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Anti-mycobacterial, Antimicrobial and Phytochemical Evaluation of *Pulicaria crispa* and *Scoparia dulcis* Plant Extracts

Adedayo Adebiyi[1,2*], Effa Emmanuel Bassey[3], Rachael Ayo[2], Isaac Bello[2], James Habila[2] and Gali Adamu Ishaku[4]

[1]Chemistry Advanced Research Centre, Sheda Science and Technology Complex (SHESTCO), P.M.B. 186, Garki, Abuja, Nigeria.
[2]Department of Chemistry, Ahmadu Bello University (ABU), P.M.B. Zaria, Nigeria.
[3]Department of Crop Science, Faculty of Agriculture, F&WRM, University of Calabar, P.M.B. 1115, Calabar, Cross River State, Nigeria.
[4]Department of Biotechnology, Modibbo Adama University of Technology, P.M.B. 2076, Yola, Adamawa, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors RA and JH designed the study. Authors AA and EEB collected all data and performed the statistical analysis. Authors AA and GAI did the literature search. Authors AA, EEB, IB and GAI wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To examine the antimicrobial activity and evaluate the anti-mycobacterial potency of *Pulicaria crispa* and *Scoparia dulcis* whole plant extracts in solvents of different polarities (n-hexane, ethyl acetate and methanol.
Study Design: Assessing the anti-microbial and anti-Mycobacterium tuberculosis activity of two Nigerian medicinal plants which have been reported according to folklore for treatment of various

**Corresponding author: E-mail: dayophillips24@gmail.com;*

ailments including respiratory tract infections.
Place and Duration of Study: The experiment was conducted at the Chemistry Department of Ahmadu Bello University, Zaria, Nigeria and at the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria between November 2014 and September 2015.
Methodology: The standard pan sensitive tuberculosis reference strain (H37Rv), eleven bacterial and four fungal clinical isolates were used. Methanol, Ethyl Acetate and Hexane extracts of *Scoparia dulcis* and *Pulicaria crispa* (whole plants) were tested at 0, 20 and 40 µg/ml using the Microplate Alamar Blue Assay. The minimum inhibitory, minimum bactericidal and minimum fungicidal concentrations of each solvent extract were assessed. Phytochemical analysis was also performed.
Results: Phytochemical compounds obtained in the methanol extract fractions where alkaloids, balsams, cardiac glycosides, glycosides, phenols, steroids and tannins in *Scoparia dulcis (Linn),* while extracts of *Pulicaria crispa* showed the presence of alkaloids, balsams, cardiac glycosides, flavonoids, glycosides, phenols, tannins and terpenoids. Saponins were detected in the n-hexane fractions for both plants but only appeared in the semi-polar fractions of *Scoparia dulcis (Linn).* Microplate Alamar Blue Assay (MABA) used for sensitivity study of *Mycobacterium tuberculosis* with 10 µg/ml rifampicin revealed that the n-hexane extract of *Puliaria crispa* and *Scoparia dulcis* gave 48.44±0.75, 12.14±0.02 mm zones of inhibition respectively, whereas the methanol and ethyl acetate extracts gave a 24.10±1.35 mm and 17.00±0.91 zones of inhibition for *Puliaria crispa* and *Scoparia dulcis* respectively in comparison to 33.70±0.64 mm obtained from the control. The minimal inhibitory content (MIC) of the methanol and n-hexane extracts for *Pulicaria crispa* were recorded at 8.01±1.70 and 10.03±1.33, while the MIC values for the ethyl acetate and n-hexane extracts of *Scoparia dulcis* was 12.03±0.86 and 20.40±0.24. The MIC value recorded for rifampicin was 0.38±1.40.
Conclusion: The results obtained suggested that the studied plants possess anti-tuberculosis and selective antimicrobial activities with the major activity tailored to the phyto-constituents.

Keywords: Anti-mycobacterial activity; Pulicaria crispa; Scoparia dulcis; phytochemicals.

1. INTRODUCTION

The world is being ravaged by the spread of infectious diseases whereby the particular onslaught of drug resistant pathogens has diminished that effectiveness of regular anti-microbial treatment options. This has further been aggravated by the occurrence of opportunistic infections and a dwindling therapeutic spectrum alongside previously non-conceived side effects of synthetic drugs [1,2]. One of such infectious diseases also classified as an opportunistic infection in the case of HIV is tuberculosis. Tuberculosis is a bacterial disease caused by the *Mycobacterium africanum, Mycobacterium bovis* and *Mycobacterium tuberculosis*; the latter being the most potent. *Mycobacterium tuberculosis* is acid-fast, aerobic, non-capsulated bacilli with mold-like morphology and waxy cell walls that contain mycolic acid [3]. Their rate of replication is slower than most other bacteria and can stay dormant within their host for long periods of time, thereby requiring lengthy drug treatment regimens; a factor that maybe responsible for multi-drug resistant variants particular when patients fail to adhere to treatment plan. Drug-resistant strains of *M. tuberculosis* also arise from spontaneous chromosomal mutations at a predictable low frequency [4,5].

A report indicated that misuse of anti-TB drugs, such as monotherapy or the addition of single drugs to failing regimens, results in the emergence of resistant mutants. *M. tuberculosis* resistance to antibiotics; Rifampicin, in 40,000 studied tuberculosis cases across Africa was as a result of the multi-drug resistant strains of *Mycobacterium tuberculosis* [6]. Screening for alternative therapies, compounds or formulations with good prospects towards tuberculosis treatment warrants adequate consideration. The unique arsenal of bioactive compounds within certain plants is conceived as a cheaper alternative to synthetic drugs in the treatment of many different bacterial infections, mainly in developing countries [7,8]. Since the therapeutic information for a lot of such plants is based on folklore, there is a need to establish a scientific rationale for their use in treatment by performing *in vivo* and *in vitro* testing.

Studies indicate that extracts of *Alpinia galanga* were active against a pan sensitive strain of

Fig. 1. *Scoparia dulcis* (Linn)

Fig. 2. *Pulicaria crispa*

Mycobacterium tuberculosis [9]. The anti-mycobacterial activity of *Adhatoda vasica*, *Aegle marmelos*, *Tectona grandis* leaves respectively as well as the whole plant of *Solanum trilobatum* have been tested and found to an effective treatment option in other developing countries [10]. Using assaying techniques, other medicinal plants found to possess anti-mycobacterial activity against pan sensitive strains of *Mycobacterium tuberculosis* by selectively inhibiting their growth include *Acalypha indica, Adhatoda vasica, Allium sativum*, *Allium cepa*, *Syzygium aromaticum* and *Cinnamomum verum* [11,12]. In Nigeria, a variety of indigenous plants possesses ethno-medicinal properties and has been utilized in traditional medical practices for the treatment of numerous diseases. However, their application towards the treatment and efficacy against *Mycobacterium tuberculosis* has not been scientifically validated. Two of such whole plants include *Scoparia dulcis (Linn)* known as *roma fada* (Hausa), *aiya* (Igbo), *bibiimbelemo* (Ijaw), *mesenmesèngogoro* (Yoruba), *ndiyang* (Efik), *ungungbuhi* (Gwari) *and Pulicaria crispa* (forssk) known as Bááfúúrè, Bâlbéélàà, or Fárár saura in Hausa, depending on the dialect. Like other parts of the world, these plants are used for treating bronchitis, gastric disorders, hemorrhoids, insect bites, skin wounds, hypertension [13]. However, very limited work has been carried out on both plant species towards establishing its phytochemical and anti-TB fingerprints. In order to increase the probability of discovering new drugs via phyto-medicine, this study was carried out to investigate and estimate two traditional Nigerian medicinal plants; *Scoparia dulcis (Linn)* and *Pulicaria crispa* (forssk) which have been used to treat respiratory infections for its anti-tuberculosis activity by the Microplate Alamar Blue Assay (MABA).

2. MATERIALS AND METHODS

The whole plant *Scoparia dulcis* and *Pulicaria crispa* were collected at Samaru (9℃45'0''N 8℃23'0''E) and Bassawa (11℃4'0''N 7℃42'0''E) regions of Zaria locality in Kaduna state, Nigeria. The plants were identified at the herbarium of Department of Biological Science, Faculty of Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, where voucher specimen numbers 1064 and 2583 were given and samples were deposited. The whole plant in each case were air-dried, powdered with the use of a mortar and pestle and then kept in airtight container until required for further laboratory analysis. Air-dried plant materials were pulverized into powder using a motar and pestle. 1kg each of ground plant samples were exhaustively extracted using maceration extraction method [14]. The marc was extracted successfully using 2.5L each of hexane, ethyl acetate and methanol to 1kg each of plant sample using batch extraction methods. The crude extracts were later concentrated to a minimum volume using rotary evaporator (Büchi Labortechnik AG, Switzerland) at 40℃ and reduced pressure.

Stock solutions of 10 mg/ml of rifampicin were prepared by dissolving 0.1 g in 10 ml of methanol from which different concentrations were obtained and used for the susceptibility tests.

2.1 Phytochemical Screening

Phytochemical screening was carried out on the hexane, ethyl acetate and methanolic extracts for the qualitative determination of major constituents using methods previously described [14,15,16].

2.2 Antimicrobial Screening

All the Media were purchased from Sigma-Aldrich and were prepared in accordance with manufacturer instructions. The bacterial isolates were collected from Medical Microbiology Department of Specialist Teaching Hospital, University of Abuja, F.C.T., Nigeria on a slant Nutrient agar. The isolates were restored on Nutrient broth and confirmed using standard biochemical tests according to the Bergey's manual of Bacteriology [17]. While the collected fungal isolates were identified using fungi chrome test kits in Specialist Teaching Hospital, University of Abuja, F.C.T., Nigeria. The isolates were collected on a Potato dextrose agar slant and restored in Potato dextrose broth. Agar diffusion method was adopted from [18] was employed.

2.3 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined on the test organisms that were sensitive to the extracts and was done by broth dilution method [19]. Mueller Hinton broth was prepared, dispersed into test tubes and the broth was sterilized at 121℃ for 15 smins, the broth was allowed to cool. Normal saline was prepared, 10 mls was dispersed into sterile test tube and the test microbes was inoculated and incubated at 39℃ for 6hrs. Dilution of the test microbes was done in the normal saline until the turbidity marched that of the McFarland's standard scale by visual comparison at this point, this test microbes has a concentration of about $1.5x10^8$ CFU/ml.

Two fold serial dilution of the extract in sterilized broth was made to obtain the concentration of 50.0 µg/ml, 25.0 µg/ml, 12.5 µg/ml, 6.25 µg/ml, and 3.2 µg/ml. The initial concentration was obtained by dissolving 6.0 mg of the extract in 10 mls of sterile broth. Having obtained the different concentration of the extracts in the sterile broth, was observed for turbidity (growth), the lowest concentration of the extract in the broth which shows no turbidity was recorded to the MIC.

2.4 Determination of Minimum Bactericidal and Fungicidal Concentrations

Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC) were evaluated by plating the bacterial suspensions from individual well at the beginning and at the end of the experiments on Mueller Hinton agar medium for estimation of MBC [19]. The culture from MIC well was taken and streaked on the surface of fresh Mueller Hinton agar in a 90-mm plate with division and incubated at 37℃ for 24 hours (bacteria) and 37℃ for 48 hours (fungi) after which the plates of the medium was observed for colony growth, the MBC/MFC were the plates with lowest concentration of the extract without colony growth.

2.5 Microplate Alamar Blue Assay (MABA)

Stock solutions of the individual plant extracts were prepared in 0.05% DMSO (Dimethyl sulfoxide) diluted to the final concentration of 20 and 40 µg/ml in sterile distilled water as part of experimental standardization. The sensitivity of *Mycobacterium tuberculosis* strain to the various extracts was demonstrated by agar diffusion method [20]. This strain; H37Rv, is the most widely used reference strain as it retains its virulent ability following laboratory passaging and is sensitive to all first and second line drugs used in treating mycobacterium tuberculosis such including Isoniazid, Rifampicin, Kanamycin, Capreomicin, Ciprofloxacin, etc. A sterile cork borer of 7 mm diameter was used to bore holes into the inoculums seeded solidified nutrient agar. A 50 µl volume of each 20 and 40 µg/ml of the plant extracts was loaded into the labelled well in the prepared media plate using sterile pipette. The test was performed in triplicates and the plates were kept in a refrigerator for pre-diffusion of the sample and incubated at 37℃ and 48 hours. Growth of *Mycobacterium tuberculosis* was observed after the incubation of 48 hr and the diameter of inhibition zone was measured subtracting the well size.

3. RESULTS AND DISCUSSION

The pharmacological activities of medicinal plants lie with the sundry of phytochemical compounds they possess, thus supporting their ethno-pharmacological use against different infectious diseases by traditional medicine practitioners in developing countries.

Susceptibility to common side effects associated with certain synthetic drugs is rare with use of phyto-medicines. Elucidating the presence of phytochemicals within targeted plants is a scientific first step aimed at unveiling which active compounds or synergy of compounds produce their antimicrobial activities thus making them effective drug candidates in addition to confirming the believe that local plants are the platform for traditional African medicine [21]. Table 1 represents the phytochemical composition from whole plants of *Scoparia dulcis (Linn) and Pulicaria crispa*. The qualitative tests carried out on the extracts showed most activity in the methanolic extract fractions whereby alkaloids, balsams, cardiac glycosides, glycosides, phenols, steroids and tannins in *Scoparia dulcis (Linn).* The methanolic extracts of *Pulicaria crispa* showed the presence of alkaloids, balsams, cardiac glycosides, flavonoids, glycosides, phenols, tannins and terpenoids. Saponins were detected in the n-hexane fractions for both plants but only appeared in the semi-polar fractions of *Scoparia dulcis (Linn).* Phlobatanins and triterpenoids were absent in all extracted fractions of both plants. The presence of these secondary metabolites will enhance its therapeutic potential against several pathogens as well as its physiological activities [22,23]. For instance, plant glycosides and cardiac glycosides possess herpato-stimulatory activity thus making them useful for increasing heart muscles contractions as well as in cancer treatment [24,25]. In tuberculosis therapy, the aminoglycoside antibiotic; streptomycin and kanamycin, are of importance as these in combination with other similar second-line drugs is used for treating tuberculosis, meningitis, pneumonia and brucellosis [26,27]. The presence therefore of both glycosides and cardiac glycosides in the methanol extract of the studied plants gives a good indication that these plants may possess important bioactive compounds of different antibiotic class. Phenols are another very important class of plant secondary metabolites whose antiseptic properties have been exploited towards several antimicrobial activities and some polyhydroxyphenols have been shown to inhibit HIV. A number of antibiotics with strong antifungal potentials are phenolic in nature. This includes amphopterin B, nystatin, natamycin and griseofulvin; the latter is used to treat systemic infections whereby it acts by preventing the infestation of new tissue [28].

It was observed that none of the fractions contain cardenolides, phlobatanins, resins and volatile oil, thereby suggesting a limited antioxidant potential that if present would boost the host immunity against pathology induced free radical generation [29].

The highest recorded anti-mycobacterial activity was observed in the n-hexane extract of *Pulicaria crispa* (40 µg/ml) against H37Rv with a zone of

Table 1. Phytochemical screening of *Scoporia dulcis (Linn) and Pulicaria crispa (Forssk)*

Phyto-constituents	N-hexane extract		Ethyl acetate extract		Methanolic extract	
	Scoporia dulcis (LINN)	***Pulicaria crispa (Forssk)***	***Scoporia dulcis (LINN)***	***Pulicaria crispa (Forssk)***	***Scoporia dulcis (LINN)***	***Pulicaria crispa (Forssk)***
Steroid	-	-	+	+	+	-
Triterpenoid	-	-	-	-	-	-
Glycoside	-	-	-	-	+	+
Saponins	+	+	+	-	-	-
Phenols	-	-	-	-	+	+
Alkaloid	-	-	+	+	+	+
Cardenolides	-	-	-	-	-	-
Terpeniods	-	-	+	-	-	+
Cardiac glycoside	-	-	-	-	+	+
Phlobatanin	-	-	-	-	-	-
Resins	-	-	-	-	-	-
Balsam	-	-	-	-	+	+
Volatile oil	-	-	+	-	-	-
Tannin	+	-	-	+	+	+
Flavonoid	-	-	+	-	-	+

(+) – Present (-) – Absent

inhibition of 48.44 mm. This was followed by the methanol extract (40 µg/ml) of the same plant at 24.10 mm. This response was positive in comparison against the 33.70 mm zone of inhibition control (10 µg/ml Rifampicin). Both the methanol extract of *Scoparia dulcis* and the ethyl acetate extract of *Pulicaria crispa* did not show any activity therefore these particular extracts were not examined further to determine their MIC values. The lowest MIC value among the tested extracts against the H37Rv M. tuberculosis was the methanol extract of *Pulicaria cripsa* (8 µg/ml). This was followed by the n-hexane extract of the same plant with a MIC of 10 µg/ml. The ethyl acetate and n-hexane extracts of *Scoparia dulcis* showed activity against the tested strain of M. *tuberculosis.* The detailed results are shown in Table 2. Rifampicin was active on the pan sensitive strain with the lowest recorded MIC value.

The mean zones of inhibition of different extract against different bacterial and fungal species are summarized in Table 3. Notably, all solvent extracts of *Scoporia dulcis (Linn)* conferred resistance against *Shigella dysenteriae* (34, 32, 30 mm), *Salmonella typhi* (27, 26, 24 mm) and *Escherichia coli* (24, 26, 28 mm), while the extracts of *Pulicaria crispa (Forssk)* were all sensitive. On the other hand, all solvent extracts of *Pulicaria crispa (Forssk)* exhibited different levels of resistance to *Staphylococcus aureus* (29, 32, 30 mm), *Streptococus pyogenes* (27, 29, 24 mm), *Neisseria gonorrheae* (29, 30, 32 mm) and *Candida stellatoidea* (16, 18, 21 mm) while *Scoporia dulcis (Linn)* appeared sensitive. Against *Vancomycin Resistant Enterococci* both plants exhibited good levels of resistance whereby the control bioactive compounds; ciproflaxin (30 µg/ml) and fluconazole (30 µg/ml) were ineffective. With the exception of *Candida albicans*, the data suggests that *Pulicaria crispa* (Forrsk) constituents possess stronger antifungal activities that *Scoparia dulcis* (Linn). For stomach upsets caused by *Shigella dysenteriae* and *Salmonella typhi*, extracts of *Scoparia dulcis* are far more suitable for treatment, thereby confirming earlier reports [30]. Ciproflaxin was used as standard for bacteria, ranging the value of zone of inhibition from 35 to 41 mm, while fluconazole was used as the control antifungal agent with a zone of inhibition ranging from 31 to 37 mm [31].

In the northern parts of Nigeria, traditional plants are consumed not only for forage but also in medicine, particularly in the treatment of dysentery, diarrhoea, stomach upsets, respiratory tract infections, certain viruses and haemorrhoids [32,33]. *Scoparia dulcis* is used mainly for the treatment of diabetes, as a birth control measure, with reports suggesting that it possesses bronchodilating and anti-asthmatic activity hence its use for treating respiratory infections within small communities [34,35]. With an increasing mortality rate each year due to different infectious disease including but not limited to upper respiratory tract infections, bacteremia, diarrhea and dysentery. Phyto-therapeutic agents that show resistance to the causative microbial agents such as *Methicillin Resistant Staphylococcus aureus*, enteric bacteria (*Enterococcus, Pseudomonas, Escherichia coli*, etc) serves as a new frontier towards the treatment of a host of respiratory and digestive infections as the latter is most often the common route to outbreaks in developing countries. Data from Table 3 suggests that extracts of *Pulicaria crispa* proffer greater resistance to disease causing agents for most respiratory tract infections than that of *Scoparia dulcis*. However, solvent extracts of *Scoparia dulcis* gave more favourable results against agents that lead to stomach upsets than *Pulicaria crispa*. One more noticeable difference between the two plants is the degree of resistance that only the extracts of *Pulicaria crispa* confers to *Neisseria gonorrheae* which leads to venereal diseases, thus suggestive other applications of this plant in ethno-medicine. The findings from Tables 2 and 3 compliments the results given in Table 1 whereby other studies have shown that bioactive compounds of flavonoid and tannin origin are both active against *Staphylococcus aureus*, *Escherichia coli* and different strains of mycobacterium [36,37]. Other studies have implicated the compounds obtained from the saponin, terpenoid, flavonoid and tannin fractions as the driving force behind the reported activity against infectious disease causing agents like *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococus pyogenes* as well as their activity against multi drug resistant strains of *M. tuberculosis* [38,39]. The success in treating diarrhoea and stomach upsets, attributed to *Staphylococcus aureus* and *Salmonella spp* infections could be due to the antibacterial effects of alkaloids, polyphenols, saponins and steroids [40]. Distorting the enzyme activity within bacteria is usually observed in the presence of phyto-tannins [32].

Table 2. Result of anti-TB activity of *Scoporia dulcis* and *Pulicaria crispa*

Plant	Fraction	Concentration (µg/ml)	Zone of inhibition	MIC (µg/ml)
Scoporia dulcis (Linn)	Hexane	40	12.14	20.0
		20	8.62	
	Ethyl acetate	40	17.00	12.0
		20	12.56	
	Methanol	40	NR	-
		20	NR	
Pulicaria crispa (Forssk)	Hexane	40	48.44	10.0
		20	15.17	
	Ethyl acetate	40	NR	-
		20	NR	
	Methanol	40	24.10	8.0
		20	20.86	
	Rifampicin (10 µg/ml)		33.70	0.4

NR = No reaction under experimental conditions. The results presented here are the mean values of three independent experiments

Table 3. Antimicrobial activity of *Scoporia dulcis (Linn) and Pulicaria crispa (Forssk) extracts against test organisms* zone of inhibition (mm)

Test microorganisms	SDN (mm)	SDE (mm)	SDM (mm)	PCN (mm)	PCE (mm)	PCM (mm)	Control[1] (mm)	Control[2] (mm)
Methicillin Resistant Staphylococcus aureus	16±0.78	20±0.34	20±0.28	29±0.12	26±0.38	24±0.44	35± 0.10	0
Vancomycin Resistant Enterococci	31±0.44	30±0.46	34±0.34	29±0.48	29±0.81	31±0.87	0	0
Staphylococcus aureus	0	0	0	29±0.98	32±0.92	30±0.69	37±0.60	0
Corynebacterium ulcerans	0	0	0	0	0	0	34±0.42	0
Shigella dysenteriae	34±0.16	32±0.62	30±0.88	0	0	0	40±0.98	0
Salmonella typhi	27±0.23	26±0.32	24±0.74	0	0	0	41±0.21	0
Streptococus pyogenes	0	0	0	27±0.66	29±0.38	24±0.34	35±0.63	0
Neisseria gonorrheae	0	0	0	29±0.71	30±0.74	32±0.72	0	0
Escherichia coli	24±0.41	26±0.86	28±0.36	0	0	0	39±0.85	0
Pseudomonas aeruginosa	26±0.66	27±0.43	27±0.94	32±0.33	32±0.50	30±0.88	0	0
Proteus mirabilis	32±0.44	35±0.60	30±0.54	31±0.45	34±0.40	30±0.63	35 ±0.91	0
Candida albicans	30±0.56	32±0.11	33±0.42	26±0.37	26±0.33	22±0.91	0	35±0.15
Candida krusei	22±0.14	20±0.28	24±0.81	22±0.55	24±0.71	25.0±0.27	0	34±0.26
Candida stellatoidea	0	0	0	16±0.74	18±0.81	21±0.41	0	37±0.42
Candida tropicalis	20±0.32	22±0.92	20±0.70	24±0.40	25.0±0.64	27±0.40	0	31±0.64

SDN = Scoparia dulcis n-Hexane extract, SDE = Scoparia dulcis Ethyl acetate extract, SDM = Scoparia dulcis methanol extract, PCN = Pulicaria crispa n-Hexane extract, PCE = Pulicaria crispa Ethyl acetate extract, PCM = Pulicaria crispa methanol extract, Control[1]= Ciproflaxin (30 µg/ml), Control[2]= Fluconazole (30 µg/ml)

Table 4. Minimum inhibitory, bacterial and fungal concentrations (MIC, MBC, MFC) of *Scoporia dulcis* (LINN) and *Pulicaria crispa (Forssk)* (mg/ml ± SD)

Test microorganisms	*Scoparia dulcis*									*Pulicaria crispa*								
	n-H (µg/ml)			E.A (µg/ml)			Me (µg/ml)			n-H (µg/ml)			E.A (µg/ml)			Me (µg/ml)		
	MIC*	MBC	MFC	MIC*	MBC	MFC	MIC*	MBC	MFC	MIC*	MBC	MFC	MIC*	MBC	MFC	MIC*	MBC	MFC
Methicillin Resistant Staphylococcus aureus	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-
Vancomycin Resistant Enterococci	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	12.5	12.5	-	12.5	25.0	-	12.5	25.0	-
Staphylococcus aureus	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	3.12	25.0	-	3.12	12.5	-	12.5	25.0	-
Corynebacterium ulcerans	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	12.5	25.0	-
Shigella dysenteriae	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	12.5	25.0	-
Salmonella typhi	3.12	25.0	-	3.12	25.0	-	3.12	25.0	-	3.12	12.5	-	3.12	12.5	-	12.5	25.0	-
Streptococus pyogenes	3.12	25.0	-	3.12	25.0	-	3.12	25.0	-	3.12	12.5	-	3.12	12.5	-	3.12	25.0	-
Neisseria gonorrheae	3.12	25.0	-	3.12	25.0	-	3.12	25.0	-	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-
Escherichia coli	3.12	25.0	-	3.12	25.0	-	3.12	25.0	-	12.5	12.5	-	12.5	25.0	-	12.5	12.5	-
Pseudomonas aeruginosa	12.5	25.0	-	12.5	25.0	-	12.5	25.0	-	12.5	25.0	-	12.5	25.0	-	12.5	25.0	-
Proteus mirabilis	12.5	25.0	-	12.5	25.0	-	12.5	25.0	-	3.12	25.0	-	3.12	12.5	-	3.12	25.0	-
Candida albicans	12.5	-	25.0	12.5	-	25.0	12.5	-	25.0	3.12	-	12.5	3.12	-	12.5	3.12	-	12.5
Candida krusei	3.12	-	12.5	3.12	-	12.5	3.12	-	12.5	3.12	-	12.5	3.12	-	12.5	3.12	-	12.5
Candida stellatoidea	12.5	-	25.0	12.5	-	25.0	12.5	-	25.0	12.5		25.0	12.5	-	25.0	12.5	-	25.0
Candida tropicalis	12.5	-	25.0	12.5	-	25.0	12.5	-	25.0	3.12	-	12.5	3.12	-	12.5	3.12	-	12.5

*KEY: SDN = Scoparia dulcis n-Hexane extract, SDE = Scoparia dulcis Ethyl acetate extract, SDM = Scoparia dulcis methanol extract, PCN = Pulicaria crispa n-Hexane extract, PCE = Pulicaria crispa Ethyl acetate extract, PCM = Pulicaria crispa methanol extract, * control results for MIC test Rimfamicin (10µg/ml) 0.38±1.40*

Table 4 data shows the MIC, MBC and MFC of the different solvent extracts of *Scoporia dulcis* (Linn) and *Pulicaria crispa* (Forrsk). The data indicated that polar and nonpolar extracts of *G. senegalensis* were most active against *Staphylococcus aureus*, with MIC and MBC values of 3.12 and 12.5 mg/ml, respectively. *Candida albicans* and *Candida tropicalis* showed high resistance, with 12.5 and 25.0 mg/ml of MIC and MBC, respectively against the all three extracts.

In general the accumulation and concentration of secondary metabolites are responsible for antibacterial activity and varies according to the plant extracts base on their polarity [41]. The sensitivity of *E. coli*, *S. aureus* and *S. dysenteriae* to some of the extracts implies that chemical compounds in the extracts can be further developed to fight against this microorganism and the use of the plant for the treatment of diarrhea, stomach pain and skin itching is justified since this bacteria are responsible for such illness [42,43]. The extracts also showed activities against *S. dysenteriae*, the bacteria responsible for bacillary dysentery [43]. Therefore all the extracts could serve as source of compounds that may be effective in the management of the ailments.

4. CONCLUSION

This study serves to authenticate the indigenous customs in developing countries in addition to contributing further depths to the growing literature on plant materials recognized as a reservoir of important novel anti-tuberculosis compounds. Furthermore, the awareness of the therapeutic effect of these plants in the treatment of tuberculosis seems to be well known in some Northern Nigerian cultures. The findings in this study have hence provided scientific support for the ethnomedical anti–TB activity of extracts of the whole plant of *Scoparia dulcis* and *Pulicaria crispa*, where the reactive solvent fractions were found to have a significantly adequate MIC values when compared to the other extracts using the standard drug (Rifampicin). The phyto-chemistry of the plant shows that the extracts contain steroids, saponin, alkaloid, terpenoids and tannin. Hence the constituent with the anti-TB activity can be drawn down to be from the above phyto-constituents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Huie CW. Review: Modern sample preparation techniques for the extraction and analysis of medicinal plants. Journal of Analytical and Bio-analytical Chemistry. 2002;373:23-30.
2. Doughart JH, Okafor B. Anti-microbial activity of *Senna alata (Linn)*. East and Central African Journal of Pharmaceutical Sciences. 2007;10:17-21.
3. Glickman MS, Jacobs WR. Microbial pathogenesis review of *Mycobacterium tuberculosis*: Dawn of a discipline. Cell Press. 2001;104:477–485.
4. Mariita RM, Ogol CKP, Oguge NO, Okemo PO. Anti-tubercular and phytochemical investigation of methanol extracts of medicinal plants used by the Samburu community in Kenya. Tropical Journal of Pharmaceutical Research. 2010;9:379-385.
5. Lawn SD, Zumla AI. Tuberculosis. Lancet. 2011;378:57-72.
6. Zignol M, Hosseini MS, Wright A, Lambregts C, Weezenbeek V, Nunn P, Watt CJ, Williams BG, Dye C. Global incidence of multidrug-resistant tuberculosis. Journal of Infectious Diseases. 2006;194:479-485.
7. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life Science Journal. 2005;78:431-441.
8. Gautam R, Saklani A, Jachak SM. Indian medicinal plants as a source of anti-mycobacterial agents. Journal of Ethnopharmacology. 2007;110:200-234.
9. Phongpaichit S, Vuddhakul V, Subhadhirasaku S, Wattanapiromsakul C. Evaluation of the anti-mycobacterial activity of extracts from plants used as self-medication by AIDS patients in Thailand. Journal of Pharmaceutical Biology. 2006; 44:71-75.
10. Purushotham KG, Arun P, Johnsy JJ, Vasantha KR. Screening of four Indian medicinal plants for *in vitro* anti-mycobacterial activity. The Bioscan. 2010; 5:103.

11. Sivakumar A, Jayaraman G. Anti-tuberculosis activity of commonly used medicinal plants of south India. Journal of Medicinal Plant Research. 2011;5:6881-6884.
12. Renu G, Bandana T, Pushpendra S, Singh HB, Sharma HD, Katoch VM, Chauhan SVS. Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates. Indian Journal of Medicinal Plant Research. 2012;131:809-813.
13. Latha M, Pari L, Sitasawad S, Bhonde R. Insulin secretagogue activity and cytoprotective role of the traditional antidiabetic plant *Scoparia dulcis*. Life Science Journal. 2004;75:2003-2014.
14. Harborne JB. Phytochemical methods: A guide to modern techniques in plants analysis. 2nd edition: Chapman and Hall/London; 1984.
15. Sofowora A. Medicinal plants and Traditional Medicine in Africa. Spectrums Books Limited: Ibadan Nigeria; 1993.
16. Trease G, Evans W. A textbook of pharmacognosy, 5th edition: Elsevier Ltd, Edinburgh; 2002.
17. Cheesbrough M. Reaction isolates on tropical diseases: The effects. Cambridge University press, London; 2002.
18. European Committee on Antimicrobial Susceptibility Testing (EUCAST), 2012;2: 12.
19. Suffredini IB, Sander HS, Goncalves AG, Reis AO, Gales AC, Varella AD, Younes RN. Screening of anti-bacterial extracts from plants native to brazilian amazon rain forest and atlantic forest. Brazilian Journal of Medical and Biological research. 2004; 37:379-384.
20. El-Mahmood MA. Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae and Pseudomonas aeruginosa.* Journal of Medicinal Plant Research. 2009;3:179-185.
21. Mann A, Banso A, Clifford LC. An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. Tanzanian Journal of Health Research. 2008;10:34-38.
22. Hassan MA, Oyewale AO, Amupitan JO, Abdullahi MS, Okonwo EM. Preliminary phytochemical and antimicrobial investigation of crude extract of root bark of *Deteriummi crocarpum. Nigerian* Journal of Chemical Science. 2004;29(1):36-49.
23. Usman H, Abdulrahman FK, Ladan AA. Phytochemical and Antimicrobial Evaluation of *Tributus.* L. *(Zygophylaceae*) Growing in Nigeria. Research Journal of Biological Sciences. 2007;2(3):244-247.
24. Afaq F, Saleem M, Aziz MH, Mukhtar H. Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-induced tumor promotion markers in CD-1 mouse skin by oleandrin.. Toxicology and Applied Pharmacology. 2004;195(3):361-369.
25. Newman RA, Yang P, Hittelman WN, Lu T, Ho DH, Ni D, Chan D, Vijeswarapu M, Cartwright C, Dixon S, Felix E, Addington C. Oleandrin-mediated oxidative stress in human melanoma cells. Journal of Experimental Therapeutics and Oncology. 2006;5(3):167-181.
26. Lawn SD, Wilkinson R. Extensively drug resistant tuberculosis. British Medical Journal. 2006;333(7568):559–560.
DOI: 10.1136/bmj.38971.587222.AB
27. Furin JJ. The clinical management of drug-resistant tuberculosis. Current opinion in pulmonary medicine. 2007;13(3):212-217.
DOI: 10.1097/MCP.0b013e3280f3c0b2
28. Thomas G. Medicinal chemistry: An Introduction. 2nd edition: Wiley, England; 2007.
29. Abdel-Hamid SG, Bakht MA, Yar MS, Al-Qasoumi SI, Samad A. Molecular properties prediction, synthesis and antimicrobial activity of some newer oxadiazole derivatives. European Journal of Medicinal Chemistry. 2010;45(12): 5862-5869.
DOI: 10.1016/j.ejmech.2010.07.069
30. Murti K, Panchal M, Taya P, Singh R. Pharmacological Properties of Scoparia Dulcis: A Review. Pharmacologia. 2012;3: 344-347.
DOI: 10.5567/pharmacologia.2012.344.347
31. Bauer AN, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized singly disc method. American Journal of Clinical Pathology. 1996;45:493-496.
32. Sallau AB, Njoki GC, Olokisi AR, Wurochekke AU, Abdukadir AA, Isah S, Abubakar MS, Ibrahim S. Effects of *Guiera senegalinsis* leaf extracts on some *Echis carinatus* venom enzymes. Journal of Medicinal Science. 2005;5:2880-2883.

33. Williams ET, Barminas JT, Akinniyi J, William A. Anti-diarrhoeal effects of the root extracts of *Guiera senegalensis* in male mice. African Journal of Pure and Applied Chemistry. 2009;3:152-157.
34. Yamamoto G. Therapeutic potential of inhibition of the NF - KB pathway in the treatment of inflammation and cancer. Journal of Clinical Investigation. 2005;135.
35. Simone Fulda. Betulinic acid for cancer treatment and prevention. International Journal of Molecular Sciences. 2008; 1097.
36. Newton SM, Lau C, Gurch SS, Besra GS, Wright CW. The evaluation of forty-three plant species for *in vitro* anti-mycobacterial activities; isolation of active constituents from *Psoralea corylifolia* and *Sanguinaria canadensis*. Journal of Ethno-pharmacology. 2002;79:57–67.
37. Dahiya P, Purkayastha S. Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. Indian Journal of Pharmaceutical Science. 2012;74:443-450.
38. Esquenazi D, Wigg MD, Miranda MMFS, Rodrigues HM, Tostes JBF, Rozental S, da Silva AJ, Alviano CS. Anti-microbial and antiviral activities of polyphenolics from *Cocos nucifera Linn.* (Palmae) husk fiber extract. Research in Microbiology. 2002; 153(10):647–652.
39. Arunkumar S, Muthuselvam M. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera L.* against clinical pathogens. World Journal of Agricultural Science. 2009;5(5):572–576.
40. Vieira RHSF, Rodrigues DP, Goncalves FA, Menezes FGR, Aragao JS, Sousa OU. Microbial effect of medicinal plant extracts (*Psdium guajava linn and Carica papaya linn)* upon bacteria isolated form fish muscle and known to induce diarrhoea in children. Revista Deinstitute de Medecina Tropical de Sao Paulo. 2001;43:145-148.
41. Sakagami Y, Kajimura K, Wijesinghe WMNM. Antibacterial activity of calozeyloxanthone isolated from *Calophyllum* species against vancomycin-resistant *Enterococci* (VRE) and synergism with antibiotics. Planta Med. 2002;68(6): 541-543.
42. Ramanathan R, Baby R, Bhuvaneswarri R, Dhandapani R. Antimicrobial activities of *Canthium parviflorum* lam. and *Pergularia daemia* (Forsk) Chiov. International Journal of Comprehensive Pharmacy. 2013;4(9):205-209.
43. Uttu AJ, Sallau MS, Hamisu I, Mubarak BD, Abdullahi YI. Phytochemical and antimicrobial screening of stem bark extracts from *Glossonema boveanum* (Decne). Journal of Pharmacognosy and Phytochemistry. 2015;4(2):86-88.

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