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Antimicrobial Activity of Root Bark Extracts of *Rhus natalensisa* and *Rhus ruspolii*

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Abstract Bacteria and fungi are frequent causes of serious opportunistic infections worldwide. Several effective antibiotic drugs are in the market. However, drug-resistance, side effects and cost of such antibiotics limits their usefulness in disease management. About 80% of the world's inhabitants still rely on traditional medicines based on herbal plants for their primary healthcare. Anti-infective agents from plant origin are preferred because they have no side effects and are readily available. *Rhus species* have several applications both in traditional and contemporary medicine yet scientific data to support such uses is scanty. The aim of this study was to determine the efficacy of *Rhus natalensis* Krauss and *Rhus ruspolii* Engl in management of microbial infections. Plant extracts were subjected to cytotoxicity, antibacterial and antifungal assays using standard methods. LC_{50} values *n*-hexane, dichloromethane, ethyl acetate and methanol extracts were 7.15, 13.35, 4.80, 16.70 and 42.53, 22.3, 19.70, 25.70 µg/ml for *R. natalensis* and *R. ruspolii*, respectively. The plant extracts showed moderate activities ($P \leq 0.05$) against the tested bacteria (Gram-positive and Gram-negative) and fungi. In all the tests, bioactivities of the extracts were lower compared to activity of standard drugs which were used as controls. These findings support the use *R. natalensis* and *R. ruspolii* in treating bacterial and fungal infections. Further studies aimed at isolation and characterization of the bioactive principles is necessary with the hope of getting better antimicrobial agents. It is also necessary to determine the mechanism of action of the extracts.

Keywords *Rhus natalensis*, *Rhus ruspolii*, Cytotoxicity, Antibacterial, Antifungal, LC_{50}

1. Introduction

Bacteria and fungi are frequent causes of serious opportunistic infections [1]. This is more so with a wide range of hosts with weakened immunity such as cancer patients, premature infants and human immunodeficiency virus (HIV) victims [2]. Many opportunistic bacteria and fungi are resistant to the antibiotics currently available in the market [3]. Antimicrobial resistance has worsened in the last decade. The increase in the number of drug-resistance cannot be matched by parallel expansion in the anti-infective agents used to treat infections [4]. In addition, side effects and cost of antibiotics limits their usefulness in disease management [4]. About 80% of the world's inhabitants still rely on traditional medicines based on herbal plants for their primary healthcare [5-7]. Previous studies have shown that in plants there are bioactive compounds that have gained increasing interest as potential therapeutic agents [8-10]. Anti-infective agents from plant origin are preferred since they have no side effects, have better patient tolerance and are relatively inexpensive [11,12].

The genus *Rhus* (sumac) which belongs to the family Anacardiaceae, consists of approximately 250 species which occur mainly in the tropics, subtropics and temperate areas of the world, especially in North America and Africa [13]. In traditional medicine, extracts of *Rhus* species are used to manage several ailments including influenza, wounds, diarrhea, abdominal pain, indigestion [14], diabetes, malaria, rheumatism [15], aching gums, toothaches, swollen legs [16], dog bites, peptic ulcer, kidney stones, skin eruptions, bruises and boils [17]. Root extracts of *R. natalensis* are used to manage venereal diseases, heartburn, cold, cough, diarrhea [18], hernia and stomach ache [19], influenza, abdominal pain and gonorrhea [14]. The leaves are used treatment of syphilis [20], cough and colds [14].

Previously studied showed that the species posses a wide range of bioactivities including antimicrobial [21,22], antiviral [14,23], inflammation [24], anticancer [25,26], antiplasmodial [27-29], and antioxidant [22,24,29,30-32]. Phytochemical studies revealed that the plants are rich in flavonoids [22,24,26,27,30], biflavonoids [28,33], anthocyanins, quinones [27], triterpenes [34], sterols, coumarin [35], and urushiol [22,33].

We report the cytotoxic, antibacterial and fungal activities of *R. natalensis* and *R. ruspoli*. This is the first report on

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biological activity of *R. ruspoli*.

2. Materials and Methods

2.1. Plant Material

The root bark of *R. natalensis* and *R. ruspolii* were collected from Kapkonga, Iten, 40 km northeast of Eldoret Town in Kenya. The plant materials were identified and the voucher specimen, reference numbers PKT/R1 and PKT/R2 for *R. natalensis* and *R. ruspolii* respectively, were deposited at the National Museums of Kenya. The plant materials were dried under the shade at room temperature for one month then ground into coarse using a mill (Christy and Norris Ltd., Chelmsford, England).

2.2. Solvent Extraction

Powdered root bark of *Rhus natalensis* (1.6 kg) and *R. ruspolii* (2.1 kg), were sequentially extracted using *n*-hexane, dichloromethane (DCM), ethyl acetate and methanol. The plant materials were soaked in cold solvent and left to stand for five days with occasional shaking. The macerates were filtered and concentrated at reduced pressure (temperature of 45℃) using rotary evaporator. The extracts (Table 1) were weighed and stored at 4℃ in brown glass bottles.

Table 1. Root bark extracts of *the plants*

Plant extracts	Mass of extract (g)	Yield %
R. natalensis		
Hexane	12	0.8
DCM	16	0.7
EtOAc	22	1.3
MeOH	400	25.0
R. ruspolii		
Hexane	30	1.4
DCM	34	1.6
EtOAc	50	2.4
MeOH	250	12.0

2.3. Brine Shrimp Lethality Test

Brine shrimp lethality bioassay is considered a useful tool for preliminary assessment of toxicity. The method is attractive, because it is simple, inexpensive and sensitive [36]. Brine shrimp eggs (*Artemia salina*) were hatched in artificial sea water prepared by dissolving sea salt (38 g/l) in distilled water. After incubation for 48 h at room temperature, the brine shrimp nauplii were attracted to one side of the vessel with a light source and collected using a pipette. The larvae were separated from eggs and placed in small beakers containing sea water [37]. Each crude extract (10 mg) was dissolved in dimethyl sulphoxide (DMSO, 50µl) and then diluted with 9.95 ml of sea water. Test solutions (100 µl) of varying concentrations (0.78 - 200 µg/ml) were obtained by the serial dilution using simulated seawater in 96-well microplates. DMSO (5 µl) in sea water (95 µl) and vincristine sulfate were used as negative and positive controls respectively [37].

A suspension of larvae (100 µl) containing 20 live nauplii was added to each well and the covered plate incubated at 29℃. After 24 h, the plates were inspected using a magnifying glass against a black background and the number of survived nauplii were counted. Percentage lethality of the nauplii was calculated for each concentration. The effectiveness of plant product is usually expressed as a median lethal concentration (LC_{50}). This represents the concentration of drug that cause death in half of the test subjects after a certain exposure time. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality was and the values of LC_{50} were calculated using Microsoft Excel [37].

2.4. Antibacterial Activity Test

Antibacterial activity of the plant extracts was tested by paper disc diffusion method [38]. The test organisms used were *Staphylococcus aureus* (ATCC no. 25923), *Bacillus subtilis* (local isolate), *Escherichia coli* (ATCC no. 25922) and *Pseudomonas aeruginosa* (ATCC no. 10622) which were obtained from Botany Department, Kenyatta University in Kenya. Test bacteria were cultured in nutrient broth medium from stock cultures and later transferred to the nutrient agar in the petri dishes [39]. Nutrients agar (28 g/l) in distilled water was sterilized at 121℃ and 15 psi pressure for 20 minutes. Sterilized nutrient agar (15 ml) was dispensed in 90 mm diameter sterile petri dishes to yield a uniform depth of 4 mm. The petri dishes were allowed to cool at room temperature until the culture medium hardened. Using a sterile wire loop, bacteria cultures from stock cultures were scooped and spread on the nutrient agar surface and incubated at 37℃ for 24 h. One loop-full of the bacterial strain from the 24 h culture was added to the sterile nutrient broth medium and incubated at 37℃ for 24 hours in a rotary shaker [39]. The 24 h broth bacteria culture (0.1 ml) was pipetted into the nutrient agar media in the petri dishes and spread evenly using a sterilized glass rod.

Plant extracts (10 µl) of concentration of 200 mg/ml in DMSO were dispensed on 6 mm sterile paper disc [38]. The oven dried (50℃, 1h) discs were firmly placed on the seed agar in the petri dishes using sterile forceps and incubated at 37℃ in inverted position. The zones of inhibition were measured after 48 h [40]. Standard antibiotic drugs were used as control.

2.5. Antifungal Activity Test

Agar-well plate diffusion method was used [41]. Three fungal species, *Candida albicans*, *Penicillium notatum* and *Aspergillus Niger* were obtained from Botany Department, Kenyatta University in Kenya. Portions of sterile potato dextrose agar (15 ml) were dispensed in sterile petri dishes under sterile conditions and left to solidify. Pure cultures of test fungi were introduced in the petri dishes from stock

cultures and incubated at 30℃ for seven days to produce a good crop of spores. The fungal inoculums were prepared by harvesting the spores from the crop of spores using a sterile spores-harvesting needle and transferred in to a sterile tube containing sterile distilled water. The spore suspension (0.5 ml) was pipetted onto the PDA surface in the petri dishes. The plates were tilted several times to spread the inoculum then left undisturbed for 10 minutes. Using a sterile cork-borer (6 mm), four agar wells were cut out from the inoculated PDA medium. Plant extracts (1 mg) were dissolved in DMSO (50 µl) then made to 1 ml using methanol. The resulting solutions (0.1 ml) were pipetted into the wells in triplicates. The petri dishes were covered, sealed and incubated at 30℃ for 72 h after which diameter of zones of inhibition was measured [41].

3. Results and Discussion

3.1. Brine Shrimp Lethality

Root extracts of *R. natalensis* and *R. ruspolii* (*n*-hexane, dichloromethane, ethyl acetate and methanol) were subjected to brine shrimp lethality bioassay for possible cytotoxic action. All the crude extracts exhibited brine shrimp larvicidal activity good (Table 2). Ethyl acetate and *n*-hexane extracts of *R. natalensis* were the most lethal, having LC_{50} values of 4.80 and 7.15 µg/ml, respectively. DCM and methanol extracts of *R. natalensis* had LC_{50} values of 13.35 and 16.70 µg/ml respectively. Brine shrimp larvicidal activity displayed by the extracts of *R. ruspolii* was lower than that of *R. natalensis* extracts. Ethyl acetate, DCM, methanol and *n*-hexane extracts of *R. ruspolii* had LD_{50} values of 19.70, 22.35, 25.70 and 42.53 µg/ml, respectively. Compared to positive control (vincristine sulphate, LC_{50} = 2.13 µg/ml), all the extracts tested showed good but lower brine shrimp larvicidal activity. Crude extracts resulting in LC_{50} values less than 240 µg/ml was considered significantly active and had the potential for further investigation [42]. The cytotoxic activity exhibited by the extracts was promising and this indicates the presence of potent bioactive compounds.

Table 2. LC_{50} of plant extracts in Brine Shrimp lethality bioassay

Test drugs	LC_{50} (µg/ml)	Regression equation	R^2
R. natalensis			
n-Hexane	7.15	y = 35.02x + 21.15	$R^2 = 0.80$
DCM	13.35	y = 31.28x + 15.15	$R^2 = 0.93$
EtOAc	4.80	y = 30.92x + 26.63	$R^2 = 0.93$
MeOH	16.70	y = 24.80x + 19.82	$R^2 = 0.91$
R. ruspolii			
Hexane	42.53	y = 20.21x + 17.96	$R^2 = 0.88$
DCM	22.35	y = 14.24x + 30.94	$R^2 = 0.93$
EtOAc	19.70	y = 13.30x + 33.20	$R^2 = 0.91$
MeOH	25.70	y = 19.82x + 22.47	$R^2 = 0.98$
vincristine sulphate	2.13	y = 24.49x + 43.23	$R^2 = 0.98$

Table 3. Antibacterial activity of plant extract

Antibacterial agents	Diameter of zone of inhibition (mm ± SD) (*n* = 3)			
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
R. natalensis extracts				
Hexane	10 ± 0.3	-	-	-
DCM	10 ±0.5	11 ±0.2	-	-
EtOAc	14 ± 0.1	15 ± 0.1	9 ± 0.3	10 ± 0.2
MeOH	11 ± 0.3	13 ± 0.4	11 ± 0.2	-
Rhus ruspolii extracts				
Hexane	10 ± 0.2	11 ± 0.2	11 ± 0.4	-
DCM	11 ± 0.2	15 ± 0.2	-	-
EtOAc	12 ± 0.3	12 ± 0.3	12 ± 0.1	11 ± 0.2
MeOH	12 ± 0.1	12 ± 0.2	-	-
Ampicillin 25 mg/disc	23 ± 0.2	23 ± 0.3	20 ± 0.2	20 ± 0.1
Tetracycline 25 mg/disc	22 ± 0.1	22± 0.2	18 ± 0.1	21 ± 0.2
Contrimoxazole 25 mg/disc	19 ± 0.2	24 ± 0.3	18 ± 0.1	22 ± 0.3
Streptomycin 10 mg/disc	20 ± 0.3	22 ± 0.2	20 ± 0.2	20 ± 0.3
Kenamycin 30 mg/disc	22 ± 0.3	21± 0.1	20 ± 0.2	18 ± 0.1
Gentamycin 10 mg/disc	20 ± 0.5	23 ± 0.3	21 ± 0.1	18 ± 0.2
Chroramphenicol 30 mg/disc	22 ± 0.1	23 ± 0.4	16 ± 0.1	18 ± 0.2
Sulphamethorazole 200 mg/disc	24 ± 0.3	22 ± 0.1	18 ± 0.3	20 ± 0.4
LSD, $P \leq 0.05$	0.2			

"-" Indicates no zone of inhibition

3.2. Antibacterial Activity of Extract

Plant extracts were subjected to antibacterial test against selected Gram-positive and Gram-negative bacteria (Table 3). All extracts showed moderate activity against Gram-positive bacteria tested except *n*-hexane extract of *R. natalensis* which was not active against *S. aureus.* Ethyl acetate extract of *R. natalensis* exhibited the highest activity ($P \leq 0.05$) against *B. subtilis* (inhibition zone of 14 ± 0.1mm) followed by ethyl acetate and methanol extracts of *R. ruspolii* which had 12 ± 0.3 and 12 ± 0.1mm inhibition zones respectively. Hexane extracts showed the least ($P \leq 0.05$) antibacterial activity against *B. subtilis*. *Rhus natalensis* EtOAc and *R. ruspolii* DCM extract exhibited the highest against *S. aureus* (inhibition zone of 15 mm) followed by *R. natalensis* MeOH extract (inhibition zone of 13 mm) then *R. ruspolii* EtOAc and MeOH extracts (inhibition zone of 12 mm). Both *R. natalensis* and *R. ruspolii* showed antibacterial activity against the Gram-negative bacteria tested.

Rhus ruspolii EtOAc and hexane extracts, and *R. natalensis* MeOH and EtOAc extracts showed moderate antibacterial activity against *E. coli* with 12 ± 0.1, 11 ± 0.4, 11 ± 0.2 and 9 ± 0.3 mm inhibition zones respectively. Only EtOAc extracts of the two plants showed antibacterial activity against *P. aeruginosa* with inhibition zone of 11 ± 0.2 and 10 ± 0.2 mm for *R. ruspolii* and *R. natalensis* extracts respectively. Gram-positive bacteria were more susceptible to the plant extracts than Gram-negative bacteria. Such results were expected since the latter are known to be more difficult to inhibit. The fact that the crude extracts were activity against both Gram-positive and Gram-negative suggests the antibacterial activity of *Rhus* species is broad spectrum. However, the antibacterial activities of the plant extracts were lower than those of standards. Antibacterial activity of *R. natalensis* extracts are in agreement with previous reports [28,43].

3.3. Antifungal Activity of Extracts

Hexane, dichloromethane, ethyl acetate and methanol root extracts of *R. natalensis* and *R. ruspolii* were tested for antifungal activity against *C. albicans*, *A. niger* and *P. notatum* (Table 4). Five out of the eight extracts tested were active against one or more or the test fungi. *Candida albicans* and *A. niger* were most susceptible to EtOAc and MeOH extracts of *R. natalensis* with inhibition zones of 10 ± 0.2 and 10 ± 0.5 mm (*C. albicans)* 9 ± 0.4 and 9 ± 0.3 (*A. niger)* respectively. *Rhus natalensis* EtOAc extract was the most active against *P. notatum (*11 ± 0.3 mm) while *R. natalensis* MeOH extract was the least active (8 ± 0.4 mm) against the pathogen. *R. natalensis* hexane extract, *R. ruspolii.* Hexane and DCM extracts did not show any antifungal activity against the test pathogens. *R. ruspolii* EtOAc extracts inhibited the growth of *C. albicans* and *P. notatum* but was ineffective in controlling the growth of *A. niger*. *Candida albicans* and *A. niger were* susceptible to MeOH extract of *R. ruspolii* but *P. notatum was* not. The antifungal activity of *R. natalensis* concur with previous reports [28,43].

Table 4. Antifungal activities of extract

Antifungal extracts	Diameter of zone of inhibition (mm ± SD) (*n* = 4)		
	C. albicans	*A. niger*	*P. notatum*
Rhus natalensis			
Hexane	-	-	-
DCM	9± 0.3	(-)	10 ± 0.1
EtOAc	10 ± 0.2	9 ± 0.4	11 ± 0.3
MeOH	10 ± 0.5	9 ± 0.3	8 ± 0.4
Rhus ruspolii			
Hexane	-	-	-
DCM	-	-	-
EtOAc	9 ± 0.3	-	10 ± 0.2
MeOH	9 ± 0.4	8 ± 0.1	-
Kenamycin 30 mg/disc	20 ± 0.2	24 ± 0.1	22 ± 0.4
LSD, $P \leq 0.05$		0.3	

"-" Indicates no zone of inhibition

4. Conclusions

The findings support the use *R. natalensis* and *R. ruspolii*, in treating bacterial and fungal infections. Further studies aimed at isolation and characterization of the bioactive principles is necessary with the hope of getting better antimicrobial agents. It is also necessary to determine the mechanism of action of the extracts.

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