

1 **Original Research Article**
2 **Anti-mycobacterial, antimicrobial and**
3 **phytochemical evaluation of *Pulicaria crispa* and**
4 ***Scoparia dulcis* plant extracts**

5 **ABSTRACT**
6

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 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 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 40µg/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 10µg/ml rifampicin revealed that the n-hexane extract of *Pulicaria 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 *Pulicaria 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.

7 **Keywords:** Anti-mycobacterial activity, *Pulicaria crispa*, *Scoparia dulcis*, phytochemicals.

8 **1. INTRODUCTION**

9 The world is being ravaged by the spread of infectious diseases whereby the particular onslaught of
10 drug resistant pathogens has diminished that effectiveness of regular anti-microbial treatment options.
11 This has further been aggravated by the occurrence of opportunistic infections and a dwindling
12 therapeutic spectrum alongside previously non-conceived side effects of synthetic drugs [1, 2]. One of
13 such infectious diseases also classified as an opportunistic infection in the case of HIV is tuberculosis.
14 Tuberculosis is a bacterial disease caused by the *Mycobacterium africanum*, *Mycobacterium bovis*
15 and *Mycobacterium tuberculosis*; the latter being the most potent. *Mycobacterium tuberculosis* is acid-
16 fast, aerobic, non-capsulated bacilli with mold-like morphology and waxy cell walls that contain
17 mycolic acid [3]. Their rate of replication is slower than most other bacteria and can stay dormant
18 within their host for long periods of time, thereby requiring lengthy drug treatment regimens; a factor
19 that maybe responsible for multi-drug resistant variants particular when patients fail to adhere to
20 treatment plan. Drug-resistant strains of *M. tuberculosis* also arise from spontaneous chromosomal
21 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 virulent strain of *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 virulent 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), *bibiimbelema* (Ijaw), *mesenmesengogoro* (Yoruba), *ndiyang* (Efik), *ungungbuh* (Gwari) and *Pulicaria crispa* (forssk) known as Bááfuúré, Bálbéélàà, 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).



Figure 1 *Scoparia dulcis* (Linn)



Figure 2 *Pulicaria crispa*

2. MATERIALS AND METHODS

The whole plant *Scoparia dulcis* and *Pulicaria crispa* were collected at Samaru and Basawa 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 a voucher specimen has been 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 mortar and pestle. 1kg each of ground plant samples were exhaustively extracted using maceration extraction method [14]. The marc

was extracted successfully using hexane, ethyl acetate and methanol using batch extraction methods. The crude extracts were later concentrated using rotary evaporator.

Stock solutions of 5.0mg/ml of rifampicin were prepared by dissolving 0.1g in 10ml of methanol.

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 [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°C for 15mins, the broth was allowed to cool. Normal saline was prepared, 10mls was dispersed into sterile test tube and the test microbes was inoculated and incubated at 39°C for 6hrs. Dilution of the test microbes was done in the normal saline until the turbidity matched that of the McFarland's standard scale by visual comparison at this point, this test microbes has a concentration of about 1.5×10^8 CFU/ml.

Two fold serial dilution of the extract in sterilized broth was made to obtain the concentration of 50.0µg/ml, 25.0µg/ml, 12.5µg/ml, 6.25µg/ml, and 3.2µg/ml. The initial concentration was obtained by dissolving 6.0mg of the extract in 10mls 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°C for 24 hours (bacteria) and 37°C 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]. A sterile cork borer of 7mm diameter was used to bore holes into the inoculum seeded solidified nutrient agar. A 50µl volume of each 20 and 40µg/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°C and 48 hours. Growth of *Mycobacterium tuberculosis* was observed

after the incubation of 48hr 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* (40µg/ml) against H37Rv with a zone of inhibition of 48.44mm. This was followed by the methanol extract (40µg/ml) of the same plant at 24.10mm. This response was positive in comparison against the 33.70mm zone of inhibition control (10µg/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 crispa* (8µg/ml). This was followed by the n-hexane extract of the same plant with a MIC of 10µg/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 virulent 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 *Scoparia dulcis* (Linn) conferred resistance against *Shigella dysenteriae* (34, 32, 30mm), *Salmonella typhi* (27, 26, 24mm) and *Escherichia coli* (24, 26, 28mm), 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, 30mm), *Streptococcus pyogenes* (27, 29, 24mm), *Neisseria gonorrhea* (29, 30, 32 mm) and *Candida stellatoidea* (16, 18, 21mm) while *Scoparia dulcis* (Linn) appeared sensitive. Against *Vancomycin Resistant Enterococci* both plants exhibited good levels of resistance whereby the control bioactive compounds; ciproflaxin (30µg/ml) and fluconazole (30µg/ml) were ineffective. With the exception of *Candida albicans*, the data suggests that *Pulicaria crispa* (Forssk) 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

171 suitable for treatment, thereby confirming earlier reports [30]. Ciproflaxin was used as standard for
 172 bacteria, ranging the value of zone of inhibition from 35 to 41 mm, while fluconazole was used as the
 173 control antifungal agent with a zone of inhibition ranging from 31 to 37 mm [31].
 174 In the northern parts of Nigeria, traditional plants are consumed not only for forage but also in
 175 medicine, particularly in the treatment of dysentery, diarrhoea, stomach upsets, respiratory tract
 176 infections, certain viruses and haemorrhoids [32, 33]. *Scoparia dulcis* is used mainly for the
 177 treatment of diabetes, as a birth control measure, with reports suggesting that it possesses
 178 bronchodilating and anti-asthmatic activity hence its use for treating respiratory infections within
 179 small communities [34, 35]. With an increasing mortality rate each year due to different infectious
 180 disease including but not limited to upper respiratory tract infections, bacteremia, diarrhea and
 181 dysentery. Phyto-therapeutic agents that show resistance to the causative microbial agents such as
 182 *Methicillin Resistant Staphylococcus aureus*, enteric bacteria (*Enterococcus*, *Pseudomonas*,
 183 *Escherichia coli*, etc) serves as a new frontier towards the treatment of a host of respiratory and
 184 digestive infections as the latter is most often the common route to outbreaks in developing
 185 countries. Data from table 3 suggests that extracts of *Pulicaria crispa* proffer greater resistance to
 186 disease causing agents for most respiratory tract infections than that of *Scoparia dulcis*. However,
 187 solvent extracts of *Scoparia dulcis* gave more favourable results against agents that lead to stomach
 188 upsets than *Pulicaria crispa*. One more noticeable difference between the two plants is the degree of
 189 resistance that only the extracts of *Pulicaria crispa* confers to *Neisseria gonorrhea* which leads to
 190 venereal diseases, thus suggestive other applications of this plant in ethno-medicine. The findings
 191 from tables 2 and 3 compliments the results given in table 1 whereby other studies have shown that
 192 bioactive compounds of flavonoid and tannin origin are both active against *Staphylococcus aureus*,
 193 *Escherichia coli* and different strains of mycobacterium [36, 37]. Other studies have implicated the
 194 compounds obtained from the saponin, terpenoid, flavonoid and tannin fractions as the driving force
 195 behind the reported activity against infectious disease causing agents like *Staphylococcus aureus*,
 196 *Pseudomonas aeruginosa*, *Streptococcus pyogenes* as well as their activity against multi drug
 197 resistant strains of *M. tuberculosis* [38, 39]. The success in treating diarrhoea and stomach upsets,
 198 attributed to *Staphylococcus aureus* and *Salmonella spp* infections could be due to the antibacterial
 199 effects of alkaloids, polyphenols, saponins and steroids [40]. Distorting the enzyme activity within
 200 bacteria is usually observed in the presence of phyto-tannins [32].
 201 Table 4 data shows the MIC, MBC and MFC of the different solvent extracts of *Scoparia dulcis* (Linn)
 202 and *Pulicaria crispa* (Forssk). The data indicated that polar and nonpolar extracts of *G. senegalensis*
 203 were most active against *Staphylococcus aureus*, with MIC and MBC values of 3.12 and 12.5 mg/ml,
 204 respectively. *Candida albicans* and *Candida tropicalis* showed high resistance, with 12.5 and 25.0
 205 mg/ml of MIC and MBC, respectively against the all three extracts.
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207 **Table 1: Phytochemical screening of *Scoparia dulcis* (LINN) and *Pulicaria crispa* (Forssk)**

Phyto-constituents	N-hexane extract		Ethyl acetate extract		Methanolic extract	
	<i>Scoparia dulcis</i> (LINN)	<i>Pulicaria crispa</i> (Forssk)	<i>Scoparia dulcis</i> (LINN)	<i>Pulicaria crispa</i> (Forssk)	<i>Scoparia dulcis</i> (LINN)	<i>Pulicaria crispa</i> (Forssk)
Steroid	-	-	+	+	+	-
Triterpenoid	-	-	-	-	-	-
Glycoside	-	-	-	-	+	+
Saponins	+	+	+	-	-	-
Phenols	-	-	-	-	+	+
Alkaloid	-	-	+	+	+	+
Cardenolides	-	-	-	-	-	-
Terpenoids	-	-	+	-	-	+
Cardiac glycoside	-	-	-	-	+	+
Phlobatanin	-	-	-	-	-	-

Resins	-	-	-	-	-	-
Balsam	-	-	-	-	+	+
Volatile oil	-	-	+	-	-	-
Tannin	+	-	-	+	+	+
Flavonoid	-	-	+	-	-	+

(+) – Present (-) – Absent

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210 **Table 2: Result of Anti-TB Activity of *Scoporia dulcis* and *Pulicaria crispa***

Plant	Fraction	Concentration (µg/ml)	Zone of Inhibition	MIC(µg/ml)
<i>Scoporia dulcis</i> (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	
<i>Pulicaria crispa</i> (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

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

213 **Table 3. Antimicrobial activity of *Scoparia 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)
<i>Methicillin Resistant Staphylococcus aureus</i>	16 ±0.78	20 ±0.34	20 ±0.28	29 ±0.12	26 ±0.38	24 ±0.44	35 ±0.10	0
<i>Vancomycin Resistant Enterococci</i>	31 ±0.44	30 ±0.46	34 ±0.34	29 ±0.48	29 ±0.81	31 ±0.87	0	0
<i>Staphylococcus aureus</i>	0	0	0	29 ±0.98	32 ±0.92	30 ±0.69	37 ±0.60	0
<i>Corynebacterium ulcerans</i>	0	0	0	0	0	0	34 ±0.42	0
<i>Shigella dysenteriae</i>	34 ±0.16	32 ±0.62	30 ±0.88	0	0	0	40 ±0.98	0
<i>Salmonella typhi</i>	27 ±0.23	26 ±0.32	24 ±0.74	0	0	0	41 ±0.21	0
<i>Streptococcus pyogenes</i>	0	0	0	27 ±0.66	29 ±0.38	24 ±0.34	35 ±0.63	0
<i>Neisseria gonorrhoea</i>	0	0	0	29 ±0.71	30 ±0.74	32 ±0.72	0	0
<i>Escherichia coli</i>	24 ±0.41	26 ±0.86	28 ±0.36	0	0	0	39 ±0.85	0
<i>Pseudomonas aeruginosa</i>	26 ±0.66	27 ±0.43	27 ±0.94	32 ±0.33	32 ±0.50	30 ±0.88	0	0
<i>Proteus mirabilis</i>	32 ±0.44	35 ±0.60	30 ±0.54	31 ±0.45	34 ±0.40	30 ±0.63	35 ±0.91	0
<i>Candida albicans</i>	30 ±0.56	32 ±0.11	33 ±0.42	26 ±0.37	26 ±0.33	22 ±0.91	0	35 ±0.15
<i>Candida krusei</i>	22 ±0.14	20 ±0.28	24 ±0.81	22 ±0.55	24 ±0.71	25.0 ±0.27	0	34 ±0.26
<i>Candida stellatoidea</i>	0	0	0	16 ±0.74	18 ±0.81	21 ±0.41	0	37 ±0.42
<i>Candida tropicalis</i>	20 ±0.32	22 ±0.92	20 ±0.70	24 ±0.40	25.0 ±0.64	27 ±0.40	0	31 ±0.64

214 SDN = *Scoparia dulcis* n-Hexane extract, SDE = *Scoparia dulcis* Ethyl acetate extract, SDM = *Scoparia dulcis* methanol extract,
215 PCN = *Pulicaria crispa* n-Hexane extract, PCE = *Pulicaria crispa* Ethyl acetate extract, PCM = *Pulicaria crispa* methanol extract,
216 Control¹= Ciproflaxin (30 µg/ml), Control²= Fluconazole (30 µg/ml)

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

Test Microorganisms	<i>Scoparia dulcis</i>									<i>Pulicaria crispa</i>								
	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 <i>Staphylococcus aureus</i>	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 <i>Enterococci</i>	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	12.5	12.5	-	12.5	25.0	-	12.5	25.0	-
<i>Staphylococcus aureus</i>	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	3.12	25.0	-	3.12	12.5	-	12.5	25.0	-
<i>Corynebacterium ulcerans</i>	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	12.5	25.0	-
<i>Shigella dysenteriae</i>	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-	12.5	25.0	-
<i>Salmonella typhi</i>	3.12	25.0	-	3.12	25.0	-	3.12	25.0	-	3.12	12.5	-	3.12	12.5	-	12.5	25.0	-
<i>Streptococcus pyogenes</i>	3.12	25.0	-	3.12	25.0	-	3.12	25.0	-	3.12	12.5	-	3.12	12.5	-	3.12	25.0	-
<i>Neisseria gonorrhea</i>	3.12	25.0	-	3.12	25.0	-	3.12	25.0	-	3.12	12.5	-	3.12	12.5	-	3.12	12.5	-
<i>Escherichia coli</i>	3.12	25.0	-	3.12	25.0	-	3.12	25.0	-	12.5	12.5	-	12.5	25.0	-	12.5	12.5	-
<i>Pseudomonas aeruginosa</i>	12.5	25.0	-	12.5	25.0	-	12.5	25.0	-	12.5	25.0	-	12.5	25.0	-	12.5	25.0	-
<i>Proteus mirabilis</i>	12.5	25.0	-	12.5	25.0	-	12.5	25.0	-	3.12	25.0	-	3.12	12.5	-	3.12	25.0	-
<i>Candida albicans</i>	12.5	-	25.0	12.5	-	25.0	12.5	-	25.0	3.12	-	12.5	3.12	-	12.5	3.12	-	12.5
<i>Candida krusei</i>	3.12	-	12.5	3.12	-	12.5	3.12	-	12.5	3.12	-	12.5	3.12	-	12.5	3.12	-	12.5
<i>Candida stellatoidea</i>	12.5	-	25.0	12.5	-	25.0	12.5	-	25.0	12.5		25.0	12.5	-	25.0	12.5	-	25.0
<i>Candida tropicalis</i>	12.5	-	25.0	12.5	-	25.0	12.5	-	25.0	3.12	-	12.5	3.12	-	12.5	3.12	-	12.5

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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 Rimfamycin (10µg/ml) 0.38 ±1.40

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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.

5. 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 (WHERE EVER APPLICABLE)

It is not applicable.

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