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Chemical composition, antioxidant and antimicrobial activities of the essential oil of *Vetiveria nigritana* (Benth.) Stapf roots from Burkina Faso

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ABSTRACT

The main objective of this study was to determine the chemical composition, antioxidant and antimicrobial properties of the essential oil of *Vetiveria nigritana* (Benth.) Stapf roots growing in Burkina Faso. The composition of the essential oil was analyzed by GC and GC / MS. DPPH radical scavenging method and FRAP test were used to demonstrate the antioxidant activity of the essential oil. Antimicrobial activity of the essential oil was determined using disk diffusion method and broth microdilution method. The major components of the essential oil of *V. nigritana* were dehydronigritene, zizanoic acid, preziza-7-(15)-en-3-ol, khusian-2-ol, ziza-6(13)-en-3-beta-ol, prezizaan-15-al and khusimone. The essential oil demonstrated weak radical scavenging power with an IC50 of 28.35±0.58µl and a low reduction capacity. The essential oil showed a relative good inhibitory action against strains of *Bacillus*, *Escherichia coli*, *Staphylococcus*, *Clostridium perfringens*, *Listeria monocytogenes*, *Micrococcus luteus*, *Enterococcus faecalis*, *Candida kefir* and *Saccharomyces cerevisiae* with inhibition diameters ranging from 11.5±0.71 mm to 23.5±0.71 mm. Minimum inhibitory concentrations of the essential oil of *V. nigritana* ranged from 1% to 8%, minimum bactericidal concentrations from 2% to 4% with minimum fungicidal concentration of 4%. These results suggest that essential oil of *V. nigritana* roots could be subjected for pharmaceutical drug formulations.

1. INTRODUCTION

Vetiveria nigritana (Benth.) Stapf (Poaceae) or black vetivergrass is a robust perennial grass growing from Mauritania to Nigeria and through to north-eastern, eastern and southern tropical Africa [1]. *V. nigritana* is a grass with short rhizomes which form compact tufts with numerous upright leaves. The leaves are very long (1 to 1.50 meters) and the flowers are arranged in racemes composed of 15 to 20 whorls [2]. In some African countries such as Senegal and Mali, *Vetiveria nigritana* roots are used to flavor and disinfect drinking water. Flavored water is specially recommended in cases of infants and children common diarrhea [2]. *V. nigritana* roots are also used in the treatment of stomach aches in Nigeria [3]. The chemical composition of the essential oil of *Vetiveria nigritana* roots from different localities has already been elucidated by some studies revealing a variability of the constituents of this essential oil [1, 4-6]. Few investigations have been found on the chemical composition and biological properties of *V. nigritana* from Burkina Faso. This study aimed to determine the proximate chemical composition, the antioxidant and antimicrobial activities of the essential oil extracted from *V. nigritana* roots from Burkina Faso.

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2. MATERIAL AND METHODS

2.1 Plant material

The roots of *Vetiveria nigritana* were collected in Loumbila near the city of Ouagadougou in July 2015. Voucher specimens are kept in the herbarium of the Biodiversity Information Center under the number ID 16964, University of Ouagadougou.

2.2 Essential oil extraction

The harvested roots of *V. nigritana* were air-dried and subjected to hydrodistillation during eight hours using a Clevenger-type apparatus [7]. The essential oil obtained was dried over anhydrous sodium sulfate and then stored at 4 °C waiting for analyzes. The extraction yield was calculated following this equation:

R (%) =V/W × 100, where V is the Volume of essential oil in ml and W the weight of dried roots in g.

2.3 Chemical analyzes

Chemical analyzes of the essential oil of *V. nigritana* roots was analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). GC analyzes were performed on a Hewlett-Packard HP 6890 equipped with a split/splitless injector (280°C), a split ratio 1:10, using a HP-5 capillary column (25 m x 0.25 mm, film thickness 0.25 m). The oven temperature was programmed from 50 to 300°C at a rate of 5°C/min. Helium was used as carrier gas at a flow rate of 1.1ml/min. The injection sample consisted of 1.0 µl of essential oil diluted to 10% (v/v) with acetone. GC/MS analyzes were carried out on a Hewlett-Packard 5973/6890 system operating in EI mode (70eV) using two different columns: a fused silica HP-5 MS capillary column (25 m x 0.25 mm, film thickness 0.25 m), and a HP-Innowax capillary column (60 m x 0.25 mm, film thickness 0.25 m). The temperature program for HP-5MS column was 50°C (5 min) rising to 300°C at a rate of 5 °C/min and for the HP-Innowax column, 50-250°C at a rate of 5°C/min. Helium was used as carrier gas at a flow rate of 1.1 ml/min. The oil components were identified by comparison of their mass spectra and their retention indices with those of reference compounds or with literature data [8-11].

2.4 Antioxidant activity

The antioxidant activity of the essential oil of *V. nigritana* roots was assessed by two methods: the DPPH radical scavenging method and the ferric reduction antioxidant power **(**FRAP) test.

2.4.1 DPPH radical scavenging assay

The radical scavenging power of the essential oil of *V. nigritana* roots was determined by the DPPH radical scavenging assay**.** This test was carried out as described previously by Joshi et al. (2010) [12]. Different amounts of the essential oil of *V. nigritana* (5, 10, 15, 20 and 25 µl) were mixed with 5 ml of an ethanolic solution of DPPH (0.004%). The mixture thus obtained was incubated in the dark for 30 min and the absorbance then read at 517 nm using a spectrophotometer (JASCO V-530 UV/VIS Spectrophotometer). BHT, ascorbic acid and quercetin at a concentration of 0.005M (5, 10, 15, 20 and 25 µL) used as reference antioxidants and a negative control were also tested under the same conditions. A low absorbance indicates a high scavenging power; the inhibition percentage was calculated according to the following equation:

% inhibition = $[(A_{blank}–A_{sample}) / A_{blank}] \times 100$ where A_{blank} is the absorbance of the negative control and A_{sample} the absorbance of the essential oil.

The antioxidant activity of the essential oil of *V. nigritana* was expressed as the inhibitory concentration 50 (IC50) which is defined as the amount of essential oil required to reduce the initial concentration of DPPH by 50%. The IC50 was calculated graphically using a linear regression [% inhibition = f (concentrations)]. Assay was done in triplicate and the standard solutions as well as the DPPH solution were prepared and used the same day.

2.4.2 Ferric reduction antioxidant power (FRAP)

The reducing power of the essential oil of *V. nigritana* was determined following the method used by Joshi et al. (2010) [12]. Different amounts of essential oil (5, 10, 15, 20 and 25 µl) were mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 30 min and then 2.5 ml of trichloroacetic acid (10%) were added to the mixture followed by centrifugation at 600g for 10 min. The supernatant was collected (5 ml) and mixed with 5 ml of distilled water and then 1 ml of iron chloride (0.1% $FeCl_3$) was added to the mixture and the absorbance measured at 700 nm using a spectrophotometer (JASCO V-530 UV/VIS Spectrophotometer). Ascorbic acid (0.1M) and quercetin (0.1M) were used as standards; a negative control (blank) was also included in each test. The standards and the blank were subjected to the same procedure as the essential oil. An increase in absorbance indicates an increase of the reducing power.

2.5 Antimicrobial activity

2.5.1 Microbial strains

Twenty (20) bacterial strains and four (04) fungal strains were used for antimicrobial testing. Gram positive bacteria used were: *Bacillus cereus* LMG 13569, *Bacillus subtilis ssp subtilis* ATCC 6051, *Clostridium perfringens*, *Enterococcus faecalis* ATCC 19433, *Listeria monocytogenes* NCTC 9863, *Micrococcus luteus* SKN 624, *Staphylococcus aureus* ATCC 2523, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* toxin (A+B) and *Staphylococcus hominis* B246. Gram negative bacteria were represented by *Escherichia coli* 81nr.149 SKN 541, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enteridis* P167807, *Salmonella infantis* SKN 557, *Salmonella nigeria* SKN 1160, *Salmonella typhimurium* SKN

1152, *Shigella dysenteria* 370, *Shigella flexneri* USCC 2007 and *Yersinia enterocolitica* 8A30 SKN 601. Fungal strains tested were *Candida albicans*, *Candida kefir*, *Candida tropicalis* and *Saccharomyces cerevisiae* KVL 013. All microorganisms were kindly provided by Food Technology Department (CNRST/IRSAT/DTA) and Center for Research in Biological, Food and Nutritional Sciences (CRSBAN) of Burkina Faso.

2.5.2 Agar disc diffusion method

The antimicrobial activity of the essential oil of *V. nigritana* roots was carried out by the agar disc diffusion method. The tests were done on Mueller Hinton agar for the bacterial strains and on Sabouraud dextrose agar for the fungal strains following the method used by Bassolé et al. (2005) [7].

Microbial cultures of 18-24 hours were prepared in nutrient broth for the bacterial strains and in Sabouraud broth for the fungal strains and then diluted with sterile saline solution (NaCl 0.9%) to adjust the density of the inoculums to that of McFarland standard 0.5. The Petri dishes containing sterile and solid Mueller-Hinton agar or Sabouraud dextrose agar were inoculated with this microbial suspension. Sterile blank discs (6 mm diameter) were impregnated with *V. nigritana* essential oil (15 µl per disc) and then placed on the surface of the agar previously inoculated. The Petri dishes were then aerobically incubated at 37 ° C for the bacterial strains and at 30 ° C for the fungal strains for 24 hours. The sensitivity of microbial strains to the essential oil of *V. nigritana* is determined by measuring the inhibition diameter (ID). For the evaluation of the inhibition diameters (ID), the criteria used by Carovic-Stanko et al. (2010) [13] were considered:

- ID >15 mm: the essential oil has a high inhibitory action
- 10 mm ≤ID ≤15 mm: the essential oil has a moderate inhibitory action
- ID <10 mm: the essential oil has a weak inhibitory action

Tetracycline (30 µg) and ciprofloxacin (5 µg) were used as positive control for bacterial strains and nystatin (100 UI) for fungal strains. All tests were performed in duplicate.

2.5.3 Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The broth microdilution method [14] was used to determine the minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC) and the minimum fungicidal concentration (MFC) of the essential oil of *Vetiveria nigritana* roots. All tests were carried out in Mueller-Hinton broth (MH) for bacterial strains and in Sabouraud broth for fungal strains. The broth was supplemented with Tween 80 at a concentration of 0.5% (v / v) in order to improve the solubility of the essential oil. A serial dilution of the essential oil of *V. nigritana* was prepared in a 96 well microplate containing MH broth or Sabouraud broth in a range of 0.03% to 8% (v / v). Overnight broth cultures (18-24 hours) of each strain were prepared in nutrient broth for the bacterial strains and in Sabouraud broth for the fungal strains. The density of the inoculums was adjusted with sterile saline solution (0.9% NaCl) to the McFarland standard 0.5 corresponding to 10^8 CFU / ml. Then 10µl of these inoculums diluted were added in the well. For each microbial strain a positive growth control (no essential oil added in the well) and a negative growth control (no inoculum, no essential oil added in the well) were included in the test. The microplate thus seeded, was incubated aerobically at 30 ° C for the fungal strains and at 37 ° C for the bacterial strains and the MICs determined after 24 hours of incubation. The MIC is considered to be the lowest concentration of essential oil which does not show visible growth after 24h of incubation. To determine the MBC, 10 µl of microbial suspension was taken from the wells of concentration greater than or equal to MIC and inoculated on the Mueller-Hinton agar or Sabouraud agar and then incubated for 24 hours at 37 ° C or 30 ° C depending on the strains. The lowest essential oil concentration at which no growth was observed on the agar after 24h of incubation is considered as MBC. The ratio MBC / MIC was used to determine the intrinsic activity (bactericidal or bacteriostatic) of the essential oil of *Vetiveria nigritana* roots, considering that:

- CMB / CMI = 1: absolute bactericidal activity
- 1 < CMB / CMI ≤ 4: bactericidal activity
- 8 < CMB / CMI < 16: bacteriostatic activity

3. RESULTS AND DISCUSSION

3.1 Chemical analysis

The hydodistillation of *Vetiveria nigritana* roots enabled us to obtain a yellowish-colored essential oil with an extraction yield of 0.22±0.038%. This yield is very low compared to those of 2% (W/W) and 1.35% (V/W) obtained respectively by Khalil and Ayoub (2011) and Champagnat et al. (2006) [4, 6]. This difference could be due to the difference of extraction methods, the extraction time, the harvesting time or the geographical location of the plant [15, 16].

The chemical composition of essential oil of *V. nigritana* roots is given in table 1. Twenty seven (27) compounds representing 75.237% of the essential oil of *V. nigritana* were identified. The relative abundance of some components of the essential oil is shown in figure 1. Dehydronigritene (24.24%), zizanoic acid (11.48%), preziza-7-(15)-en-3-ol (6.42%), khusimol + Preziza-7(15)-en-12-ol (5.83%), khusian-2-ol (5.09%), ziza-6(13)-en-3-beta-ol (3.21%), prezizaan-15-al (3.00%) and khusimone (2.69%) are the major constituents of this essential oil. The chemical composition of the essential oil of *V. nigritana* from Burkina Faso differs from that of Mali, whose major compounds were prezizanoic acid (15%), preziza-7-(15)-en-12-ol (9.5%), cedren-8-en-15-ol (6.2%), preziza-7-(15)-en-3α-ol (6%) and zizanoic acid (5.9%) [6]. Longifolene D (25.1%), 2-hydroxycyperol (9.7%) and aromadendrene oxide (8.8%) were identified by Khalil and Ayoub (2011) [4] as the major constituents of the essential oil of *V. nigritana* roots from Sudan.

Table 1: Chemical composition of the essential oil of *Vetiveria nigritana* roots.

No.	Retention time (mn)	Component	Proportion (%)
01	12.13	Nor Nigritene isomer	2.152
02	12.38	Dehydronigritene	24.246
03	12.71	Nigritene	0.467
04	12.87	Acoradiene	0.117
05	12.97	Beta-Funebrene	0.567
06	13.14	Beta-Gurgunene	0.202
07	13.42	Prezizaene	0.206
08	13.48	Zizanene	0.188
09	13.69	Cis-Eudesma-6-11-diene	0.142
10	13.75	Delta-Selinene	0.086
11	13.93	Delta-Amorphene	0.252
12	14.20	Delta-Cadinene+Inconnu MW204	1.132
13	14.35	Zonarene	0.169
14	14.53	Elemol	0.378
15	14.66	Béta-Vetivenene	0.582
16	15.03	15-Nor-prezizaan-7-one	1.166
17	15.24	Cis Dihydro-Mayurone	0.977
18	15.28	Khusimone	2.693
19	15.49	Epi-Cedrol	1.601
20	15.72	Alpha-Copaen-ol	1.87
21	15.86	Prezizaan-15-al	3.007
22	16.00	Preziza-7(15)-en-3-ol	6.425
23	16.23	Khusian-2-ol	5.099
24	16.31	Ziza-6(13)-en-3-beta-ol+inconnu MW 220	3.217
25	16.84	Khusimol+Preziza-7(15)-en-12-ol	5.838
26	17.49	Zizanoic acid	11.488
27	17.58	Prezizanoic acid	0.97
		Total	**75.237**

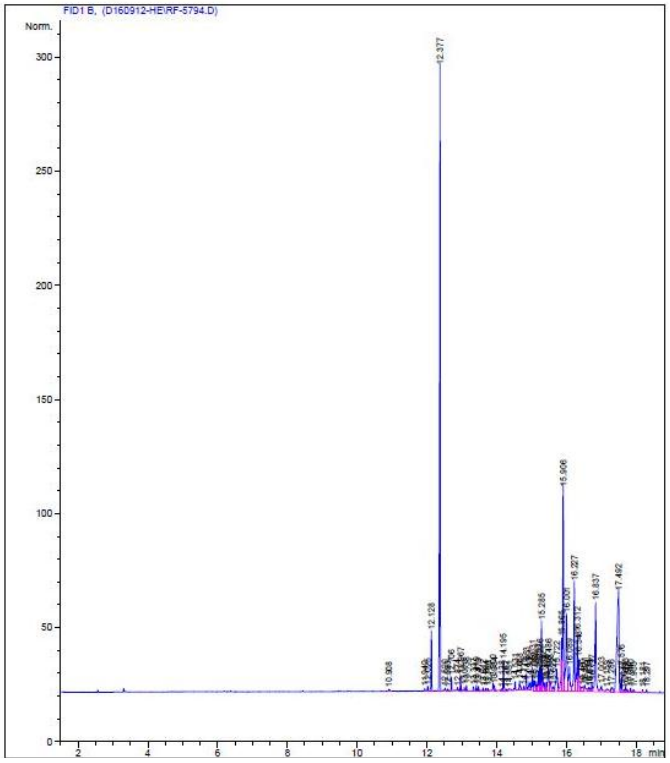

Fig. 1: Chromatogram of the essential oil of *Vetiveria nigritana* roots.

According to some authors, principal factors which determine the composition and yield of the essential oil obtained could include seasonal and maturity variation, geographical origin, genetic variation, growth stages, and postharvest drying and storage [17-19].

3.2 Antioxidant activity

The antioxidant capacity of the essential oil of *V. nigritana* was determined by comparison with the activities of known antioxidants, such as BHT, ascorbic acid, and quercetin.

DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. The radical scavenging power of the essential oil of *V. nigritana* roots and reference antioxidants is showed in figure 2. The results presented in figure 2 show that as the amount of essential oil increases, the DPPH radical inhibition percentage also increases. Thus, the DPPH radical inhibition power of the essential oil is concentration dependent. The inhibitory concentrations (IC50) of the essential oil of *V. nigritana* roots and standard antioxidants are shown in Table 2. The highest IC50 was obtained with *V. nigritana* (28.35µl) essential oil and the lowest one with quercetin (12.45µl). Essential oil of *Vetiveria nigritana* possessed a radical scavenging power, but this activity is lower as compared to quercetin (12.45µl) and ascorbic acid (18.33µl) but quite similar to BHT (26.94µl).

Table 2: Inhibitory concentration (IC50).

Essential oil and standards	Regression equation	R^2	IC50 (µl)
Quercetin (0.005M)	y=2.7792x+15.388	0.99	12.45±0.25
Ascorbic acid (0.005M)	y=2.3558x+6.8831	0.99	18.33±0.65
BHT (0.005M)	y=1.8254x+0.831	0.99	26.94±0.32
Vetiveria nigritana	y=1.65x+3.2306	0.99	28.35±0.58

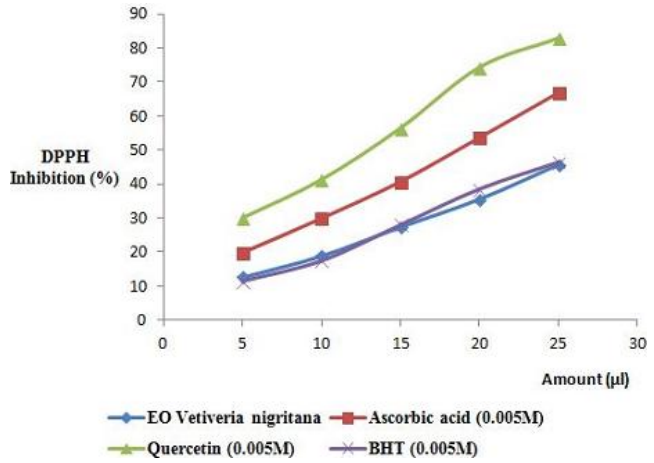

Fig. 2: DPPH radical scavenging power.

The results obtained with the FRAP test are shown in figure 3. The essential oil of *V. nigritana* roots showed a very lower reducing power comparing to ascorbic acid and quercetin. These results confirm those of the DPPH test. The antioxidant activity of the essential oil of *V. nigritana* roots is evaluated for the first time.

Antioxidant properties of essential oils such as lipid peroxidation, scavenging of free radicals, chelating metal ions, and

reducing power are often come from their monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes [20, 21]. Some studies supported that phenolic components in the essential oil were the main source of antioxidant activity [22-24]. The essential oil of *V. nigritana* roots is poor in monoterpenes, oxygenated monoterpenes and phenolic components, which can explain its weak antioxidant activity. However Özkan and Erdoğan (2011) [25] showed that the essential of *Origanum onites* (Lamiaceae) had a free radical scavenging power greater than its two major phenolic component carvacrol and thymol.

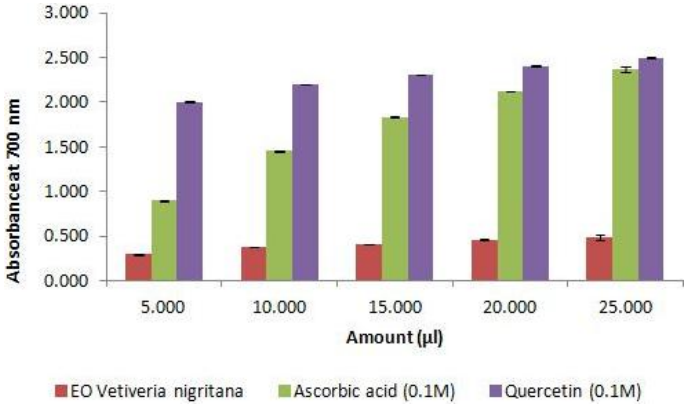

Fig. 3: Reducing power of the essential oil of *Vetiveria nigritana* roots.

3.3 Antimicrobial activity

The antimicrobial activity of the essential oil of *Vetiveria nigritana* roots has been evaluated by the disk diffusion method. The results presented in table 3 show that the essential oil of *V. nigritana* roots was active on all the microbial strains tested but at different levels.

The inhibition diameters (ID) of the essential oil were between 8.5 mm (*Salmonella infantis*, *Salmonella typhimurium*, *Shigella flexnerii*) and 23.5 mm (*Bacillus subtilis*). Applying the criteria of Carovic-Stanko et al. (2010) [13], the essential oil of *V. nigritana* roots had:

- Strong inhibitory action (ID > 15 mm) on the strains of *Bacillus*, *Clostridium perfringens*, *Escherichia coli* ATCC 25922 and *Staphylococcus hominis*;
- Moderate inhibitory action (10 mm ≤ ID ≤ 15 mm) on the strains of *Enterococcus faecalis*, *Escherichia coli* 81 nr.149 SKN 541, *Listeria monocytogenes*, *Micrococcus luteus*, *Salmonella enteridis*, *Salmonella nigeria*, *Shigella dysenteria*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Candida albicans*, *Candida kefir* and *Saccharomyces cerevisiae*;
- Weak inhibitory action (ID <10 mm) on the strains of *Pseudomonas aeruginosa*, *Salmonella infantis*, *Salmonella typhimurium*, *Shigella flexnerii* and *Candida tropicalis*.

Table 3: Inhibition zone diameters (mm) of the essential oil of *Vetiveria nigritana* roots (15µl) and standard antibiotics including the disc diameter (6mm).

Microbial strains			Inhibition diameters (mm)			
Bacterial strains	**Gram**	**Origin**	**EO *V. nigritana***	**Tetracyclin (30µg)**	**Ciprofloxacin (5µg)**	**Nystatin (100UI)**
Bacillus cereus LMG13569	Positive	Culture collection of London Metropolitan University	19± 1.41	20 ± 1.41	27 ± 1.41	-
Bacillus subtilis ssp subtilis ATCC 6051	Positive	ATCC	23.5±0.71	30.5 ± 0.71	34.5 ± 0.71	-
Clostridium perfringens	Positive	CRSBAN	16±1.41	27±1.41	16.5 ± 0.71	-
Enterococcus faecalis ATCC 19433	Positive	ATCC	12±1.41	24.5 ± 0.71	25 ± 1.41	-
Escherichia coli 81 nr.149 SKN 541	Negative	Culture collection of Copenhagen University	15±1.41	16±1.41	33 ± 1.41	-
Escherichia coli ATCC 25922	Negative	ATCC	17±1.41	33.5 ± 2.12	22.5 ± 0.71	-
Listeria monocytogenes NCTC 9863	Positive	Culture collection of London Metropolitan University	14±1.41	22±1.41	31.5 ± 0.71	-
Micrococcus luteus SKN 624	Positive	Culture collection of Copenhagen University	12±0.00	17±1.41	32 ± 1.41	-
Pseudomonas aeruginosa ATCC 9027	Negative	ATCC	9.5±0.71	12.5 ± 0.71	32.5 ± 0.71	-
Salmonella enteridis P167807	Negative	Culture collection of London Metropolitan University	10±0.00	23±1.41	31 ± 1.41	-
Salmonella infantis SKN 557	Negative	Culture collection of Copenhagen University	8.5±0.71	21.5 ± 2.12	28 ± 1.41	-
Salmonella typhimurium SKN 1152	Negative	Human	8.5±0.71	20±1.41	26.5 ± 0.71	-
Salmonella nigeria SKN 1160	Negative	Cocoa beans	10±0.00	18±1.41	30.5 ± 0.71	-
Shigella dysenteria 370	Negative	Culture collection of London Metropolitan University	10±2.83	23.5 ± 2.12	36.5 ± 0.71	-
Shigella flexneri USCC 2007	Negative	Culture collection of London Metropolitan University	8.5±0.71	22.5 ± 0.71	31.5 ± 0.71	-
Staphylococcus aureus ATCC 2523	Positive	ATCC	12±0.00	20.5 ± 0.71	24.5 ± 0.71	-
Staphylococcus aureus ATCC 25923	Positive	ATCC	15±0.00	24±1.41	27 ± 1.41	-
Staphylococcus aureus toxine A+B	Positive	Culture collection of Copenhagen University	15±1.41	10.5 ± 0.71	06±00	-
Staphylococcus hominis B246	Positive	Maari (fermented baobab seeds)	17±1.41	30±1.41	33.5 ± 0.71	-
Yersinia enterocolitica 8A30 SKN 601	Negative	Culture collection of Copenhagen University	10±1.41	15.5 ± 0.71	37.5 ± 0.71	-
Fungal strains	-	**Origin**	-	-	-	**Nystatin (100UI)**
Candida albicans	-	Blood	10±0.00	-	-	22.5±0.5
Candida kefir	-	Fura (fermented millet food)	12.5±0.71	-	-	24.5±0.5
Candida tropicalis	-	Fura (fermented millet food)	9±1.41	-	-	20.5±0.5
Saccharomyces cerevisiae KVL 013	-	Culture collection of Copenhagen University	11.5±0.71	-	-	27.5±0.5

EO: Essential Oil.
ATCC: American Type Culture Collection.
CRSBAN: Centre de Recherche en Sciences Biologiques Alimentaires et Nutritionnelles.

3.4 MIC, MBC and MFC

Minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (CMBs) and minimum fungicidal concentrations (CMFs) of the essential oil of *Vetiveria nigritana* roots are shown in table 4. MICs of the essential oil ranged from 1% to 8% (v/v) for twelve bacterial strains and greater than 8% for the other tested strains. The lowest MIC is obtained with strains of *Bacillus*, *C. perfringens*, *S. aureus* ATCC 25923, *S. aureus* (toxin A + B) and *S. hominis*. The MBCs of essential oil of *V. nigritana* were 2% and 4% (v/v) for ten bacterial strains and greater than 8% for the other bacterial strains. The essential oil had a bactericidal action ($1< MBC / MIC \leq 4$) on the strains of *Bacillus*, *C. perfringens*, *E. coli*, *L. monocytogenes*, *M. luteus*, *S. aureus* ATCC 25923, *S. aureus* (toxin A + B) and *S. hominis*.

All the fungi strains tested were sensitive to the essential oil of *V. nigritana* with MIC value of 2% (v/v) for *Candida kefir* and *Saccharomyces cerevisiae*, 4% for *Candida albicans* and 8% for *Candida tropicalis*. The MFCs of the essential oil were 4% for *C. kefir* and *S. cerevisiae* and greater than 8% for *C. albicans* and *C. tropicalis*. The essential oil had a fungicidal action ($1 < MFC / MIC \leq 4$) on the strains of *Candida kefir* and *Saccharomyces cerevisiae*.

The essential oil of *V. nigritana* from Mali is active against *Staphylococcus aureus*, *Enterococcus faecalis and Escherichia coli*, less active against *Pseudomonas aeruginosa* and active against *Candida albicans* with MIC value of 1300 to 1400 µg/ml [26]. The results of the present study are in accordance with these results. However Adamua et al. (2005) [3] reported that *Escherichia coli* was resistant, *Bacillus subtilis* and *Staphylococcus aureus* were less susceptible and *Pseudomonas aeruginosa* was sensible to the essential oil of *V. nigritana* from Sudan. The variation of the antimicrobial activity of essential oils could be correlated to chemical composition variability [27, 28].

In recent years, several researchers have reported that monoterpenes or sesquiterpenes hydrocarbon and their oxygenated derivatives, which are the major components of essential oils, exhibit potential antimicrobial activity [27, 29, 30]. The relative good antimicrobial activity of the essential oil of *V. nigritana* roots can be attributed to the presence of sesquiterpene alcohols which represented about 23% of this essential oil. The essential oil of *V. nigritana* roots from Burkina Faso had a better action on Gram positive bacterial strains than Gram negative bacterial strains except for *Escherichia coli* strains. *V. nigritana* and *V. zizanoïdes* oils have a low activity against Gram-negative bacilli but are capable of inhibiting Gram-positive cocci growth with much higher efficiency [26]. The high concentrations of alcohols and ketones in these essential oils could explain their antibacterial activity. Essential oil of *V. nigritana* roots is rich in sesquiterpene components which have low antibacterial activity against Gram negative bacteria as described by some authors [31-33].

Table 4: Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) of the essential oil of *Vetiveria nigritana* roots 0.03% to 8% (v/v).

Microbial strains			**EO *V. nigritana* (v/v)**		
Bacterial strains	**Gram**	**Origin**	**MIC**	**MBC**	**MBC/MIC**
Bacillus cereus LMG13569	Positive	Culture collection of London Metropolitan University	1%	4%	4
Bacillus subtilis ssp subtilis ATCC 6051	Positive	ATCC	1%	4%	4
Clostridium perfringens	Positive	CRSBAN	1%	4%	4
Enterococcus faecalis ATCC 19433	Positive	ATCC	8%	> 8%	>1
Escherichia coli 81 nr.149 SKN 541	Negative	Culture collection of Copenhagen University	2%	4%	2
Escherichia coli ATCC 25922	Negative	ATCC	2%	4%	2
Listeria monocytogenes NCTC 9863	Positive	Culture collection of London Metropolitan University	2%	4%	2
Micrococcus luteus SKN 624	Positive	Culture collection of Copenhagen University	2%	4%	2
Pseudomonas aeruginosa ATCC 9027	Negative	ATCC	> 8%	> 8%	>1
Salmonella enteridis P167807	Negative	Culture collection of London Metropolitan University	> 8%	> 8%	>1
Salmonella infantis SKN 557	Negative	Culture collection of Copenhagen University	> 8%	> 8%	>1
Salmonella typhimurium SKN 1152	Negative	Human	> 8%	> 8%	>1
Salmonella nigeria SKN 1160	Negative	Cocoa beans	> 8%	> 8%	>1
Shigella dysenteria 370	Negative	Culture collection of London Metropolitan University	> 8%	> 8%	>1
Shigella flexneri USCC 2007	Negative	Culture collection of London Metropolitan University	> 8%	> 8%	>1
Staphylococcus aureus ATCC 2523	Positive	ATCC	8%	> 8%	>1
Staphylococcus aureus ATCC 25923	Positive	ATCC	1%	2%	2
Staphylococcus aureus toxine A+B	Positive	Culture collection of Copenhagen University	1%	4%	4
Staphylococcus hominis B246	Positive	Maari (fermented baobab seeds)	1%	4%	4
Yersinia enterocolitica 8A30 SKN 601	Negative	Culture Collection of Copenhagen University	> 8%	> 8%	>1
Fungal strains	-	**Origin**	**MIC**	**MFC**	**MFC/MIC**
Candida albicans	-	Blood	4%	> 8%	>2
Candida kefir	-	Fura (fermented millet food)	2%	4%	2
Candida tropicalis	-	Fura (fermented millet food)	8%	> 8%	>1
Saccharomyces cerevisiae KVL 013	-	Culture collection of Copenhagen University	2%	4%	2

EO: Essential oil.
ATCC: American Type Culture Collection.
CRSBAN: Centre de Recherche en Sciences Biologiques Alimentaires et Nutritionnelles.

4. CONCLUSION

The essential oil of *Vetiveria nigritana* roots had an antioxidant activity but this activity is low compared to that of standard antioxidants such as quercetin and ascorbic acid.

The essential oil of *V. nigritana* also possessed good antimicrobial activity against most of the gram-positive bacterial strains tested. These results confirm the traditional usage of *V. nigritana* roots. The essential oil of *V. nigritana* roots could be used as potential natural antimicrobial agent.

Hence the use of essential oil of V. nigritana roots will be a cheaper and natural drug formulation to replace the commercially available chemical drug and also without any side effects.

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