

Methanol Extract of Three Medicinal Plants from Samburu in Northern Kenya Show Significant Antimycobacterial, Antibacterial and Antifungal Properties

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ABSTRACT

We determined the antimycobacterial, antibacterial, and antifungal potential of medicinal plants used by the Samburu Community of Northern Kenya, following an ethnobotanical survey. Using BACTEC MGIT 960 system, we assessed plant extract effects on four mycobacterial strains, i.e., *Mycobacterium tuberculosis*, *M. Kansasii*, *M. fortuitum*, and *M. smegmatis*. For *Candida albicans*, *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia*, we determined zones of inhibition, Minimum Inhibitory Concentrations (MICs) and minimum bactericidal/fungicidal concentrations (MBCs/MFCs) using standard procedures. Preliminary phytochemistry on the extracts was also carried out using standard procedures. The extracts from *Scadoxus multiflorus* and *Acacia nilotica* gave strong antimycobacterial activity (zero GUs) against slow growing mycobacteria strains in all the concentrations tested. *Scadoxus multiflorus* was also active against *M. tuberculosis*. *Boscia angustifolia* was active against *M. tuberculosis* (183 GUs). *Acacia nilotica* showed strong antimicrobial activity against *E. coli* (with of MIC 4.69 mg mL⁻¹ and MBC of 18.75 mg mL⁻¹), *P. aeruginosa* (with both MIC and MBC of 18.75 mg mL⁻¹), *K. pneumoniae* and *C. albicans* (with MIC of 9.38 mg mL⁻¹ and MBC of 18.75 mg mL⁻¹). *Thylachium africanum* showed good antimicrobial activity against *S. aureus* (with MIC of 18.75 mg mL⁻¹ and MBC of 37.5 mg mL⁻¹) and *P. aeruginosa* (with both MIC and MBC of 4.69 mg mL⁻¹). Preliminary phytochemistry identified six phytochemicals to which tannins was common to all plant extracts. The data suggests that methanolic extracts of at least three plant species could be a rich source of antimicrobial agents. These results provide an indication of merit in their ethnomedicine use by the local communities.

Key words: BACTEC MGIT 960, zone of inhibition, MIC, MBC, phytochemical

INTRODUCTION

Plants form an integral part of life in many indigenous African communities as a readily and cheaply available alternative to allopathic medicines (Wagate *et al.*, 2010). They have been used since prehistoric times to alleviate and treat diseases (Potterat and Hamburger, 2008). In Africa, traditional medicine is of great value and more than 70% of the people refer to traditional healers concerning health issues (Tijjani *et al.*, 2009). Traditionally, the Samburu community of Northern

Kenya utilizes plants for both food and therapeutic purposes. It is estimated that about 85% of the Samburu people use medicinal plants for primary medicare (Omwenga *et al.*, 2009).

With the emergence of new diseases and drug resistance to infections such as HIV/AIDS, malaria, tuberculosis, diarrheal diseases and skin problems; traditional medicine should be given more attention in modern research and development (Asres *et al.*, 2001; Jeruto *et al.*, 2008; Ani *et al.*, 2009). Multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infections (Aliero *et al.*, 2008). In addition to this problem antibiotics are sometimes associated with adverse effects on host including hypersensitivity, immune suppression and allergic reactions (Nebedum *et al.*, 2009). Among such infections is TB which is a deadly infectious disease that annually kills about 3 million people worldwide (Camacho-Corona *et al.*, 2008). TB is now highly associated infection of persons suffering from Human Immunodeficiency Virus (HIV). There is also a major therapeutic problem due to the worldwide emergence of *Escherichia coli*, *Klebsiella pneumoniae*, *Haemophilus* and many other β -lactamase producers (Khan *et al.*, 2009).

A number of studies have been conducted in different countries in the last few years to prove that plant has antimicrobial activity (Khanna and Kannabiran, 2008). Because of unmatched availability of chemical diversity, natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads (Parekh and Chanda, 2007). Now with 78% of the new chemical entities being natural or natural product-derived molecules, there has been a promising alternative treatment of infectious diseases using medicinal plants (Lokhande *et al.*, 2007).

Traditionally, herbalists use plant extracts to treat ailments but with no knowledge of scientific base of their activities (Andy *et al.*, 2008). The Samburu pastoralists are among the few communities in Kenya that have retained immense knowledge on ethnobotany. This knowledge is however dwindling rapidly due to changes towards a less traditional lifestyle, overgrazing and overexploitation of plant resources. In an earlier survey (Omwenga *et al.*, 2009), a number of plants were identified as important to the Samburu community for medicinal purposes. Present study thus aimed at assessing the effect of plants' extracts from selected candidate species on common pathogens. Specifically, we investigated antimycobacterial (antituberculosis), antibacterial and antifungal activities of methanol crude extracts from eight plant species. We further undertook a preliminary phytochemical assessment to provide clues of active secondary compounds in the plants.

MATERIALS AND METHODS

Study site: The ethnobotanical survey was undertaken among five community groups inhabiting eastern Samburu District in northern Kenya between June 2008 and July 2009 (Fig. 1). Samples were collected between Ewaso Nyiro River and the Mathews Range from five communities namely Lodungokwe, Namunyak, Ngilai West, Ngutuk Ongiron, and Nkaroni in July 2009 with the help of the volunteers from different countries.

Plant material: An ethnobotanical survey was carried out between 2008 and 2009 through the use of questionnaires, in-depth interviews and market visits (Omwenga *et al.*, 2009). The herbalists were identified with the help of the local administration. Information gathered from the survey included plant vernacular names, the parts used and the diseases treated. The plants were identified and taxonomically grouped at the Department of Pharmacy and Complimentary

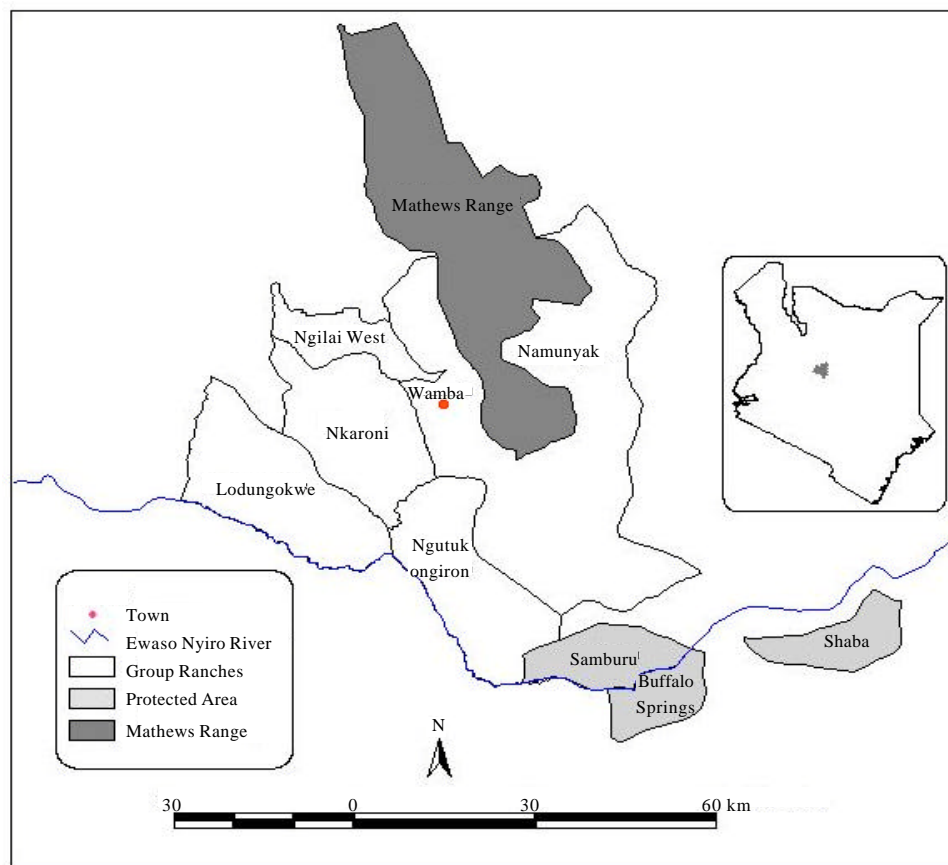


Fig. 1: Map of the study area showing Samburu community groups studied

Alternative Medicine, Kenyatta University, Nairobi, Kenya; where voucher specimens were also deposited. From this initial study, we selected eight species (Appendix 1) for extraction and analysis.

Preparation of plant extracts: The plant samples collected were chopped into small pieces, shade dried and grounded using hammer type milling machine (Meecan, CM/L-1364548, India) at the Department of Pharmacy/CAM, Kenyatta University, Nairobi, Kenya. The powdered material was transferred into and extracted in the soxhlet extractor using methanol for 72 h (Aiyelaagbe and Osamudiamen, 2009). The extracts were filtered through a Whatmann filter paper No. 42 (125 mm) and concentrated using a rotary evaporator (Laborota 4000, SN 090816862, Germany) with the water bath set at 40°C (Edeoga *et al.*, 2005), then dried in a dessicator over anhydrous CuSO_4 . The powdered residue were transferred into vials and stored at 4°C in airtight vials before analysis.

Test microorganisms: The four species of mycobacteria used for the assays were obtained from the Center for Respiratory Diseases Research (CRDR), Kenya medical Research Institute (Kemri), Nairobi, Kenya. These included *Mycobacterium tuberculosis*, *M. kansasii*, *M. smegmatis* and *M. fortuitum*. *Salmonella typhi* (clinical isolate), *Klebsiella pneumoniae* (clinical isolate),

Pseudomonas aeruginosa (ATCC 25852), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 20591) and *Candida albicans* (ATCC EK138), a yeast like fungi, were obtained from Kenyatta National Hospital in Nairobi, Kenya and used in the antibacterial and antifungal activity tests, respectively.

Growth media: Mycobacteria strains were inoculated in parallel solid medium (Lowenstein Jensen) and the liquid (mycobacterial growth indicator tube: MGIT 960) media. Bacteria were grown in Mueller Hinton agar (Oxoid) and *C. albicans* in Potato Dextrose Agar (PDA).

Antimycobacterial susceptibility test using BACTEC MGIT™ 960 system: This was used in the antimycobacterial activity assessment of the plant extracts. The extracts were dissolved in 0.01% DMSO to final concentrations of 0.5, 1.0 and 2.0 mg mL⁻¹. A stock solution of 2.0 mg mL⁻¹ of isoniazid was used as the positive and 0.01% DMSO as the negative control, respectively. Into the 7 mL BBL™ MGIT™ tubes, 0.8 mL of the mixture of growth OADC (containing Oleic acid, Bovine albumen, Dextrose and Catalase) supplement (added to provide essential substances for rapid growth of mycobacteria) and BBL™ MGIT™ PANTA (a mixture of antimicrobial agents) were added. Then 1 mL of the extract was added into the BBL™ MGIT™ tubes containing the supplement to attain appropriate concentrations of 0.5, 1.0 and 2 mg mL⁻¹. Mycobacterium suspension (adjusted to 0.5 McFarland standard) was introduced into the BBL™ MGIT™ tubes. The strains included *M. tuberculosis* (Mtb), *M. kansasii* (Mk), *M. fortuitum* (Mf) and *M. smegmatis* (Ms). The BACTEC MGIT™ 960 system was loaded using manufactures' instructions and incubated at 37°C. Culture vials which remained negative for a minimum of 42 days (maximum 56 days) were removed and recorded as negative, while growth units (GUs) for the positive ones were recorded appropriately (Becton and Company, 2007). The same was done for the controls. Results were provided as positive/negative and numerical Growth Units (GUs) using a non-radiometric evaluation technique (Becton and Company, 2007).

Evaluation of antibacterial and antifungal activity: The antibacterial and antifungal activities of extracts from the 8 plant species were assayed *in vitro* by agar Disc Diffusion (DD) method (Parekh and Chanda, 2007). Filter paper discs (6 mm) were impregnated with the plant extracts. Mueller Hinton agar and Potato Dextrose Agar (PDA) were prepared using manufactures' instructions for purposes of culturing the bacteria and fungi respectively. Normal saline solution was used to dilute a 24 h culture of the bacterial type culture or clinical isolate to attain a 0.5 McFarland standard. Spread plate method was used to culture 100 µL of the microbial suspension that was introduced into the Petri dishes (Meite *et al.*, 2009). Eighteen dry sterile discs (6 mm diameter) were soaked in the plant extract (made by dissolving 300 mg of the extracts in 1000 µL of methanol) air dried and placed on the spread plates at reasonable distances. Discs impregnated with methanol and air dried were used as negative controls and various standard conventional antibiotics (Amoxicillin (Hangzhou Ruijian Chemical Co., Ltd., batch 490805241); Ciprofloxacin (Chengdu Ware Yuanheng Pharmaceutical Co., Ltd., batch 20070907); Fluconazole (Pfizer Ltd., UK batch 30) as positive controls. The plates were then incubated at 35°C for 24 h. This was replicated three times for each pathogen.

Candida albicans was cultured by taking 100 µL from the broth and spreading on PDA. The culture was incubated at 25°C for 72 h. The cork boarer was used to pick a section of the young mycelium which was placed at the centre of the PDA plate and the dry discs which were

impregnated with 100 μ L of the plant extracts placed at a distance around the inoculum mycelium. The inoculum was incubated at 25°C for 72 h. Fluconazole and dry discs treated with methanol were also used as positive and negative controls, respectively. All tests were performed in triplicate. Microbial growth inhibition was determined by measuring the zones of inhibition using a transparent ruler.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC):

The Minimum Inhibitory Concentration (MIC) which is the least amount of antimicrobial agent that will inhibit visible growth of an organism after an overnight incubation was determined using the microtitre dilution broth method in 96-well micro plates. This was done only where the plant extract showed strong antibacterial activity by the disk diffusion method (≥ 9 -15 mm) (Rani and Khullar, 2004). The wells were filled with 50 μ L of the Nutrient broth for bacterial strains and Potato dextrose broth for *C. albicans*. The extract was then prepared by taking 300 mg of the plant extract and mixing it with 1000 μ L of DMF (0.01% Dimethyl formamide) for complete dissolution of the extract. Then 50 μ L of the plant extract was dispensed into the first well before serial dilutions were done by transferring 50 μ L of nutrient or potato dextrose broth containing the extract from the first well to the second well, and from the second well to the third well through the fourth well. Fifty microlitres of the test isolate was then dispensed into each well. One well (without extract or drug) was used as negative control of the growth of the microorganisms in the medium whereas another well with 50 μ L of the antibiotic (Amoxicillin/Ciprofloxacin/fluconazole) was used as positive control. Incubation was done at 37°C for 24 h. The MIC values were determined as the lowest concentrations of the extract capable of inhibiting microbial growth.

For the determination of MBC/MFC, wells where there was no growth were subcultured on nutrient agar and PDA. The lowest concentration of the plant extracts that did not yield any colony on the solid medium (Nutrient or PDA agar) after sub culturing and incubating for 24 h for bacterial strains and 72 h for *C. albicans* was taken as the MFC/MBC. All tests were performed in triplicates.

Preliminary phytochemistry

Test for alkaloids (Wagner's method): Alkaloids presence was determined by dissolving and filtering 200 mg plant extract in 10 mL methanol followed by filtration using Whatmann filter paper No. 42 (125 mm) filters. One thousand microlitres (1 mL) of the filtrate was then mixed with 6 drops of Wagner's reagent (Obadoni and Ochuko, 2001). Creamish, brownish-red or orange precipitate indicated the presence of alkaloids. A low (+) reaction was recorded if the addition of the reagent produced a faint turbidity; a moderate (++) reaction was recorded if alight opalescence precipitate was observed and a high (+++) reaction was recorded if a heavy yellowish-white precipitate was observed.

Test for cardiac glycosides (Keller-Killani test): Five milliliter of each extracts were treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 mL of concentrated sulphuric acid. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides. A (+) reaction was recorded when a faint green-blue color was observed (indicating low concentrations of detectable cardiac glycosides); a (++) reaction was recorded when a medium green-blue colour was observed (indicating moderate concentrations of

detectable cardiac glycosides) and a (+++) reaction was recorded when a deep green-blue colour was observed (indicating high concentrations of detectable cardiac glycosides) (Aiyelaagbe and Osamudiamen, 2009).

Test for flavonoids: Five milliliters of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4 . A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing. A (+) reaction was reported in pale yellow colour; (++) in moderate yellow and (+++) in strong yellow coloration, indicating low, moderate or high concentration of flavonoids respectively in the plant extract (Edeoga *et al.*, 2005).

Test for saponins: To 0.5 mg of extract was added 5 mL of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, after which it was observed for the formation of an emulsion (Aiyelaagbe and Osamudiamen, 2009). A (+) sign was recorded when the froth reached a height of 50 mm; a (++) sign with the height of 0.6-1 cm and a (+++) sign with a height of more than 100 mm to indicate low, moderate or high concentration of saponins, respectively in the plant extract.

Test for tannins: About 0.5 mg of the extract was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration (Edeoga *et al.*, 2005). A (+) reaction was recorded when a slight precipitate was observed; a (++) reaction was recorded when a medium precipitate was observed and a (+++) reaction was recorded when a heavy precipitate was observed. The reactions were used to indicate the presence of different concentrations of detectable tannins, with (+) representing low, (++) moderate and (+++) high levels of tannins.

Test for terpenoids (Salkowski test): To 0.5 mg each of the extract was added 2 mL of chloroform. Concentrated H_2SO_4 (3 mL) was carefully added to form a layer. A reddish brown coloration at the interface indicated the presence of terpenoids (Aiyelaagbe and Osamudiamen, 2009; Edeoga *et al.*, 2005). A (+) reaction was recorded when a faint reddish brown coloration was observed; a (++) reaction was recorded when a medium reddish brown coloration was observed and a (+++) reaction was recorded when a deep reddish brown coloration was observed.

RESULTS

At the highest concentration of 2.0 mg mL^{-1} , all plant extracts showed high activity (with Zero GUs) against mycobacteria strains tested (Table 1), except for *M. smegmatis* which had 17 GUs against *C. africana*. *Acacia nilotica* and *S. multiflorus* extracts were effective against fast growing mycobacteria at all concentrations. The *S. multiflorus* extract was also active against *M. tuberculosis* at 1.0 mg mL^{-1} concentrations. At 0.5 mg mL^{-1} , *B. angustifolia* gave appreciable inhibition against *M. kansasii* (501 GUs) and *M. tuberculosis* (183 GUs) compared to the negative control (10597 and 18683 GUs, respectively). *Grewia simi* and *C. africana* showed measurable effects at 1.0 or 0.5 mg mL^{-1} .

Table 1: Antimycobacterial activity (GUs) of eight plant species identified to have medicinal properties by Samburu herbalists in Northern Kenya. The test used was BACTEC MGIT™ 960 system

Plant specimen	Slow growers						Fast growers					
	Concentrations (mg mL ⁻¹)						Concentrations (mg mL ⁻¹)					
	2		1		0.5		2		1		0.5	
	Mk	Mtb	Mk	Mtb	Mk	Mtb	Mf	Ms	Mf	Ms	Mf	Ms
<i>T. africanum</i>	0	0	6432	2515	ND	ND	0	0	0	0	189	2477
<i>B. angustifolia</i>	0	0	0	0	501	183	0	9	0	120	618	13801
<i>C. quadrangularis</i>	0	0	256	900	ND	ND	0	0	0	0	1041	120
<i>G. simi</i>	0	0	247	0	9025	1848	0	0	99	67	406	125
<i>A. etbaica</i>	0	0	7404	2170	ND	ND	0	0	0	0	564	1507
<i>S. multiflorus</i>	0	0	468	0	10258	0	0	0	0	0	0	0
<i>C. africana</i>	0	0	958	2557	ND	ND	0	17	112	4660	870	12400
<i>A. nilotica</i>	0	0	3656	19613	ND	ND	0	0	0	0	0	0
Positive control	0	0	0	0	0	0	0	0	0	0	0	0
Negative control	745	2002	37611	3862	10597	18683	187	2957	212	5266	893	16017

Gus: Numerical growth units, Mk: *Mycobacteria kansasii*, Mtb: *M. tuberculosis*, Mf: *M. fortuitum*, Ms: *M. smegmatis*, 0: indicates complete inhibition, ND: Not done, Positive control: Isoniazid, Negative control: Dimethyl sulphoxide. *Note: The higher the growth index, the less inhibitory the extract is to mycobacteria (compared to negative control)

Table 2: Antibacterial activity of five plant species identified to have medicinal properties by Samburu herbalists in northern Kenya

Plant specimen	Diameter of inhibition zone (mm)					
	1	2	3	4	5	6
<i>T. africanum</i>	7.67	10.66	8.00	10.00	7.33	6.00
<i>B. angustifolia</i>	8.00	9.00	7.33	6.66	7.00	6.00
<i>G. simi</i>	6.66	6.66	8.00	7.00	7.33	6.00
<i>S. multiflorus</i>	9.00	8.33	8.33	6.33	7.33	7.00
<i>A. nilotica</i>	8.66	8.00	12.00	11.66	9.00	10.33
Positive control	16.00	21.33	20.22	17.33	17.66	13.00
Negative control	6.00	6.00	6.00	6.00	6.00	6.00

1: *S. typhi*, 2: *S. aureus*, 3: *E. coli*, 4: *P. aeruginosa*, 5: *K. pneumoniae*, 6: *C. albicans*. Positive controls: Fluconazole for *C. albicans*, Zeftazidime for *S. typhi*, Ciprofloxacin for *K. pneumoniae* and Amoxicillin for *S. aureus*, *E. coli* and *P. aeruginosa*. Values are means of triplicates

All the extracts showed varying degrees of antibacterial and antifungal activity against the test organisms (Table 2) with some plant extracts showing strong antimicrobial activity with zones of inhibition of between 9.00 and 12.00 mm. *Acacia nilotica* extract showed strong antimicrobial activity against *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *C. albicans* with inhibition zones of between 9.00 and 12.0 mm. *Thylachium africanum* showed strong antibacterial activity against *S. aureus* and *P. aeruginosa* (zones of inhibition of 10.66 and 10.00 mm, respectively). The extracts of *B. angustifolia* and *S. multiflorus* gave strong antibacterial activity only against *S. aureus* (zone of inhibition of 9.00 mm) and *S. typhi* (zone of inhibition of 9.00 mm), respectively, but *G. simi* gave weak antimicrobial activity (zones of inhibition of between 6.00-8.00 mm) against all the test microorganisms.

Table 3: Minimum inhibitory concentrations and minimum bactericidal/fungicidal concentrations (mg mL⁻¹) produced by the medicinal plants against various bacterial test cultures

Plant specimen	<i>S. typhi</i>		<i>S. aureus</i>		<i>E. coli</i>		<i>P. aeruginosa</i>		<i>K. Pneumoniae</i>		<i>C. albicans</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>T. africanum</i>	ND	ND	18.75	37.50	ND	ND	4.69	4.69	ND	ND	ND	ND
<i>B. angustifolia</i>	ND	ND	37.50	75.00	ND	ND	ND	ND	ND	ND	ND	ND
<i>S. multiflorus</i>	37.50	75.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>A. nilotica</i>	ND	ND	ND	ND	4.69	18.75	18.75	18.75	18.75	18.75	9.38	18.75
Positive control	4.69	4.69	4.69	4.69	4.69	4.69	4.69	4.69	4.69	4.69	4.69	4.69
Negative control	Growth observed in all tubes											

ND: Not done

Table 4: Preliminary phytochemical screening of eight plant species identified to have medicinal properties by Samburu herbalists in Northern Kenya

Plant specimen	Constituents					
	Alkaloids	Cardiac glycosides	Flavonoids	Saponins	Tannins	Terpenoids
<i>T. africanum</i>	+++	++	++	+++	+++	-
<i>B. angustifolia</i>	+	-	-	-	+	-
<i>C. quadrangularis</i>	+	+	+	+	+++	+++
<i>G. simi</i>	-	+	+	+++	+++	-
<i>A. etbaica</i>	+	++	++	++	+	+++
<i>S. multiflorus</i>	+++	++	+++	+++	+++	+
<i>C. africana</i>	+++	+++	-	+	++	-
<i>A. nilotica</i>	+++	+	++	++	+++	++

+++ : Present in high concentration, ++: Moderately present, +: Trace, -: Absent. Four medicinal plant species (*C. quadrangularis*, *A. nilotica*, *A. etbaica* and *S. multiflorus*) exhibited presence of all the six phytochemicals with moderate to high concentrations being recorded in *A. nilotica* and *S. multiflorus*

The *A. nilotica* extract showed strong MICs (4.69 mg mL⁻¹) and MBC of 18. mg mL⁻¹ for *E. coli*, MIC and MBC of 18.75 mg mL⁻¹ for *P. aeruginosa* and *K. pneumoniae* respectively (Table 3). *Thylachium africanum* was most active against *P. aeruginosa* with an MIC of 4.69 mg mL⁻¹ and an MBC of similar concentration. *Boscia angustifolia* and *S. multiflorus* were the least active extracts against *S. aureus* and *S. typhi* with MICs of 37.5 mg mL⁻¹ and MBCs of 75 mg mL⁻¹, respectively.

Preliminary phytochemistry indicated that the extracted samples showed presence of most six phytochemicals tested for (Table 4), including alkaloids, cardiac glycosides, flavonoids, saponins, tannins and terpenoids. Tannins were present in extracts of all the eight while terpenoids in only four plant species.

DISCUSSION

The activity of *S. multiflorus* and *A. nilotica* extracts against mycobacterial strains showed that the plants contain pharmacologically active substances. The results were comparable to those of the standard drug (Isoniazid) in BACTEC MGIT 960 system. The two extracts appeared particularly active against *M. smegmatis* and *M. fortuitum* where they were potent at 0.5 mg mL⁻¹. *Scadoxus multiflorus* was also active against *M. tuberculosis* at 0.5 mg mL⁻¹. Other plant extracts gave varying results, with *B. angustifolia* giving moderate activity.

General antibacterial and antifungal results were also notable (Table 2), with *A. nilotica* extracts showing strong antimicrobial activity against *E. coli* (inhibition zone of 12.00 mm), *P. aeruginosa* (inhibition zone of 11.66 mm), *K. pneumoniae* (inhibition zone of 9.00 mm) and *C. albicans* (inhibition zone of 10.33 mm). Minimum inhibitory concentrations and minimum bactericidal/fungicidal concentrations (mg mL^{-1}) produced by the medicinal plants against various bacterial test cultures showed strong antimicrobial activity (Table 3), with *A. nilotica* extracts showing strong antimicrobial activity against *E. coli* (MIC of 4.69 mg mL^{-1} and MBC of 18.75 mg mL^{-1}), *P. aeruginosa* (both MIC and MBC of 18.75 mg mL^{-1}), *K. pneumoniae* (both MIC and MBC of 18.75 mg mL^{-1}) and *C. albicans* (MIC of 9.38 mg mL^{-1} and MBC of 18.75 mg mL^{-1}).

A zone of inhibition $\geq 9\text{-}15 \text{ mm}$ is an indication of strong antimicrobial activity (Rani and Khullar, 2004). These findings are in agreement with the findings of Tijjani *et al.* (2009), whereas they contradict those they reported as being hardly susceptible to plant extracts whose doses were as low as 200 mg mL^{-1} . The activity of *A. nilotica* is in agreement with the findings of Khan *et al.* (2009) where it was found to give the most potent antimicrobial extract against *K. pneumoniae*, *C. albicans*, *P. aeruginosa* and *E. coli*, but contrasts with their findings that it is active against *S. aureus* and *S. typhimurium*. From a study carried out by Haj Ali and Yagoub (2007), the inhibitory effects of the *A. nilotica* fruit extracts on *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were compared with those of selected antibiotics. The ethanol extract of *A. nilotica* fruit was either equally or more effective than the test antibiotics, which contrasts our current study.

The eight species used as medicinal plants by Samburu communities showed presence of four to six phytochemicals. Present study is in concurrence with others (Aliero *et al.*, 2008) that have shown *S. multiflorus* bulbs to contain alkaloids, flavonoids, tannins, saponin and cardiac glycosides. All the six phytochemicals identified are known to show medicinal activity as well as exhibiting physiological effects (Edeoga *et al.*, 2005). For instance, flavonoids have antidiarrhoeal effects (Meite *et al.*, 2009), while plants rich in saponins have immune boosting and anti-inflammatory properties (Aliero *et al.*, 2008; Meite *et al.*, 2009). Tannins have antibacterial potential due to their ability to react with proteins to form stable water soluble compounds that kill bacteria by directly damaging their cell membranes (Aliero *et al.*, 2008). The MspA in *M. fortuitum* and *M. smegmatis* which is absent in slow-growers could be the reason for low inhibitory activity of the *A. nilotica* and *S. multiflorus* extract against the fast growers (Sharbati-Tehrani *et al.*, 2005).

The high microbial effects exhibited by *S. multiflorus* and *A. nilotica* may thus be attributed to the high concentration of phytochemicals detected in their extracts, particularly alkaloids and flavonoids. The presence of these phytochemical components in the two plant species is an indication that they may have medicinal properties as used by the Samburu herbalists.

CONCLUSION

The results of the investigation revealed that most of the medicinal plants contain pharmacologically active substances with antimycobacterial, antibacterial and antifungal properties. It also points out that there is a possibility of getting effective compounds from natural sources, which can be of value in the fight against tuberculosis and other infectious diseases. The study also provides support for the use of these plants in the management of infectious diseases in the Samburu community and elsewhere.

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APPENDIX

Appendix 1: Selected Medicinal plants used by the Samburu Community of Kenya to treat mycobacterial, selected bacterial and fungal diseases

Botanical name	Family name	Local name	Where collected from	Part(s) used	Diseases treated
<i>Thylachium africanum</i> Lour.	Capparaceae	Loimugi	Namunyak	Bark	Diarrhoea, TB
<i>Boscia angustifolia</i> Guill. and Perr	Capparaceae	Lororoi	Nkaroni	Bark	Diarrhoea, chest problems, Gonorrhea
<i>Cissus quadrangularis</i> L.	Vitaceae	Sukurtut	Namunyak	Stem	Stomach aches, chest pains
<i>Grewia simi</i> L.	Tiliaceae	Lngalayoi	Nkaroni	Roots	Diarrhoea
<i>Acacia etbaica</i> Schweinf.	Mimosaceae	Lchakwai	Namunyak	Leaves	Stomach ache, Asthma
<i>Scadoxus multiflorus</i> (Martyn) Raf.	Amaryllidaceae	Loilei	Westgate	Bulbs	Gastrointestinal problems, coughs, wounds
<i>Commiphora africana</i> (A. Rich) Engl. Var.	Burseraceae	Lcheni-ngiro	Namunyak	Bark	Eye problems, Stomach ache, Asthma, Polio, arthritis
<i>Acacia nilotica</i> (L.) Del.	Mimosaceae	Lkiloriti	Namunyak	Stem bark	Stomach ache, TB

REFERENCES

- Aiyelaagbe, O.O. and P.M. Osamudiamen, 2009. Phytochemical screening for active compounds in *Mangifera indica* leaves from Ibadan, Oyo State. Plant Sci. Res., 2: 11-13.
- Aliero, A., B.L. Aliero and U. Buhari, 2008. Preliminary phytochemical and antibacterial screening of *doxus multiflorus*. Int. J. Pure Applied Sci., 2: 13-17.
- Andy, I.E., M.E. Eja and C.I. Mboto, 2008. An evaluation of the antimicrobial potency of *Lasianthera africana* (BEAUV) and *Heinsia crinata* (G. Taylor) on *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans*. Malaysian J. Microbiol., 4: 25-29.
- Ani, A., J. Idoko, Y.B. Dalyop and S.L. Pitmang, 2009. Drug resistance profile of *Mycobacterium tuberculosis* isolates from pulmonary tuberculosis patients in Jos, Nigeria. Trans. Royal Soc. Trop. Med. Hyg., 103: 67-71.
- Asres, K., F. Bucar, S. Edelsbrunner, T. Kartnig, G. Hoyer and W. Thiel, 2001. Investigations on antimycobacterial activity of some ethiopian medicinal plants. Phytoth. Res., 15: 323-326.
- Becton and D. Company, 2007. BBL MGIT Mycobacteria Growth Indicator Manual. Maryland, USA., pp: 1-23.
- Camacho-Corona, M.D.R., M.A. Ramírez-Cabrera, O. González-Santiago, E. Garza-González, I.D.P. Palacios and J. Luna-Herrera, 2008. Activity against drug resistant-tuberculosis strains of plants used in mexican traditional medicine to treat tuberculosis and other respiratory diseases. Phytother. Res., 22: 82-85.

- Edeoga, H.O., D.E. Okwu and B.O. Mbaebie, 2005. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4: 685-688.
- Haj Ali, A.M. and S.O. Yagoub, 2007. Anti-microbial activity of *Acacia nilotica* extracts against some bacteria isolated from clinical specimens. Res. J. Medicinal Plant, 1: 25-28.
- Jeruto, P., C. Lukhoba, G. Ouma, D. Otieno and C. Mutai, 2008. An ethnobotanical study of medicinal plants used by the Nandi people in Kenya. J. Ethnopharmacol., 116: 370-376.
- Khan, R., B. Islam, M. Akram, S. Shakil and A. Ahmad *et al.*, 2009. Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules, 14: 586-597.
- Khanna, V.G. and K. Kannabiran, 2008. Antimicrobial activity of saponin fraction from the roots of *Hemidesmus indicus*. Res. J. Medicinal Plant, 2: 39-42.
- Lokhande, P.D., K.R. Gawai, K.M. Kodam, B.S. Kuchekar, A.R. Chabukswar and S.C. Jagdale, 2007. Antibacterial activity of isolated constituents and extract of roots of *Inula racemosa*. Res. J. Medicinal Plant, 1: 7-12.
- Meite, S., J.D. N'guessan, C. Bahi, H.F. Yapi, A.J. Djaman and F.G. Guina, 2009. Antidiarrheal activity of the ethyl acetate extract of *Morinda morindoides* in rats. Trop. J. Pharm. Res., 8: 201-207.
- Nebedum, J., K. Ajeigbe, E. Nwobodo, C. Uba, O. Adesanya, O. Fadare and D. Ofusori, 2009. Comparative study of the ethanolic extracts of four Nigerian plants against some pathogenic microorganisms. Res. J. Med. Plant, 3: 23-28.
- Obadoni, B.O. and P.O. Ochuko, 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global J. Pure Applied Sci., 8: 203-208.
- Omwenga, E.O., P.O. Okemo, P.K. Mbugua and C.K.P. Ogot, 2009. Ethnobotanical survey and antimicrobial evaluation of medicinal plants used by the samburu community (Kenya) for treatment of Diarrhoea. Pharm. Maga., 4: 165-176.
- Parekh, J. and S.V. Chanda, 2007. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk. J. Biol., 31: 53-58.
- Potterat, O. and M. Hamburger, 2008.. Drug Discovery and Development with Plant-derived Compounds. In: Natural Compounds as Drugs, Petersen, F. and R. Amstutz (Eds.). Springer, New York, ISBN: 978-3-7643-8098-4.
- Rani, P. and N. Khullar, 2004. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi*. Phytother. Res., 18: 670-673.
- Sharbati-Tehrani, S., J. Stephan, G. Holland, B. Appel, M. Niederweis and A. Lewin, 2005. Porins limit the intracellular persistence of *Mycobacterium smegmatis*. Microbiology, 151: 2403-2410.
- Tijjani, M.B., I.A. Bello, A.B. Aliyu, T. Olurische, S.M. Maidawa, J.D. Habila and E.O. Balogun, 2009. Phytochemical and antibacterial studies of root extract of *Cochlospermum tinctorium* A. rich. (Cochlospermaceae). Res. J. Med. Plant, 3: 16-22.
- Wagate, C.G., J.M. Mbaria, D.W. Gakuya, M.O. Nanyingi and P.G. Kareru *et al.*, 2010. Screening of some kenyan medicinal plants for antibacterial activity. Phytoth. Res., 24: 150-153.