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Antimicrobial and antioxidant activities of leaves and flowers essential oils of Egyptian *Lantana camara* L.

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ABSTRACT

The essential oil of flowers (FLCO) and leaves (LLCO) of *Lantana camara* species growing in Egypt were obtained by hydrodistillation, and analyzed by GC/MS. Twenty six constituents were detected in the FLCO representing 95.33% of the total oil, and eighteen in the LLCO representing 95.56% of the total oil. The main constituents were: davanone (28.59%), α -caryophyllene (11.21%), α -curecumene (10.26%), β -copaene and humulene (12.29%) in FLCO, and Davanone (23.37%), α -caryophyllene (22.96%) and humulene (14.32%) in LLCO. Both oils exhibited *in vitro* antimicrobial activity against *Bacillus cereus* and *Bacillus subtilis*. Bioautography on thin-layer chromatography plates demonstrated antimicrobial of the FLCO and LLCO with an R_f values of 0.30 and 0.55. A total of 7 compounds, 3 sesquiterpenoids (humulene, davanone and caryophyllene) were detected and identified using gas chromatography–mass spectrometry analysis. However, transmission electron microscopy micrographs of tested *B. cereus* spores confirmed the effects of these oils on morphological and ultra-structural alterations in the treated spores. Moreover, the oils showed moderate antioxidant activity and this effect was increased by increasing their concentrations. The significant antimicrobial and antioxidant activities of both oils suggest that it could serve in medical purposes, food and perfumery industries.

Keywords: *Lantana camara*, essential oil, antimicrobial and antioxidant activity

INTRODUCTION

For thousands of years, it has been known that medical agents and an impressive number of drugs have been isolated and manufactured from natural sources. 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. Also, increasing food borne diseases resulted from consumption of food contaminated with pathogenic microorganisms or the microbial toxins [1,2], and increasing concern over the presence of chemical residue in foods and the demand for non-toxic natural preservatives stimulated the research effort towards the development of natural alternatives [3].

Medicinal and aromatic plants are considered as a good alternative source of natural preservatives and costly antibiotics against which microbes are developing resistance rapidly. Most of the medicinal plants are safe with little or no side effects, cost effective and have ability to affect a wide range of antibiotic resistant microorganisms [4,5]. However, approximately 20% of the plants in the world have been submitted to pharmacological and biological tests. The natural products extracted from the medicinal plants play an important role in drug and natural preservative development in the pharmaceutical and food industry, respectively [3,6,7,8].

Essential oils (EOs) are natural aromatic oily liquids extracted from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). EOs or their components have been shown to exhibit antibacterial [6,7,9], antiviral [9], antimutagenic [10], antiparasitic [11], and insecticidal [12] properties. Currently the EOs are used (as flavorings), perfumes (as fragrances and aftershaves) and pharmaceuticals (for their functional properties). The modern use of essential oils will be continued to grow rapidly as health scientists and medical practitioners continue to research and validate the numerous health and wellness benefits of therapeutic-grade essential oils [13, 14, 15].

Lantana camara L. (Verbanaceae), commonly known as wild or red sage, is the most widespread species of this genus and regarded both as a notorious weed and a popular ornamental garden plant [16]. *Lantana camara* L. is listed as one of the important medicinal plants of the world. Several reports have described antifungal [17,18] and antimicrobial activities of *L. camara* [19, 20]. In popular medicine, it is used as carminative, antispasmodic, antiemetic agents, and to treat respiratory infections as cough, cold, asthma, and bronchitis [21]. All the parts of this plant are traditionally used for several ailments. Leaves of the plant are antiseptic, antitumoural, and antimicrobial [22] whereas, roots are used in the treatment of malaria, rheumatism, and skin rashes [23].

Consequently, the objective of the present study was to evaluate the chemical composition and antioxidant and antimicrobial efficacy of essential oils extracted from the leaves and flowers of Egyptian *Lantana camara*. Also, the effect of FLCO and LLCO on *Bacillus cereus* spores was measured using transmission electron microscopy (TEM).

MATERIALS AND METHODS

1. Extracts preparation

The leaves and flowers of *lantana canmara* were obtained from the garden of Faculty of Agriculture, Cairo University. A known amount (250 g) of each plant part were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous Na₂SO₄, filtered and stored at -10 °C in a sealed vial until tested and analyzed.

2. Gas chromatography/ mass spectrometry (GC-MS) analysis.

The analysis of the essential oils were performed using GC/MS system consisted of a HP 5890 series II gas chromatograph, HP 5972 mass detector. A TEMS-MS capillary column (75% cyanosiloxane, 30 m x 0.25 mm i.d., 0.25 µm film thickness) was used with helium as the carrier gas, at a flow rate of 1.2 ml/min. GC oven temperature was programmed at an initial temperature of 60°C for 5 min, then heated up to 140°C at 5°C/min and held at 140°C for 5 min, then heated to 280°C at 9°C/min and held for 5 additional minutes. Injector and detector temperatures were 250°C. Diluted sample (1/100, v/v in heptane) of 1.0 µL were injected. Mass spectrometry was run in the electron impact mode (EI) at 70 eV. The components were identified based on the comparison of their GC retention times, interpretation of their mass spectra and confirmed by mass spectral library search using the National Institute of Standards and Technology (NIST, 2010) database [24, 25].

3. Antioxidant activity

3.1. DPPH scavenging assay

The ability of the essential oil to scavenge DPPH radical was assessed as described by Tagashira and Ohtake [26]. Briefly, different concentrations of tested oil (ranged 5-200 µg) in one ml of ethanol were added to 25 ml of methanolic DPPH (100 mmol/L⁻¹) solution. The mixtures were incubated in the dark at 27 °C ± 1°C, then the absorbance was measured at 517 nm for 90 min, at 15 min intervals, against a blank (pure methanol). BHT, BHA and α-tocopherol (2-40 µg/mL) were used as reference standards. The radical scavenging activity of the each oil was calculated from a calibration curve. All tests were run in triplicate and averaged. The oil concentration providing 50% inhibition (IC₅₀) was calculated from a graph representing the inhibition percentage against oil concentration. For the calculation of these values, Microsoft Excel software was used.

3.2. β -Carotene-linoleic acid bleaching

The ability of the oils to prevent the bleaching of β -carotene-linoleic acid was assessed as described by Abd El Baky and El Baroty [27]. An aliquot, β -carotene (0.2 mg) in chloroform (2 mL), linoleic acid (30 μ L) and Tween-20 (200 mg) were transferred into round-bottom flask. Once the solvent was evaporated, 250 mL ultra-pure water was added and the resulting mixture was stirred vigorously. One mL of each essential oil (containing 5–200 μ g/mL of 1% Tween-20) was added to 50 mL reagent mixture, and tested samples were subjected to thermal auto-oxidation at 50°C. At intervals up to 300 min, the absorbance of reaction mixture (4 mL) was measured at 470 nm against a blank (1% Tween-20 solution). All samples were done in triplicates. Antioxidant capacities of algal extracts were compared with those of BHT, BHA and α -tocopherol (at 2–100 μ g mL⁻¹) and control. Inhibition of bleaching β -carotene (I %) was calculated as follows: Inhibition percentage (%I) = $(\text{Abs}_{\text{initial}} - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{initial}}) \times 100$

3.3. TLC rapid antioxidant assay:

TLC plates were used to detect the most active constituents in both essential oils based on spraying the separated compounds on TLC plates either with β -carotene/ linoleic acid reagent as described by Abd El Baky and El-Baroty [27] or with 0.05 % DPPH radical in methanol [28], to locate the antioxidant compounds. The protection against the bleaching β -carotene gave orange spots, and scavenging DPPH radical gave pale yellow colored spots were considered positive results [29].

4. Antimicrobial assay

4.1. Indicator microorganisms

The microorganisms used as indicators to assay the antagonistic effect of oil extracted from *L. camara* leaves and flowers included three G⁺ bacteria; *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 33018) and *Bacillus subtilis* (ATCC 6633), three G⁻ bacteria; *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 9027) and two fungal strains; *Aspergillus niger* (nrrl 326) and *Candida albicans* (ATCC 10231) representing a mold and yeast strain, respectively. A loopful of each bacterial strain was added to 50 ml sterile nutrient broth in a 100 ml conical flask. The flasks were then incubated at the optimum temperature (37°C for *Staph. aureus*, *Sal. typhimurium*, *E. coli* and *Ps. aeruginosa*, and 30°C for *B. cereus* and *B. subtilis*) overnight to activate the growth. The same procedure was used to activate the growth of fungal strains using different media; potato dextrose broth and glucose broth for *Aspergillus niger* and *Candida albicans*, respectively. The selected microbial strains were obtained from the Microbiology labs, Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University.

4.2. Screening of oil extracted from *L. camara* for the antimicrobial activity

The antimicrobial effect of tested oils was evaluated employing the disc–diffusion assay [30]. Filter paper discs of oils and positive reference standards were screened against selected microorganisms. Table (1) shows the positive reference standard used for each microbial type. The oils were emulsified in dimethyl sulfoxide (DMSO) to a concentration of 10 mg/ml. To carry out the disc diffusion assay, agar medium (1.5% agar) was inoculated with 10% v/v of bacterial or fungal culture broth. The sterile paper disc with a diameter of 5 mm was saturated with 13 μ l of the emulsified oil and placed on the inoculated agar medium. The plates were incubated at the optimum temperature for each indicator strain and tested after 24, 48 and 72 h. Growth inhibition was scored positive in presence of a detectable clear zone around the disc.

Table 1. Positive antimicrobial reference standard used for each microbial type

Microbial Type	Positive Reference Standard
<i>Pseudomonas aeruginosa</i> (ATCC 9027)	Polymyxin (disc loaded with 130 units)
<i>E. coli</i> (ATCC 8739)	Ampicillin (disc loaded with 10 μ g)
<i>Sal. typhimurium</i> (ATCC 14028)	Penicillin G (disc loaded with 10 units)
G ⁺ bacteria	Penicillin G (disc loaded with 10 units)
Fungal strains	Nystatin (disc loaded with 100 units)

4.3. Quantification of the oil activity (Determination of MIC)

The antimicrobial activity of tested oils was quantified by determination of minimum inhibitory concentration (MIC) using the critical dilution assay and disc diffusion assay [31]. The MIC was determined as the lowest concentration of tested oils inhibiting the visible growth of each microorganism on the agar plate. The emulsified oil was serially diluted two fold. The saturated disc of each dilution was placed on the inoculated agar medium as mentioned before.

4.4 Thin layer chromatography (TLC) and bioautography

A TLC bioautographic assay was used to detect the components of oil extracted from both leaves and flowers of *L. cammara* and to identify the most bioactive constituents as antibacterial agent [27]. Aliquot of 5 μ l of each oil were applied to 2 TLC plates (15 x 20 cm, silica gel G, 60F 254 Merck, Darmstadt, Germany). The plates were then developed with toluene-ethyl acetate (95:5, v/v). The dried TLC plates were then cut with a diamond into three strips. One of the strips was inspected under UV light (254 nm) and also by visualization with 1% vanillin-sulfuric acid reagent and then heated at 110°C for 3 min; the second was used for the bio-autographic assay, while the active constituents on the third strip were eluted with hexane for GS/MS analysis. Modified contact bio-autography is used to identify the antimicrobial constituents separated from the tested oils against *Bacillus cereus* and *Bacillus subtilis*. In contact bio-autography, the antimicrobials diffuse from the TLC plate to an inoculated agar plate. The chromatogram is placed face down onto the inoculated agar layer and left for some minutes or hours to enable diffusion. Then, the chromatogram is removed and the agar layer is incubated. The inhibition zones are observed on the agar surface in the places where the spots of antimicrobials are stuck to the agar. In modified contact bio-autography applied in this study, the chromatogram was placed face down on inoculated agar layer supplemented with 0.1% iodinitrotetrazolum chloride reagent. Then, the inoculated medium was incubated at 30°C, without removing the chromatogram. After incubation for 24-48 h, the inhibition zones (light yellow circle) were shown as clear areas against a pink background. TLC zones that showed inhibition activities of the third strip of the TLC plate were scraped from the plates and eluted with dichloromethane, filtrated, concentrated by nitrogen to a final volume of 10 μ l and analyzed by GC/MS [5,7].

5. Effect of extracted oils on *Bacillus cereus* spores

The effect of FLCO and LLCO on *Bacillus cereus* spores was conducted using transmission electron microscopy (TEM). *Bacillus cereus* was grown in nutrient broth supplemented with both oils in a concentration of 2.5 and 5.0 mg /ml, respectively and incubated at 30°C for 24 h. The control samples were prepared similarly without any treatment. The spores were collected after centrifugation at 4000 rpm for 10 minutes at room temperature, fixed in glutaraldehyde and osmium tetroxide, dehydrated in alcohol and embedded in an epoxy resin. Microtome sections prepared at approximately 500-1000 μ m thickness with a Leica Ultracut UCT ultramicrotome. Thin sections were stained with toluidin blue (1X) then sections were examined by camera Leica IC₅₀ HD. Ultra thin sections, prepared at approximately 75-90 μ m thickness, were stained with uranyl acetate and lead citrate then, examined by transmission electron microscope JEOL (JEM-1400 TEM) at the candidate magnification. Images were captured by CCD camera model AMT, Optronics camera with 1632 x 1632 pixel format as side mount configuration. This camera uses a 1394 fire wire board for acquisition.

6. Statistical analysis

All results are expressed as mean values \pm S.D. Statistical differences between experimental groups were assessed by analysis of variance (ANOVA) using the COSTAT software package (Cohort Software, CA, USA). The main values were compared with LSD test ($P < 0.05$).

RESULTS AND DISCUSSION

1. Chemical composition of *Lantana camara* essential oil

The chemical composition of the obtained essential oil from flower and leaves of *Lantana camara* are presented in Table 2. The components are listed in order of their elution on the column. Twenty six compounds were identified in FLCO representing 94.5% of the total oil. The oil was found to be containing 17 sesquiterpenoids (92.1%) compounds: davanone (28.59%), α -caryophyllene (11.21%), β -curecumene (10.26%), E-caryophyllene (8.03%), β -copaene and humulene (12.29%). The similar profile was obtained for LLCO, which it containing high quantity of sesquiterpenoids (91.86%) compounds. Of which, davanone (23.37%), E-caryophyllene (22.96%), humulene (14.32%) α -caryophyllene (8.18%), α -curcumene (6.33%) and β -copaene (4.43%) were detected. However, the composition of the both LLCO and FLCO oils are different from the chemical composition of the essential oil obtained from species growing in different organs. *L. camara* grown in different location in Brazil was found to be differing in oil composition. Accordingly, oil from Anjouan (Comoro Archipelago) contained mainly humulene (22%) and caryophyllene (15%), whereas, oil from Reunion, caryophyllene (35%) and davanone (15%) were identified as major constituents. Also, oil samples from Benin, Madagascar and Central India were more variable both in the relative amounts of these constituents [32]. The main compounds of essential oil of *L. camara* aerial parts, that were grow in France containing: camphor (44%), germacrene D (16%), *trans*-pinocarveol (11%), β -selinene (9%), β -caryophyllene (8.9%) and Artemisia ketone (3%). Therefore, the geographic location, agro-climatic

condition (climatic and seasonal), stage of maturity, adaptive metabolism of plants, distillation conditions, the plant part analyzed and some other factors had greatly influences on the type and concentration of the main constituents [13, 27,33].

2. Antioxidant DPPH scavenging activity

The antioxidant activity of FLCO and LLCO oils on DPPH radicals scavenging was conducted to thought their hydrogen donating ability. DPPH is stable free radicals and accept an electron or hydrogen radical to become a stable diamagnetic molecule. Both FLCO and LLCO oil showed a moderate scavenging ability against DPPH with concentration dependent manner. The IC₅₀ values were found to be 55.43 and 48.36 µg/ml, respectively (Fig. 2). Therefore, antioxidant activity of both oils may be due to the hydrogen donating ability. However, antioxidant activity of both oils was less that of standard antioxidants (α-tecophrol 17.23 µg/ml, BHT 15.28 µg/ml and BHA 12.36 µg/ml). It is known well that these antioxidant compounds had a high antioxidant activity, due to its ability to donate a hydrogen atom to a free radical, that terminating free radical reaction [13, 34].

3. Antimicrobial activity of *L. camara* leaves and flowers oil

The currently available screening methods for the detection of antimicrobial activity of natural products fall into three groups, including diffusion, bioautographic and dilution methods. The bioautographic and diffusion methods are known as qualitative techniques since these methods will only give an idea of the presence or absence of substances with antimicrobial activity. On the other hand, dilution methods are considered as quantitative assays as they determine the minimal inhibitory concentration [35].

The antimicrobial activity of *L. camara* leaves and flowers oil is shown in Table 3. All tested G⁻ bacteria, fungi and *Staphylococcus aureus* ATCC 25923 were resistant to oils extracted from both leaves and flowers. On the other hand, *B. subtilis* (ATCC 6633) and *Bacillus cereus* (ATCC 33018) were susceptible to the tested oils.

The flowers oil was considered to be the most effective than the corresponding leaves oil against *B. subtilis*, since the largest inhibition zone (Ø, 11±0.50 mm) and the highest MIC value (1.25 mg/ml) were determined with the flowers oil, whereas the smallest inhibition zone (Ø, 8.3±1.53mm) and the lowest MIC (5 mg/ml) were determined with the leaves oil.

For *B. cereus*, the flowers oil showed the highest inhibitory effect comparing with leaves oil with a value of inhibition zone (Ø) of 10.3±0.53 and 8.0 mm, respectively, whereas the highest MIC value (2.5 mg/ml) was recorded with the leaves oil.

The resistance of G⁻ bacteria to the tested oils as antimicrobial agents was obvious, as these bacteria have a unique outer membrane that determines permeability and susceptibility of the cells to certain drugs and antibiotics [36]. Several new antibacterial agents are currently developed in view of ever increasing the bacterial resistance to existing drugs. New phytochemical sources presenting antimicrobial activity and low toxicity could be a potential alternative. Antibacterial activity of *L. camara* has been demonstrated against phytopathogenic *Xanthomonas campestris* [37]. Crude extract of *L. camara* root has been found to be active against *Staphylococcus aureus*, *Bacillus cereus*, and *Cladosporium sphaerosperum* [22]. Essential oil of much aromatic plant has been proved to be antimicrobial agent [9,13,38].

4. Bioautography

As shown in Fig. 3A and B, clear inhibition zone was observed against *B. subtilis* with an R_f value of 0.30 and 0.50, suggesting that the substances responsible for antimicrobial activity are monoterpene and sesquiterpenoid constituents in both oils. The antibacterial zones were eluted from TLC plates and detected with GS/MS. Based on the interpretation of mass spectrums, in FLCO the band located at R_f 0.3 contained mainly citral (49.89%) and terpinene-4-ol (14.64%), whereas citral (42.56%) and davanone (39.47%) were identified as major constituent in 0.50 R_f band. In LLCO bands at 0.30 and 0.50 were characterized by present of citral (69.21%) and spathulenol (14.24%), and citral (34.71%), caryophyllene oxide (30.41%) and davanone (15.46%) as promenade constituents. These compound have been reported in literature have antimicrobial activities [27,38].

Table 2. Qualitative and quantitative composition of *Lantana camara* leaf and flowers essential oils.

Components	Relative area %	
	Flowers	Leaf
α -Pinene	0.12	0.38
Sabinene	0.06	1.33
β -Pinene	0.5	1.03
α -Terpineol	0.29	0.0
α -Copaene	0.80	0.0
β -Elemene	0.27	0.0
β -Patchoulene	0.52	2.3
γ -Patchoulene	0.85	0.0
E-Caryophyllene	8.03	22.96
Z-Caryophyllene	11.21	8.18
α -Curcumene	10.26	6.33
B-Copaene	11.38	4.43
Humulene	12.29	14.32
α -Elemene	3.55	1.99
β -Elemene	2.89	3.51
α -Farnesene	0.02	0.00
β -Bisabolene	0.62	0.34
(-) Caryophyllene oxide	0.47	0.99
B-Guaiene	0.86	0.34
Davanone	28.59	23.37
β -Curcumene	0.32	0.65
P-Menth-3-ene-9-ol	0.40	1.07
α -Cadinol	0.33	0.00
Iso-Caryophyllene	0.60	0.00
t-Nerolidol	0.00	1.05
Caryophyllene acetate	1.10	0.00
Total of identified compounds %	95.33	95.56
Monoterpene	0.97	2.74
Sesquiterpenes	94.36	91.81
Oil yield (g/100 g)	0.42	0.56

^a Identification based on retention time authentic sample and mass spectrum data

^b Trace: relative area % is less than 0.05%.

5. Effect of extracted oils on *Bacillus cereus* spores

The microbial mode of action of tested oils was experimented for *Bacillus cereus* because of its importance as one of the causative agents of food poisoning. *Bacillus cereus* is responsible for a minority of food poisoning (2–5%), causing severe nausea, vomiting and diarrhea. *Bacillus cereus* food poisoning occurs due to survival of the bacterial endospores when food is improperly cooked. Cooking temperatures less than or equal to 100 °C allows *B. cereus* spores to survive. This problem is magnified when food is then improperly refrigerated, allowing the endospores to germinate with production of enterotoxins.

Table 3. Antimicrobial activity of oils extracted from *Lantana camara* flowers and leaves

Microorganism	Diameter of inhibition zone (mm)		
	Flowers oil	Leaves oil	Positive control
<i>Staphylococcus aureus</i> ATCC 25923	R	R	16.7±0.58
<i>Bacillus cereus</i> ATCC 33018	10.3±0.53	8.0±0.0	10.0±1.0
<i>Bacillus subtilis</i> ATCC 6633	11.0±0.50	8.3±1.53	8.0±0.0
<i>Salmonella typhimurium</i> ATCC 14028	R	R	13.7±0.58
<i>Escherichia coli</i> ATCC 19404	R	R	14.7±0.58
<i>Pseudomonas aeruginosa</i> ATCC 9027	R	R	12.0±1.0
<i>Aspergillus niger</i> nrrl 326	R	R	10.0±0.0
<i>Candida albicans</i> ATCC 10231	R	R	13.7±0.58

R, resistant

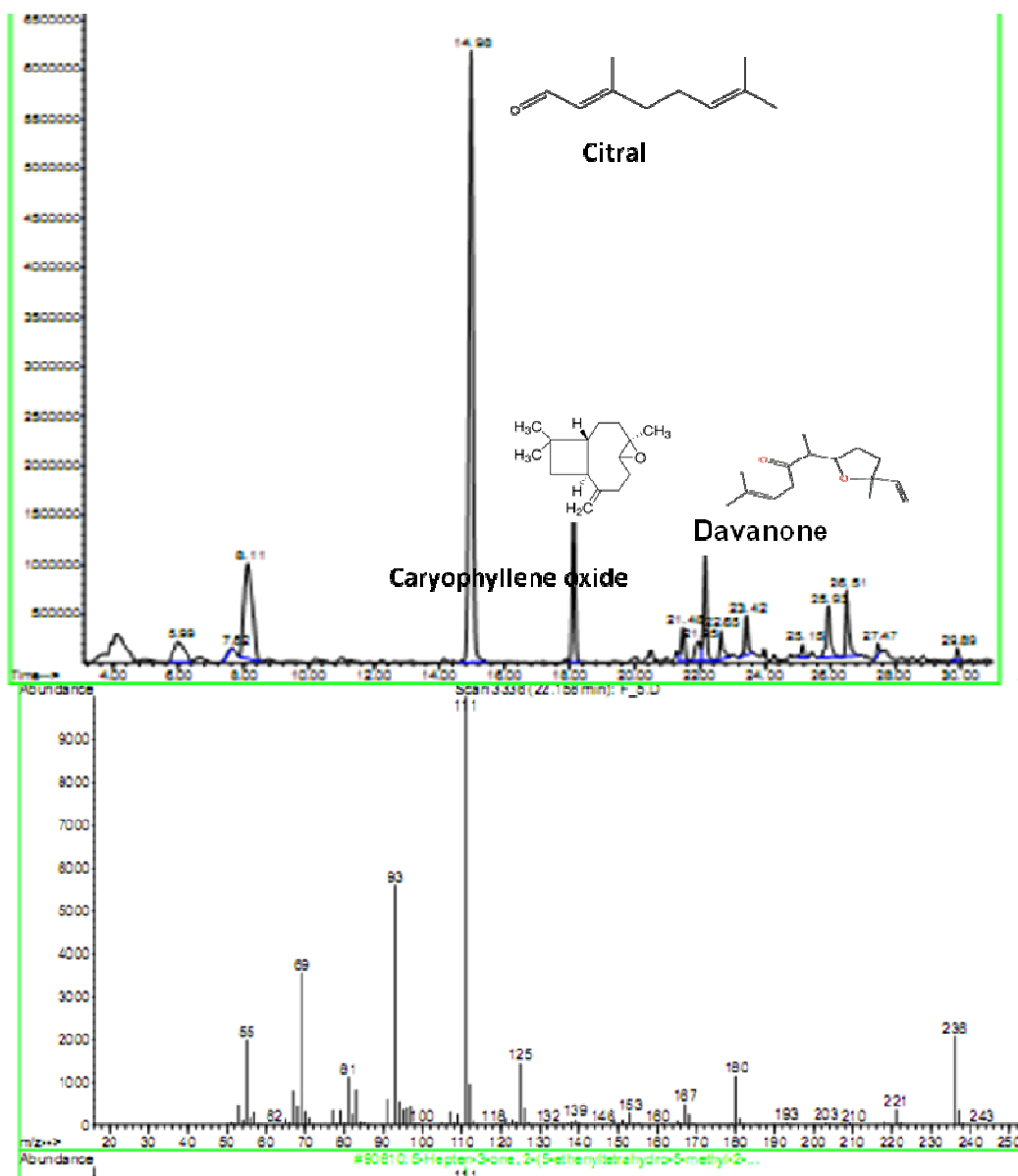


Fig.1. Main constancies of *Lantana camara* leaf and flower essential oils GC/MS

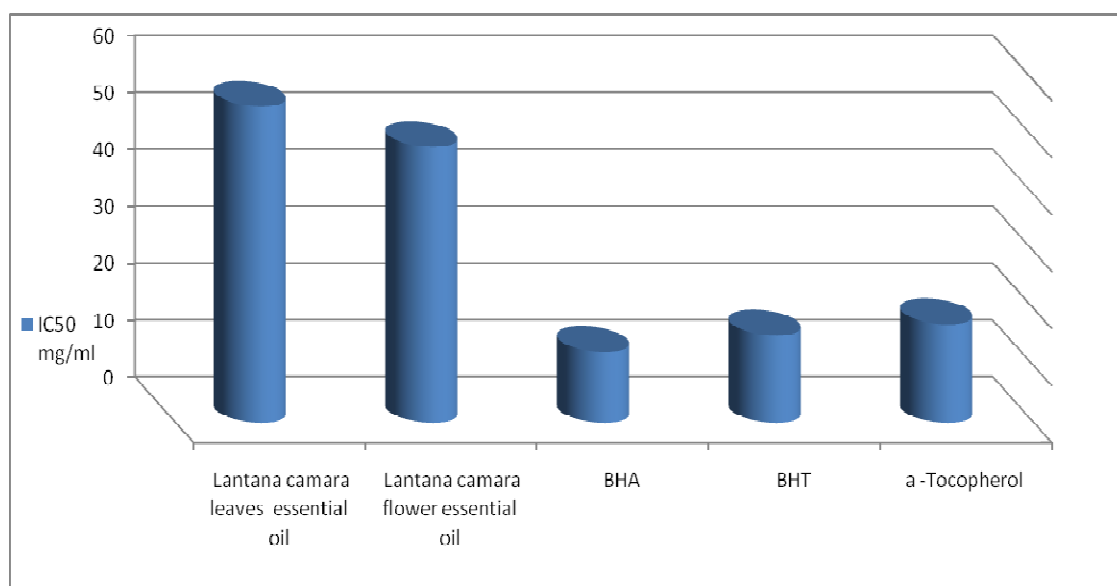


Fig 2: Scavenging activity of *Lantana camara* leaves and flower essential oils on DPPH

Bacillus cereus produces two different types of enterotoxins. The commonest is the emetic or vomiting toxin, cereulide. This toxin causes nausea and vomiting, sometimes accompanied by abdominal cramps and diarrhea. Symptoms do not usually last for more than 24 hours and complications are rare. Foods involved in *B. cereus* emetic food poisoning cases are usually starchy, such as boiled or fried rice, potatoes, pasta and noodles. The toxin is extremely heat-stable and withstands cooking.

The second type of *B. cereus* toxin is an enterotoxin causing a diarrheal type of food poisoning. At least two different enterotoxins have been identified, a haemolysin and a non-haemolytic enterotoxin. Symptoms include watery diarrhea, abdominal pain and occasional vomiting. Recovery typically takes place within 24 hours. Foods associated with this form of food poisoning are varied, but include meat and vegetable dishes, soups and sauces. *B. cereus* enterotoxins are acid-labile and easily destroyed by heating.

The mode of action of FLCO and LLCO on *B. cereus* spores was detected using TEM. The spores were TEM - imaged after treating with leaves and flowers oil in a concentration of 2.5 and 5.0 mg / ml, respectively at 30°C / 24 h (Fig. 3).

The spore preparations examined by TEM as a control revealed that the spores were structurally typical. They have fully developed distinct layers; cortex, spore coat and exosporium (Fig. 3A). As shows in Fig. 3, the LLCO had a strong effect on the spore structure as comparing with the FLCO. The treatment with LLCO resulted in a complete destruction of the spore core and layers (Fig. 3 E and F). Almost all spores looked less structure (Fig. 3G). The FLCO had a slighter effect on the spore structure; clear distortion of the spore layers. The exosporium was either malformed (Fig. 3B) and/or dissolved (Fig. 3 C). For other cases, the spore core itself looked abnormal, being thinner and elongated (Fig. 3 D).

IC₅₀ concentration (µg/ml) for a 50% inhibition was calculated from the plot of inhibition (%) from the plot of inhibition (%) against *Lantana camara* leaves and flower essential oils concentration Tests were carried out in triplicate.

Since, the exact of antimicrobial mechanism of essential oils have not been completely elucidated. However, Farag et al. [33] and Daw et al. [38] proposed that lipophilicity or Hydrophobicity and chemical structure of essential oil or their main compounds such as the presence of functional polar groups and aromaticity could play an important role for the antimicrobial activity, which enable them to partition the lipids of the bacterial or fungal cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable which will lead to death [13, 39].

Thus, as can be seen from Figure 2, some the major components present in FLCO and LLCO could be penetrate the membrane of the microorganisms and react with the membrane enzymes and proteins as well as phospholipids bilayer, which cause an impairment of microbial enzyme system and/or disturb genetic material functionality [9,27,29,33,40]. However, Bang *et al.* [41] reported that volatiles constituents may inhibit the microbial-cell-wall synthesizing systems through the reaction with sulfhydryl groups (-SH) present in active site of these enzymes.

This study high light on the potential use of flower and leaves of *Lantana camara* essential oils in ethno-medicine as a preventer of cellular damage, and in food industries as preserver against spoilage bacteria and fungi.

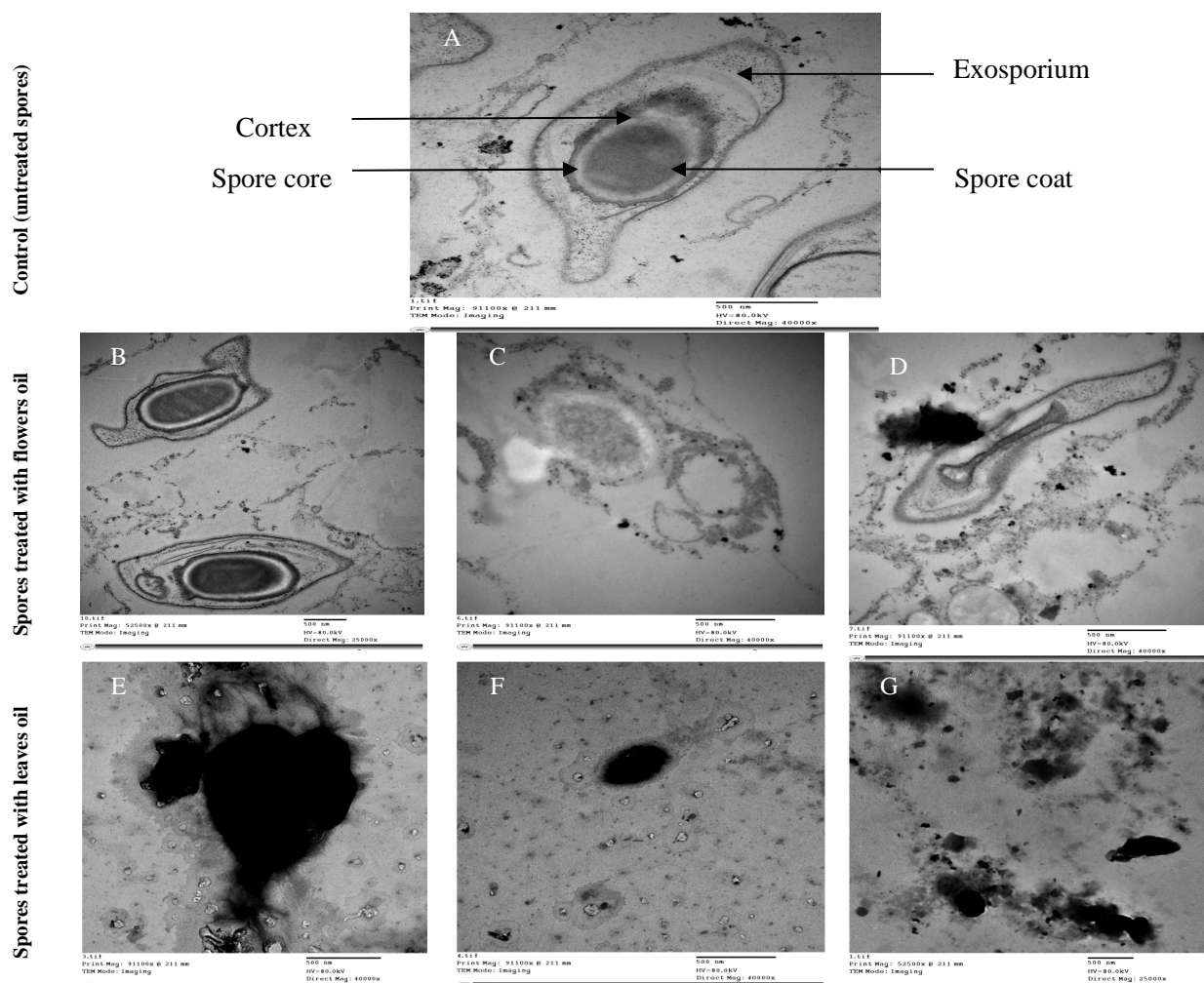


Fig. 3. TEM micrographs present ultra – structure of treated and untreated *B. cereus* spores; A: typical ultra-structure of untreated spores, B, C and D: spores treated with flowers oil, E, F and G: spores treated with leaves oil

REFERENCES

- [1] F Anwar, M Ali, A I Hussain, M Shahid, *Flavour Fragrance J*, **2009**, 24: 170-176.
- [2] H H Abd El Baky, G S El-Baroty, *Food & Function*, **2012**, 3, 381-388.
- [3] C Gupta, A P Garg, C Ramesh, S Gupta, *The Inter J Microbiology*, **2009**, 7 (1): 10.5580/297f
- [4] G S El Baroty, F K El Baz, I Abd-Elmoein, H H Abd El Baky, M M Ail, E A Ibrahim *J. Envi Agric Food Chem*, **2011**, 10(4) 2114-21218.
- [5] B Sharma, P Kumar, *Indian J Pharm Sci*, **2009**, 71(5): 589–593.
- [6] S A Saleh, M H Belal, G S EL-Baroty, *J Environ Sci & Health (B)*, **2006**, 41: 237-244.

- [7] A Shahat, G S El-Baroty, R. A. Hassan, F. M. Hammouda, F. H. Abdel-Rahman, M.A. Saleh. *J Enviro Sci Health, Part B*, **2008**, 43, 519–525.
- [8] H H Abd El Baky, G S El-Baroty, *J Aquatic Food Product Technol.*, **2013**, 4, 392-406.
- [9] R S Farag, G S El-Baroty, M A El Marsafy, A E Abd El-Gawad, *J. Biol. Chem. Environ. Sci*, **2007**, 2(1)348-361.
- [10] M Mari, P Bertolini, G Pratella, *J Appl Microbioly*, **2003**, 94:761-766.
- [11] L Pessoa, S Morais, C Bevilaqua, J Luciano, *Veterinary Parasitol* , **2002**, 109:59-63.
- [12] I Karpouhtsis, E Pardali, E Feggou, S Kokkini, Z Scouras, P Mavragani,. *J Agric Food Chem*. **1998**, 46:1111-1115.
- [13] G S El Baroty, R S Farag, H H Abd El Baky, M A Saleh, *Afr J Biochem Res*, **2010**, 4(6): 167-17.
- [14] C Jones, Essential oils: A perfect example of alternative medicine exaggeration, *skeptoid.com* **2013**.
- [15] H H Abd El Baky, F K El Baz, G S El-Baroty, *Acta Physiol. Plant*, **2009**, 31 (3)623-631.
- [16] O P Sharma, R K Dawra, H Makkar, *Toxicol Letters*, **1987**, 37: 165-172.
- [17] A K Tripathi, B Shukla, *J Mycol Plant Pathol*, **2002**, 32: 266-267.
- [18] V P Kumar, S C Neelam, P Harish, P., *J Ethopharmacol*, **2006**, 107: 182-188.
- [19] H R Juliani, F Biurum, A R Koroch, A. R., *Planta Medica*, **2002**, 68: 762-764.
- [20] A Kasali, O Ekundayo, A Oyedeji, *J Essential Oil Bearing Plants*, **2002**, 5: 108-110.
- [21] K Ravinder, R Daizy, H Batish, P Singh, S Kuldip, *Biological Invasions*, **2000**, 8:1501–1510.
- [22] K Taoubi, M Fauvel, J Gleye, C Moulis, *Planta Medica*, **1997**, 63:192–3.
- [23] S Chhabra, R Mahunnah, E Mshiu, *J Ethnopharmacol*, **1993**, 39:83–103.
- [24]. R P Adams, Identification of Essential Oils Components by Gas Chromatography/Quadruple Mass Spectroscopy, 4th edition. Allured Publishing Corporation, Carol Stream, Illinois, USA, **2007**.
- [25] Y Massada, Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry. Wiley, New York. USA **1976**.
- [26] M Tagashira, Y Ohtake, *Planta Med.*, **1998**, 64: 555-558.
- [27] H H Abd El Baky, G S El-Baroty, G.S. (2008). *Inter J Essential Oil Therapeutics*, **2008**, 2: 76-81.
- [28] L Jaime, J A Mendiola, M Herrero, C Soler-Rivas, C., S Santoyo, F J Senorans, A Cifuentes, E Ibanez, E, *Sep. Sci.*, **2005**, 28:2111–2119.
- [29] H H Abd El Baky, F K El Baz, G S El-Baroty, *Inter J Food Sci Technol*. **2009**, 44, 1688–1695 1688.
- [30] Bauer, A. W.; Kirby, W. M.; Sherris, J. C. and Turck, M. (1966). *American Journal of Clinical Pathology*, 45:493-496.
- [31] Yamamoto, Y.; Togawa, Y.; Shimosaka, M. and Okazaki, M. (2003). *Applied and Environmental Microbiology*, 69:5746 -5753.
- [32] G Alitonou, F Avlessi, D C K Sohounhloue, J M Bessiere, C Menut, *J Essential Oil Res*, **2010**, 22, 138
- [33] R S Farag, A S Shalaby, G S El Barotys, N A Ibrahim, E M Hassan, *Phytother. Res*. **2004**, 18:30-35.
- [34] F Shahidi, P K Janitha, M Wanasundara, *Critical Rev Food Sci Nutrition*, **2004**, 32(1),67–103.
- [35] C Valgas, S Machado, E Smânia, A Smânia, *Braz. J. Microbiol*, **2007**, 38:369-380.
- [36] S Yokota, N Fujii, *Comp Immunol Microbiol Infect. Dis*, **2007**, 30:97–109.
- [37] S Satish, K A Raveesha, G R Janardana, *Lett Appl Microbiol*, **1999**, 28:145–7.
- [38] Z Y Daw, G S EL-Baroty, A E Mahmoud, *Chem Microbiol Technol Lebensm*, **1994**, 16 (5/6): 129-135.
- [39] J Sikkema, J A M De-Bont, B Poolman, *J Biological Chem*, **1994**, 269, 8022–8028
- [40] H H Abd El Baky, M M Hussein, El-Baroty, *Electronic J Enviro Agric Food Chem*, **2008**, 7(4): 2812 –2832.
- [41] K H Bang, D W Lee, H M Park, Y H Rhee, *Bioscience, Biotechnol Biochem*, **2000**, 64: 1061-63.