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Original Article

Chemical analysis, antioxidant and antimicrobial activities of leaves essential oil of *Annona senegalensis pers.* from Burkina Faso

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ABSTRACT

The aim of this study was to determine the chemical composition, antioxidant and antimicrobial properties of leaves essential oil of *Annona senegalensis* from Burkina Faso. Essential oil was obtained by hydrodistillation and analyzed by GC and GC/MS. Antioxidant activity of the essential oil was evaluated by DPPH radical scavenging assay and FRAP test and antimicrobial activity by disk diffusion method and microdilution method. Caryophyllene oxide (32.565%), humulen-1, 2-epoxide (5.85%), spathulenol (3.62%), linalool (2.525%)**,** β-caryophyllene (2.446%) and δ-cadinene (2.012%) were the major compounds of *A. senegalensis* essential oil. The oil showed good radical scavenging power and moderate reducing power compared with quercetin, ascorbic acid and BHT. Essential oil of *A. sengalensis* exhibited high to weak antibacterial activity with inhibition diameters ranging from 08±00mm to 15.5±0.71mm and MIC value of 4% to 8%; MBCs were higher than the highest concentration tested. The oil inhibited all fungal strains with inhibition diameters between 10.5±0.71mm and 13.5±0.71mm, MIC value of 0.125% and 4% with MFC of 1% and 8%.
Plants with radical antioxidant capacity are useful for medicinal applications and as food additive. Hence the essential oil of *Annona senegalensis* leaves could be a potential antioxidant and antimicrobial agent.

Keywords: *Annona senegalenlis*, essential oil, DPPH, FRAP, disk diffusion, broth microdilution

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INTRODUCTION

Recently, the considerable research interest towards the discovery of new antimicrobial agents has been initiated with spreading drug resistant pathogens that are one of the most threats to successful treatment of microbial diseases [1]. *Annona senegalensis* Pers. is a tropical plant species also known as 'wild custard apple' or 'wild soursop' [2] belonging to the *Annonaceae* family. It is a Shrub up to 4 m tall, with irregular and open crown often from many suckers (wasteland and fallow). The bark is smooth, gray with pink slice. Boughs are more or less pubescent, gray to brown. Leaves are alternate, largely oval or oblong (7-20 x 6-12 cm), fragrant with crumpling. Flowers are solitary or arranged in groups of two or three under the armpit of a leaf, suspended under the twigs by a pedicel about 2 cm long. The fruit is a globular and fleshy berry (4 x 1 cm), orange at maturity, with many smooth protuberances and having an odor of pineapple [3]. *A. senegalensis* Pers. is a multipurpose plant with a high traditional and medicinal uses for the maintenance of free health life. Traditionally the plant is used as stimulant, pain reliever etc. Several uses of the plant species is reported for example anti-oxidant, antimicrobial, antidiarrheal, antiinflammatory, antiparasitic, anticonvulsant, antimalarial, antitrypanosonal, anti-snake venom and antinociceptive properties and many other biomedical properties of pharmaceutical relevance [2, 4, 5]. These properties of the plant are due to its important phytochemical constituents like triterpenes, anthocyanes, glucids, coumarins, flavonoids and alkaloids etc. [6]. *A. senegalensis* root bark aqueous extract is used in traditional treatment of epilepsy and convulsions in Burkina Faso [7]. The essential oil of *A. senegalensis* from different regions of the world has been studied. Ameen et al. [8] found citronellal (30%), citronellol (14.8%), geranial (17.2%), thymol (8.1%), caryophyllene (7.8%) and cavacrol (6.92%) as major compounds of the essential oil of *A. senegalensis* dried leaves from Nigeria. β-eudesmol (34.5%), elemol (29%), γ-eudesmol (13.8%), β-caryophyllene oxide (6.2%), E-β-caryophyllene (4.9%) and β-elemene (2.2%) were found as major compounds of dried leaves essential oil of *A. senegalensis* from Congo by Nkounkou-Loumpangou et al. [10]. Nébié et *al.* [10] highlighted germacrene D (19.2%), β-caryophyllene (19.1%), γ-cadinene (11.1%), and α-humulene (9.7%) as main components of essential oil of dried leaves of *A. senegalensis* from Burkina Faso.

However, we have found few studies on the essential oils of *A. senegalensis* growing in Burkina Faso. The purpose of this study was to determine chemical composition, antioxidant activity and antimicrobial activity of essential oil of leaves of *A. senegalensis*.

MATERIAL AND METHODS

Plant material: Plant material consisted of *A. senegalensis* leaves. The leaves of *A. senegalensis* were collected in Peyiri, a village near the town of *Koudougou* during the months of July and August 2014. After identification at the Laboratory of Plant Biology and Ecology, voucher specimens were kept in the herbarium of the Biodiversity Information Center under the number ID16963. The harvested leaves were dried in the laboratory at room temperature and powdered.

Extraction of the essential oil: The extraction of the essential oil from the dried leaves of *A. senegalensis* was done by hydrodistillation using a Clevenger-type apparatus [11] for 4 h. The obtained essential oil was dried over anhydrous sodium sulfate and then stored at 4° until analyzes. The extraction yield was determined using the following equation:

$R\ (\%) = V / W \times 100$ where V is the volume of essential oil (ml) and W the weight of dry plant material (g).

Chemicals:2,2-Diphenyl-1-picrylhydrazyl (DPPH), quercetin, ascorbic acid, Butylhydroxytoluene (BHT), iron (III) chloride, potassium ferricyanide, trichloroacetic acid, phosphate buffer, tween 80 were obtained from Sigma-Aldrich, Germany. Ciprofloxacin (5 µg), tetracycline (30 µg), nystatin (100 IU), blank discs (6 mm), nutrient broth, Sabouraud dextrose broth, Mueller Hinton agar, Mueller Hinton broth were purchased from Liofilchem, Italy; Sabouraud agar chloramphenicol 2 from Biomérieux, France; sodium chloride, sodium sulfate and ethanol from Prolabo. All the solvents were of analytic grade.

Chemical analysis: Chemical composition of essential oil of dried leaves of *A. senegalensis* was determined by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS). Gas chromatography analysis was carried out using a Hewlett-Packard HP 6890 type apparatus equipped with a split / splitless injector (280°), a 1:10 division ratio, using an HP-5 capillary column (25 m x 0.25 mm, film thickness 0.25 m). The oven temperature was programmed from 50 to 300° at a rate of 5°/min. Helium was used as carrier gas at a flow rate of 1.1 ml/m. The injected sample consisted of 1.0 µl of essential oil diluted 10% (V/V) with acetone. The GC/MS analysis was performed 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 an HP-Innowax capillary column (60 m x 0.25 mm, film thickness 0.25 m). The temperature program for the HP-5 MS column was 50° (5m) rising to 300° at a rate of 5°/min and for the HP-Innowax column from 50-250° at a rate of 5°/m. Helium was used as carrier gas at a flow rate of 1.1 ml/m. Identification of *A. senegalensis* leaves essential oil constituents was done by comparison of their mass spectra and their retention indices with those of reference compounds and with literature data [12, 13, 14, 15].

Antioxidant activity: The antioxidant activity of essential oil of *A. senegalensis* leaves was evaluated using two methods: DPPH radical scavenging assay and Ferric Reduction Antioxidant Power (FRAP) test.

DPPH radical scavenging assay: The radical scavenging power of the essential oil of *A. senegalensis* leaves was determined by the DPPH radical scavenging assay. This test was performed as described previously by Singh et *al.* [16]. Different amounts of the essential oil of *A. senegalensis* (5, 10, 15, 20 and 25 µl) were mixed with 5 ml of an ethanolic solution of DPPH (0.004%). This mixture was incubated in the dark for 30 min and the absorbance read at 517 nm using a spectrophotometer (JASCO V-530 UV/VIS Spectrophotometer). BHT (0.005 mol), ascorbic acid (0.005 mol) and quercetin (0.005 mol) used as reference antioxidants and a negative control were included in each test. Low absorbance indicates high inhibitory power; the inhibition percentage of the DPPH radical is 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 or reference antioxidants.

The antioxidant activity of the essential oil of *A. senegalensis* leaves was expressed as the inhibitory concentration 50 (IC50) which is defined as the amount of essential oil necessary to reduce by 50% the initial concentration of the DPPH. The IC50 was calculated graphically using a linear regression (% inhibition = f [essential oil concentrations]). The assay was performed in triplicate.

Ferric reduction antioxidant power (FRAP) test: The reducing power of the leaves essential oil of *A. senegalensis* was determined by the FRAP test. The test was carried out as describe by Singh et *al.* [17]. Different amounts of the essential oil (5, 10, 15, 20 and 25 µl) were mixed with 2.5ml of phosphate buffer (200 mmol, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was then incubated at 50° for 30 min and then 2.5 ml of trichloroacetic acid (10%) were added to the mixture followed by centrifugation at 600 G for 10 min. The supernatant was collected (5 ml), mixed with 5 ml of distilled water and 1 ml of iron chloride (0.1% $FeCl_3$) was added and the absorbance immediately measured at 700 nm with a spectrophotometer (JASCO V-530 UV/VIS Spectrophotometer). Ascorbic acid (0.1 mol) and quercetin (0.1 mol) were used as positive control and a negative control was included in each test. The higher the absorbance measured, the greater the reduction power.

Antimicrobial activity: Antimicrobial activity of the essential oil of *A. senegalensis* leaves was determined using two methods, agar disc diffusion method and broth microdilution method.

Microbial strains: Ten Gram-positive bacterial strains, ten Gram-negative bacterial strains and four fungal strains were used for antimicrobial testing.

Agar disc diffusion method: Antimicrobial activity of the essential oil of *A. senegalensis* leaves was demonstrated by the agar disc diffusion method. The tests were carried out on Mueller Hinton Agar for bacterial strains and on Sabouraud Agar for fungal strains [18].

Overnight broth cultures (18-24 h) 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. Petri dishes containing sterile Mueller Hinton Agar (for bacteria) or sterile Sabouraud Agar (for fungal strains) were inoculated with this microbial suspension. Sterile neutral discs (6 mm diameter) were impregnated with the essential oil of *A. senegalensis* leaves (15 µl per disc) and then placed on the surface of the previously inoculated agar. The dishes were then aerobically incubated at 30° for the fungal strains and at 37° for the bacterial strains for 24 h. The microbial strains sensitivity to the essential oil was assessed by measuring the diameter of the inhibition zone appearing around the disc. The criteria used by Carovic-Stanko et *al.* [19] were considered to evaluate the inhibition diameters (ID) of the essential oil:

- ID > 15 mm: the essential oil had high inhibitory action
- 10 ≤ ID ≤ 15 mm: the essential oil had moderate inhibitory action
- ID < 10 mm: the essential oil had low inhibitory action

Tetracycline (30 µg), ciprofloxacin (5 µg) was used as a positive control for bacterial strains and nystatin (100 IU) for fungal strains. The tests were carried out in duplicate.

Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC): Broth microdilution method was used to determine the MIC, the MBC and the MFC [20]. The tests were carried out in Mueller Hinton broth for bacterial strains and in Sabouraud broth for fungal strains. A double serial dilution of the essential oil of *A. senegalensis* was done in a 96-well microplate to obtain concentrations of 0.03 to 8% (V/V). 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.
Overnight broth cultures (18-24 h) 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 10 µl of these diluted inoculums 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° for the fungal strains and at 37° for the bacterial strains and the MICs determined after 24 h of incubation. The lowest concentration of the essential oil showing no visible growth in the broth after 24 h of incubation is considered to be the MIC.
For the determination of MBC or MFC, 10 µl of microbial suspension was collected from wells with no visible growth and seeded on Mueller Hinton Agar for bacterial strains and on Sabouraud Agar for fungal strains and then incubated for 24 h at 30° or 37°. The lowest concentration of essential oil at which no growth is observed on the agar after 24 h of incubation is considered to be MBC or MFC. The antimicrobial activity of the essential oil of *A. senegalensis* was evaluated, considering that:

- CMB / MIC = 1: the essential oil had absolute bactericidal activity
- 1 < CMB / CMI ≤ 4: the essential oil had bactericidal activity
- 8 < CMB / CMI < 16: the essential oil had bacteriostatic activity

RESULTS AND DISCUSSION

Chemical analysis: The hydrodistillation of the leaves of *A. senegalensis* gave a yellowish-colored essential oil with an extraction yield of 0.14 ± 0.04% (v/w). This yield is similar to the yield of 0.19% obtained in Cameroon by Ngamo et *al.* [21] and greater than the 0.02% yield obtained by Ameen et *al.* [8] in Nigeria and the yield of 0.022% obtained by Noudogbessi et *al.* [22] in Benin. However, this yield is lower than the yields of 0.60% and 0.73% respectively obtained by Nkounkou-Loumpangou et *al.* [9] in Congo and Nebié et *al.* [10] in Burkina Faso.
The results of the chemical analysis of dried leaves essential oil of *A. senegalensis* are presented in table 1. A total of forty five (45) compounds accounting for 72.009% of the essential oil were identified. The relative abundance of the components is shown in figure 1. The main components of *A. senegalensis* dried leaves essential oil were caryophyllene oxide (32.565%), humulen-1,2-epoxide (5.85%), spathulenol (3.62%), linalol (2.525%), β-caryophyllene (2.446%), δ-cadinene (2.012%) and paracymene (1.93%). The chemical composition of the essential oil of *A. senegalensis* leaves from Burkina Faso differs from the chemical compositions reported by several authors. Ameen et *al.* [8] found citronellal (30%), citronellol (14.8%), geranial (17.2%), thymol (8.1%), caryophyllene (7.8%) and cavacrol (6.92%) as major compounds of the essential oil of *A. senegalensis* dried leaves from Nigeria. β-eudesmol (34.5%), elemol (29%), γ-eudesmol (13.8%), β-caryophyllene oxide (6.2%), E-β-caryophyllene (4.9%) and β-elemene (2.2%) were found as major compounds of dried leaves essential oil of *A. senegalensis* from Congo [9]. Nébié et *al.* [10] highlighted germacrene D (19.2%), β-caryophyllene (19.1%), γ-cadinene (11.1%), and α-humulene (9.7%) as main constituents of essential oil of dried leaves of *A. senegalensis* from Burkina Faso.
Climatic, geographic conditions, environmental factors, genetic variation, vegetative cycle stage, part of plant utilized, post-harvest drying and storage and type of extraction are among the factors that could explain such differences [23-26].

Antioxidant activity: Antioxidant activity of the essential oil of *Annona senegalensis* was evaluated by DPPH radical scavenging assay and FRAP test. Quercetin, ascorbic acid and BHT were used for comparison.

DPPH radical scavenging power: DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radical scavenging assay was used to evaluate free radical scavenging power of the investigated essential oil. The DPPH radical scavenging power of the essential oil~~s~~ of *A. senegalensis* and reference antioxidants are shown in figure 2. These results show that when the concentration of essential oil or antioxidants increases, the DPPH radical scavenging power also increases. The DPPH radical scavenging power of the essential oil of *A. senegalensis* therefore depends on the concentration. The inhibitory concentrations 50 (IC50) of the studied essential oil and the standards are shown in table 2. The lowest IC50 (10.64 µl) was obtained with the essential oil of *A. senegalensis* and the highest one (26.93 µl) with BHT. Referring to Table 2, the essential oil of *A. senegalensis* has a better radical scavenging power than BHT (0.005 mol), ascorbic acid (0.005 mol) and quercetin (0.005 mol). However, considering figure 2, at the high concentrations, the DPPH radical scavenging powers of the essential oil and quercetin are similar.

FRAP Test: Figure 3 shows the results of the FRAP test. These results indicate that the reducing power of essential oil and standards increases as the concentration increases. The reducing power of essential oil of *A. senegalensis* is therefore dependent on concentration. Contrary to the results of the DPPH radical scavenging assay, in this test the essential oil had a low reducing power compared to ascorbic acid (0.1 mol) and quercetin (0.1 mol). This difference in results could be attributed to the concentration of standards which is higher.

Aqueous, aqueous methanol and ethyl acetate extracts of leaves of *A. senegalensis* from different countries have shown antioxidant activity when testing by different methods [27, 28]. Antioxidant properties are influenced by several factors, including the species, part of the plant, season of harvesting, geographical origin and extraction method, which also influence the chemical composition of plant essential oils [29]. Some studies have been conducted to clarify the possible substances involved in antioxidant properties of essential oils. 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 [30, 31]. Antioxidant activity of the essential oil of *A. senegalensis* dried leaves could be attributed to its main compounds which are caryophyllene oxide (32.565%), humulen-1,2-epoxide (5.85%), spathulenol (3.62%), linalol (2.525%), β-caryophyllene (2.446%), δ-cadinene (2.012%) and paracymene (1.93%). Indeed caryophyllene oxide, spathulenol and β-caryophyllene have been reported to possess strong radical scavenging power [32-34]. In addition, linalol is a monoterpenoid possessing biological properties such as antibacterial and antioxidant activities [35]. However, we cannot attribute the antioxidant effect of a total essential oil to the major compounds only; minor molecules may make significant contributions to the oil activity [36].

Antimicrobial activity: The antimicrobial activity of the essential oil of *A. senegalensis* was demonstrated by the disk diffusion method. The results are presented in table 3. Essential oil of *A. senegalensis* was active on all bacterial strains with inhibition diameters of 08±00 mm (*Salmonella infantis*) to 15.5±0.71 mm (*Shigella flexneri*). All fungal strains were inhibited by the essential oil of *A. senegalensis* with inhibition diameters ranging from 10.5±0.71 mm (*Candida albicans*) to 13.5±0.71 mm (*Saccharomyces cerevisiae*).

According to Carovic-Stanko et *al.* (2010) criteria, essential oil of *A. senegalensis* had:

- strong inhibitory action (ID>15 mm) on *Shigella flexneri;*
- moderate inhibitory action (10 ≤ ID ≤15 mm) on *Bacillus* strains, *Escherichia coli* strains, *Staphylococcus* strains, *Clostridium perfringens*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shygella dysenteria*, *Yersinia enterocolitica* and all fungal strains;
- and low inhibitory action (ID<10mm) on *Salmonella enteritidis*, *Salmonella infantis* and *Salmonella nigeria.*

MIC, MBC and MFC: MICs, MBCs and MFCs of *A. senegalensis* essential oil were determined by broth microdilution method. The results are shown in Table 4. The MICs of *A. senegalensis* essential oil were 4% (V/V) for five bacterial strains, 8% for nine bacterial strains and greater than 8% for the others. The MBCs of *A. senegalensis* essential oil were all higher than the highest concentration tested (8%).

The essential oil of *A. senegalensis* had inhibitory action on all the fungal strains tested with MICs of 0.125% (Saccharomyces cerevisiae) to 4% (*Candida albicans*). The MFCs were 1% (*Saccharomyces cerevisiae*) and 8% (*Candida kefir* and *Candida tropicalis*). The essential oil of *A. senegalensis* had a fungicidal action (1< CMB/CMI ≤4) on *Candida kefir*, *Candida tropicalis* and a fungistatic action on *Saccharomyces cerevisiae*.

Antimicrobial properties of leaf extracts of *Annona senegalensis* have been reported by other studies. Traoré et *al.* [37] found that leaf extracts of *A. senegalensis* had bactericidal action on *Streptococcus pneumoniae* and fungicidal action on *Aspergillus fumigatus*. *A. senegalensis* root extracts possessed antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [4, 38]. Chalchat et *al.* [39] found that the essential oil of *A. senegalensis* fruit had inhibitory action against *Staphylococcus aureus* and *Klebsellia pneumoniae* with MIC value of 3.60mg/ml and 3.40mg/ml respectively.

Antimicrobial activity of dried leaves essential oil of *A. senegalensis* could be attributed to its major components, but also to other components present in small quantities or to synergy among them. Indeed several researchers have reported that monoterpene or sesquiterpene hydrocarbons and their oxygenated derivatives, which are the major components of essential oils, exhibit potential antimicrobial activity [40]. More essential oils with a high quantity of sesquiterpenes have been reported to have antibacterial and antifungal properties. δ-cadinene, (*Z*)-β-farnesene, γ-muurolene, caryophyllene oxide, (*E*)-caryophyllene, *α*-eudesmol, β-eudesmol, spathulenol, hexahydrofarnesyl acetone and *α*-selinene have been identified as main active components [41, 42]. In addition β-caryophyllene, caryophyllene oxide and spathulenol have been reported to exhibit moderate to strong antimicrobial activities [32, 43, 44]. Moreover, linalol has been known to possess antimicrobial activities [35, 45, 46]. *A. senegalensis* leaves essential oil is more active on Gram-positive bacteria than Gram-negative bacteria as many studies on antimicrobial activities of essential oils have pointed out [25, 47, 48].

CONCLUSION

This study provides data on the chemical composition and biological activities of essential oil of *A. senegalensis* from Burkina Faso. Essential oil extracted from the dried leaves of this plant showed good antioxidant activity and good antimicrobial activity against several microorganisms. These results support the traditional uses of *A. senegalensis*.

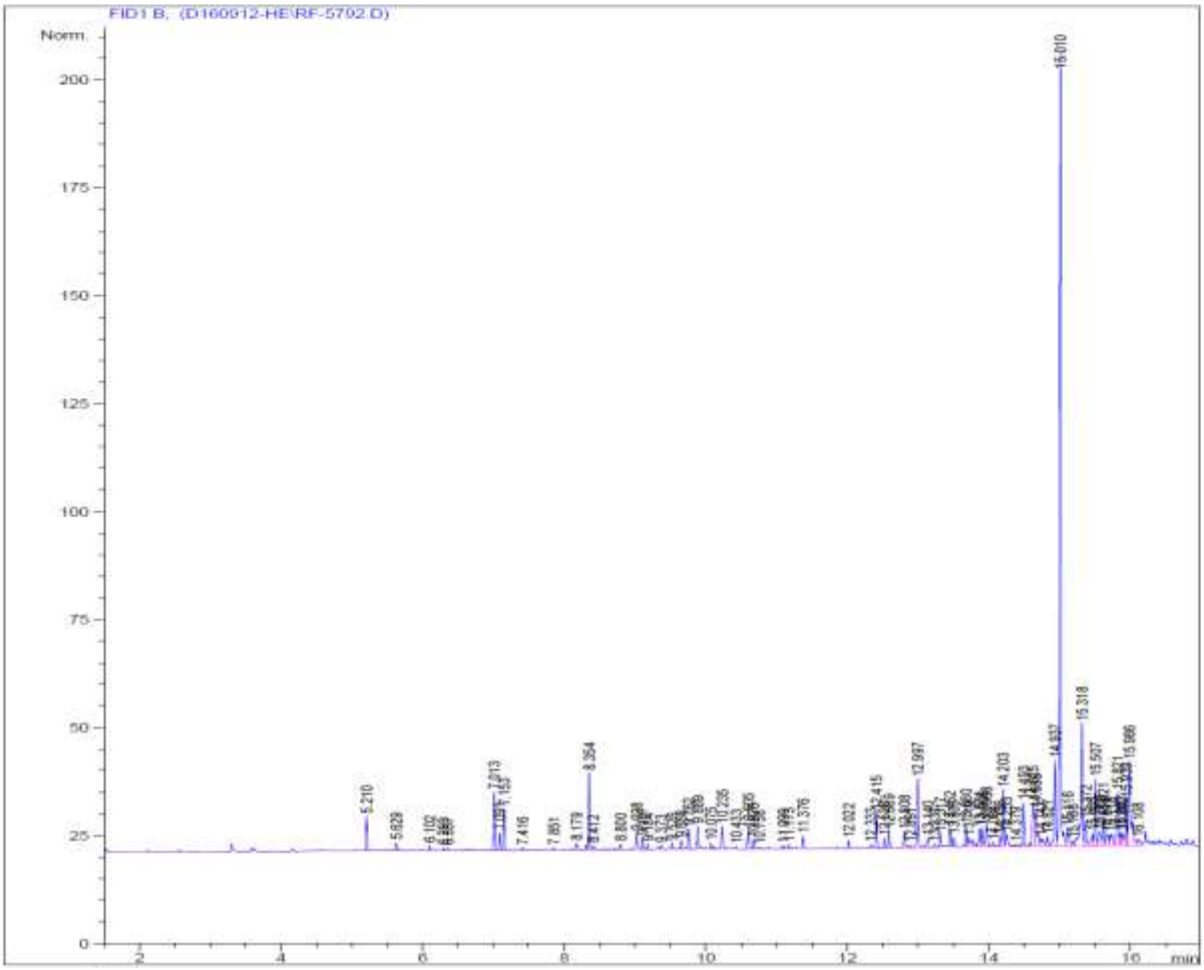

Figure 1: Chromatogram of the essential oil of *Annona senegalensis* dried leaves

TABLE 1: CHEMICAL COMPOSITION OF DRIED LEAVES ESSENTIAL OIL OF *ANNONA SENEGALENSIS*

Number	Retention time (min)	Components	Proportion (%)
1	5.21	α-Pinene	1.214
2	5.63	Thuja-2,4(10)-Diene	0.249
3	6.1	β-Pinene	0.171
4	6.29	3-Octanone	0.091
5	6.36	Myrcene	0.13
6	**7.01**	**Para-Cymene**	**1.93**
7	7.09	Limonene	0.61
8	7.15	Eucalyptol	1.402
9	7.42	(E)-β-Ocimene	0.091
10	7.85	Cis-Oxide Linalol	0.109
11	8.18	Para-Cymenene	0.267
12	**8.35**	**Linalol**	**2.525**
13	9.03	Trans-Pinocarveol	0.682
14	9.11	Trans-Verbenol	0.377
15	9.18	Mentha-1,5-Diene-8-ol para	0.222
16	9.37	Pinocarvone	0.138
17	9.53	Borneol	0.241
18	9.66	Terpinene-4-ol	0.341
19	9.76	Para-Cymene-8-ol	0.722
20	9.89	α-Terpineol	0.887
21	10.07	Verbenone	0.237
22	10.24	trans-Carveol	0.891
23	10.6	Cuminaldehyde+Carvone	0.838
24	10.68	Geraniol	0.328
25	11.38	Carvacrol	0.408
26	12.02	Alpha-Cubebene	0.259
27	12.42	Alpha-Copaene	1.148
28	12.53	β-Bourbonene	0.308
29	12.59	β-Elemene	0.606
30	12.81	Ylanga-2,4(15)-Diene	0.668
31	**13**	**β-Caryophyllene**	**2.446**
32	13.14	α-Copene	0.586
33	13.23	α-Trans-Bergamotene	0.183
34	13.45	Neryl Acetone	0.612
35	13.5	α-Humulene	0.398
36	13.68	γ-Muurolene	0.743
37	13.87	β-Selinene	0.73
38	13.97	α-Muurolene	1.116
39	14.16	γ-Cadinene	0.408
40	**14.2**	**δ-Cadinene**	**2.012**
41	14.49	α-Calacorene	1.62
42	**14.94**	**Spathulenol**	**3.622**
43	**15.01**	**Caryophyllene oxide**	**32.565**
44	**15.32**	**Humulene-1,2-epoxide**	**5.85**
45	15.62	Intermedeol	1.028
		Total	**72.009**

TABLE 2: INHIBITORY CONCENTRATION 50 (IC50) OF DRIED LEAVES ESSENTIAL OIL OF *ANNONA SENEGALENSIS* AND STANDARDS

Essential oil / Standards	**Regression equation**	**R^2**	**IC50 (µl)**
Annona senegalensis	y= 2.3419x + 25.108	0.99	10.64 ± 0.51
BHT (0.005M)	y= 1.8255x+0.8298	0.99	26.93 ± 0.32
Ascorbic acid (0.005M)	y= 2.3558x+6.8831	0.99	18.33 ± 0.65
Quercetin (0.005M)	y= 2.7794x+15.387	0.99	12.19 ± 0.41

TABLE 3: INHIBITION ZONE DIAMETERS (mm) OF DRIED LEAVES ESSENTIAL OIL OF *ANNONA SENEGALENSIS* (15µL) AND STANDARD ANTIBIOTICS

Microbial strains			**Inhibition zones diameters (mm) including disk diameter (6mm)**			
Bacterial strains	***Gram***	***Origin***	***A. senegalensis***	***Tetracycline (30 µg)***	***Ciprofloxacin (5 µg)***	***Nystatin (100 UI)***
Bacillus cereus LMG13569	Positive	CCLMU	14±00	19±1.41	26.5±2.12	-
Bacillus subtilis ssp subtilis ATCC 6051	Positive	ATCC	13±1.41	30±00	34±1.41	-
Clostridium perfringens	Positive	CRSBAN	12±00	26.5±212	16±1.41	-
Enterococcus faecalis ATCC 19433	Positive	ATCC	12±00	24.5±0.71	24.5±2.12	-
Escherichia coli 81 nr.149 SKN 541	Negative	CCCU	13±1.41	15.5±2.12	32.5±2.12	-
Escherichia coli ATCC 25922	Negative	ATCC	12.5±0.71	32.5±3.54	22.5±0.71	-
Listeria monocytogenes NCTC 9863	Positive	CCLMU	14±1.41	21.5±2.12	31±1.41	-
Micrococcus luteus SKN 624	Positive	CCCU	11±1.41	16.5±2.12	31.5±2.12	-
Pseudomonas aeruginosa ATCC 9027	Negative	ATCC	10±1.41	12±1.41	32.5±0.71	-
Salmonella enteridis P167807	Negative	CCLMU	8.5±0.71	22.5±2.12	30.5±2.12	-
Salmonella infantis SKN 557	Negative	CCCU	8±00	20.5±2.12	27.5±2.12	-
Salmonella typhimurium SKN 1152	Negative	CCCU	10.5±0.71	19.5±2.12	26±1.41	-
Salmonella nigeria SKN 1160	Negative	Cocoa beans	8.5±0.71	17±1.41	30±00	-
Shigella dysenteria 370	Negative	CCLMU	12±1.41	22±2.83	36±1.41	-
Shigella flexneri USCC 2007	Negative	CCLMU	15.5±0.71	22.5±0.71	31±1.41	-
Staphylococcus aureus ATCC 2523	Positive	ATCC	11.5±0.71	20±1.41	24±1.41	-
Staphylococcus aureus ATCC 25923	Positive	ATCC	15±1.41	23.5±0.71	26.5±2.12	-
Staphylococcus aureus toxine A+B	Positive	CCCU	12±00	10.5 ± 0.71	ND	-
Staphylococcus hominis B246	Positive	Maari (fermented baobab seeds)	12±00	29±1.41	33±1.41	-
Yersinia enterocolitica 8A30 SKN 601	Negative	CCCU	14±1.41	15.5±0.71	37±1.41	-
Fungal strains		***Origin***	***A. senegalensis***	-	-	***Nystatin (100 UI)***
Candida albicans		Blood sample	10.5±0.71	-	-	22±00
Candida kefir		Fura (fermented millet food)	12±00	-	-	24±00
Candida tropicalis		Fura (fermented millet food)	12±00	-	-	20.5±0.71
Saccharomyces cerevisiae KVL 013		CCCU	13.5±0.71	-	-	27.5±0.71

ATCC: American Type Culture Collection
CCCU: Culture Collection of Copenhagen University
CCLMU: Culture Collection of London Metropolitan University
CRSBAN: Centre de Recherche en Sciences Biologiques Alimentaires et Nutritionnelles

TABLE 4: MIC, MBC AND MFC OF *ANNONA SENEGALENSIS* DRIED LEAVES ESSENTIAL OIL (0.03% TO 8% (V/V))

Microbial strains			***Annona senegalensis***		
Bacterial strains	***Gram***	***Origin***	***MIC***	***MBC***	***MBC/MIC***
Bacillus cereus LMG13569	Positive	CCLMU	8%	>8%	>1
Bacillus subtilis ssp subtilis ATCC 6051	Positive	ATCC	>8%	>8%	>1
Clostridium perfringens	Positive	CRSBAN	4%	>8%	>2
Enterococcus faecalis ATCC 19433	Positive	ATCC	8%	>8%	>1
Escherichia coli 81 nr.149 SKN 541	Negative	CCCU	4%	>8%	>2
Escherichia coli ATCC 25922	Negative	ATCC	4%	>8%	>2
Listeria monocytogenes NCTC 9863	Positive	CCLMU	4%	>8%	>2
Micrococcus luteus SKN 624	Positive	CCCU	4%	>8%	>2
Pseudomonas aeruginosa ATCC 9027	Negative	ATCC	8%	>8%	>1
Salmonella Enteridis P167807	Negative	CCLMU	>8%	>8%	>1
Salmonella Infantis SKN 557	Negative	CCCU	>8%	>8%	>1
Salmonella Typhimurium SKN 1152	Negative	CCCU	8%	>8%	>1
Salmonella nigeria SKN 1160	Negative	Cocoa beans	>8%	>8%	>1
Shigella dysenteria 370	Negative	CCLMU	>8%	>8%	>1
Shigella flexneri USCC 2007	Negative	CCLMU	8%	>8%	>1
Staphylococcus aureus ATCC 2523	Positive	ATCC	8%	>8%	>1
Staphylococcus aureus ATCC 25923	Positive	ATCC	>8%	>8%	>1
Staphylococcus aureus toxine A+B	Positive	CCCU	8%	>8%	>1
Staphylococcus hominis B246	Positive	Maari (Fermented baobab seeds)	8%	>8%	>1
Yersinia enterocolitica 8A30 SKN 601	Negative	CCCU	8%	>8%	>1
Fungal strains		**Origin**	**MIC**	**MFC**	**MFC/CMI**
Candida albicans		Blood sample	4%	>8%	>2
Candida kefir		Fura (fermented millet food)	2%	8%	4
Candida tropicalis		Fura (fermented millet food)	2%	8%	4
Saccharomyces cerevisiae KVL 013		CCCU	0.125%	1%	8

ATCC: American Type Culture Collection
CCCU: Culture Collection of Copenhagen University
CCLMU: Culture Collection of London Metropolitan University
CRSBAN: Centre de Recherche en Sciences Biologiques Alimentaires et Nutritionnelles

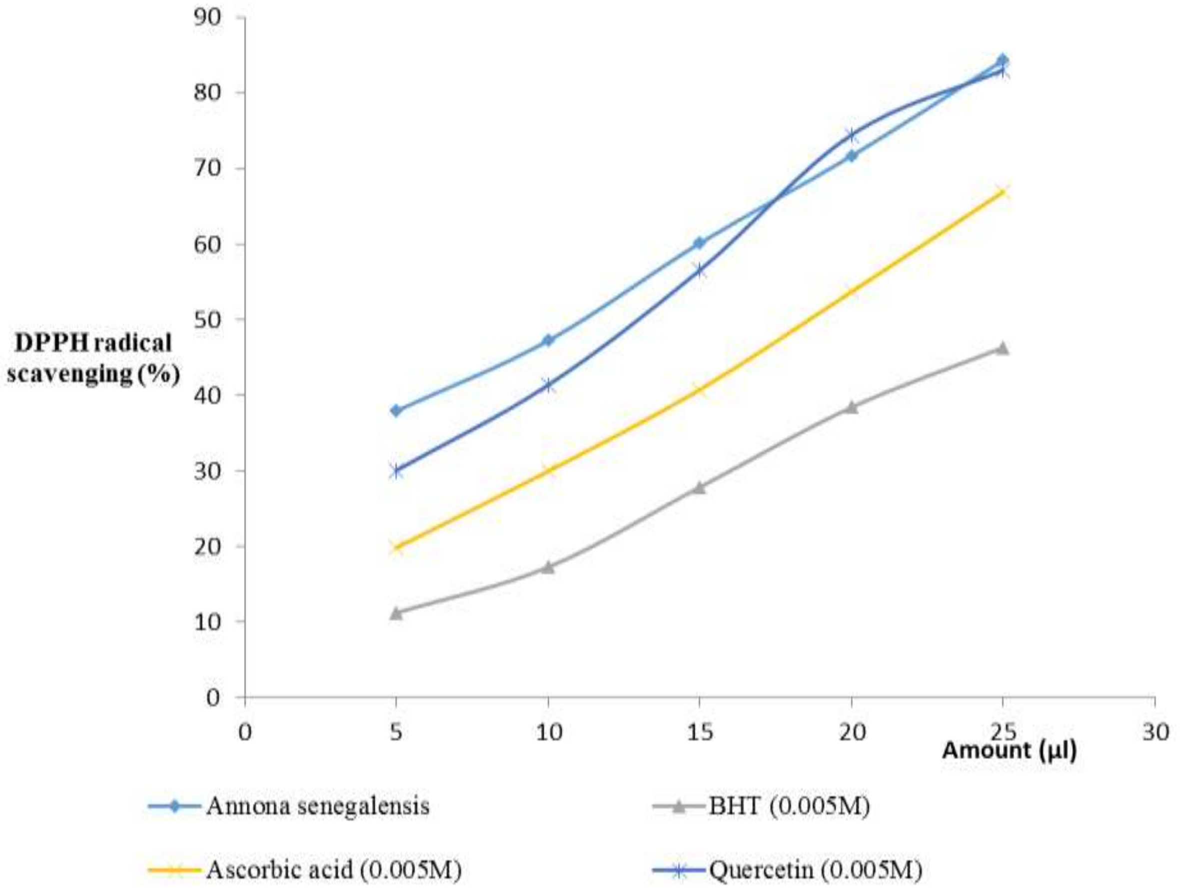

Figure 2: Radical scavenging power of the essential oil of *Annona senegalensis* and standards

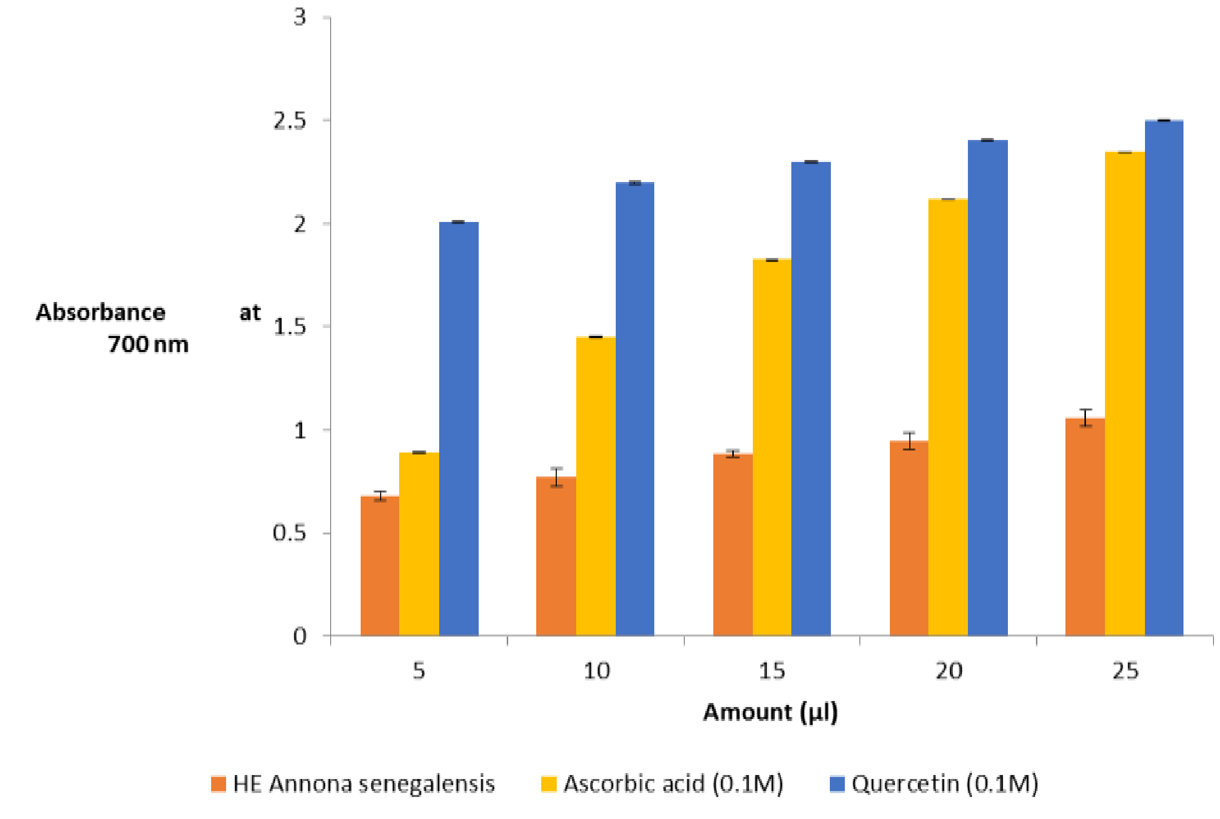

Figure 3: Ferric reducing antioxidant power of the essential oil of *Annona senegalensis* and standards

REFERENCES

1. El Baroty GS et al. Antimicrobial and antioxidant activities of leaves and flowers essential oils of Egyptian *Lantana camara* L. Der Pharm Chem 2014; 6(6): 246-255.
2. Ngbolua KN et al. A mini-review on the pharmacognosy and phytochemistry of a tropical medicinal plant: *Annona senegalensis* Pers. (Annonaceae). Trop Plant Res 2017; 4(1): 168-175.
3. Arbonnier M. Trees, shrubs and vines in the dry lands of West Africa, 2nd ed.; CIRAD-MNHN: France, 2002 [in French].
4. Apak L, Olila D. The *in-vitro* antibacterial activity of *Annona senegalensis*, *Securidacca longipendiculata* and *Steganotaenia araliacea* - Ugandan medicinal plants. Afr Health Sci 2006; 6 (1).
5. Ajaiyeoba E et al. *In vivo* antimalarial and cytotoxic properties of *Annona senegalensis* extract. Afr J Tradi Complem Altern Med 2006; 3(1): 137-141.
6. Okhale SE et al. *Annona senegalensis* Persoon (Annonaceae): a review of its ethnomedicinal uses, biological activities and phytocompounds. J Pharmaco Phytochem 2016; 5(2): 211-219.
7. Konate A et al. Phytochemical and anticonvulsant properties of *Annona senegalensis* Pers. (Annonaceae), plant used in Burkina folk medicine to treat epilepsy and convulsions. Brit J Pharm Toxico 2012; 3(5): 245-250.
8. Ameen OM et al. Chemical composition of leaf essential oil of *Annona senegalensis* Pers. (Annonaceae) growing in North Central Nigeria. Int J Biol Chem Sci 2011; 5(1): 375-379.
9. Nkounkou-Loumpangou C et al. Comparative study of the chemical composition of the essential oils from organs of *Annona senegalensis* Pers. *oulotricha* le Thomas subspecies (Annonaceae). Afr J Biotechnol 2010; 9 (6): 887-891.
10. Nébié RCH et al. Chemical composition of leaf essential oil of *Annona senegalensis* Pers. from Burkina Faso. J Essent Oil Res 2005; 17: 331-332.
11. Lograda T et al. Chemical analysis and antimicrobial activity of *Teucrium polium* L. essential oil from Eastern Algeria. Amer J Advanced Drug Deliv 2014; 2(6): 697-710.
12. Adams RP. Identification of essential oils components by Gas Chromatography-Quadrupole Mass Spectrometry. Allured Publishing Corp., Card Stream: Illinois, USA, 2001.

13. Joulain D, König WA. The Altlas of spectral data of sesquiterpene hydrocarbons. E. B. Verlag.: Hamburg, 1998.
14. Mc Lafferty FW, Stauffer DB. The Wiley NBS registry of Mass Spectral Data, 2nd ed.; Wiley and Sons: New York, 1989.
15. Van Den Dool H, Kratz PDJ. A generation of the retention index system inclg linear temperature programmed gas-liquid partition chromatography. Chromatog. 1963; 11: 463-471.
16. Singh S et al. Composition, in vitro antioxidant and antimicrobial activities of essential oil and oleoresins obtained from black Cumin seeds (*Nigella sativa* L.). BioMed Res Inter 2014.
17. Singh G et al. Antioxidative and antibacterial potentials of essential oils and extracts isolated from various spice materials. J Food Safety 2005; 25: 130-145.
18. Bassolé IHN et al. Composition and antimicrobial activities of *Lippia multiflora* Moldenke, *Mentha* x *piperita* L. and *Ocimum basilicum* L. essential oils and their Major monoterpene alcohols alone and in combination. Molecules 2010; 15: 7825-7839.
19. Carovic-Stanko K et al. Composition and antibacterial activities of essential oils of seven Ocimum taxa. Food Chem 2010; 119: 196-201.
20. Obame ELC et al. Antifungal and antibacterial activities of *Aucoumea klaineana* Pierre essential oil from Gabon. VRI Phytomedicine 2014; 2(1).
21. Ngamo TSL et al. Persistence of insecticidal activities of crude essential oils of three aromatic plants towards four major stored product insect pests. Afr J Agric Res 2007; 2(4): 173-177.
22. Noudogbessi JP et al. Chemical composition of the essential oils extracted from two Annonaceae required in Beninese pharmacopeia. Aust J Basic and Appl Sci 2011; 5(2): 34-40.
23. Anwar F et al. Changes in composition and antioxidant and antimicrobial activities of essential oil of fennel *(Foeniculum vulgare* Mill.) fruit at different stages of maturity. J Herbs Spices Med Plants 2009; 15: 1-16.
24. Vidic D et al. Influence of the continental climatic conditions in the essential-oil composition on *Salvia Brachyodon* Vandas transferred from Adriatic Coast. Chem Biodivers 2010; 7: 1208-1216.
25. Casiglia S et al. Influence of harvesting time on composition of the essential oil of *Thymus capitatus* (L.) Hoffmanns. & Link. growing wild in northern Sicily and its activity on microorganisms affecting historical art crafts. Arab J Chem 2015.
26. Zekri N et al. Effect of geographic locations on chemical composition of *M. Spicata* L. essential oils from Moroccan Middle-Atlas. Der Pharm Lettre 2016; 8(4): 146-150.
27. Ajboye TO et al. Antioxidant and drug detoxification potential of aqueous extract of *Annona senegalensis* leaves in carbon tetrachloride-induced hepatocellular damage. Pharm Biol 2010; 48(12): 1361-1370.
28. Potchoo Y et al. Antioxidant activity of aqueous methanol and ethyl acetate extract of leaves of *Annona senegalensis* Pers from Togo versus the one originates from Burkina Faso. Int J Pharmacol 2008.
29. Teixeira B et al. Chemical composition and antibacterial and antioxidant properties of commercial essential oils. Ind Crop Prod 2013; 43: 587-595.
30. Tepe B et al. Antimicrobial and antioxidant activities of essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chem 2005; 90: 333-3340.
31. Loizzo MR et al. *Salvia leriifolia* Benth (Lamiaceae) extract demosntrates *in vitro* anti-oxidant properties and cholinesterase inhibitory activity. Nutr Res 2010; 30: 823-830.
32. Dahham SS et al. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-Caryophyllene from the essential oil of *Aquilaria crassna.* Molecules 2015; 20: 11808-11829.
33. Donati M et al. Radical scavenging and antimicrobial activities of Croton zehntneri, Pterodon emarginatus and Schinopsis brasiliensis essential oils and their major constituents: estragole, trans-anethole, β-caryophyllene and myrcene. Nat Prod Res 2014.
34. Kaurinovic B et al. Antioxidant properties of *Marrubium peregrinum* L. (Lamiaceae) essential oil. Molecules 2010; 15: 5943-5955.
35. Liu K et al. Isolation and biological activities of decanal, linalool, valencene and octanal from sweet orange oil. J Food Sci 2012; 77: 1156-1161.
36. Laggoune S et al. Chemical composition, antioxidant and antibacterial activities of the essential oil of *Mentha spicata* L. from Algeria. J. Mater. Environ. Sci. 2016; 7(11): 4205-4213.
37. Traoré Y et al. Research of antifungal and antibacterial activities of leaves of *Annona senegalensis* Pers. (Annonaceae). J Appl Biosci 2012; 58: 4234-4242 [in French].
38. Okoye TC et al. Antimicrobial effects of a lipophilic fraction and kaurenoic acid isolated from the root bark extracts of *Annona senegalensis*. Evid-Based Compl Alt Med 2012.
39. Chalchat JC et al. Correlation between chemical composition and antimicrobial activity of some African essential oils. J Essent Oil Res 1997; 9: 67-75.

40. Bajpai VK et al. Chemical composition analysis and antibacterial mode of action of *Taxus cuspidata* leaf essential oil against foodborne pathogens. J Food Safety 2014; 34: 9-20.
41. Negri M et al. Early state research on antifungal natural products. Molecules 2014; 19: 2925-2956.
42. Koroch AK et al. Bioactivity of essential oils and their components. In: Berger R.G. (Ed.), Flavours and Fragrances Chemistry, Bioprocessing and Sustainability. Springer-Verlag, Berlin: Heidelberg, 2007: 87-115.
43. Ngassapa OD et al. Chemical composition and antimicrobial activity of *Geniosporum rotundifolium* Briq and *Haumaniastrum villosum* (Bene) AJ Paton (Lamiaceae) essential oils from Tanzania. Trop J Pharm Res 2016; 15(1): 107-113.
44. Schmidt E et al. Antimicrobial activities of single aroma compounds. Nat Prod Commun 2010; 5(9): 1365-1368.
45. Park S et al. Antimicrobial effect of linalool and α-terpineol against periodontopathic and cariogenic bacteria. Anaerobe 2012; 18: 369-372.
46. Soković M et al. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. Molecules 2010; 15: 7532-7546.
47. Costa R et al. Antimicrobial activity and chemical composition of Citrus aurantifolia (Christm.) Swingle essential oil from Italian organic crops. J Essent Oil Res 2014; 26(6): 400-408.
48. Giweli A et al. Antimicrobial and antioxidant activities of essential oils of *Satureja thymbra* growing wild in Libya. Molecules 2012; 17: 4836-4850.