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Anti-proliferative effect of *Scoparia dulcis L.* against bacterial and fungal strains

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ABSTRACT

Scoparia dulcis L. was sequentially extracted with hexane, chloroform and methanol and soaked with aqueous-acetone (80%) to check for its antimicrobial activities against five bacterial and four fungal strains. 250 µg of each extract loaded on a whatman paper disc exhibited significant antimicrobial activities on all the fungus and against Gram-negative and Gram-positive bacteria. Therefore, *P. mirabilis* is less sensitive to all the extracts while *B. cereus*, a β-lactamase producer bacterium, was resistant to the activity of the polar methanol and aqueous-acetone extracts. By the microdilution method, the most active extracts were chloroform extract on *B. cereus* with minimal inhibitory concentration (MIC) of 1.56 mg/ml and Aqueous-acetone extract on *S. typhimurium* (MIC = 1.56 mg/ml); the antifungal activity was strongest for hexane extract (MIC = 6.25 mg/ml) on both *A. niger* and *P. roquefortii*.

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Keywords: *Scoparia dulcis*, antibacterial, antifungal, polyphenols.

INTRODUCTION

Microorganisms including bacteria and fungus are responsible for various infections in plants and animals. Many efforts were made to fight against these microbial agents and a number of antibiotics have been approved until now. Therefore, there is an increasing resistance of microorganisms against available antimicrobial agents; then, it is more and more difficult to treat pathogenic viruses, bacteria, fungi, and protozoa with the existing drugs (Koomen et al.; 2002). In this scheme, it remains a great need to search for new antimicrobial agents with greater efficacy. Several researches are focusing on screening medicinal plants to develop new antimicrobial drugs (Eloff et al., 2005; Aliero and Afolayan, 2006) and many plant extracts

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showed excellent activities against plant fungal pathogens (Masoko et al., 2007).

Scoparia dulcis L. (Scrophulariaceae), commonly known as sweet broomweed, is a perennial and widespread herb in tropical regions.

Some investigations previously evidenced its use to help with the symptoms of several diseases such as arterial hypertension and diabetes mellitus (Satyanarayana, 1969). A number of the medicinal properties of *S. dulcis* have been previously demonstrated including its anti-diabetic, anti-inflammatory and antioxidant activities *in vivo* (Pari and Latha, 2005; Adaikpoh et al., 2007), its potent inhibition of DPPH radicals (Babincová and Sourivong, 2001), its antimicrobial activity (Latha et al., 2006) and its impact on lipid peroxidation (Ratnasooriya et al., 2005; Coulibaly et al., 2011b). Furthermore a few phenolic and terpenic compounds have been isolated from *S. dulcis* including scoparic acid A, scoparic acid B, scopadulin (Hayashi et al., 1991), scopadulcic acid A and B, scopadulciol, scopadulin (Hayashi et al., 1990) and were identified as contributing to the observed medicinal effect of the plant. Among them, scopadulcic acid B and scopadulciol show antiviral activity by their inhibitory activity on replication of herpes simplex virus type 1 (Hayashi et al., 1991).

This study reports the antibacterial and antifungal activities of *S. dulcis* on stereotypes fungal and bacterial strains and the contribution of phytochemicals as tannins in this antimicrobial activity.

MATERIALS AND METHODS

Plant material

Scoparia dulcis L (whole plant) was collected at Gampela (25 km, East of Ouagadougou, Burkina Faso). Taxonomic identification was verified by the Laboratoire de Biologie et Ecologie Végétales (University of Ouagadougou, Burkina Faso) where a voucher specimen ($SD_{\text{-ca }001}$) was deposited and archived.

Extraction

Air-dried grounded *Scoparia dulcis* (25 g) was sequentially extracted with 250 ml of hexane, chloroform and methanol using a Soxhlet apparatus. The extracts were then concentrated to dryness in a vacuum evaporator and stored for the different investigations. Another powder of *Scoparia dulcis* plant (25 g) was soaked for 36 hours in 250 ml of acetone containing 20% of water; the mixture was then filtered and evaporated to dryness to constitute the aqueous-acetone extract.

Chemicals

Ferric ammonium citrate, Ammonium hydroxide, Sodium chloride, Barium chloride and Dimethylsulfoxyde were purchase from Sigma-Aldrich (Germany), Mueller Hinton broth and Mueller-Hinton agar (DIFCO, Becton Dickinson, USA), Ampicliin and Gentamicin were purchase from MediMark (France).

Microbial strains

All the strains were obtained from Hôpital pédiatrique Charles De Gaulle, Ouagadougou, Burkina Faso.

Bacterial strains: Five strains of bacteria from the American Type Culture Collection (ATCC, Rockville) were tested: *Staphylococcus aureus* (Gram+, ATCC 6538), *Bacillus cereus* (Gram+, ATCC 13061), *Escherichia coli* (Gram-, ATCC 25922), *Proteus mirabilis* (Gram-, ATCC 35659) and *Salmonella thyphimurium* (Gram-, ATCC 13311).

Fungal strains: Aspergillus niger (DSM737), *Mucor rouxii* (DSM1151), *Penicillium roquefortii* (DSM1080) *and Fusarium oxysporum* (NCIM 1008).

Determination of tannins content

Tannins content was evaluated by the method of European Community (2004). Briefly, distilled water (1 ml) was mixed with extract solution (0.2 ml; 10 mg/ml), ferric

ammonium citrate (0.2 ml; 3.5 mg/ml in water) and ammonium hydroxide (0.2 ml; 0.8%). The mixture was incubated in darkness at room temperature for 15 min. A blank without extract was also prepared. The absorbance was measured at 525 nm against a standard curve of tannic acid. Tannin content was expressed as mg tannic acid equivalent (TAE)/100 mg of dried extract.

Antibacterial and antifungal activities

Preparation of microbial inoculums

Bacterial and fungal strains grown on nutrient agar at 37 °C for 24 hours were suspended in a saline solution (0.9% NaCl) and adjusted to the turbidity of the 0.5 MacFarland standard (0.5 ml of $BaCl_2$, $5H_2O$, 11.7 mg/ml mixed with 99.5 ml of H_2SO_4 1%) to obtain approximately 10^6 colony-forming units/ml.

Preparation of culture medium and inoculation

Thirty-eight grams of Mueller-Hinton agar was mixed with 1000 ml of sterile distilled water. The mixture was then sterilized by autoclaving at 120 °C for 20 minutes. Under aseptic conditions in the laminar flow hood, 15 ml of agar medium was uniformly dispensed into sterilized Petri dishes. They were then covered and allowed to cool at room temperature until the culture medium hardened. The inoculation of the microbial culture on the agar surface was done by the spread plating technique.

Disc application and incubation

Discs (6 mm in diameter) were prepared from Whatman No. 1 filter paper and were sterilized by autoclaving. The sterile discs were placed on the Mueller-Hinton agar surface with flamed forceps and then impregnated with 10 µl of the extract solution (25 mg/ml in water containing 10% DMSO) before being gently pressed down to ensure contact with the agar surface.

Standard commercial antibiotic discs of ampicillin (10 µg) and gentamicin (10 µg) and a prepared solution of nystatin (5 mg/ml) were used as positive controls while a water solution containing 10% DMSO was used as negative control.

The Petri dishes were finally incubated for 24 hours (for bacteria) and 96 h (for fungus) at 37 °C in an inverted position for optimal growth. Then, the diameter (in mm) of the inhibition zone around each disc was measured. Antimicrobial activities were indicated by a clear zone of growth inhibition. Each test was repeated three times.

Microdilution method

The microdilution method was used for antibacterial and antifungal activities. 100 µl of Mueller Hinton broth medium (21 mg/ml) were placed into each 96 wells of the microplates. The extract solutions (100 µl, 100 mg/ml) were added into first rows of microplates and two-fold dilutions (100 to 0.78 mg/ml) were made by dispensing the solutions to the remaining wells. 10 µl of microbial inoculum was inoculated into all the wells. For each dilution, a negative control without inoculum (100 µl of medium, 100 µl of extract solution and 10 µl of saline solution 0.9%) was prepared for optical comparison. A control without extract solution (100 µl of medium, 100 µl of DMSO 10% and 10 µl of inoculum) was prepared to ensure bacterial growth. Another well was filled with 100 µl of medium, 100 µl of DMSO 10% and 10 µl of saline solution 0.9% as control to ensure their sterility. Each test was carried out in triplicate. The sealed microplates were incubated at 37 °C for 24 h (for bacteria) and 48 h (for fungus). The lowest concentration of the extract that completely inhibited macroscopic growth was determined by optical comparison and noted as minimum inhibitory concentration (MIC).

Statistical analysis

All the reactions were performed in triplicate and data were presented as mean ± standard deviation. Data were examined by one-way analysis of variance (ANOVA)

followed by Tukey multiple-comparison test using XLSTAT7.1. $P < 0.05$ was used as the criterion for statistical significance.

RESULTS

Tannin content

The yield of each extract was obtained by calculating the ratio of the dried extract by the weight of the initial powder of the plant material. Methanol extract showed the highest yield (15.12%) followed by aqueous-acetone extract (11.23%). Hexane extract (4.74%) and chloroform extract (2.98%) showed the lowest yields.

Tannins (Table 1) are undetectable in the apolar hexane extract but they are concentrated in the non polar chloroform extract (3.29 ± 0.37 mg TAE/100 mg). They are contained in a lesser extent in the polar methanol (2.36 ± 0.11 mg TAE/100 mg) and aqueous-acetone (1.74 ± 0.04 mg TAE/100 mg) extracts.

Antimicrobial activities

The antimicrobial activities of the extracts from *Scoparia dulcis* were checked through its antibacterial and antifungal effects.

The antibacterial activity was evaluated by the disc diffusion method using five strains of bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Proteus mirabilis* and *Salmonella thyphimirium*).

The diameter of inhibition zone was measured around the disc containing 250 µg of the extract (10 µl, 25 mg/ml). The results are showed in Table 2. All the extracts exhibited antibacterial activity ($d > 6$ mm) and are less active on *P. mirabilis* ($d \leq 9$ mm) than the other bacteria. All the extracts significantly inhibited *E. coli* and *S. typhimurium* ($d \geq 12$ mm) except methanol extract.

For gram positive bacteria, only chloroform extract significantly inhibited *S. aureus* (d = 14 mm); *B. cereus* is resistant to methanol and aqueous-acetone extracts but is sensitive to hexane and chloroform extracts.

The positive controls ampicillin (10 µg) and gentamycin (10 µg) significantly inhibited bacterial growth on the agar. Ampicillin inhibition zones are comparable to those of all the extracts excepted for *P. mirabilis*. The inhibition zones of gentamycin are more than that of all the extracts.

Table 3 reported the result of antifungal activities of the different extracts of *Scoparia dulcis*. Four fungal strains (*Aspergillus niger*, *Mucor rouxii*, *Penicillium roquefortii and Fusarium oxysporum*) were used to evaluate this activity.

M. rouxii and *F. oxysporum* are sensitive to all the extracts at sensibly the same level ($d = 13 \pm 1$ mm) except methanol extract that exhibited a two-fold inhibition on *F. oxysporum*. Methanol and aqueous-acetone extracts have similar and significant inhibitions on fungi *A. niger* and *P. roquefortii* (13 ± 2 mm). The non polar hexane and chloroform extracts strongly inhibited growth of *P. roquefortii* (40 ± 1 mm). Chloroform extract has a weak inhibition on *A. niger* while hexane extract has the strongest one. The standard nystatin is relatively more active than the extracts; therefore it inhibited weakly *P. roquefortii* than hexane and chloroform extracts.

The most active extracts ($d \geq 14$ mm) were considered for the determination of the minimal concentration that inhibits microbial growth. The minimal inhibitory concentrations (MIC) varied from 1.56 mg/ml to 12.5 mg/ml. In accordance with the diameter of inhibition, chloroform extract is most active on Gram positive bacteria *B. cereus* (MIC = 1.56 mg/ml) and *S. aureus* (MIC = 3.12 mg/ml). *S. typhimurium* is most sensitive to hexane extract (MIC= 3.12 mg/ml) and Aqueous-acetone extract (MIC = 1.56 mg/ml).

Hexane extract is the most active on fungal strains with a minimal inhibitory concentration of 6.25 mg/ml on both *A. niger* and *P. roquefortii*.

Table 1: Tannin contents in *Scoparia dulcis* extracts.

Extracts	Tannins (mg TAE/100 mg)
Hexane	nd
Chloroform	3.29 ± 0.37^{a}
Methanol	2.36 ± 0.11^{b}
Aqueous-acetone	1.74 ± 0.04^{c}

Tannins amount was expressed as mg Tannic Acid Equivalent (TAE)/100 mg of extract. Data are expressed as mean values ± standard deviation (n = 3). Values within each column with different superscript letters (a, b, c) are significantly different ($P < 0.05$) as determined using ANOVA.

Table 2: Antibacterial activity of *Scoparia dulcis* extracts.

	Gram negative bacteria			Gram positive bacteria	
Extracts	***E. coli***	**P. mirabilis**	***S. typhimurium***	***B. cereus***	***S. aureus***
Hexane	12 ± 1^{b}	9 ± 1^{b}	15 ± 1^{b}	22 ± 2^{b}	9 ± 1^{c}
Chloroform	13 ± 1^{b}	7 ± 1^{b}	13 ± 2^{b}	16 ± 1^{c}	14 ± 1^{b}
Methanol	21 ± 3^{a}	9 ± 2^{b}	8 ± 1^{c}	R^{d}	10 ± 1^{c}
Aqueous-acetone	15 ± 2^{b}	8 ± 1^{b}	14 ± 1^{b}	R^{d}	10 ± 1^{c}
Ampicillin	18 ± 2^{a}	24 ± 2^{a}	12 ± 1^{b}	R^{d}	R^{d}
Gentamycin	nd	20 ± 2^{a}	36 ± 3^{a}	32 ± 3^{a}	27 ± 2^{a}

Antibacterial activity of *Scoparia dulcis* extracts (25 mg/ml) are expressed as diameter of inhibition zone (in mm). Data are mean values (n = 3) ± standard deviation. Ampicillin (10 µg) and gentamycin (10 µg) were used as standards. R= Resistant ($d \leq 6$ mm). nd = not determined. Values within each column with different superscript letters (a, b, c, d) are significantly different ($P < 0.05$) as determined using ANOVA.

Table 3: Antifungal activity of *Scoparia dulcis* extracts.

Extracts	***A. niger***	***P. roquefortii***	***M. rouxii***	***F. oxysporum***
Hexane	23 ± 3^{a}	43 ± 3^{a}	13 ± 3^{b}	13 ± 1^{c}
Chloroform	9 ± 1^{b}	40 ± 1^{a}	14 ± 2^{b}	12 ± 1^{c}
Methanol	12 ± 2^{b}	13 ± 4^{b}	12 ± 1^{b}	27 ± 2^{b}
Aqueous-acetone	14 ± 1^{b}	13 ± 1^{b}	13 ± 2^{b}	13 ± 2^{c}
Nystatin	29 ± 2^{a}	12 ± 1^{b}	18 ± 2^{a}	37 ± 2^{a}

Antifungal activity of *Scoparia dulcis* extracts (25 mg/ml) are expressed as diameter of inhibition zone (in mm). Data are mean values (n = 3) ± standard deviation. Nystatin (5 mg/ml) was used as standard. Values within each column with different superscript letters (a, b, c,) are significantly different ($P < 0.05$) as determined using ANOVA.

Table 4: Minimal inhibitory concentrations of *Scoparia dulcis* extracts.

	Microbes	Extracts			
		Hexane	**Chloroform**	**Methanol**	**Aqueous-acetone**
Bacteria	*E. coli*	nd	nd	12.5	12.5
	S. typhimurium	3.12	nd	nd	1.56
	B. cereus	12.5	1.56	nd	nd
	S. aureus	nd	3.12	nd	nd
Fungi	A.niger	6.25	nd	nd	12.5
	P. roquefortii	6.25	12.5	nd	nd
	M. rouxii	nd	12.5	nd	nd
	F. oxysporum	nd	nd	12.5	nd

Minimal inhibitory concentrations are expressed in mg/ml. nd = not determined.

DISCUSSION

The different extracts from *Scoparia dulcis* globally showed a significant antimicrobial activity on both bacterial and fungal strains.

Gram positive bacteria (*B. cereus* and *S. aureus*) are relatively most sensitive to non polar extracts (Hexane and chloroform) comparatively to polar extracts (Methanol and Aqueous-acetone). Therefore, the sensitivity of each bacterium to the different extracts is variable, due to the structure of each bacterium and also to the mechanism by which the extracts exert their antibacterial action. Indeed, Gram negative bacteria possess an external double-bond membrane which made them more impermeable and then resistant to antibiotics (Lopez et al., 2005 ; Tian et al., 2009). That could explain why the Gram negative bacterium *P. mirabilis* is particularly not sensitive to the extracts of *Scoparia dulcis*.

Gram-positive bacteria have no external membrane but some of them as *B. cereus* and *S. aureus* can develop resistance to antibiotics by producing β-lactamase enzymes. These enzymes are penicillinases that can inactivate some antibiotic drugs by hydrolyzing their β-lactame structure (Xian-Zhi et al., 2007). This could justify why *B. cereus* and *S. aureus* are resistant to ampicillin, a penicillin-derived drug used as standard. *B. cereus* is moreover resistant to the methanol and aqueous-acetone extracts while *S. aureus* is sensitive to them; so, the antibacterial compounds in these two extracts (methanol and aqueous-acetone) may contain β-lactame structure. Therefore, these two extracts may also contain other antibacterial compounds without β-lactame structure, as tannins and other polyphenols, which are active on *S. aureus.*

Except *E. coli*, the methanol extract is not significantly active on the other bacteria ($d \leq 9$ mm). This observation is in contradiction with previous studies (Eloff, 1998; Muthumani et al., 2010) which pointed methanol to be a suitable solvent for extraction of antimicrobial compounds. Chloroform extract with the highest amount in tannins, exhibited significant inhibition ($d \geq 13$ mm) of the growth of all the bacteria excepted on *P. mirabilis* which was ever showed to be relatively insensitive to all the extracts ($d \leq 9$ mm). In regard of their lowest content in tannins, hexane and aqueous-acetone extracts have approximately the same sensitivity to all the bacteria excepted to *B. cereus*. Then, the antibacterial activity of *S. dulcis* extracts could be attributed to the quantity and the nature of tannins they contain. Previous studies pointed polyphenols as catechins, anthocyanidins and hydrolysable tannins to be anti-infective agents (Papadopoulou et al., 2005; Daglia et al., 2007). The sensitivity of bacteria to these polyphenols depends on the polyphenol

structure and the bacterial species (Campos et al., 2003; Taguri et al., 2004). This could explain the difference in the activity on *P. mirabilis* and *B. cereus* previously stated.

The antimicrobial activity of the compounds in *S. dulcis* extracts may be of four mechanisms as prior indicated (Latha et al., 2006): they hamper cell wall synthesis, they inhibit microbial protein and nucleic acid synthesis, they disrupt microbial membrane structure and function or they block metabolic pathways through inhibition of key enzymes. Indeed, the extracts of *S. dulcis* were showed to possess potentials for inhibition of various enzymes (Coulibaly et al., 2011a) and tannins in these extracts are known to bind proteins with suppression of their enzymatic activity (Fingerman and Nagabhushanam, 2006).

The four strains used for antifungal activity are all sensitive to the extracts of *S. dulcis*. *A. niger* and *P. roquefortii* are most sensitive to non polar hexane and chloroform extracts. This is in accordance with previous studies which pointed non polar extracts to be particularly active on *Aspergillus* sp, *Penicillium* sp and *Fusarium* sp (Mahlo et al., 2010). The strong activity of these non-polar extracts could be related to their high content in terpenes such as β-carotene and lycopene (Coulibaly et al., 2011b) as previously suggested (Gudzic et al., 2002; Cakir et al., 2004).

Fungi are responsible for major losses in agricultural production. *Aspergillus* sp cause spoilage of mangoes and produce also highly toxic aflatoxins that contaminate cotton seed, corn, peanuts and tree nuts during harvesting or storage (Wilson and Payne, 1994). *Fusarium* sp are common fungi affecting wheat and barley worldwide and *Fusarium oxysporum* particularly causes vascular wilt disease in tomato crops (Parry et al., 1995; Mahlo et al., 2010). Moreover, many fungi can produce mycotoxins which cause immunological, neurological and gastrointestinal toxicity in animals (Desjardin, 2006). That show all the economical, nutritional, environmental and sanitary problems cause by fungi. Many fungicides as amphotericin B and fluconazol exist but are sometimes expensive for most poor rural farmers and fungi often develop resistances; they may also have adverse effects including toxicity to humans and organisms in the environment (Lingk, 1991). Various extracts from plants are traditionally used to protect crops and seeds (Shai et al., 2009). Some of these extracts and other metabolites as flavonoides showed significant antifungal activity than standards as fluconazol (Masoko et al., 2005; Orhan et al., 2010). Then, the significant antifungal activity of *S. dulcis* could be helpful for sanitary purpose in humans, plants and animals.

Conclusion

The four extracts from *S. dulcis* presented significant inhibition of bacterial and fungal proliferation *in vitro*. This antimicrobial activity is related to the amount of tannins and other phytochemicals in each extract. The activity of the extracts from *S. dulcis* on resistant bacteria as *B. cereus* and *S. aureus* could be useful for the management of various multi-resistant infections.

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