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Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders

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Abstract

Hydroalcoholic extracts of eight species of medicinal plants, namely, *Acokanthera schimperi* (Apocynaceae), *Calpurnia aurea* (Leguminosae), *Kalanchoe petitiana* (Crassulaceae), *Lippia adoensis* (Verbenaceae), *Malva parviflora* (Malvaceae), *Olinia rochetiana* (Oliniaceae), *Phytolacca dodecandra* (Phytolaccaceae) and *Verbascum sinaiticum* (Scrophulariaceae), traditionally used in the treatment of various skin disorders were screened for antimicrobial activity against different strains of bacteria and fungi which are known to cause different types of skin infections. The tests were carried out using agar well diffusion method at three concentration levels (100, 50 and 25 mg/ml) of the crude extracts. The MICs of the crude extracts of *Lippia adoensis* and *Olinia rochetiana* were determined by agar dilution method. Furthermore, the powdered leaves of *Lippia adoensis* and *Olinia rochetiana* were fractionated into different solvents of wide ranging polarity and the resulting fractions were screened for antimicrobial activity against the same organisms. Of all the plants tested, *Lippia adoensis* and *Olinia rochetiana* were found to be the most active species against bacterial and fungal strains, respectively. In addition, almost all species of plants were found to have activity on at least one microbial strain. The antimicrobial activity profile also showed that *Staphylococcus aureus* and *Trichophyton mentagrophytes* were the most susceptible bacterial and fungal strains, respectively. The results indicate the potential of these herbal drugs in treating microbial infections of the skin, thus, justifying their claimed uses in the treatment of various skin disorders, the majority of which are of infectious origin.

Keywords: Medicinal plants; Crude extracts; Antimicrobial activities; Minimum inhibitory concentration; Skin disorders

1. Introduction

Medicinal plants have been used as sources of medicine in virtually all cultures (Baquar, 1995). During the last decade, the use of traditional medicine (TM) has expanded globally and is gaining popularity. It has continued to be used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system (Lanfranco, 1999). According to WHO, herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries (WHO, 2001). In Ethiopia, traditional remedies represent not only part of the struggle of the people to fulfill their essential drug needs but also they are integral components of the cultural beliefs and attitudes (Abebe, 1996).

The Ethiopian flora is estimated to contain between 6500 and 7000 species of higher plants of which about 12% are endemic. Ethiopia is also a home for many languages, cultures and beliefs that have in turn contributed to the high diversity of traditional knowledge and practice of the people, which, among others include the use of medicinal plants. More than 95% of traditional preparations in the country are of plant origin (Gidey, 2001; Demissew and Dagne, 2001). Some of the common uses of the medicinal plants sold in markets include fumigation, vermifuge, pain relief and treating skin infections. Antimicrobial and wound healing plants

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are among some of the major medicinal plants that are commonly available in markets (Dagne, 1996). Despite its significant contribution to society, TM has received very little attention in modern research and development and less effort has been paid to upgrade the traditional health practices in the country. But, the long history of use of medicinal plants in Ethiopia and its huge biotic riches can be of paramount importance in future research and drug discovery.

In this study, eight species of plants namely *Acokanthera schimperi* (Apocynaceae), *Calpurnia aurea* (Leguminosae), *Kalanchoe petitiana* (Crassulaceae), *Lippia adoensis* (Verbenaceae), *Malva parviflora* (Malvaceae), *Olinia rochetiana* (Oliniaceae), *Phytolacca dodecandra* (Phytolaccaceae) and *Verbascum sinaiticum* (Scrophulariaceae), having traditional claims for the treatment of various skin disorders were investigated for their antimicrobial activities on bacterial and fungal strains, which are known to be common pathogens of the skin. The ethnobotanical data on the traditional uses of these plant species (Table 1) and selection of the plant parts to be tested were complemented with literature review and information from traditional healers.

2. Materials and methods

2.1. Chemicals and drugs

Methanol (LOBA CHMIE Pvt. Ltd., India), acetone (Fisher Scientific International Company, UK), chloroform (E. Merk, Stockholm) and petroleum ether (80–100 °C), were obtained from School of Pharmacy, Addis Ababa University. Gentamycin sulphate and ketoconazole (working standards) were kindly supplied by the Department of Quality Control, Drug Administration and Control Authority (DACA), Addis Ababa.

2.2. Media

Nutrient agar (DEFCO Laboratories, USA), peptone bacteriological (BDH Chemicals Ltd., England), tryptone soya broth and Sabauraud dextrose agar (both from OXOID Ltd., England), yeast extract (UNIPATH Ltd., England) and sodium chloride (East Anglia Chemicals, UK) were all obtained from the Department of Microbiol-

Table 1
Botanical names, voucher specimen numbers, plant part extracted, local names and traditional medicinal uses of the plant species studied

Genus-species (family)	Voucher number	Part extracted	Local name	Traditional medicinal uses(s)
Acokanthera schimperi (Apocynaceae)	HT05	Leaf	Mrenz	For the treatment of headache, epilepsy, amnesia, eye disease, syphilis, rheumatic pain, elephantiasis, scabies, leprosy, Tinea capitis, wound, eczema, warts and swelling (Abebe and Ayehu, 1993)
Calpurnia aurea (Leguminosae)	HT04	Leaf	Digita	For the treatment of amoebic dysentery and diarrhoea in animals, killing head lice in humans and ticks in animals, syphilis, diarrhoea, leishmaniasis, tapeworm, trachoma, Tinea capitis, wound, scabies, elephantiasis and different swellings (Asres, 1986; Abebe and Ayehu, 1993; Fullas, 2001)
Kalanchoe petitiana (Crassulaceae)	HTO2	Leaf	Endohahila	To expel tapeworm, and to treat trachoma, syphilis and different swellings (Abebe and Ayehu, 1993)
Lippia adoensis (Verbenaceae)	HT03	Leaf	Kessie	To treat various skin diseases including eczema and superficial fungal infections (Abate, 1989)
Malva parviflora (Malvaceae)	HT06	Root	Lit	To treat asthma and wounds (Abate, 1989)
Olinia rochetiana (Oliniaceae)	HT08	Leaf	Tife	For the treatment of eczema, acne and scabies (Abebe and Ayehu, 1993)
Phytolacca dodecandra (Phytolaccaceae)	HT07	Fruit	Endod	To treat ascariasis, gonorrhoea, malaria, rabies, sore throat, rheumatic pain, jaundice, syphilis pruritus, eczema, and vitiligo (Abate, 1989; Abebe and Ayehu, 1993; Fullas, 2001)
Verbascum sinaiticum (Scrophulariaceae)	HT03	Leaf	Ketetina	To treat anthrax, postpartum diarrhoea, haemorrhage, rheumatic pain, elephantiasis, measles, superficial fungal infections and wounds (Abate, 1989; Abebe and Ayehu, 1993; Fullas, 2001)

ogy, Ethiopian Health and Nutrition Research Institute (EHNRI).

2.3. Test strains

Staphylococcus aureus (ATCC 6538), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), all American Type Culture Collections were obtained from the Department of Microbiology, EHNRI. *Aspergillus niger* (ATCC 10535), *Trichophyton mentagrophytes* (ATCC 18748) all American Type Culture Collections and *Candida albicans* (clinical isolate) were obtained from the Department of Drug Research, EHNRI.

2.4. Collection and preparation of the plant material

The leaves of *Lippia adoensis*, *Olinia rochetiana*, and *Verbascum sinaiticum*, the fruits of *Phytolacca dodecandra* and the roots of *Malva parviflora* were collected from Northern Shoa, Lallo Mama Woreda (260 km north of Addis Ababa) in November 2002. The leaves of *Kalanchoe petitiana* were collected from Addis Ababa, Entoto area in October 2002 and the leaves of *Calpurnia aurea* and *Acokanthera schimperi* were collected from around Debrezeit (50 km south of Addis Ababa) in December 2002. All parts of the plant materials were dried in an open air protected from direct exposure to sunlight. The dried plant materials were separately powdered to suitable size. The identity of each plant specimen was confirmed at the National Herbarium, Addis Ababa University where a voucher specimen was deposited.

2.5. Preparation of crude extracts

One hundred grams of each powdered plant material were extracted with 80% methanol by maceration for 48 h with frequent agitation and the resulting liquid was filtered (Whatman No. 3 filter paper, Whatman Ltd., England). Extraction was repeated five times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation using rota vapor (BÜCHI Rota-vapor R-205, Switzerland) at not more than 40 °C. The aqueous residue was then placed in an oven at 40 °C for about 48 h to remove the water. The resulting dried mass was then powdered, packed into a glass vial and stored in a desiccator over silica gel until use.

2.6. Preparation of fractions

Lippia adoensis (100 g) and *Olinia rochetiana* (100 g) were sequentially extracted with petroleum ether, chloroform, acetone and methanol using Soxhlet apparatus. The solvent was evaporated under reduced pressure and the fractions were then placed in a vacuum oven at not more than 40 °C for about 24 h to remove any residual solvent. The resulting semisolid mass of each fraction was stored in a desiccator until use in the same way as the crude extract.

2.7. Antimicrobial screening

The antibacterial and antifungal activities of the hydroalcoholic extracts of all the plant species were determined using agar well diffusion method (Boakye-Yiadom et al., 1977). The tests were conducted at three concentration levels (100, 50 and 25 mg/ml) in case of crude extracts and at two concentration levels (25 and 5 mg/ml) in case of fractions. Eighty percent methanol, 100% methanol, chloroform and distilled water were used as negative controls during the whole test on bacteria and fungi. The results are averages of triplicate tests. The zone of inhibitions reported in all cases includes the diameter of the wells. The procedures followed for the antibacterial and antifungal tests are described below.

2.7.1. Screening for antibacterial activity

One Gram-positive (*Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) standard bacterial strains of human pathogens were used. All bacterial cultures were first grown on 5% sheep red blood agar plates at 37 °C for 18–24 h prior to inoculation onto the nutrient agar. Few colonies (4–5) of similar morphology of the respective bacteria were transferred with a sterile inoculating loop to a liquid medium (TSY broth) and this liquid culture was then incubated until adequate growth of turbidity equivalent to McFarland 0.5 turbidity standard was obtained. The inocula of the respective bacteria were streaked on to the nutrient agar plates using a sterile swab in such a way as to ensure thorough coverage of the plates and a uniform thick lawn of growth following incubation. Wells of 11 mm in diameter were formed on to nutrient agar plates using a sterile cork borer. The wells were filled with the test agents (100 μl each) and the plates were then allowed to stay for 1–2 h at room temperature. Finally, the plates were incubated at 37 °C (Heraeus GmbH, D-6450, Germany) for 18–24 h and the resulting diameters of zones of inhibition were measured. Gentamycin was used as a positive control at a concentration of 0.1 mg/ml.

2.7.2. Screening for antifungal activity

The fungal strains used in this study were *Candida albicans*, *Trichophyton mentagrophytes* and *Aspergillus niger*. The required amounts of each fungal strain were removed from the stock and suspended in 2 ml of Sabauraud dextrose broth. This suspension was uniformly spread on petriplates containing Sabauraud dextrose agar media using sterile swabs. After applying the samples into the wells formed by using the same technique for tests on bacteria, the plates were incubated at 25 °C for 3 days in case of *Candida albicans* and *Aspergillus niger* and 7 days in case of *Trichophyton mentagrophytes*. The plates were then examined for the presence of zones

Table 2
Percentage yields of the 80% methanol extracts of the dried and powdered plant materials ($n = 3$)

Plant species	Part extracted	Percentage yield (w/w) (average ± S.D.)
Acokanthera schimperi	Leaf	35.3 ± 3.1
Calpurnia aurea	Leaf	29.5 ± 2.4
Kalanchoe petitiana	Leaf	16.4 ± 1.2
Lippia adoensis	Leaf	20.7 ± 0.8
Malva parviflora	Root	15.2 ± 1.5
Olinia rochetiana	Leaf	32.6 ± 1.6
Phytolacca dodecandra	Fruit	39.5 ± 2.8
Verbascum sinaiticum	Leaf	24.8 ± 2.4

of inhibition and the results were recorded. Ketoconazole was used as a positive control at a concentration of 0.3 mg/ml.

2.8. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the crude extracts of the leaves of *Lippia adoensis* and *Olinia rochetiana* were determined by agar dilution method (EUCAST Definitive Document, 2000; Mukherjee, 2002). The growth media, nutrient agar (for bacteria) and Sabauraud dextrose agar (for fungi) were first prepared in a usual fashion and sterilized by autoclaving (Webco GmbH & Co. KG Bad Schwartau, Germany). The sterilized media were allowed to cool to 50 °C and 18 ml of the molten agar was added to test tubes which contained 2 ml of different concentration of the test drugs (crude extract) and the control (80% methanol). The mixture of the media and the test drugs were thoroughly mixed and poured into pre-labeled sterile petridishes on a level surface. Additional petridishes containing only the growth media were prepared in the same way so as to serve for comparison of growth of the respective organisms. The concentrations of the extracts used in this test ranged from 20 to 0.156 mg/ml. The plates were dried at room temperature. The suspensions of the respective microorganisms having density adjusted to 0.5 McFarland turbidity standard were inoculated onto the series of agar plates using standard loop. Three loopful of the suspension were transferred into each plate. The plates were then incubated at 37 °C for 24 h in case of bacteria and 25 °C for 3–7 days in cases of fungi. The lowest concentration which inhibited the growth of the respective organisms was taken as MIC. All tests were carried out in triplicate.

Table 3
Antimicrobial activities of the crude extracts against different strains of bacteria and fungi

Test samples	Concentration (mg/ml)	Zone of inhibition (mm)					
		Bacterial strains			Fungal strains		
		Sa	Ec	Pa	Ca	Tm	An
Acokanthera schimperi	100	24 ± 0.5	–	17 ± 0.0		22 ± 0.5	–
	50	20 ± 0.3	–	16 ± 0.6	–	17 ± 0.6	–
	25	18 ± 1.3	–	14 ± 0.2	–	15 ± 0.8	
Calpurnia aurea	100	21 ± 0.8	21 ± 1.0	18 ± 0.3	–	–	–
	50	21 ± 1.3	19 ± 0.3	18 ± 0.6	–	–	–
	25	19 ± 1.0	15 ± 0.3	17 ± 0.5	–	–	–
Kalanchoe petitiana	100	26 ± 2.8	14 ± 0.8	21 ± 0.6	–	–	–
	50	20 ± 0.8	13 ± 0.6	20 ± 1.3	–	–	–
	25	19 ± 1.0	–	19 ± 0.5	–	–	–
Lippia adoensis	100	31 ± 0.5	16 ± 0.3	24 ± 1.3	–	22 ± 0.8	–
	50	27 ± 0.8	14 ± 0.9	22 ± 0.3	–	16 ± 0.3	–
	25	20 ± 2.4	14 ± 0.4	20 ± 1.5	–	15 ± 0.4	–
Malva parviflora	100	20 ± 0.0	–	16 ± 0.3	–	28 ± 0.8	–
	50	17 ± 0.9	–	–	–	19 ± 1.0	–
	25	15 ± 0.0	–	–	–	16 ± 0.8	–
Olinia rochetiana	100	25 ± 0.0	19 ± 0.8	22 ± 1.0	18 ± 1.5	54 ± 4.0	–
	50	20 ± 0.3	15 ± 1.0	19 ± 0.8	15 ± 1.0	31 ± 1.5	–
	25	19 ± 0.9	13 ± 0.8	17 ± 0.9	14 ± 0.7	18 ± 1.2	–
Phytolacca dodecandra	100	–	–	16 ± 0.9	–	–	–
	50	–	–	15 ± 0.5	–	–	–
	25	–	–	14 ± 0.3	–	–	–
Verbascum sinaiticum	100	25 ± 0.6	–	20 ± 0.5	–	–	–
	50	21 ± 1.0	–	18 ± 1.0	–	–	–
	25	19 ± 0.6	–	16 ± 0.3	–	–	–
Gentamycin[a]	0.1	29 ± 0.0	22 ± 1.3	21 ± 0.5	NT	NT	NT
Ketoconazole[a]	0.3	NT	NT	NT	37 ± 1.6	23 ± 2.4	–

Sa: *Staphylococcus aureus*, Ec: *Escherichia coli*, Pa: *Pseudomonas aeruginosa*, Ca: *Candida albicans*, Tm: *Trichophyton mentagrophytes*, An: *Aspergillus niger*, NT: not tested, –: no activity.
[a] Positive controls.

3. Results and discussion

The percentage yields of the hydroalcoholic extracts of the eight plant species are given in Table 2. Maximum and minimum yields were obtained from the fruits of *Phytolacca dodecandra* and roots of *Malva parviflora*, respectively. From among the leaves, *Acokanthera schimperi* afforded maximum yield (35.3%) followed by *Olinia rochetiana* (32.6%) and *Calpurnia aurea* (29.5%), while minimum yield was obtained from *Kalanchoe petitiana* (16.4%), which is almost comparable to the yield obtained from the roots of *Malva parviflora* (15.2%). The fresh leaves of this plant have very high water content and shrink extremely to a light weight dried mass with a partial loss of their green colour. In general, the yields obtained from these plants are quite adequate thereby making further development of these herbal drugs economically feasible.

The results of the antimicrobial screening assay of the crude extracts of all species of plants are shown in Table 3. As can be seen from the results, *Lippia adoensis* is the most active species against bacteria and *Olinia rochetiana* against fungi. All species of plants included in the present study were also found to be active on at least one of the selected microbial strains.

The antibacterial activities of most of the herbal drugs (e.g. *Calpurnia aurea*, *Kalanchoe petitiana*, *Lippia adoensis* and *Olinia rochetiana* at a concentration of 100 mg/ml) were found to be almost comparable to the standard gentamycin (0.1 mg/ml). Similarly, at a concentration of 100 mg/ml, *Lippia adoensis* and *Malva parviflora* exhibited comparable activity to that of the standard (ketoconazole, 0.3 mg/ml) against *Trichophyton mentagrophytes*. The negative controls 80% methanol, 100% methanol, chloroform and distilled water were devoid of any antimicrobial activity.

The antimicrobial activity profile of all species of plants (except *Phytolacca dodecandra*) against the tested strains indicated that *Staphylococcus aureus* was the most susceptible bacterium of all the bacterial test strains. Similarly, *Trichophyton mentagrophytes* was found to be the most sensitive fungus although some species such as *Calpurnia aurea*, *Kalanchoe petitiana*, *Phytolacca dodecandra* and *Verbascum sinaiticum* were found to be inactive against it. Of all the fungal strains included in the test, *Aspergillus niger* was found to be virtually insensitive to all plant extracts and *Candida albicans*, which is an isolate, was found to be the least inhibited fungus.

Escherichia coli was the most insensitive strain of all the bacteria used in this study. In fact, Gram-negative bacteria are frequently reported to have developed multi drug resistance to many of the antibiotics currently available in the market of which *Escherichia coli* is the most prominent (Alonso et al., 2000; Sader et al., 2002). Therefore, it is not surprising to learn that *Escherichia coli* is the least responding bacterial strain to the tested plant extracts. However, some species of plants are still of special interest for further investigations in this regard as in the case of *Calpurnia aurea*, which showed exceptionally stronger activity against *Escherichia coli* than other plant extracts yet having poor activity on Gram-positive bacteria, a trend not observed for other species of plants.

In general, among the tested microbial strains, bacteria were found to be more sensitive to many of the test agents than fungi. The antibacterial activity was more pronounced on the Gram-positive bacteria (*Staphylococcus aureus*) than the Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The reason for the difference in sensitivity between Gram-positive and Gram-negative bacteria might be ascribed to the differences in morphological constitutions between these microorganisms, Gram-negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances. The Gram-positive bacteria on the other hand are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of Gram-negative organisms which are more complex than the Gram-positive ones act as a diffusional barrier and making them less susceptible to the antimicrobial agents than are Gram-positive bacteria (Nostro et al., 2000; Hodges, 2002). In spite of this permeability differences, however, some of the extracts have still exerted some degree of inhibition against Gram-negative organisms as well.

Several reports (Dagnew and Gunther, 1990; Dagnew and Erwin, 1991; Figueroa et al., 1996; Sheibeshi, 2000) indicate that infectious skin disorders are very common in Ethiopia. Among the pathogens most commonly known to cause infectious disorders of the skin is *Staphylococcus aureus* (Jones et al., 2003; Rennie et al., 2003). Thus, the fact that all species of the tested plants except *Phytolacca dodecandra* showed activity against *Staphylococcus aureus* might justify the extensive use of these agents for the treatment of skin disorders.

Literature review on the phytochemical constituents of these plants revealed that *Acokanthera schimperi* contains very potent cardiotonics of which the principal compound is ouabain (Cassels, 1985; Iwe, 1993). Similarly, quinolizidine alkaloids, lectins, non-protein amino acids and tannins are the major components of *Calpurnia aurea* (Radema et al., 1979; Asres et al., 1986a, 1986b). The presence of 24-alkylsterols in *Kalanchoe petitiana* (Toshihiro et al., 1992) and essential oils in *Lippia adoensis* (Elakovich and Oguntimein, 1987; Abegaz et al., 1993; Asres and Bucar, 2002) has been reported. *Malva parviflora* and *Olinia rochetiana* were reported to contain antifungal proteins (Wang and Bunkers, 2000), and the cyanogenic glucoside, prunasin (Nahrstedt and Rockenbach, 1993), respectively. A series of saponins has been isolated from *Phytolacca dodecandra* (Goll et al., 1983; Hostettmann, 1984; Ndamba et al., 1989; Birrie et al., 1998), and *Verbascum sinaiticum* contains flavonolignans and flavones (Afifi et al., 1993).

Previous reports have demonstrated that the alkaloid virgiline isolated from *Calpurnia aurea* possesses a potent molluscicidal activity against *Biomphalaria glabrata* (Isao et al., 1984). The essential oils of *Lippia adoensis* were also

Table 4
Minimum inhibitory concentration (MIC) values of the 80% methanol extracts of *Lippia adoensis* and *Olinia rochetiana* on the tested strains

Plant species	Concentration (g/ml)	Presence/absence of growth				
		Bacterial strains			Fungal strains	
		Sa	Pa	Ec	Ca	Tm
Lippia adoensis	20	–	–	–	NT	–
	10	–	–	–	NT	–
	5	–	–	+	NT	–
	2.5	–	–	+	NT	+
	1.25	+	+	+	NT	+
	0.625	+	+	+	NT	+
Olinia rochetiana	20	–	–	–	–	–
	10	–	–	–	–	–
	5	–	–	+	+	–
	2.5	+	–	+	+	–
	1.25	+	+	+	+	–
	0.625	+	+	+	+	+

+: presence of growth, –: absence of growth, Sa: *Staphylococcus aureus*, Ec: *Escherichia coli*, Pa: *Pseudomonas aeruginosa*, Ca: *Candida albicans*, Tm: *Trichophyton mentagrophytes*, NT: not tested.

shown to possess a significant radical scavenging property (Asres and Bucar, 2002). The saponins of *Phytolacca dodecandra* exhibited potent molluscicidal and spermicidal properties (Goll et al., 1983; Hostettmann, 1984; Ndamba et al., 1989; Birrie et al., 1998). All compounds isolated from *Verbascum sinaiticum* displayed dose dependent cytotoxicity against leukemia cells (Afifi et al., 1993). The ethanolic extracts of in vitro cultures of *Verbascum sinaiticum* are also reported to possess broad-spectrum antibacterial activity (Khafagi, 1999). The present work also revealed that this species has strong antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. It appears that there is no report in the literature concerning the biological activities of *Acokanthera schimperi*, *Kalanchoe petitiana* and *Olinia rochetiana*.

Based on the initial antimicrobial screening test, *Lippia adoensis* and *Olinia rochetiana* were selected for further studies for the determination of MIC, because they were found to be most active against bacterial and fungal strains, respectively. The MICs of the extracts are shown in Table 4.

The MIC values indicate that the extracts of *Lippia adoensis* are more potent against bacteria than against fungi and similarly, extracts of *Olinia rochetiana* found to be more active against fungi than against bacteria. The results were in agreement with the initial antimicrobial screening test results. The lowest MIC value observed was 1.25 mg/ml, which was the MIC value of the hydroalcoholic extracts of *Olinia rochetiana* on *Trichophyton mentagrophytes*. On the other hand, the highest MIC value was registered for *Escherichia coli* (the least sensitive bacterial strain) to the crude extracts of both *Lippia adoensis* and *Olinia rochetiana*, i.e. 10 mg/ml. *Pseudomonas aeruginosa* was more sensitive to the antimicrobial agents from among the Gram-negative bacteria being inhibited at 2.5 mg/ml by both *Lippia adoensis* and *Olinia rochetiana* crude extracts.

The MIC values of the two extracts on *Staphylococcus aureus* were found to be 2.5 mg/ml (*Lippia adoensis*) and 5 mg/ml (*Olinia rochetiana*). *Candida albicans* was inhibited by *Olinia rochetiana* at a concentration of 10 mg/ml. The MIC values of the two species seem to be relatively higher. But, being crude extracts, the overall antimicrobial activity screening results are still indicative of the potential of these herbal drugs as effective medicaments in the treatment of infectious skin disorders.

The crude extracts *Lippia adoensis* and *Olinia rochetiana*, which showed better activity against the selected strains of bacteria and fungi were also further fractionated into different solvents. The percentage yields obtained from successive extraction of these plants (Table 5) indicated that increasing polarity of the extracting solvent increases the yield except for chloroform which provided lower yield compared to petroleum ether. As a result, methanol, which is the most polar of all solvents used for fractionation, afforded the maximum yield.

The antimicrobial activities of these fractions against the selected bacterial and fungal strains are indicated in Table 6. The results illustrated that the non-polar fractions (i.e. petroleum ether and chloroform) were stronger in their activity compared to the relatively polar fractions (i.e. acetone and methanol). Antimicrobial activities were found to decrease with increasing polarity indicating that the active

Table 5
Percentage yields of different fractions of *Lippia adoensis* and *Olinia rochetiana*

Plant species	Part extracted	Solvent	Percentage yield (w/w)
Lippia adoensis	Leaf	Petroleum ether	2.76
		Chloroform	1.62
		Acetone	7.29
		Methanol	13.3
Olinia rochetiana	Leaf	Petroleum ether	6.15
		Chloroform	2.63
		Acetone	15.12
		Methanol	23.33

Table 6
Antimicrobial activities of different fractions of *Lippia adoensis* and *Olinia rochetiana* against selected strains of bacteria and fungi

Test sample	Fraction	Concentration (mg/ml)	Zone of inhibition (mm)				
			Bacterial strains			Fungal strains	
			Sa	Ec	Pa	Ca	Tm
Lippia adoensis	Petroleum ether	25	27 ± 1.2	17 ± 3.0	15 ± 1.9	NT	18 ± 2.0
		5	24 ± 1.7	15 ± 2.6	15 ± 1.8	NT	14 ± 1.2
	$CHCl_3$	25	26 ± 1.2	15 ± 1.0	16 ± 1.0	NT	16 ± 0.0
		5	22 ± 1.0	13 ± 0.3	14 ± 1.3	NT	13 ± 0.5
	Acetone	25	24 ± 1.5	15 ± 0.5	17 ± 0.9	NT	–
		5	17 ± 0.6	13 ± 0.6	14 ± 1.3	NT	–
	Methanol	25	20 ± 0.5	14 ± 0.8	16 ± 0.3	NT	–
		5	13 ± 0.6	–	14 ± 1.3	NT	–
	Crude	25	20 ± 2.4	14 ± 0.4	20 ± 1.5	NT	15 ± 0.4
		5	15 ± 1.3	–	15 ± 0.6	NT	–
Olinia rochetiana	Petroleum ether	25	27 ± 0.3	21 ± 0.6	21 ± 2.3	21 ± 1.9	32 ± 1.8
		5	22 ± 2.3	20 ± 0.6	19 ± 1.8	15 ± 0.4	18 ± 0.8
	$CHCl_3$	25	27 ± 2.3	20 ± 1.0	19 ± 1.0	15 ± 0.3	44 ± 3.6
		5	22 ± 0.8	19 ± 1.0	15 ± 0.5	–	17 ± 1.3
	Acetone	25	24 ± 1.0	15 ± 0.5	18 ± 0.9	–	16 ± 1.6
		5	17 ± 0.6	13 ± 0.3	13 ± 0.5	–	13 ± 1.0
	Methanol	25	23 ± 1.6	14 ± 0.3	16 ± 0.8	–	–
		5	15 ± 0.5	–	14 ± 0.5	–	–
	Crude	25	19 ± 0.9	13 ± 0.8	17 ± 0.9	14 ± 0.7	18 ± 1.2
		5	16 ± 0.3	–	15 ± 0.6	–	13 ± 0.3

Sa: *Staphylococcus aureus*, Ec: *Escherichia coli*, Pa: *Pseudomonas aeruginosa*, Ca: *Candida albicans*, Tm: *Trichophyton mentagrophytes*, NT: not tested, –: no activity.

compounds responsible for antibacterial and antifungal activities of the extract reside in the non-polar fractions in relatively higher concentrations.

Comparison of the antimicrobial activities of the fractions with that of the crude extract indicated that the non-polar fractions in many cases are stronger in activity at the two concentration levels than the crude extract. These results are expected because 80% methanol, being highly polar, is unable to extract as much of the active compounds (which are likely to be non polar) as can be extracted with non-polar solvents like petroleum ether and chloroform.

The antimicrobial activity of the non-polar fractions of the two species showed similar activity profile on the selected strains to that of the crude extract, i.e. the petroleum ether and chloroform fractions of *Lippia adoensis* were more active against the bacteria and that of *Olinia rochetiana* against the fungi. In addition, the activity of the petroleum ether fraction of *Olinia rochetiana* at 25 mg/ml is almost equivalent to the activity of the crude extract at 100 mg/ml. This result supports the fact that the active compounds are concentrated more in this fraction. It also underlines the importance of testing activities of the different fractions before reporting that such type of herbal drugs are inactive by simply looking at the results of the crude extract, especially for those drugs having a long history of use in traditional medical practices.

In conclusion, all the plants investigated possessed activity against at least one strain of bacteria and/or fungi. The extensive use of this herbal drugs by the local people in treating various types of skin disorders might therefore be justified by their antimicrobial activities against different strains of bacteria and fungi, which are known to be responsible for causing various skin infections. The results also indicate that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. Further studies aimed at the isolation and identification of active substances from the petroleum ether and chloroform fractions of *Lippia adoensis* and *Olinia rochetiana* could also disclose compounds with better therapeutic value. It is believed that screening of all the investigated plants for other biological activities including anti-inflammatory, wound healing and antioxidant activities is essential.

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