**		
		M.R.S. aureus	***E. coli***	***C. albicans***
-ve control	DMSO	6±0.8	6±0.3	6±0.5
+ve control	Flucloxacillin	10±0.1	14±0.7	6±0.2
	Nyastatin	6±0.4	6±0.1	16±0.6
SR/N. 001	Leaves	6±0.4	6±0.7	14±0.4
SR/N. 003	Leaves	6±0.8	6±0.9	12±0.9
SR/N. 006	Leaves	6±0.7	6±0.6	6±0.1
SR/N. 007	Leaves	6±0.5	6±0.1	10±0.8
SR/N. 008	Stem bark	14±0.7	6±0.2	6±0.7

	Roots	10±0.3	8±0.7	16±0.9
SR/N. 009	Leaves	6±0.8	12±0.1	8±0.6
SR/N. 010	Leaves	6±0.3	6±0.6	6±0.5
	Roots	8±0.7	6±0.3	12±0.2
SR/N. 011	Leaves	6±0.9	6±0.2	10±0.4
	Stem bark	8±0.7	6±0.9	8±0.8
	Roots	10±0.7	6±0.2	12±0.5
SR/N. 014	Stem bark	10±0.7	6±0.9	6±0.4

6±0.0mm = diameter of agar well = no inhibition band (not sensitive)

-ve control = Negative control (Dimethyl sulfoxide – DMSO)

+ve control = Bactericidal and fungicidal drugs (Flucloxacillin and Nystatin respectively)

Activity against fungal strain was by the samples SR/N 001-L, SR/N 003-L, SR/N 007-L, SR/N 009-L, SR/N 010-R, SR/N 011-L, SR/N 011-S and SR/N 011-R with minimum diameter of inhibition at 8mm and highest as 16mm which was interestingly equivalent to the diameter displayed by the standard antifungal drug. Although the results attest and justifies the ethno-pharmacologic utilization be the Maasai, it is difficult to compare the findings directly to previous studies. The witnessed antimicrobial activity cuts across the various species of plants in similar families. The sensitivity results nevertheless are sufficient to warrant next level investigations of the plants utilized as antimicrobial alternatives by Maasai of Narok Kenya

3.4 Phytochemical screening

Spotted and developed TLC plates were sprayed by the visualization agents and upon observation under the ultraviolet light both positive and negative results were realized for the phytochemical groups in the various plant extracts. This were appropriately tabulated as follows:

Sample code	Part utilized	Phytochemical group				
		Glycosides	***Flavonoids***	***Terpenoids***	***Steroids***	***Alkaloids***
SR/N. 001	Leaves	-	++	-	+	++
SR/N. 003	Leaves	-	+	-	++	++
SR/N. 006	Leaves	-	+	++	+	+
SR/N. 007	Leaves	-	-	-	+	+
SR/N. 008	Stem bark	+	+	+	+	-
	Roots	++	+	-	++	++
SR/N. 009	Leaves	-	++	+	-	-
SR/N. 010	Leaves	-	+	++	-	++
	Roots	++	-	+	+	++
SR/N. 011	Leaves	-	+	-	++	-
	Stem bark	+	+	+	-	-
	Roots	++	-	++	-	++
SR/N. 014	Stem bark	+	-	-	+	-

- negative results (phytochemical group absent)

+ positive results (phytochemical group present)

++ positive results (high amounts of phytochemical group)

Glycosides were mostly present in stem barks and roots of the plant samples while the other phytocompounds groups including flavonoids, terpenoids, steroids and alkaloids were distributed across different plant parts to varying degrees. The presence of flavonoids and Terpenoids are found to correlate well with the witnessed antibacterial and antifungal activities. This is found to be in line with other reports. The array of antimicrobial activity has been variously attributed to these phytochemicals [17-19].

4. CONCLUSION

Extensive achievement has been attained in the development of synthetic medicines, yet the demand for antimicrobials remains unmet particularly in developing countries, and due to antimicrobial resistance. This has warranted exploration of the flora which continues to play an important role in drug discovery. Application was the main rationales for plant selection by the Maasai of Narok as herbal alternatives of antibiotics. This resonates well with confirmatory antibacterial and antifungal efficacies witnessed in screening of the aqueous plants extracts. The extracts of *Toddalia asiatica* stem bark and roots; *Rhamnus staddo* roots; *Tarchonanthus camphoratus* stem bark and roots; and *Zanthroxyleum chelybeum* stem bark, all exhibited well defined inhibition diameters against *M.R.S. aureus* in the range 8mm to 14mm as compared to the standard drug (10mm). All these were extracts of non-leafy samples. The significant antimicrobial activity corresponded to presence of flavonoids and alkaloids as seen on TLC plates during phytochemical screening. The results could be regarded as sufficient rationale for utilization of the plants identified as alternatives to antibiotics for management of antimicrobial infections.

COMPETING INTERESTS

The authors wish to to declare no competing interests exist for this work.

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