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Phytochemical screening and antimicrobial activity of extracts of five aromatic and medicinal plants from Burkina Faso

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ABSTRACT

Antimicrobial resistance is a global concern word wild. New antibacterials are urgently needed to fight this problem. Indeed, medicinal plants are a potential source of new antimicrobial molecules. The objective of this study was to evaluate the antimicrobial properties of extracts of five aromatic and medicinal plants from Burkina Faso. Phytochemical screening by solution reactions in test tubes and by Thin Layer Chromatography was performed. The antimicrobial activity of the extracts was evaluated by the impregnated disc diffusion method and the microdilution method. Phytochemical screening of the extracts of the different plants studied allowed the identification of secondary metabolites such as tannins, flavonoids, and saponosides. The methanol extract of *E. camaldulensis* showed the best antimicrobial activity against Gram + bacteria, especially against *Staphylococcus saprophyticus* with an inhibition zone diameter of 16.33 mm and a Minimum Inhibitory Concentration (MIC) of 156 µg/ml. Then, the methanol extract of *C. nardus* showed the best inhibitory activity on the Gram- *Escherichia coli* ATCC 35218 with an inhibition zone diameter of 11.66 ± 0.57 mm. These results could justify the traditional use of the studied plants in the treatment of certain pathologies in public health. In perspective, a bioguided fractionation of the active extracts will be performed to isolate and elucidate the structure of the compounds responsible for the activity.

Keywords: Tanins, flavonoid, *Eucalyptus camaldulensis*, *Cymbopogon nardus*, antibacterial activity.

INTRODUCTION

Infectious diseases caused by bacteria and fungi affect a high number of people worldwide and cause heavy economic losses related to decreased productivity caused by disease and increased cost of treatment (OMS, 2016). In recent decades, the study of microorganisms has seen an increasing interest following the emergence and progression of resistant and multi-resistant germs to the most commonly used antimicrobials, due to their intensive and irrational use. This antimicrobial

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resistance is a global public health threat of concern (Hawkey, 2008; Michael et al., 2014; OMS, 2016).

Aromatic and medicinal plants, which represent a rich source of bioactive molecules, have been used for many decades in the treatment of several pathologies (Akakpo et al., 2022; Ombouma et al., 2021; Sombié et al., 2018). According to the WHO, nearly 80% of the African population relies on traditional medicine for their primary health care due to the high cost of imported drugs and the geographical inaccessibility of medicines (OMS, 2002). Plants contain a very high number of bioactive chemical compounds such as alkaloids, flavonoids, tannins, carotenoids, saponins, essential oils, etc. These chemical compounds, with a wide range of physicochemical properties, exhibit a wide range of biological activities such as analgesic, antitumor, antimicrobial, antioxidant, insecticidal, anti-inflammatory, etc. (Launay, 2017).

The use of aromatic and medicinal plants in several fields thus makes local flora an important reservoir of active substances of interest in several sectors of activity. Indeed, the search for natural substances with antimicrobial activity derived from plants is an important scientific issue. In this goal, this study was initiated with the general objective to evaluate the antimicrobial properties of methanolic extracts of five aromatic and medicinal plants from Burkina Faso.

MATERIALS AND METHODS

Plant material

The plant material consisted of five aromatic and medicinal plants from the flora of Burkina Faso: *Cymbopogon nardus*, *Eucalyptus camaldulensis*, *Hyptis suaveolens*, *Lantana camara*, collected in the botanical garden of the Institut de Recherche en Sciences Appliquées et Technologique in Ouagadougou (N 12°25'28.2"; W 1°29'15.06") and *Lippia multiflora* collected in Zorgho (N 12°11.211'; W 000°43.454') during October 2018. This plant material was identified and authenticated by KOURA S. Paulin, botanist at the herbarium of the "Centre National de la Recherche Scientifique et Technologique (CNRST)". Reference specimens have been deposited at the CNRST herbarium.

Biological material

The microbial strains used in this study are listed in Table 1. These different strains belong to four families: two bacterial families (Enterobacteriaceae and Staphylococcaceae), one fungal family (Eurotiaeae) and one yeast family (Saccharomycetaceae).

Methods

Preparation of extracts

The plant material, previously washed with water and dry, was dried in the laboratory at room temperature and protected from the sun, then ground into powder and stored in clean bags. The extraction was carried out by maceration with solvents of increasing polarity. Thus, 40 grams of the plant material were successively extracted with 400 ml of hexane, dichloromethane (DCM) and methanol (MeOH) respectively under stirring for 24 hours. After filtration and dry concentration, the extraction yield was determined. The extracts were stored in a refrigerator at 4°C until use (Landoulsi, 2018).

Phytochimical screening

Phytochemical screening by solution reactions in test tubes and by Thin Layer Chromatography was performed according to the classical methods of (Ciulei) 1982 and Pachaly (1997).

Diffusion in agar medium

The methanolic extracts were tested for their antimicrobial bioactive potential by the impregnated disk diffusion method (Bauer et al., 1966). The extracts were solubilized in DMSO to obtain a solution of concentration equal to 200 mg/ml. Tests were performed on Mueller Hinton (MH) agar for bacteria, Sabouraud agar for yeast and Potato Dextrose Agar (PDA) for mold.

Sterile Wattman paper discs (6 mm in diameter) were individually impregnated with a volume of 20 µL of extract and then deposited with sterile forceps on the surface of the agar medium, previously seeded with a microbial suspension at the standardized

concentration (0.5 McFarland) using a sterile swab. Petri dishes were stored at 4°C for 2 hours and then incubated at 37°C for 24 hours for bacteria, at 30°C for 24 hours for yeast, and at 28°C for 48 h to 72 hours for molds. The antimicrobial activity of the extracts was determined by the presence of inhibition zone around the extract-impregnated disk. These inhibition zones were measured, and results obtained were expressed in millimeters (mm).

Only microbial strains showing susceptibility to active extracts were selected for determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) by the liquid microdilution method (Abedinia, 2013).

Determination of the Minimum Inhibitory Concentration (MIC)

It consisted in introducing a volume of 100 µL of sterile culture medium (Mueller-Hinton Broth) into the test wells. Then, 100 µL of the extract were added to well 1 and then a cascade dilution was performed in the different test wells in a geometric progression of the reason of 2 (10 to 0.07 mg/ml). A volume of 100 µl of standardized microbial inoculum (10^6 CFU/ml) was tested by adding it to the mixture.

The microplates were incubated in the oven for 24 hours at 37°C. Reading was done by colorimetric assay using 40 µl of iodonitrotetrazolium reagent (INT at the concentration of 2 mg/ml) after incubation for thirty minutes.

Determination of the Minimum Bactericidal Concentration (MBC)

It was determined by inoculating a volume of 100 µl of the wells containing MIC, MIC × 2, and MIC × 4 on new sterile culture media, which were incubated in the incubator for 24 hours at 37°C. The MBC/MIC ratios were calculated, to assess the intrinsic activity (bactericidal or bacteriostatic) of the active extracts.

Statistical analysis

Data were entered into Microsoft Office Excel 2016, and then means and standard deviations were calculated. Results are presented as mean ± standard deviation.

Analyses of variance (ANOVA) were performed using SPSS version 20 software. Means were compared using Fisher's least significant difference (LSD) method with a 95% confidence level.

Table 1: Microbial strains used for the antimicrobial activity test.

	Microbial strains	Gram	Family
Bactérial strains	*Escherichia coli* ATCC 35218	-	*Enterobacteriaceae*
	Klebsiella pneumoniae ATCC 700603	-	*Enterobacteriaceae*
	Klebsiella pneumoniae	-	*Enterobacteriaceae*
	Staphylococcus aureus ATCC 25923	+	*Staphylococcaceae*
	Staphylococcus aureus ATCC 6538	+	*Staphylococcaceae*
	Staphylococcus epidermidis ATCC 1532	+	*Staphylococcaceae*
	Staphylococcus saprophyticus	+	*Staphylococcaceae*
Yeast strain	*Candida albicans* CIP 4872		*Saccharomycetaceae*
Fungal strain	*Aspergillus* sp		*Eurotiaeae*

RESULTS

Extraction yield

Fifteen (15) extracts were obtained by successive exhaustion using solvents of increasing polarity with hexane, dichloromethane and methanol. The values are presented in Table 2. The extraction yield varies between 1.40% and 10.21% depending on the plant and the solvent used. The methanol extract of *E. camaldulensis* recorded the highest yield with a value of 10.21% followed by the methanol extract of *C. nardus* with a yield of 8.25%. The hexane extract of *L. multiflora* recorded the lowest yield with a value of 1.40%.

Phytochemical screening

Phytochemical screening of extracts from the different plants studied revealed the presence of a wide range of secondary metabolites, including tannins, flavonoids, saponosides, terpenes, and sterols. The results of these phytochemical tests are reported in Table 3. It is evident that chemical compounds such as tannins, flavonoids, and saponosides were present in most of the methanolic extracts and absent in the hexanolic extracts, except for the methanol extract of *C. nardus*, where saponosides were not detected. In addition, flavonoids were detected in all dichloromethane extracts. On the other hand, compounds such as terpenes and sterols were only detected in the hexane and dichloromethane extracts. In contrast, tube reactions and observation of TLC plates after using Dragendorff's reagent did not reveal detectable amounts of alkaloids in the extracts.

Diameters of the inhibition zones

The results of the antimicrobial activity of the methanol extracts of the studied plants are recorded in Table 4. Results shows that some extracts, especially the extracts of *E. camaldulensis* and *C. nardus*, show activity against the growth of some bacterial strains to a different degree. The methanol extract of *E. camaldulensis* produced larger inhibition zones against Gram+ bacteria with the inhibition zone diameters of 16.33 ± 0.57 mm; 13.33 ± 2.08 mm; 12.66 ± 1.52 mm and 11.66 ± 1.52 mm on *Staphylococcus saprophyticus; Staphylococcus epidermidis* ATCC 1532; *Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* ATCC 6538 respectively. The methanol extract of *C. nardus* produced smaller inhibition zones against Gram + bacteria with inhibition zone diameters of 09.33 ± 1.52 mm; 10.66 ± 1.15 mm; 09.66 ± 0.57 mm and 09.00 ± 1.00 mm on *Staphylococcus saprophyticus; Staphylococcus epidermidis* ATCC 1532; *Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* ATCC 6538 respectively.

MIC and MBC of extracts

The results of these two parameters in determining the antimicrobial properties of the extracts are reported in Table 5. The MIC varies between 0.156 to 5 mg/ml from one extract to another depending on the bacterial strain. The methanol extract of *E. camaldulensis* showed the best activities against *Staphylococcus saprophyticus* and *Staphylococcus aureus* ATCC 25923 strains with MICs of 156 µg/ml and 312 µg/ml respectively. The highest MICs, 5 mg/ml, were recorded with the methanol extract of *E. camaldulensis* on *Escherichia coli* strain ATCC 35218 and the methanol extract of *C. nardus* on *Staphylococcus epidermidis* strain ATCC 1532 respectively.

Table 2: Extraction yield of different plants.

Plants	Solvent	Yield (%)
E. camaldulensis	Hexan	1.74
	DCM	3.48
	MeOH	10.21
C. nardus	Hexan	3.25
	DCM	2.5
	MeOH	8.25
H. suaveolens	Hexan	2.96
	DCM	1.97
	MeOH	5.43
L. camara	Hexan	1.75
	DCM	1.50
	MeOH	5.10
L. multiflora	Hexan	1.40
	DCM	3.50
	MeOH	1.53

DCM: Dichloromethane, MeOH : methanol.

Table 3: Phytochimical screening of extracts.

Plants	Solvent	Chemical groups tested				
		Tanins	**Flavonoids**	**Alkaloids**	**Terpens et sterols**	**Saponosids**
E. camaldulensis	Hexan	-	-	-	+	-
	DCM	-	+	-	+	-
	MeOH	+	+	-	-	+
C. nardus	Hexan	-	-	-	+	-
	DCM	-	+	-	+	-
	MeOH	+	+	-	-	-
H. suaveolens	Hexan	-	-	-	+	-
	DCM	-	+	-	+	-
	MeOH	+	+	-	-	+
L. camara	Hexan	-	-	-	+	-
	DCM	-	+	-	+	-
	MeOH	+	+	-	-	-
L. multiflora	Hexan	-	-	-	+	-
	DCM	-	+	-	+	-
	MeOH	+	+	-	-	+

+: presence of the chemical group; - : absence of the chemical group; DCM: Dichloromethane, MeOH: methanol.

Table 4: Diameters of the inhibition zones of the extracts towards the studied microbial strains.

Microbial strains		**Diameters of the inhibition zones (mm)**					
Bacterial strains	**Gram**	***E. camaldulensis***	***C. nardus***	***H. suaveolens***	***L. camara***	***L. multiflora***	**Ciprofloxacine (5µg)**
Escherichia coli ATCC 35218	-	08.33 ± 1.15	11.66 ± 0.57	< 8	< 8	< 8	28.33 ± 0.57
Klebsiella pneumoniae ATCC 700603	-	< 8	< 8	< 8	< 8	< 8	18.33 ± 0.57
Klebsiella pneumoniae	-	< 8	< 8	< 8	< 8	< 8	18.00 ± 1.00
Staphylococcus aureus ATCC 25923	+	12.66 ± 1.52	09.66 ± 0.57	< 8	< 8	< 8	25.66 ± 0.57
Staphylococcus aureus ATCC 6538	+	11.66 ± 1,52	09.00 ± 1.00	< 8	< 8	< 8	23.33 ± 0.57
Staphylococcus epidermidis ATCC 1532	+	13.33 ± 2.08	10.66 ± 1.15	< 8	< 8	< 8	21.00 ± 1.00
Staphylococcus saprophyticus	+	16.33 ± 0,57	09.33 ± 1.52	< 8	< 8	< 8	30.33 ± 0.57
Yeast and Fungal strains		***E. camaldulensis***	***C. nardus***	***H. suaveolens***	***L. camara***	***L. multiflora***	**Nystatine (100UI)**
Candida albicans CIP 4872		<8	<8	<8	<8	<8	22.00 ± 1.00
Aspergillus sp		<8	<8	<8	<8	<8	20.66 ± 0.57

Values are expressed as mean of three trials ± standard deviation; < 8: no activity.

Table 5: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of active extracts.

		CMI (mg/mL)		**CMB (mg/mL)**		**CMB/CMI**	
Bacterial strain	**Gram**	***E. camaldulensis***	***C. nardus***	***E. camaldulensis***	***C. nardus***	***E. camaldulensis***	***C. nardus***
Escherichia coli ATCC 35218	-	5	2.5	>10	>10	-/-	-/-
Staphylococcus aureus ATCC 25923	+	0.31	1.25	0.62	5	2	4
Staphylococcus aureus ATCC 6538	+	1.25	2.5	2.5	10	2	4
Staphylococcus epidermidis ATCC 1532	+	2.5	5	>10	>10	-/-	-/-
Staphylococcus saprophyticus	+	0.15	2.5	0.31	10	2	4

All experimental measurements were performed in triplicate; -/- : means not determined.

DISCUSSION

The quality and quantity of the extracts depend on the natural sources, the structures of the target compounds, the nature of the solvent, and the type of process used (Karacabey et al., 2013).

Previous studies have reported that methanolic extracts of the aerial part of *C. nardus* were detected in addition to tannins, flavonoids, and other chemical groups such as iridoids, proanthocyanidins, and phenolic acids (Gebashe et al., 2020). Petroleum ether, methanol and ethanol extracts of *E. camaldulensis* leaves contain in addition to tannins, flavonoids, and other chemical groups such as alkaloids, saponins, steroids, carbohydrates, and cardiac glycosides (Chuku et al., 2016 ; Dianda et al., 2020). Phytochemical screening of methanolic extracts of *H. suaveolens* leaves revealed chemical groups such as coumarins, mucilages, sterols, triterpenes, cardiotonic heterosides, oses, and holosides in addition to tannins, flavonoids, and saponosides (Kone, 2009). Chemical groups such as tannins, saponins, and sterols/triterpenes were detected in methanolic extracts of *L. camara* twigs (Bangou, 2012). Phytochemical analysis of the DCM extract of *L. multiflora* revealed the presence of chemical groups such as alkaloids, carbohydrates, and tannins (Dabire et al., 2015).

The differences observed in phytochemical composition could be explained by the fact that the phytochemical composition of the same plant collected from different locations is influenced by environmental factors (Borokini and Ayodele, 2012).

On the other hand, the methanol extract of *C. nardus* exhibited the best inhibitory activity on Gram- bacteria - *Escherichia coli* ATCC 35218 with an inhibition zone diameter of 11.66 ± 0.57 mm versus 08.33 ± 1.15 mm for the methanol extract of *E. camaldulensis*. However, the inhibitory activity of these extracts against bacteria is lower than that of the reference antibiotic (Ciprofloxacin, 5 µg).

Authors reported that methanol extracts of *E. camaldulensis* exerted high activity against *Bacillus subtilis; Klebsiella* spp; *Pseudomonas aeruginosa*; *Salmonella typhi*; *Staphylococcus aureus* and *Yernisia enterocolitca* with inhibition zone diameters of 16 mm; 14 mm; 15 mm; 16 mm; 15 mm and 14 mm respectively (Olayemi and Adeniyi, 2008). The methanolic extracts of *L. camara* exhibited antibacterial activity against *Bacillus cereus* ATCC 9144; *Escherichia coli ATCC* 25922; *Klebsiella pneumoniae*; *Pantoea* sp; *Pseudomonas aeruginosa*; *Shigella flexneri*; *Staphylococcus aureus* ATCC 25923 and *Streptococcus agalactiae* with inhibition zone diameters of 11 mm; 11.33 mm; 10 mm; 12 mm; 11 mm; 12.33 mm; 11.33 mm and 11.16 mm respectively (Bangou, 2012). The methanolic extracts of *H. suaveolens* exhibited antimicrobial activity against *Escherichia coli; Pseudomonas aeruginosa; Staphylococcus aureus; Proteus vulgaris; Aspergillus niger; Aspergillus flavus; Fursarium* sp and *Rhizopus* sp with inhibition zone diameters of 14 mm; 13 mm; 12 mm; 13 mm; 10 mm; 15 mm; 11 mm and 14 mm respectively (Mozhiyarasi and Anuradha, 2016).

The composition of active substances in the extracts could explain the observed variation in sensitivity, on the one hand. Indeed, the phytochemical screening performed on the different extracts revealed the presence of numerous phytochemical compounds such as tannins and flavonoids, which could be responsible for the observed antibacterial activity (Shan et al., 2007; Askun et al., 2009). On the other hand, the sensitivity of Gram + bacteria to the tested extracts compared to Gram - bacteria could explain the observed variation in sensitivity. This difference in sensitivity would be due to the differences in characteristics between the

walls of the two types of bacteria (Landoulsi, 2018).

Authors found a MIC of 1.25 mg/mL for methanol extracts of *E. camaldulensis* against *Staphylococcus aureus* (Olayemi and Adeniyi, 2008).

In addition, authors have reported that:

- extracts with a MIC lower than 100 µg/ml, present a good antibacterial activity;
- extracts with a MIC between 100 and 500 µg/ml, have moderate antibacterial activity;
- extracts with a MIC between 500 and 1,000 µg/ml, have poor antibacterial activity;
- extracts with MIC > 1,000 µg/ml, are inactive (Toyang et al., 2012; Ramla, 2017).

Based on this classification, the methanol extract of *E. camaldulensis* therefore exhibits moderate activity against *Staphylococcus saprophyticus* and *Staphylococcus aureus* ATCC 25923 strains. The richness of this extract in chemical compounds such as tannins and flavonoids, known for their antibacterial activity (Shan et al., 2007; Askun et al., 2009), could justify this observed antimicrobial activity.

Regarding the BMC, it appears from the analysis of Table 5, that the methanol extracts of *E. camaldulensis* and *C. nardus* show a bactericidal power, for a BMC/MIC ratio = 2 and BMC/MIC = 4 respectively, on most of the *Staphylococcus* strains studied with the exception of *Staphylococcus epidermidis* ATCC 1532 for which this parameter could not be established because the BMC was higher than the range of concentration used. Similarly, this parameter could not be established for the *Escherichia coli* ATCC 35218 strain, as the BMC was also higher than the concentration range used.

Conclusion

The results obtained indicated the existence of some chemical groups with high therapeutic values in some methanol extracts. The methanol extracts of *E. camaldulensis* and *C. nardus* contain active compounds against Gram+ bacteria of the genus *Staphylococcus* and Gram- bacteria *Escherichia coli*. These results could therefore justify the traditional use of the studied plants in the treatment of certain pathologies in public health. It would be interesting to perform a bioguided fractionation of the extracts in order to isolate and elucidate the structure of the compounds responsible for the activity.

COMPETING INTERESTS

The authors declare that they have no competing interests in the publication of this work.

AUTHORS' CONTRIBUTIONS

OLP and NCHR defined the theme and objectives of this study. They participated in the statistical analysis. BBV, BKR, ZC and SA defined methodology approach. OLP, MI and ZM carried out the experiences and wrote the first draft of manuscript. All authors read and approved this manuscript.

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