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CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF THE ESSENTIAL OIL OF *DETARIUM MICROCARPUM* GUILL. AND PERR. LEAVES FROM BURKINA FASO

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ABSTRACT: Chemical composition and biological activities of the essential oil of *Detarium microcarpum* leaves were assessed *in vitro*. The essential oil was obtained using a Clevenger type apparatus and analyzed by GC and GC/MS. The essential oil was investigated for antioxidant power using the DPPH radical scavenging method and the FRAP test. Antimicrobial activity of the essential oil was also evaluated by agar disk diffusion and broth micro dilution methods. Chemical analysis showed that the major components of the essential oil of *D. microcarpum* were caryophyllene oxide (28.186%), β-caryophyllene (11.894%), humulene-1, 2-epoxide (4.05%), neryl acetone (2.452%), salvia-4(14)-ene-1-one (2.423%) and linalol (2.098%). The essential oil showed a low radical scavenging power with an IC_{50} of 21.99 ± 0.17 µl and a weak reducing power. The essential oil exhibited strong to moderate antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Bacillus cereus*, *Clostridium perfringens*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Micrococcus luteus*, *Staphylococcus*, *Candida kefir* and *Saccharomyces cerevisiae* with inhibition zone diameters ranging from 12 ± 0.00 mm to 22 ± 1.41 mm. Minimum inhibitory concentrations of the essential oil were between 0.5% and 8% (v/v) with minimum bactericidal concentrations of 1% to 8% and minimum fungicidal concentrations of 1% to 8%. The leaves essential oil of *D. microcarpum* had an absolute bactericidal action against *Staphylococcus aureus* ATCC2523. This study provided data on the proximate composition and biological activities of the essential oil of *D. microcarpum* leaves and these results confirmed some traditional usage of this plant. The essential oil of *D. microcarpum* could be a good candidate in the research of novel antimicrobial compound.

INTRODUCTION: Herbal medicines, traditional treatments, and traditional practitioners are the main source of health care, and sometimes the only source of care for many millions of people. This is care that is close to homes, accessible and affordable, it is also culturally acceptable and trusted by large numbers of people [1].

Detarium microcarpum Guill. and Perr. (Caesalpiniaceae) is a well-known wild edible fruit species growing in Saharan and sub-Saharan countries and is found mostly in savannah forest of dried type [2, 3]. *D. microcarpum* is a small straight trunk tree of 8 - 10 m high with spherical, fairly dense top. The leaves are alternate, imparipinnated with 3 - 6 pairs of alternate or sub-opposed, oval,

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oblong or elliptic leaflets with rounded apex. The petal flowers have 4 sepals and 8 - 10 prominent cream-white stamens. The fruit is an ovoid or globular drupe, more or less flattened, 2.5 - 5 cm in diameter, with a cracked surface at maturity, containing a large central nucleus surrounded by a greenish, fibrous and sweet farinaceous pulp [4]. The leaves, flowers, fruits and seeds of *D. microcarpum* are used in human food. The fruits are rich in Vitamin C and can be eaten raw or cooked, leaves and flowers are used as condiments and vegetables for the preparation of sauces [4, 5]. Leaves, bark, roots, fruits and seeds of *D. microcarpum* are also used as a pharmacopoeia for the treatment of stomach pain, dysentery, malaria, jaundice, furunculosis, panaris, mining and sexually transmitted diseases [4, 5]. The leaves, roots and trunk bark of *D. microcarpum* are used in Burkina Faso by the Sanan in the treatment of infections and infestations, musculoskeletal, skin, digestive, nutritional and pregnancy disorders [6].

Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pyogenes, Bacillus subtilis, Corynebacteria and *Candida albicans* were susceptible to ethyl acetate extract of *D. microcarpum* stem bark [7].

D. microcarpum stem bark extract show antifungal activity when tested by the agar diffusion method against dermatophytes [8]. Phytochemical analysis, antimicrobial, antioxidant activities, nutritional, toxicological and enzymes inhibition properties of *D. microcarpum* have been investigated by several authors [2, 3, 9, 10]. However we have found least literature data on *D. microcarpum* essential oils. This study aimed to determine the chemical composition, antioxidant and antimicrobial properties of the essential oil of *Detarium microcarpum* leaves from Burkina Faso.

MATERIAL AND METHODS:

Plant Material: *Detarium microcarpum* leaves were collected in the bush of Peyiri, a village located on the outskirts of the town of Koudougou during the months of July and August 2014. A voucher specimen is deposited under the number ID 16965, at the herbarium of Biodiversity Information Center of University Ouaga I. J. K. Z. The harvested leaves were dried in the laboratory at room temperature during two weeks and reduced to powder before extraction.

Essential oil Extraction: The dried and ground leaves of *Detarium microcarpum* were subjected to hydro-distillation for 4 h using a Clevenger-type apparatus [11]. The obtained essential oil was then dried over anhydrous sodium sulfate and stored at 4°C waiting for analyzes. The extraction yield was determined by the following equation:

$$R\ (\%) = V / W \times 100$$

Where V is the volume of essential oil (ml) and W the weight of dried leaves (g).

Chemical Analyzes: Chemical analyzes of leaves essential oil of *D. microcarpum* was carried out by gas chromatography (GC) and gas chromatography / mass spectrometry (GC / MS). GC analysis was 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 × 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 1.1 mL / min. The injected sample consisted of 1.0 µl of oil diluted to 10% (v/v) with acetone.

GC/MS analysis was done 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 × 0.25 mm, film thickness 0.25 m), and a HP-Innowax capillary column (60 m × 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 components of the essential oil of *D. microcarpum* leaves were identified by comparison of their mass spectra and their retention indices with those of reference compounds or with literature data [12, 13].

Antioxidant Activity: The DPPH radical scavenging assay and the ferric reduction antioxidant power (FRAP) test were used to evaluate the antioxidant activity of leaves essential oil of *Detarium microcarpum*.

DPPH Radical Scavenging Assay: Radical scavenging power of *D. microcarpum* leaves essential oil was assessed by DPPH radical scavenging assay [14]. Different amounts of the essential oil of *D. microcarpum* (5, 10, 15, 20 and 25 µl) were added to 5 ml of an ethanolic solution of DPPH (0.004%) and mixed. The mixture obtained was incubated in the dark for 30 min and the absorbance read at 517 nm using a spectrophotometer (JASCO V-530 UV / VIS Spectrophotometer). Reference antioxidants, ascorbic acid (0.005 M) and quercetin (0.005 M) and a negative control were also included in each test. The DPPH inhibition percentage was calculated according to the following equation:

$$\% \text{ inhibition} = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

Where A_{blank} is the absorbance of the negative control and A_{sample} the absorbance of the essential oil.

Antioxidant activity of the essential oil of *D. microcarpum* leaves was expressed as inhibitory concentration 50 (IC_{50}), which is the amount of essential oil required to cause a 50% decrease in initial DPPH concentration. A linear regression was used to calculate the IC_{50}.

All tests were performed in triplicate; the standards and DPPH solutions were prepared and used daily.

Ferric Reduction Antioxidant Power (FRAP) Assay: Reducing power of the essential oil of *D. microcarpum* leaves was assessed by the FRAP test [15]. Different amounts of *D. microcarpum* leaves 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 2.5 ml of trichloroacetic acid (10%) were added followed by centrifugation at 600 g 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 and the absorbance recorded at 700 nm using a spectrophotometer (JASCO V-530 UV/VIS Spectrophotometer). Ascorbic acid (0.1 M) and quercetin (0.1 M) were used as positive control and a negative control was also included in each test. An increase in absorbance indicates an increase of the reducing power.

Antimicrobial Activity:

Microbial Strains: The essential oil of *Detarium microcarpum* leaves was tested against a panel of microorganisms including ten Gram positive bacteria, ten Gram negative bacteria and four fungal strains.

Agar Disc Diffusion Method: The antimicrobial activity of the essential oil of *D. microcarpum* leaves was evaluated by the agar disc diffusion method. Mueller Hinton agar was used for the bacterial strains and Sabouraud dextrose agar for the fungal strains [11].

Microbial cultures of 18 - 24 hours were prepared in nutrient broth for the bacterial strains and in Sabouraud broth for the fungal strains. The inoculums density was then adjusted to that of McFarland standard 0.5 with sterile saline solution (NaCl 0.9%). The Petri dishes containing sterile and solid Mueller-Hinton agar or Sabouraud dextrose agar were inoculated with this microbial suspension. The essential oil of *D. microcarpum* was impregnated on sterile blank discs of 6 mm diameter (15 µl per disc) and the discs were then placed on the surface of the agar previously inoculated. The Petri dishes were aerobically incubated at 37 °C for the bacterial strains and at 30 °C for the fungal strains for 24 hours. The inhibitory action of the essential oil of *D. microcarpum* on the tested microorganisms was determined by measuring the inhibition zone diameter (ID). Inhibition zone diameters (ID) were evaluated using the following criteria [16]:

- ID > 15 mm: high inhibitory action.
- 10 mm ≤ ID ≤ 15 mm: moderate inhibitory action.
- ID < 10 mm: weak inhibitory action.

Tetracycline (30 µg) and ciprofloxacin (5 µg) were used as reference antibiotics for bacterial strains and nystatin (100 UI) for fungal strains. The assay was performed in duplicate.

Determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC): The broth micro-dilution method [11] 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 *D. microcarpum* leaves. The tests were performed 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) to enhance the solubility of the essential oil. A serial double dilution of the essential oil of *D. microcarpum* leaves was prepared in a 96 well microplate containing MH broth or Sabouraud broth in a range of 0.03% to 8% (v/v). Microbial cultures of 18 - 24 hours were prepared in nutrient broth for the bacterial strains and in Sabouraud broth for the fungal strains and the inoculums density adjusted to that of McFarland standard 0.5 with sterile saline solution (0.9% NaCl). Then 10 µl of these inoculums was added to each well, positive and negative growth controls were included in each assay.

The microplate was then incubated aerobically at 30 °C for the fungal strains and at 37 °C for the bacterial strains. The lowest concentration of the essential oil which did not demonstrate visible growth after 24 h of incubation was considered to be the MIC. To determine the MBC or MFC, 10 µl of microbial suspension was taken from the wells of concentration greater than or equal to MIC and inoculated on sterile Mueller-Hinton agar or Sabouraud agar and incubated for 24 hours at 37 °C or 30 °C. The lowest concentration of the essential oil at which no growth was recorded on the agar after 24 h of incubation was considered to be the MBC or MFC. The ratio MBC/MIC or MFC/MIC was used to determine the intrinsic activity (bactericidal or bacteriostatic) of the essential oil of *D. microcarpum* leaves, considering that:

- MBC / MIC = 1: absolute bactericidal activity.
- 1 < MBC / MIC ≤ 4: bactericidal activity.
- 8 < MBC / MIC < 16: bacteriostatic activity.

Statistical Analysis: The data were entered and processed on Microsoft excel. The results are presented as mean ± SD (standard deviation).

RESULTS AND DISCUSSION:

Chemical Composition: The hydro-distillation of *D. microcarpum* dry leaves gave a yellowish-colored essential oil with a yield of 0.052 ± 0.002 % (v/w).

Chemical analysis of the essential oil of *D. microcarpum* leaves allowed the identification of thirty compounds representing 69.11% of the essential oil. The relative abundance of some components of the essential oil is shown in **Fig. 1**. Chemical composition of the essential oil is presented in **Table 1**, the major components were caryophyllene oxide (28.186%), β-caryophyllene (11.894%), humulene-1,2-epoxide (4.05%), neryl acetone (2.452%), salvia-4(14)-ene-1-one (2.423%), linalol (2.098%), spathulenol (1.886%), γ-muurolene (1.589%), α-copaene (1.238%), intermedeol (1.208%), β-selinene (1.108%), α-muurolene (1.027%), γ-cadinene (1.13%) and δ-cadinene (1.254%).

In our best knowledge, it is the first time that the chemical composition of the essential oil of *D. microcarpum* leaves is determined.

TABLE 1: CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *DETARIUM MICROCARPUM* LEAVES

Number	Retention time (mn)	Components	Proportion (%)
01	6.29	3-Octanone	0.229
02	6.37	Myrcene	0.064
03	8.36	Linalol	2.098
04	9.5	Borneol	0.161
05	9.89	α-Terpineol	0.351
06	10.38	Safranal	0.407
07	10.68	Geraniol	0.227
08	12.03	α-Cubebene	0.357
09	12.14	1,2-Dihydro-Trimethyl-Naphtalene	0.339
10	12.42	α-Copaene	1.238
11	12.54	β-Bourbonene	0.477
12	12.6	β-Bourbonene	0.785
13	13.01	β-Caryophyllene	11.894
14	13.13	β-Copaene	0.883
15	13.24	α-Trans-Bergamotene	0,368
16	13.46	Neryl Acetone	2.452

17	13.51	α-Humulene	0,605
18	13.69	γ-Muurolene	1.589
19	13.77	Germacrene-D	0,597
20	13.88	β-Selinene	1.108
21	13.97	α-Muurolene	1.027
22	14.16	γ-Cadinene	1.13
23	14.21	δ-Cadinene	1.254
24	14.5	α-Calacorene	0.73
25	14.95	Spathulenol	1.886
26	15.02	Caryophyllene oxide	28.186
27	15.12	Salvia-4(14)-ene-1-one	2.423
28	15.33	Humulene-1,2-epoxide	4.05
29	15.58	Iso-Spathulenol	0.989
30	15.63	Intermedeol	1.208
		Total	69.112

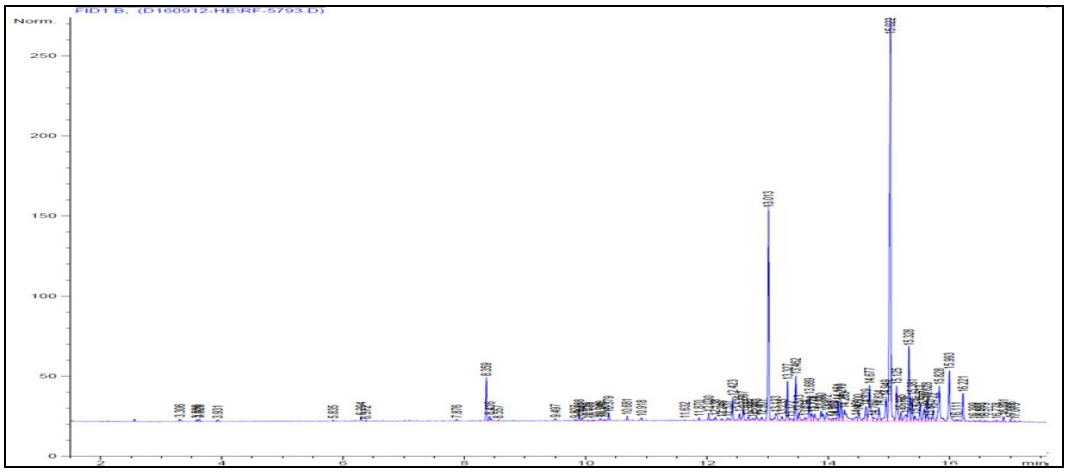

FIG. 1: CHROMATOGRAM OF *DETARIUM MICROCARPUM* LEAVES ESSENTIAL OIL

Antioxidant Activity: Antioxidant activity of the essential oil of *D. microcarpum* leaves was assessed by the DPPH radical scavenging assay in comparison with ascorbic acid and quercetin, two standard antioxidants. DPPH is a stable free radical which can readily experience reduction in the presence of an antioxidant. DPPH radical scavenging power of the essential oil of *D. microcarpum* and the standards is shown in **Fig. 2**.

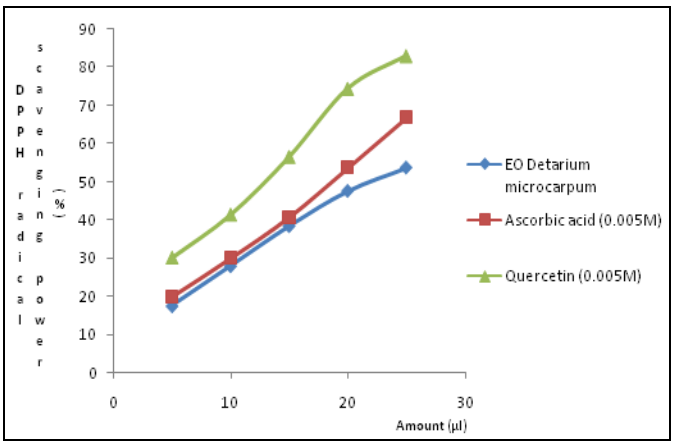

FIG. 2: DPPH RADICAL SCAVENGING POWER

The inhibitory concentrations 50 (IC_{50}) of DPPH were calculated using a linear regression and are presented in **Table 2**. The IC_{50} was 21.99 ± 0.17 µl for the essential oil, 18.33 ± 0.65 µl for ascorbic acid and 12.45 ± 0.25 µl for quercetin. The essential oil of *D. microcarpum* leaves had a low radical scavenging power relative to ascorbic acid and quercetin.

TABLE 2: IC_{50} RADICAL SCAVENGING POWER

Essential oil and standards	Regression equation	R^2	IC_{50} (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
Detarium microcarpum	Y = 1.8428x +9.488	0.99	21.99 ±0.17

The results of the FRAP test are represented by **Fig. 3**. Reducing power of the essential oil of *D. microcarpum* leaves was increased with increasing concentration but was low compared with ascorbic acid and quercetin.

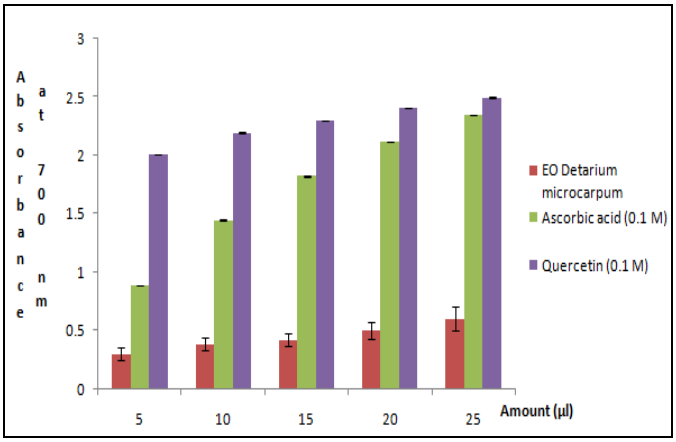

FIG. 3: REDUCING POWER OF THE ESSENTIAL OIL OF *DETARIUM MICROCARPUM* LEAVES AND STANDARDS

Phenolic compounds are major antioxidant constituents in selected plants, and there are direct relationships between their antioxidant activity and total phenolic content [17]. Oxygenated monoterpenes and monoterpene hydrocarbons are mainly responsible for the antioxidant potential of essential oil [18]. Some structural features, such as the presence of strongly activated methylene groups in the molecule, are probably the reason for antioxidant activity of monoterpene hydrocarbons [19].

In the DPPH test, the ability of the essential oil to act as the donor of hydrogen atoms or electrons in transformation of DPPH into its reduced form DPPH-H was measured spectrophotometrically [20]. The absence of antioxidant activity observed for the terpene compounds in the DPPH reduction can be explained by the fact that they are not capable of donating an hydrogen atom and the low solubility provided by them in the reaction medium of the assay, because this test utilizes methanol or ethanol as solvent [21].

Hence the low antioxidant power of the essential oil of *D. microcarpum* leaves which contains a small proportion of monoterpenes and phenolic compounds.

Antimicrobial Activity: The inhibition diameters of the essential oil of *D. microcarpum* leaves are shown in **Table 3**. The essential oil of *D. microcarpum* was active on all the microbial strains tested with inhibition diameters ranging from 08 mm (*Salmonella infantis*, *Salmonella nigeria*, *Yersinia enterocolitica*) to 22 mm (*Bacillus subtilis*). According to the criteria [16], the essential oil of *D. microcarpum* had:

- Strong inhibitory action (ID > 15 mm) on the strains of *Bacillus subtilis* and *Escherichia coli*;
- Moderate inhibitory action (10 ≤ ID ≤ 15 mm) on the strains of *Bacillus cereus*, *Clostridium perfringens*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Shigella flexnerii*, *Staphylococcus*, *Candida albicans*, *Candida kefir* and *Saccharomyces cerevisiae*;
- Weak inhibitory action (10 mm < ID) on the strains of *Salmonella*, *Shigella dysenteria*, *Yersinia enterocolitica* and *Candida tropicalis*.

MIC, MBC and MFC: Minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) of the essential oil of *D. microcarpum* leaves are given in **Table 4**. MICs of the essential oil ranged from 0.5% to 8% (v/v) for eighteen (18) microbial strains and greater than 8% for the other tested strains. The lowest MIC was obtained with *Bacillus subtilis*, *Staphylococcus aureus* ATCC 25923, *Staphylococcus hominis* and *Saccharomyces cerevisiae*.

The essential oil of *D. microcarpum* had MBCs values ranging from 1% to 8% (v/v). The lowest MBC was determined with *Staphylococcus aureus* ATCC 25923 and *Staphylococcus hominis*.

The essential oil of *D. microcarpum* leaves was absolute bactericidal (MBC / MIC = 1) against *Staphylococcus aureus* ATCC 2523 and bactericidal (1< MBC / MIC ≤ 4) against the strains of *Bacillus*, *Clostridium perfringens*, *Escherichia coli*, *Listeria monocytogenes*, *Micrococcus luteus*, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* toxin (A + B) and *Staphylococcus hominis*.

The MFCs of the essential oil of *D. microcarpum* ranged from 1% to 8%, the lowest MFC was given by *Saccharomyces cerevisiae*. The essential oil of *D. microcarpum* leaves had a fungicidal action (1< MFC / MIC ≤ 4) on *Candida albicans*, *Candida kefir* and *Saccharomyces cerevisiae*.

TABLE 3: INHIBITION ZONE DIAMETERS (MEAN ± SD) OF THE ESSENTIAL OIL OF *DETARIUM MICROCARPUM* LEAVES (15 µL) INCLUDING THE DISC DIAMETER (6 mm)

Microbial strains			Inhibition zones diameters (mm)			
Bacterial strains	**Gram**	**Origin**	**EO *D. microcarpum***	**Tetracycline (30 µg)**	**Ciprofloxacin (5 µg)**	**Nystatin (100 UI)**
Bacillus cereus LMG13569	Positive	Culture collection of London Metropolitan University	14.5±0.71	20 ± 1.41	27 ± 1.41	-
Bacillus subtilis ssp subtilis ATCC 6051	Positive	ATCC	22±1.41	30.5 ± 0.71	34.5 ± 0.71	-
Clostridium perfringens	Positive	CRSBAN	14±1.41	27±1.41	16.5 ± 0.71	-
Enterococcus faecalis ATCC 19433	Positive	ATCC	10±0.00	24.5 ± 0.71	25 ± 1.41	-
Escherichia coli 81 nr.149 SKN 541	Negative	Culture collection of Copenhagen University	18.5±2.12	16±1.41	33 ± 1.41	-
Escherichia coli ATCC 25922	Negative	ATCC	21±2.83	33.5 ± 2.12	22.5 ± 0.71	-
Listeria monocytogenes NCTC 9863	Positive	Culture collection of London Metropolitan University	12±0.00	22±1.41	31.5 ± 0.71	-
Micrococcus luteus SKN 624	Positive	Culture collection of Copenhagen University	13±0.00	17±1.41	32 ± 1.41	-
Pseudomonas aeruginosa ATCC 9027	Negative	ATCC	11±1.41	12.5 ± 0.71	32.5 ± 0.71	-
Salmonella enteridis P167807	Negative	Culture collection of London Metropolitan University	9.5±0.71	23±1.41	31 ± 1.41	-
Salmonella infantis SKN 557	Negative	Culture collection of Copenhagen University	8±0.00	21.5 ± 2.12	28 ± 1.41	-
Salmonella typhimurium SKN 1152	Negative	Culture collection of Copenhagen University	9±1.41	20±1.41	26.5 ± 0.71	-
Salmonella nigeria SKN 1160	Negative	Cocoa beans	8±0.00	18±1.41	30.5 ± 0.71	-
Shigella dysenteria 370	Negative	Culture collection of London Metropolitan University	9±0.00	23.5 ± 2.12	36.5 ± 0.71	-
Shigella flexneri USCC 2007	Negative	Culture collection of London Metropolitan University	10.5±0.71	22.5 ± 0.71	31.5 ± 0.71	-
Staphylococcus aureus ATCC 2523	Positive	ATCC	14±2.83	20.5 ± 0.71	24.5 ± 0.71	-
Staphylococcus aureus ATCC 25923	Positive	ATCC	14.5±3.54	24±1.41	27 ± 1.41	-
Staphylococcus aureus toxin A+B	Positive	Culture collection of Copenhagen University	14±1.41	10.5 ± 0.71	06 ± 00	-
Staphylococcus hominis B246	Positive	Maari (fermented baobab seeds)	15±3.54	30±1.41	33.5 ± 0.71	-
Yersinia enterocolitica 8A30 SKN 601	Negative	Culture collection of Copenhagen University	8±0.00	15.5 ± 0.71	37.5 ± 0.71	-
Fungal strains	**-**	**Origin**	**EO *D. microcarpum***	**-**	**-**	**Nystatin**
Candida albicans	-	Blood sample	10.5±0.71	-	-	22.5±0.5
Candida kefir	-	Fura (fermented millet food)	12±0.00	-	-	24.5±0.5
Candida tropicalis	-	Fura (fermented millet food)	9.5±0.71	-	-	20.5±0.5
Saccharomyces cerevisiae KVL 013	-	Culture collection of Copenhagen University	14±1.41	-	-	27.5±0.5

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

TABLE 4: MIC, MBC AND MFC OF THE ESSENTIAL OIL OF *D. MICROCARPU* LEAVES 0.03% TO 8% (v/v)

Microbial strains			EO *D. microcarpum* (v/v)		
Bacterial strains	**Gram**	**Origin**	**MIC**	**MBC**	**MBC/MIC**
Bacillus cereus LMG13569	Positive	Culture collection of London Metropolitan University	1%	2%	2
Bacillus subtilis ssp subtilis ATCC 6051	Positive	ATCC	0.50%	2%	4
Clostridium perfringens	Positive	CRSBAN	2%	4%	2
Enterococcus faecalis ATCC 19433	Positive	ATCC	8%	>8%	>1
Escherichia coli 81 nr.149 SKN 541	Negative	Culture collection of Copenhagen University	1%	2%	2
Escherichia coli ATCC 25922	Negative	ATCC	1%	2%	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	4%	8%	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	Culture collection of Copenhagen University	>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	0.50%	1%	2
Staphylococcus aureus toxine A+B	Positive	Culture collection of Copenhagen University	1%	2%	2
Staphylococcus hominis B246	Positive	Maari (fermented baobab seeds)	0.50%	1%	2
Yersinia enterocolitica 8A30 SKN 601	Negative	Culture Collection of Copenhagen University	>8%	>8%	>1
Fungal strains	-	Origin	MIC	MFC	MFC/MIC
Candida albicans	-	Blood sample	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	0.50%	1%	2

The antimicrobial activity of volatile constituents was decreased in this decreasing order: phenols (highest active) > alcohols > aldehydes > ketones > ethers > hydrocarbons [22].

Antimicrobial activity of the essential oil of *D. microcarpum* leaves could be attributed to its major components, caryophyllene oxide (28.186%), β-caryophyllene (11.894%), humulene-1,2-epoxide (4.05%), neryl acetone (2.452%), salvia-4(14)-ene-1-one (2.423%) and linalool (2.098%). β-caryophyllene and caryophyllene oxide are known to have a good to high antimicrobial activity [23]. β-caryophyllene and caryophyllene oxide possessed antibacterial activity and caryophyllene oxide had a high activity against *Candida albicans* [24]. Essential oil of *D. microcarpum* leaves is more active on Gram-positive bacterial strains than Gram-negative bacterial strains except *Escherichia coli* strains. Most studies investigating the action of whole essential oils against food spoilage organisms and food borne pathogens agree that, generally, essential oils are slightly more active against gram-positive than gram-negative bacteria [25, 26, 23, 27, 28].

CONCLUSION: This study provided data on chemical composition, antioxidant and anti-microbial properties of the essential oil of *Detarium microcarpum* dry leaves. The major components of this essential oil were caryophyllene oxide, β-caryophyllene and humulene-1, 2-epoxide. The essential oil of *D. microcarpum* showed a good antimicrobial activity but exhibited a weak antioxidant activity. These results confirmed the traditional usage of *D. microcarpum*. The essential oil of *D. microcarpum* leaves could serve as a basis for the research of new antimicrobials.

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