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CHEMICAL COMPOSITION AND ANTIMICROBIAL POTENTIAL OF SELECTED MEDICINAL PLANTS

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ABSTRACT

Different extracts from five medicinal plants were investigated for their antibacterial and antifungal activities by agar diffusion and microdilution methods. The extracts selectively exhibited antibacterial effect and the most active was the aqueous-acetone extract of *Euphorbia balsalmifera* against the Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* with diameters of inhibition zones (d) exceeding 9mm and the minimal inhibitory concentrations less than 5mg/ml. This extract also displayed significant antifungal activity against brown rot *Fomitopsis palustris* and carbamic acid methyl ester was identified by gas chromatography/mass spectrometer as a main contributor to this antimicrobial activity. The aqueous-acetone extract of *Sporobolus pyramidalis* was significantly active against all the fungal strains (d≥9mm) with an exception on *Pycnoporus sanguineus*. *Scoparia dulcis* was the most active against both white rot and brown rot fungi (d≥14mm). The antifungal activities of these extracts were found to be comparable to those of the standard Glycyrrhizin Acid Dipotassium Salt, foreseeing their use as sources of fungistats.

KEY WORDS: medicinal plant, resistant bacteria, fungistat, *Sporobolus pyramidalis*, *Euphorbia balsamifera*, wood decay

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1. INTRODUCTION

Microorganisms, including bacteria and fungi are responsible for various infections in humans, animals and plants. Because microorganisms are continuously developing resistances to current antibiotics, infectious diseases are still the world's leading cause of premature deaths[1]. *Bacillus licheniformis, Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphyloccocus aureus* are among the most widespread and resistant bacteria involving in various diseases. Beside this, plant pathogens and especially fungi, are responsible for yield reductions in crops and biodegradation of wood worldwide, causing a serious problem for both wood structure and forest management[2]. Brown-rot and white-rot basidiomycetes fungi cause the most destructive type of wood decay; the brown-rot fungi selectively removes cellulose and hemicelluloses compounds, whereas the white-rot fungi causes degradation of all cell wood components[3]. White-rot *Pycnoporus sanguineus, Schizophyllum commune, Trametes versicolor* and brown-rot *Fomitopsis palustris, Gloeophyllum trabeum* are basidiomycetes fungi responsible for different infections in plants and even in human. *Schizophyllum commune* for example, can cause invasive infections like brain abscesses[4] and sinusitis[5]. Owing to control resistance to current antibiotics and to manage infectious diseases, natural products are getting greater interest and many research projects are targeting plants as sources of antimicrobial drugs[6,7]. Various plant extracts have been traditionally used to protect crops and seeds[8]. Ethno-pharmacological surveys indicated that *Euphorbia balsamifera* is used to treat chronic wound[9] while *Fadogia agrestis, L. anobrya, Scoparia dulcis and Sporobolus pyramidalis* were reported to have been implicated in various anti-infective remedies[10]. Therefore, there is a lack of information about the effect of these plants on various microbial strains that are pathogenic in human and plants. Then the current study was carried out to investigate about the potential of these tropical plant species to fight most common pathogenic bacteria and fungi in animals and plants along with the analysis of their volatile components by Gas Chromatography-Mass Spectrometer.

2. MATERIALS AND METHODS

2.1. Plant material

Aerial parts of five herbaceous plant species *Euphorbia balsamifera* Ait. (Euphorbiaceae), *Lepidaghathis anobrya* Nees (Acanthaceae), *Fadogia agrestis* Schweinf .Ex Hiern. (Rubiaceae), *Sporobolus pyramidalis* P. Beauv .(Poaceae) and *Scoparia dulcis* L. (Scrophulariaceae) were freshly collected on June 2013 at Gampela located at 25 km east of ouagadougou, Burkina Faso. Taxonomic identification was verified by Prof. Jeanne F. Millogo (Laboratoire de Biologie et Ecologie Vegetales, University of Ouagadougou, Burkina Faso) and a voucher specimen was deposited for each plant under the following numbers: *Euphorbia balsamifera* (EB_aca 001), *Lepidaghathis anobrya* (LA_nca 001), *Fadogia agrestis* (FA_sca 001), *Sporobolus pyramidalis* (SP_pca 001) and *Scoparia dulcis* (SD_ca 002). The plants were air-dried in the laboratory and then reduced into powder for future use.

2.2. Chemicals

All the chemicals were at analytical grade. Acetone, methanol and dimethylsulfoxide were purchased from Fisher, nutrient agar medium and nutrient broth medium from Oxoid LTD England, p-iodonitrotetrazolium (INT) and sodium chloride from Sigma, Glycyrrhizin Acid Dipotassium Saltfrom from WAKO Tokyo, malt extract from Becton Dickinson France, potato dextrose agar from Merck Germany, tetracycline and chloramphenicol from Sigma-Aldrich.

2.3. Extraction

An amount of 25g of powder from each plant was boiled for 45 minutes to make a decoction which was air-dried at 50°C to give the water extract. Another 25g of powder from each plant was soaked in 250ml of a mixture of acetone/water (70/30 v/v) for 48h, filtered and evaporated under reduced pressure (132 hPa, 40°C) and then air-dried at 50°C to give a dried extract (aqueous-acetone extract). Then, both

of the two extraction processes was repeated three times.

2.4. Antibacterial activity

The antibacterial activity of the plant extracts was assayed as described previously[11] with slight modifications.

Preparation of bacterial inoculum

Five bacterial strains (*Bacillus licheniformis* ATCC 12759, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853 and *Staphyloccocus aureus* ATCC 12600 were obtained from School of Biological Sciences, Universiti Sains Malaysia. Each strain was grown separately on nutrient agar at 37°C for 24 hours, and then an aliquot was suspended in nutrient broth and stand overnight at 37°C. The bacterial inoculum was then prepared by diluting this bacterial suspension in a sterile saline solution (0.9% NaCl) to get approximately 10^6 colony-forming units/ml as compared to the turbidity of 0.5 MacFarland standards.

Preparation of culture medium

Twenty-eight grams of nutrient agar were mixed with 1000 ml of sterile distilled water. The mixture was then sterilized by autoclaving at 121°C for 20 minutes. Under aseptic conditions in the laminar flow hood, 20 ml of agar medium was uniformly dispensed into sterile Petri dishes. They were then covered and cooled down at room temperature until the culture medium hardened. The inoculation of the bacterial culture on the agar surface was done by the spread plating technique.

Disc application of the samples

The sample solution (20, 40 and 60mg/ml in water containing 10% DMSO) was sterilized by filtration through microfilter (0.2µm) and 10µl was impregnated on sterilized paper disc (6 mm in diameter, Advantec Tokyo Inc). After drying in aseptic conditions, the discs were placed on the inoculated nutrient agar surface and then gently pressed down to ensure contact with the agar surface. The Petri dishes were then incubated for 18 hours at 37°C. Then, the diameter (in mm) of the inhibition zone around each disc was measured. Antibacterial activities were indicated by a clear zone of growth inhibition around the paper disc. Each test was repeated three times. Tetracycline and chloramphenicol (10µl, 1mg/ml in water containing 10% DMSO) were used as positive controls while a water solution containing 10% DMSO was used as negative control.

Microdilution method

The microdilution method was used to determine the minimal inhibitory concentration (MIC) along the five bacterial strains. 100µl of nutrient broth medium (21mg/ml) were placed into each 96 wells of the microplates. The extract solutions (100µl, 20 mg/ml) were added into the first rows of microplates and two-fold dilutions (10 to 0.0097mg/ml) were made by dispensing the solutions to the remaining wells. Then 100µl of nutrient broth and 10µl of bacterial inoculum were added into all the wells. For each dilution, a negative control without inoculum (100µl of medium, 100µl of extract solution and 10µl of saline solution 0.9%) was prepared for optical comparison. A control without sample solution (100µl of medium, 100µl of DMSO 10% and 10µl of inoculum) was prepared to ensure bacterial growth. Another well was filled with 100µl of medium, 100µl of DMSO 10% and 10µl of saline solution 0.9% as a control to ensure their sterility. Each test was carried out in triplicate. The sealed microplates were incubated at 37°C for 18h. Then, p-iodonitrotetrazolium violet (10µl, 0.5mg/ml in water) was added. The lowest concentration of the extract that completely inhibited macroscopic growth (with no purple colour) was determined by optical comparison with the corresponding negative control and was noted as minimum inhibitory concentration (MIC).

2.5. Antifungal activity

The antifungal activity was assayed as described in a previous study[12] with some modifications. Five fungal strains were used: *Gloeophyllum trabeum* (L.: Fr.) Murr. (MI-102), *Pycnoporus sanguineus* (Pers.: Fr.) Murr. (KUM 70097), *Fomitopsis palustris* (Berk Et Curt) Gilbn.&Ryv. (FFPRI 1030), *Schizophyllum commune*(Fr.) (KUM 790036) and *Trametes versicolor* (L.Fr.) Pilat. (FFRPI 0507). Each strain was grown separately on potato dextrose agar at 25°C and 70% relative

humidity for 7 days, and then an aliquot was suspended in a liquid malt extract medium and incubated for another 7 days. The hypha was then homogenized and dilute in a sterile saline solution (0.9% NaCl) as compared to the turbidity of 0.5 MacFarland standard. Sterilized potato dextrose agar medium was then poured into the petri dishes and after cooling down, it was impregnated with the diluted hypha by the spread plating technique. The paper discs were then impregnated with 10µl of the sample solution (20, 40 and 60mg/ml in water containing 10% DMSO) and apply on the agar surface as described above. Glycyrrhizin Acid Dipotassium Salt (GADS) was used as positive control and 10% DMSO as negative control. Antifungal activity was detected by a clear zone of growth inhibition around the paper disc after 72h incubation at 25°C.

2.6. Gas Chromatography-Mass Spectrometer analysis of the samples

Shimadzu-GC-9A gas chromatograph, FID at 220, N 2 at 1.0 ml/min, SPB-5 capillary column (30 m × 0.53 mm ID; 0.3 µmdf), split ratio 1:30 injector temperature 240°C, column temperature maintained at 50°C for the first five minutes and then raised to 235°C (5°C per minute) followed by five minutes at 235°C. GC-MS: Hewlett-Packard 5890 gas chromatograph, combined with a Jeol JMS-HX 110 mass spectrometer with source at 270°C at 70 eV. Injector was set at 270°C with splitting ratio 1:30. The analysis was performed on the aforementioned program on equivalent column HP-5 (25 m × 0.22 mm and 0.25 µmdf). A mass spectral survey was performed using the NIST mass spectral search program 2008 with similarity indices more than 90%.

2.7. Statistical analysis

All the tests were run in triplicate and data are presented as mean ± standard deviation. Data were analyzed by 1-way analysis of variance followed by the Tukey multiple-comparison test with XLSTAT 2013.4.08. A p-value less than 0.05 was used to evaluate statistical significance of the findings.

3. RESULTS AND DISCUSSION

The antimicrobial potential of the five selected plant species was assayed on five bacterial strains. The antibacterial activity was evaluated by disc diffusion method and the diameter of inhibition zone (d) was measured around the paper disc loaded with different amount of sample solution (200µg, 400µg and 600µg) as indicated in Table 1. The different extracts showed selective antibacterial activities along the five strains. Increasing the dose of the extract did not showed significant change in the antibacterial effect. The range of the different doses tested may not affect the bacterial growth, but higher amounts may involve greater inhibition. Therefore, significant changes can be observed across the different dose of the aqueous-acetone extract of *E. balsamifera* on the Gram-negative bacteria *E. coli* and *K. pneumoniae* (d≥15mm); it also got significant effect on Gram negative bacteria *P. aeruginosa* (d= 10mm) but not dose-dependently. The activity of this extract on *E. coli* was also noted as the most biocidal effect along the five bacteria and among all the extracts. Therefore Gram-positive bacteria (*Bacillus licheniformis* and *Staphylococus aureus*) are less sensitive to this extract (d=7±0mm) as compared to Gram-negative bacteria. This plant sample may have high diffusion potential to reach the external double-bond membrane of Gram-negative bacteria since this membrane make them impermeable and resistant to antibiotics[13]. The most abundant compound found in this active aqueous-acetone extract of *E. balsamifera* was n-decane but this only cannot justified its antibacterial effect since n-decane is also present in all the other extracts and in greater amount (29.57 to 42.26%) but did not displayed greater antibacterial activity (Table 4). The similar results were also observed for n-undecane which is present in the extracts (13.22 to 24.13 %) in significant amount. Moreover, these hydrocarbons and derivatives were cited to possess lower antimicrobial properties because of their limited hydrogen bound capacity and their low water solubility that limits their diffusion through the medium[14].

Furthermore, the components present in the substantial proportions are not necessarily responsible for a great share of the total activity and the antibacterial activity of this extract from *E. balsamifera* can be explained by either the synergistic effect of the different components and/or by the presence of other

components that may be active even in small proportions. Then, the presence of carbamic acid methyl ester in this extract is noteworthy and could involve greater antibacterial and antifungal effects since this compound and its derivatives were considered as potent antimicrobial agents[15,16] against numeral microbes including the Gram-negative bacteria tested in this study. At any tested dose, all the five bacterial strains had resistance to the water extract of *S.pyramidalis* (Table 1). Moreover, at the highest dose (600µg/disc) *K. pneumonia* was not sensitive to the aqueous-acetone extract of *L. anobrya*, *B. licheniformis* to the water extract of *F. agrestis*, *S. aureus* to the water extracts of *F. agrestis* and *L. anobrya*, *P.aeruginosa* to the water extracts of *E. balsamifera* and *S. dulcis* and also to the aqueous-acetone extract of *L. anobrya*. Chloramphenicol and tetracycline were used as standards at a lower amount (10µg/disc) and they exhibited stronger antibacterial effect along the different strains as compared to plant samples. Therefore, *P. aeruginosa* was found to be totally resistant to chloramphenicol while it was significantly sensitive to the aqueous-acetone extract of *E. balsamifera*. *Pseudomonas aeruginosa* was cited to develop resistance to chloramphenicol through acetyltransferase enzymes that inactivate the drug or by inducing a blockage of bacterial permeability[17]. The minimal inhibitory concentrations (MIC) as determined by the microdilution method are presented in Table 2. Gram-positive bacteria and also the Gram negative bacterium *K. Pneumonia are* globally less sensitive (MIC≥10mg/ml) along the different extracts as compared to the two Gram negative bacteria (*E. coli* and *P. aeruginosa*), except for the aqueous-acetone extracts of *E. balsamifera*, *S. dulcis* and *L. anobrya* which got MICs inferior to 10 mg/ml. In general, these two sensitive Gram negative bacteria (*E. coli* and *P. aeruginosa*) are more sensitive to aqueous-acetone extract of *E. balsamifera*, *F. agrestis* and *S. dulcis* (MIC≤2.5mg/ml). This is in accordance with the results of inhibition zones as displayed in Table 1 where the Gram-negative bacteria were also the most sensitive as compared to Gram-positive bacteria. The slight variations across the two methods might be related to the difference due to the diffusion and solubility in the agar and broth media.

The antifungal activities of the plant extracts were assayed on five fungal strains by the disc diffusion method (Table 3). Different amounts of sample solution (200, 400 and 600µg) were loaded on the paper disc and the diameter of inhibition was measured after 72h. All the fungi are sensitive to the standard Glycyrrhizin Acid Dipotassium Salt (GADS) with d≥ 8mm, but they were selectively sensitive to the different extracts except *Pycnoporus sanguineus* which is apart from the others since it was not sensitive to the different extracts at any dose. The extracts of *Fadogia agrestis* were also not active on any fungal strain. Therefore the aqueous-acetone extract of *S. pyramidalis* exhibited significant antifungal activity against all the strains (d≥ 9mm) in regard to the standard GADS (d≥ 9mm). This plant commonly known as giant rats tail grass is considered as an undesirable pasture species for grazing animals[18] and also as a serious agricultural and environmental weed in tropical and subtropical areas[19]. However, such findings on the antifungal properties of *S. pyramidalis* in the present study might be considered as valuable for animals and plants welfare since it can involve beneficial effects as anti-infective agent and also protect from brown-rot and white-rot wood decay. The greater antifungal activity was observed with the water extract of *S. dulcis* against *Gloeophyllum trabeum* and *Trametes versicolor* (d= 31±1 mm and d=23±2 mm respectively). It is difficult to attribute the activity of a complex mixture to a particular component and the main compound identified in this water extract of *S. dulcis* as 2,2-dimethoxybutane (61.23%) might involve greater fungicidal effect in synergy with other non-volatile compounds as polyphenols that were previously quantified in *S. dulcis*[20] and also known to exert an antimicrobial effect in relation with their hydrogen donating ability[21]. The observed antifungal potential of these plants can help to increase yields in crops and forest management.

Table 1
Diameters of inhibition zones (mm) of bacterial growth by water and aqueous-acetone extracts

			E.bal		***F.agrestis***		***L.anobrya***		***S.dulcis***		***S.pyr***		**Standards**	
		Amounts (µg/disc)	**W**	**W-A**	**W**	**W-A**	**W**	**W-A**	**W**	**W-A**	**W**	**W-A**	**C**	**T**
Gram negative bacteria	***E. c***	200	8 ± 1^{b}	9 ± 2^{c}	8 ± 0^{b}	9 ± 1^{d}	8 ± 0^{c}	7 ± 0^{b}	8 ± 0^{c}	R^{a}	R^{a}	R^{a}	29 ± 0^{c}	42 ± 0^{b}
		400	8 ± 1^{b}	15 ± 1^{d}	8 ± 1^{b}	9 ± 1^{d}	8 ± 0^{c}	7 ± 1^{b}	8 ± 1^{c}	R^{a}	R^{a}	R^{a}	nt	nt
		600	10 ± 0^{c}	20 ± 1^{e}	8 ± 1^{b}	10 ± 1^{e}	8 ± 1^{c}	12 ± 2^{c}	8 ± 1^{c}	10 ± 1^{c}	R^{a}	9 ± 0^{bc}	nt	nt
	K. p	200	R^{a}	7 ± 0^{b}	R^{a}	8 ± 1^{c}	R^{a}	R^{a}	R^{a}	8 ± 0^{b}	R^{a}	R^{a}	29 ± 0^{c}	30 ± 0^{b}
		400	7 ± 0^{b}	9 ± 0^{c}	R^{a}	8 ± 1^{c}	7 ± 0^{b}	R^{a}	R^{a}	8 ± 0^{b}	R^{a}	R^{a}	nt	nt
		600	7 ± 0^{b}	15 ± 2^{d}	7 ± 0^{b}	10 ± 1^{e}	9 ± 0^{d}	R^{a}	7 ± 0^{b}	8 ± 0^{b}	R^{a}	R^{a}	nt	nt
	P. a	200	R^{a}	10 ± 0^{c}	R^{a}	8 ± 0^{c}	R^{a}	R^{a}	R^{a}	7 ± 1^{b}	R^{a}	7 ± 0^{b}	R^{a}	16 ± 0^{a}
		400	R^{a}	10 ± 0^{c}	7 ± 0^{b}	8 ± 0^{c}	R^{a}	R^{a}	R^{a}	7 ± 0^{b}	R^{a}	7 ± 0^{b}	nt	nt
		600	R^{a}	10 ± 0^{c}	8 ± 0^{b}	9 ± 0^{d}	7 ± 0^{b}	R^{a}	R^{a}	7 ± 0^{b}	R^{a}	12 ± 0^{c}	nt	nt
Gram positive bacteria	***B. l***	200	R^{a}	7 ± 0^{b}	R^{a}	7 ± 0^{b}	R^{a}	R^{a}	R^{a}	8 ± 1^{b}	R^{a}	7 ± 0^{b}	19 ± 2^{b}	30 ± 0^{b}
		400	10 ± 0^{c}	7 ± 0^{b}	R^{a}	8 ± 0^{c}	9 ± 1^{d}	7 ± 0^{b}	R^{a}	8 ± 1^{b}	R^{a}	7 ± 0^{b}	nt	nt
		600	13 ± 0^{d}	7 ± 0^{b}	R^{a}	12 ± 1^{f}	9 ± 1^{d}	$9\pm0^{b,c}$	9 ± 1^{d}	10 ± 0^{c}	R^{a}	7 ± 1^{b}	nt	nt
	S. a	200	7 ± 0^{b}	R^{a}	R^{a}	R^{a}	R^{a}	8 ± 0^{b}	7 ± 0^{b}	R^{a}	R^{a}	R^{a}	34 ± 1^{c}	33 ± 1^{b}
		400	7 ± 0^{b}	7 ± 0^{b}	R^{a}	7 ± 0^{b}	R^{a}	8 ± 0^{b}	7 ± 0^{b}	8 ± 0^{b}	R^{a}	R^{a}	nt	nt
		600	11 ± 0^{c}	7 ± 0^{b}	R^{a}	9 ± 0^{d}	R^{a}	8 ± 0^{b}	7 ± 0^{b}	10 ± 0^{c}	R^{a}	R^{a}	nt	nt

Values are expressed as mean of 3 replicates ±standard deviation. Values (a, b, c, d, e, f) within each column with different superscripted letters differ significantly (P ≤0.05). R= no inhibition (d≤ 6mm). W= water extract, W-A= aqueous-acetone extract. E.c= Escherichiacoli, K.p= Klebsiella pneumonia, P.a= Pseudomonas aeruginosa, B.l= Bacillus licheniformis, S.a= Staphylococusaureus. C= chloramphenicol (10µg/disc), T= tetracycline (10µg/disc). nt= not tested

Table 2
Minimal inhibitory concentrations (mg/ml) of water and aqueous-acetone extracts

			Gram-negative			**Gram-positive**	
			E. c	**K. p**	**P. a**	**B. l**	**S. a**
Plant species	**Extracts**	**Yields (%)**					
E. balsamifera	Water	22.04 ± 0.62^{c}	10	>10	5	10	>10
	H_2O-acetone	34.9 ± 8.57^{d}	2.5	5	1.25	2.5	10
F. agrestis	Water	17.2 ± 4.58^{b}	2.5	>10	>10	>10	10
	H_2O-acetone	$13.86\pm1.10^{a,b}$	1.25	10	2.5	10	10
L. anobrya	Water	10.54 ± 1.89^{a}	5	>10	2.5	>10	>10
	H_2O-acetone	$13.56\pm4.69^{a,b}$	2.5	10	5	10	2.5
S. dulcis	Water	12.06 ± 0.59^{a}	10	>10	5	>10	>10
	H_2O-acetone	12.36 ± 2.77^{a}	1.25	5	1.25	2.5	5
S. pyramidalis	Water	$14.36 \pm 2.48^{a,b}$	>10	>10	>10	>10	>10
	H_2O-acetone	10.24 ± 0.39^{a}	>10	>10	5	>10	>10

Yield values are expressed as mean of 3 replicates ±standard deviation. Values (a, b, c, d) within each column with different superscripted letters differ significantly (P ≤0.05). E.c= Escherichia coli; K.p= Klebsiella pneumonia; P.a= Pseudomonas aeruginosa; B.l= Bacillus licheniformis; S.a= Staphylococusaureus

Table3
Inhibition diameters (mm) of fungal growth by water and aqueous-acetone extracts

Amounts (µg/disc)		*E.bal*		*F.agrestis*		*L.anobrya*		*S.dulcis*		*S.pyr*		Standard
		W	W-A	W	W-A	W	W-A	W	W-A	W	W-A	GADS
F.p	200	R^{a}	13±1^{b}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	14±1^{b}	R^{a}	11±1^{c}	10±1^{b}
	400	R^{a}	13±1^{b}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	14±1^{b}	R^{a}	10±1^{c}	nt
	600	R^{a}	14±1^{b}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	15±1^{b}	R^{a}	12±1^{c}	nt
G.t	200	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	31±1^{c}	R^{a}	R^{a}	12±1^{c}	9±1^{a}
	400	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	33±1^{c}	R^{a}	R^{a}	12±1^{c}	nt
	600	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	33±1^{c}	R^{a}	R^{a}	12±1^{c}	nt
P. s	200	R ^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	8±1^{a}
	400	R ^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	nt
	600	R ^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	nt
S.c	200	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	9±1^{bc}	12±1^{c}
	400	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	9±1^{bc}	nt
	600	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	R^{a}	9±1^{bc}	nt
T.v	200	R^{a}	R^{a}	R^{a}	R^{a}	9±0^{b}	R^{a}	23±2^{b}	R^{a}	10±0^{b}	10±1^{c}	9±1^{a}
	400	R^{a}	R^{a}	R^{a}	R^{a}	9±1^{b}	R^{a}	26±2^{b}	R^{a}	10±1^{b}	12±1^{c}	nt
	600	R^{a}	R^{a}	R^{a}	R^{a}	9±1^{b}	R^{a}	27±2^{b}	R^{a}	10±0^{b}	12±0^{c}	nt

Values are expressed as mean of 3 replicates ±standard deviation. Values (a, b, c, d, e, f) within each column with different superscripted letters differ significantly ($P \leq 0.05$). R= no inhibition (d≤ 6mm). F.p= Fomitopsispalustris; G.t= Gloeophyllumtrabeum; P.s= Pycnoporussanguineus; S.c= Schizophyllum commune; T.v= Trametesversicolor.nt= not tested

Table 4
List of the identified compounds in the aqueous-acetone extracts

No	RT	Name	E.b	F.a	L.a	S.d	S.p
			Area (%)				
1	3.42	2,2-Dimethoxybutane	-	-	12.75	-	-
2	8.21	Carbamic acid methyl ester	5.71	-	-	-	-
3	11.86	Decane <n->	29.57	38.4	31.18	41.74	42.26
4	13.19	4-Chloro-1-azabicyclo[2.2.2]octane	14.58	-	-	-	-
5	13.89	2,2-Dimethyl-propyl 2,2-dimethyl-propane-thiosulfinate	-	-	3.33	-	-
6	15.34	Undecane <n->	14.4	21.19	13.22	20.6	24.13
7	17.31	Benzenehexanenitrile, .beta.,.beta.-dimethyl-.epsilon.-oxo-	-	-	4.5	-	-
8	26.15	o-Hydroxyacetophenonylidene-4,5-dimethyl-O-phenylenediamine	-	32.34	-	-	30.3
9	26.20	Ethyne, fluoro-	-	-	-	-	3.31

E.b = Euphorbiabalsamifera; F.a= Fadogiaagrestis; L.a= Lepidagathisanobrya;
S.d= Scopariadulcis; RT= Retention Time (min); -= not identified

CONCLUSION

Based on the findings in this work, the aqueous-acetone extract of *E. balsamifera* resulted in significant antimicrobial activity on both Gram-negative bacteria and brown-rot fungus *Fomitopsis palustris.* The antifungal activities of *Sporobolus pyramidalis* and *S. dulcis* are significant on both brown rot and white rot fungi except *Pycnoporus sanguineus.* These plants can be considered as potential sources of fungistat for the management of infections in both animals and plants.

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REFERENCES

1. Carey J, Fighting superbugs with superdrugs. Drug Disc. Today 9: 637-640 (2004).
2. Mahlo SM, McGaw LJ, Eloff JN, Antifungal activity of leaf extracts from South African trees against plant pathogens. Crop Protection, 29: 1529-1533 (2010).
3. Han M-J, Choi H-T, Song H-G, Degradation of phenanthrene by Trametes versicolor and its laccase. J. Microbiol, 42, 94-98 (2004).
4. Rihs JD, Padhye AA, and Good CB, “Brain abscess caused by Schizophyllum commune an emerging basidiomycete pathogen,” Journal of Clinical Microbiology, 34(7): 1628-1632 (1996).
5. Sigler L, Estrada S, Montealegre NA, Jaramilo E, Arango M, de Bedout C, “Maxillary sinusitis caused by Schizophyllum commune and experience with treatment,” Journal of Medical and Veterinary Mycology, 35(5): 365-370 (1997).
6. Eloff JN, Famakin JO, Katerere DRP, Isolation of an antibacterial stilbene from Combretumwoodii (Combretaceae) leaves. Afr. J. Biotechnol. 4: 1167-1171 (2005).
7. Aliero AA, Afolayan AJ, Antimicrobial activity of Solanumtomentosum. Afr. J. Biotechnol. 5:369-372 (2006).
8. Shai LJ, Bizimenyera ES, McGaw LJ, Eloff JN, Extracts of the leaves and twigs of the threatened tree Curtisiadentata (Cornaceae) are more active against Candida albicans and other microorganisms than the stem bark extract. S. Afr. J. Bot., 75: 363-366 (2009).
9. Inngjerdingen K, Nergård CS, Diallo D, Mounkoro PP, Paulsen BS, An ethnopharmacological survey of plants used for wound healing in Dogonland, Mali, West Africa. J. Ethnopharmacol. 92: 233-244 (2004).
10. Nacoulma OG, Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso. Cas du plateau central. Thèse d’état. Université de Ouagadougou, Tome II, 261p (1996).
11. Dev Sharma SC, Shovon MS, SarowarJahan MG, Asaduzzaman AKM, Rahman Md A, Biswas KK, Abe N, Roy N, Antibacterial and cytotoxic activity of bacillus methylotrophicus-scs2012 isolated from soil. J. Microbiol. Biotech.Food Sci. 2: 2293- 2307 (2013).
12. Kawamura F, Mahamud A, Sulaiman O and Hashim R, Antifungal activities of extracts from heartwood, sapwood and bark of 11 malaysian timbers against gloeophyllum trabeum and pycnoporus sanguineus, J. Tropical Forest Sci. 22(2): 170- 174 (2010).
13. Tian F, Li B, Ji B, Yang J, Zhang G, Chen Y, Luo Y, Antioxidant and antimicrobial activities of consecutive extracts from Gallachinensis: The polarity affects the bioactivities. Food Chem. 113: 173-179 (2009).
14. Griffin GS, Markham LJ, Leach ND, An agar dilution method for the determination of the minimum inhibitory concentration of essential oils. J. Ess. Oil Res. 12: 149-255 (2000).
15. Gautam N, Chourasia OP, Synthesis, antimicrobial, insecticidal and anthelmintic activity studies of some new alkyl/aryl 6-(4-(4-(4-methyl1-1H-1,2,3-triazol-1-yl)phenyl amino)phenyl)-N2,N4-bis(4-methoxyphenyl)-1,3,5-triazin-2,4-diamine carbamic acid ester derivatives. Indian Journal of Chemistry, 51B: 1020-1026 (2012).
16. Hernandez V, Crépin T, Palencia A, Cusack S, Akama T, Baker SJ, Bu W, Discovery of a Novel Class of Boron-Based Antibacterials with Activity against Gram-Negative Bacteria. Antimicrobial Agents and Chemotherapy 57(3): 1394-1403 (2013).
17. Nitzan Y, Rushansky NM, Chloramphenicol Acetyltransferase from Pseudomonas aeruginosa- a New Variant of the Enzyme. Curr.Microbiol. 5:261-265 (1981).
18. Rochinsky R, Mulindwa H, Galukande E, Wurzinger M, Mpairwe D, Okeyo AM, Sölkner J, Pasture use and management strategies in the Ankole

pastoral system in Uganda. Grass and Forage Science 67(2): 199-209 (2012).
19. Vogler WD, Bahnisch LM, Effect of growing site moisture stress and seed size on viability and dormancy of Sporobolus pyramidalis (giant rats tail grass) seed. Australian Journal of Experimental Agriculture 46 (11): 1473-1479 (2006).
20. Coulibaly AY, Kiendrebeogo M, Kehoe PG, Sombie PAED, Lamien CE, Millogo JF, Nacoulma, OG, Antioxidant and Anti-Inflammatory Effects of Scoparia dulcis L. J. Med. Food, 14(12): 1576-1582 (2011).
21. Soković M, van Griensven LJLD, Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, Agaricusbisporus. Europ. J. Plant Path. 116: 211-224 (2006).