

Research Article

Antitubercular and Phytochemical Investigation of Methanol Extracts of Medicinal Plants Used by the Samburu Community in Kenya

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Abstract

Purpose: To determine the potential benefits of nine medicinal plants used by the Samburu community for the treatment of tuberculosis.

Methods: The extract was tested against four strains of Mycobacteria namely; *Mycobacterium tuberculosis* (Mtb), *M. Kansasii* (Mk), *M. fortuitum* (Mf), and *M. smegmatis* (Ms) using BACTEC MGIT 960 system. The crude extracts were also analyzed for the presence of phytochemical constituents.

Results: Both the extracts of *Scadoxus multiflorus* and *Acacia nilotica* showed strong antimycobacterial activity against the four tuberculosis-causing strains. *Eurphobia scarlatina* was the most active against both the slow (Mtb and Mk) and the fast (Mf and Ms) growers with Zero GUs at 0.5mg/ml. Phytochemical screening indicated presence or absence of tannins, saponins and flavonoids, terpenoids, cardiac glycosides and alkaloids in the extracts.

Conclusion: The data suggest that some of the methanol extracts could be a rich source of antituberculosis agents. The results further show that there is some merit in the use of some of the plants studied in alternative medical practice. Pharmacological and toxicological studies of the active plants are still under investigation.

Keywords: Medicinal plants, Methanol extract, Antituberculosis, Samburu.

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INTRODUCTION

With the rising prevalence of microorganisms showing resistance to antibiotics, there is an urgent need to develop new antimicrobial compounds [1]. Man has used plants to treat common infectious diseases, and some of the traditional medicines are still part of the habitual treatment for various maladies [2]. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity [3]. Scientific interest in medicinal plant has burgeoned in recent times due to increased efficiency of new plant derived drugs and rising concerns about the side effects of modern medicine, and hence the need to look for new molecular structures as lead compounds from the plant kingdom [2,4].

Infectious diseases are the leading cause of death worldwide [3]. Increased antibiotic resistance has become a global concern [3]. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidermidis of human immunodeficiency virus (HIV) infections [5].

There is a major global health problem attributable to diseases, such as tuberculosis (TB), which are complicated due to drug resistance. This is coupled with the problem of mycobacterial persistence, thus highlighting the need to develop novel TB drugs that are not only active against drug resistant bacteria, but more importantly, kill persistent bacteria and shorten the length of treatment [6]. Apart from significant toxicity, lengthy therapy also creates poor patient compliance. Non-compliance is a frequent cause for selection of drug resistant, and often, deadly multidrug resistant TB (MDR-TB) bacteria, and recently, extensively drug resistant tuberculosis (XDR-TB). The aim of the present study was to determine the antituberculosis activity of the methanol

extracts of various medicinal plants and to investigate the variety of secondary metabolites present.

EXPERIMENTAL

Collection of plant materials

Various parts of the nine mature healthy plants were collected, as indicated in our previous study [16], from the various conservancies in Samburu (Table 1). Information gathered from the survey included vernacular names and the parts used in the preparation of herbal antibacterial remedies and the diseases they are used to treat. The plant materials were botanically authenticated by Mr. Lucas Karimi (Department of Pharmacy and Complimentary Alternative Medicine (CAM), Kenyatta University, Nairobi, Kenya.). Voucher specimens were deposited in the Department of Pharmacy and CAM herbarium.

Preparation of extracts of plant materials

The plant materials collected were sliced into small pieces, shade-dried and ground using a hammer type milling machine (Meecan, CM/L-1364548, India). The powdered material was Soxhlet-extracted using methanol at 80 °C for 72 h [7]. The extracts were filtered through a Whatmann filter paper No. 42 (125 mm) and concentrated using a rotary evaporator (Laborota 4000, SN 090816862, Germany) in a water bath set at 40 °C [8]. The extract was dried in a dessicator over anhydrous CuSO₄. The powdered residues were transferred into vials and stored pending analysis.

Test micro-organisms

The test mycobacteria strains were: *Mycobacteria tuberculosis*, *M. kansasii*, *M. fortuitum* and *M. smegmatis*, all sourced from the Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. Mycobacteria strains were rejuvenated on Lowenstein Jensen (LJ) slants for 14 days at 37 °C using standard procedures.

Table 1: Nine medicinal plants used by the Samburu Community of Kenya to treat respiratory tract infections

Botanical Name	Family name	Vernacular name	Where collected from	Voucher specimen no.	Part(s) used	Diseases treated
<i>Salvadora persica</i> L. var. <i>persica</i>	Salvadoraceae	Sokotei	Nkaroni	TB/MR/036/07	Roots/branches	Chest problems, Stomachache, teeth problems,
<i>Acacia senegal</i> (L.) Willd. Var.	Mimosaceae	Lderekesi	Nkaroni	TB/MR/045/07	Bark	Stomachache, TB
<i>Acokanthera friesiorum</i> Markgr.	Apocynaceae	Chipilikwa	Namunyak	TB/MR/046/07	leaves	Diarrhoea, Asthma, TB
<i>Plumbago dawei</i> Rolfe.	Plumbaginaceae	Lkiarianthus	Namunyak	TB/MR/054/07	Bark	Malaria, Diarrhoea, TB
<i>Loranthus acaciae</i> Zucc.	Loranthaceae	Lardenyai	Nkaroni	TB/MR/033/07	Whole plant	Stomach ache, Chest problems
<i>Cordia sinensis</i> Lam.	Boraginaceae	Silapani	Nkaroni	TB/MR/056/07	Bark	Diarrhoea, chest pains
<i>Acacia horrida</i> (L.) Willd.	Mimosaceae	Lerai	Westgate	TB/MR/047/07	Bark	Diarrhoea, TB
<i>Albizia anthelmitica</i> Brongn.	Leguminosae	Lamurtana	Nkaroni	TB/MR/055/07	Bark	Deworming, Diarrhoea, Asthma, TB
<i>Euphorbia scarlatina</i> (L) O. Ktze	Euphorbiaceae	Mpopongi	Lodungokwe	TB/MR/043/07	Stem	Stomach ache, common cold, TB

*Source: [16]

Assessment of antimycobacterial activity of the extracts

The evaluation was carried out with the aid of BACTEC MGIT™ 960 system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD). The extracts were dissolved in 0.01% DMSO to achieve desired final concentrations of 2, 1 and 0.5 mg/ml). A stock solution of 2.0 mg/ml of isoniazid was used as the positive control, and 0.01% DMSO as the negative control. Into 7ml BBL™ MGIT™ tubes, 0.8 ml of the mixture of growth OADC supplement (added to provide essential substances for rapid growth of mycobacteria) and BBL™ MGIT™ PANTA (a mixture of antimicrobial agents) was added. Thereafter, 1 ml of the extract was added to the BBL™ MGIT™ tubes to attain appropriate concentrations (2, 1 and 0.5 mg/ml) followed

by the introduction of 0.5 ml of the mycobacterium suspension. The strains used were *Mycobacterium tuberculosis* (Mtb), *M. kansasii* (Mk), *M. fortuitum* (Mf) and *M. smegmatis* (Ms). The tubes were loaded into the facility (BACTEC MGIT™ 960 system), following the manufacturer's instructions, and then incubated at 37 °C for 6 weeks. 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 [9]. The same procedure was followed for the controls.

Test for alkaloids

Wagner's method was used [10]. The presence of alkaloids 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. 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 a light opalescence precipitate was observed; and a high (+++) reaction was recorded if a heavy yellowish-white precipitate was found.

Test for cardiac glycosides

Keller-Killani test was used [7,10]. Five millilitres (5 ml) of each extract were treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered 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 colour 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)

Test for flavonoids

Five millilitres of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H₂SO₄. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. A (+) reaction was reported for pale yellow colour; (++) for moderate yellow and (+++) for strong yellow colouration, indicating low, moderate and high concentrations of flavonoids, respectively, in the plant extract [7,8].

Test for saponins

To 0.5 g of the 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 [7,8]. A (+) sign was recorded when the froth reached a height of 0.5 cm; a (++) sign for a height of 0.6 - 1 cm; and a (+++) sign for a height of more than 1cm, indicating low, moderate or high concentrations of saponins, respectively, in the plant extract.

Test for tannins

About 0.5 g 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 colouration [8]. 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 g each of the extract was added 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration at the interface indicated the presence of terpenoids [7,8]. A (+) reaction was recorded when a faint reddish brown colouration was observed; a (++) reaction was recorded when a medium reddish brown colouration was observed; and a (+++) reaction was recorded when a deep reddish brown coloration was observed.

Table 2: Antimycobacterial activity (GUs) of 9 medicinal plants

Plant specimen	Slow growers						Fast growers					
	Concentration (mg/ml)											
	2		1		0.5		2		1		0.5	
	Mk	Mtb	Mk	Mtb	Mk	Mtb	Mf	Ms	Mf	Ms	Mf	Ms
<i>S. Persica</i>	0	0	144	0	257	10	0	0	0	0	0	0
<i>A. Senegal</i>	0	0	1208	0	ND	4569	0	0	0	0	653	12407
<i>A. friesiorum</i>	0	0	773	0	ND	15098	0	0	76	48	450	11608
<i>P. dawei</i>	0	0	15663	0	ND	3891	0	0	0	0	775	996
<i>L. acaciae</i>	0	0	147	0	5660	783	0	0	0	75	601	1871
<i>C. sinensis</i>	0	0	0	0	0	0	0	0	209	178	986	15651
<i>A. horrida</i>	0	0	4896	0	ND	198	0	0	0	0	209	178
<i>A. anthelmitica</i>	0	0	1702	0	3114	1603	0	0	0	0	701	14761
<i>E. scarlatina</i>	0	0	0	0	0	0	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

Key: GUs-Numerical growth units, Mk = *Mycobacteria kansasii*, Mtb = *M. tuberculosis*, Mf = *M. fortuitum*, Ms = *M. smegmatis*, ND-Not done, Positive control = Isoniazid, Negative control = Dimethyl sulphoxide, 0 = indicates complete inhibition

***Note:** The higher the growth index, the less inhibitory the extract was to mycobacteria (compared to negative control). Tests performed in duplicate

RESULTS

The present study was conducted to investigate the antituberculosis activity of 9 medicinal plant extracts against some *Mycobacteria* species. The results are shown in Table 2. Some of the medicinal plant extracts showed strong antituberculosis activity against slow growers (*M. tuberculosis* and *M. kansasii*). *S. persica* (10 GUs at 0.5 mg/ml), *C. sinensis* (Zero GUs at 0.5mg/ml) and *E. scarlatina* (Zero GUs at 0.5 mg/ml) showed strong activity against *M. tuberculosis*. Both *C. sinensis* and *E. scarlatina* showed strong activity against *M. kansasii* with zero GUs at 0.5 mg/ml. *A. horrida* gave appreciable inhibition (257 GUs) against *M. tuberculosis* (198 GUs) at the concentration of 0.5 mg/ml.

Against the fast growers (*M. fortuitum* and *M. smegmatis*), *S. persica* and *E. scarlatina*

were the most active with zero GUs at 0.5 mg/ml. *A. horrida* produced substantial inhibition of *M. Kansasii* (178 GUs) at a concentration of 0.5 mg/ml.

The extracts were found to contain various phytochemical constituents. For instance, *E. scarlatina*, which was the most active against all test microorganisms, had high concentrations of flavonoids and terpenoids (Table 3). It had alkaloids and cardiac glycosides in moderate amounts. Tannins were in trace amounts while saponins were absent.

DISCUSSION

The activity of plant extracts against bacteria have been studied for over a century, but work in this area has intensified in the last 3 decades [11].

Table 3: Preliminary phytochemical screening

Plant specimen	Constituents					
	Alkaloids	Cardiac glycosides	Flavonoids	Saponins	Tannins	Terpenoids
<i>S. persica</i>	+	+	+	-	-	+
<i>A. senegal</i>	-	+	+	++	+	++
<i>A. friesiorum</i>	-	+	++	-	++	-
<i>P. dawei</i>	-	+	++	-	++	-
<i>L. acaciae</i>	+	+	+	+	+++	-
<i>C. sinensis</i>	-	-	+	+	+++	+
<i>A. horrida</i>	+++	++	-	++	++	++
<i>A. anthelmitica</i>	++	-	+	++	+	-
<i>E. scarlatina</i>	++	++	+++	-	+	+++

Key: +++ = Present in high concentration, ++ = Moderately present, + = Trace, - = absent, tests performed singly

The results obtained showed the strong antimycobacterial activity of some of the extracts, namely, those from *S. persica*, *C. sinensis*, *E. scarlatina* and *A. horrida*. The results were comparable to those of the standard drug (Isoniazid). This may be due to the bioactive constituents, such as alkaloids, tannins and flavonoids, present in the extracts [8]. A previous study has shown that the aqueous and methanol extracts of *S. persica* inhibited most bacteria, including *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, and the fungi *Candida albicans* though with varying effectiveness [11]. This was attributed to various chemical constituents of the extracts, such as sodium chloride and potassium chloride, salvadorene, salvadorine, saponins, tannins vitamin C, silica, and resin, as well as cyanogenic or lignan glycosides, alkaloids, terpenoids, and oleic and stearic acids [11]. Phytoalexins which tend to fall into several classes including terpenoids, glycosteroids and alkaloids have been found to exert antibacterial effects in previous studies [12]. They probably contributed to the observed activity of the medicinal plants studied. These findings are in agreement with the findings of Saludes et al [13] where *Morinda citrifolia* Linn. (Rubiaceae) showed antitubercular activity.

The high inhibitory activity of *C. sinensis* extract against the two fast growers used (Zero GUs) unlike in the fast growers which had 986 and 15615 GUs for *M. fortuitum* and *M. smegmatis* respectively at 0.5mg/ml could be due to the fact that fast growers are more susceptible to acidic pH and weak acids than slow growers [14]. For instance, *M. tuberculosis* was found to be less able to maintain its internal pH and membrane potential at acid pH than *M. smegmatis*. Incidentally from the preliminary phytochemistry, *C. sinensis* had tannins which have been known for acidic property [15].

A study carried out by Omwenga et al., [16] indicated that *A. friesiorum*, *A. horrida*, *A. senegal*, and *A. anthelmitica* had strong antimicrobial properties against *S. aureus*, *B. subtilis*, *S. typhi*, *P. aeruginosa* and *E.coli*. The phytochemical results were similar to those from the current study.

CONCLUSION

The present study exhibited various antituberculosis effects of various extracts of the nine medicinal plants used by the Samburu community of Kenya. The inhibitory effects of some extracts justify their medicinal use. The present investigation provides important baseline information for further research. Pharmacological and toxicological studies of the active plants and investigation

of the antimicrobial mechanisms of action are underway and will be soon be reported.

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REFERENCES

1. Maneemegalai S, Naveen T. Evaluation of Antibacterial Activity of Flower Extracts of *Cassia auriculata*. *Ethnobotanical Leaflets* 2010; 14: 8-20.
2. Sathiya M, Muthuchelian K. Phytochemical Investigation and Antibacterial Screening of Ethanolic Leaf Extract of *Sapindus emarginatus* Vahl. *Ethnobotanical Leaflets*, 2008; 12: 891-895.
3. Parekh J, Chanda SV. In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. *Turk J Biol.* 2007; 31: 53-58.
4. Nair R, Kalariya T, Chanda S. Antibacterial Activity of Some Selected Indian Medicinal Flora. *Turk J Biol.* 2005; 29: 41-47.
5. Vaghasiya Y, Chanda SV. Screening of Methanol and Acetone Extracts of Fourteen Indian Medicinal Plants for Antimicrobial Activity. *Turk J Biol.* 2007; 31: 243-248.
6. Zhang Y, Post-Martens K, Denkin S. New drug candidates and therapeutic targets for tuberculosis therapy. *Drug Discovery Today.* 2006; 11: 1-7.
7. Aiyelaagbe OO, Osamudiamen PM. Phytochemical screening for active compounds in *Mangifera indica* leaves from Ibadan, Oyo State. *Plant Sci. Res.*, 2009; 2(1): 11-13.
8. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.* 2005; 4 (7): 685-688.
9. Becton, Dickinson Company. BBL MGIT Mycobacteria growth indicator. Maryland, USA. 2007; 1-23.
10. Obdoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. *Global J. Pure Appl. Sci.* 2001; 8b: 203-208.
11. Al-bayati FA, Sulaiman KD. In Vitro Antimicrobial Activity of *Salvadora persica* L. Extracts against Some Isolated Oral Pathogens in Iraq, *Turk J Biol.* 2008; 32: 57-62.
12. Camacho-Corona MDR, Ramírez-Cabrera MA, González-Santiago O, Garza-González E, Palacios IDP, Luna-Herrera J. Activity against Drug Resistant-Tuberculosis Strains of Plants used in Mexican Traditional Medicine to treat Tuberculosis and Other Respiratory Diseases. *Phytother. Res.* 2008; 22: 82-85.
13. Saludes JP, Garson MJ, Franzblau SG, Aguinaldo AM. Antitubercular Constituents from the Hexane Fraction of *Morinda citrifolia* Linn. (Rubiaceae). *Phytother. Res.* 2002; 16: 683-685.
14. Zhang Z, Zhang H, Sun Z. Susceptibility of *Mycobacterium tuberculosis* to weak acids. *J. Antimicrob. Chemother.* 2003; 52: 56-60.
15. Fontoin F, Saucier C, Teissedre PL, Glories Y. Effect of pH, Ethanol and Acidity on Astringency and Bitterness of Grape Seed Tannin Oligomers in Model Wine Solution. *Food Qual. Prefer.* 2008 ; 19: 286-291.
16. Omwenga EO, Okemo PO, Mbugua PK, Ogol CKP. Ethnobotanical Survey and Antimicrobial Evaluation of Medicinal Plants used by the Samburu Community (Kenya) for treatment of Diarrhoea. *Pharmacognosy Magazine* 2009; 4(18): 165-176.