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(RESEARCH ARTICLE)



# Chemical composition, antimicrobial and free- radical scavenging activity of essential oils from *Khaya senegalensis* (Desr) A. Juss

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# Abstract

Investigations were conducted to evaluate the chemical composition, free-radical scavenging activity and the antimicrobial efficacy of essential oils obtained from the leaf and stem bark of *Khava senegalensis* (Desr) A. Juss. The plant materials (leaf and stem bark) were dried under shade, blended and sieved to obtain their respective powders. The essential oils obtained by subjecting the powders to hydro distillation method for 3hr in a Clevenger type apparatus were then investigated using standard methods. The average percentage yields of the oils were  $0.85 \pm 0.01\%$ ) and  $0.50 \pm$ 0.00% for the leaf and stem bark respectively. The major constituents of the essential oils were khayasinine (39.11%). 7-diacetyl-7oxogedunine (27.09%) and methyl- angolensate (11.51%) for the leaf oil and ivorenolide (28.77%), khivorine (16.33) and 3-diacetyl khivorine (16.10%) for the stem oil. The inhibitory concentration (IC<sub>50</sub>) of the free radical scavenging activity of the oils were 1.40± 0.29 and 1.00± 0.02 for the leaf and stem oils respectively. The mean diameter of the zones of inhibition exhibited by the oils at 50  $\mu$ g/ml was between 7.67± 0.67mm and 16.33 ±0.33mm while the MIC was between 5  $\mu$ g/ml and 40 $\mu$ g/ml for the plant materials. The plant oils remarkably inhibited the growth of Bacillus subtilis ATCC6633, Candida albicans ATCC10231, Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus. The activity of the plant oils compared favourably with the antibiotics used as positive control. The demonstration of significant antimicrobial and high free radical scavenging activities in addition to the presence of bioactive compounds in the plant materials could explain the basis for the use of K. senegalensis in the traditional treatment of diseases caused by the test microorganisms.

Keywords: Chemical composition; Antimicrobial; Antioxidant; K. senegalensis

# 1. Introduction

Medicinal plants are rich in secondary metabolites which are potential sources of drugs of therapeutic importance. The world Health Organisation (WHO) estimates that a considerable number of people in Sub-Saharan African rely on traditional complementary and alternative medicine to meet their primary health needs [1]. The emergence and spread of antibiotic resistance, as well as the evolution of new strains of diseases entails the development of some potential source of novel drugs [2]. The evaluation of various plants according to their traditional uses and medical value based on their therapeutic efficacy leads to the discovery of newer and recent drugs for treating various ailments [3, 4]. Pharmaceutical studies have also accepted the value of essential oils from medicinal plants as potential source of bioactive compounds in antimicrobial discovery. The essential oils obtained from them have been traditionally used for the treatment of infections and diseases all over the world [5, 6].

*Khaya senegalensis* (Desr) A. Juss commonly known as Dry Zone Mahogany is a species of plant in the Meliaceous family found in many African countries. It is a savanna tree, easily recognized by its round evergreen crown of dark, shining foliage pinnacle leaves and characteristics round capsules. A tree of 30m high and 3m girth with dense crown and short

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bole covered with dark grey scaly bark. The leaves are with 3-4 pairs of leaflets, 5-10 cm long by 2.5-5 cm broad, more or less elliptic, round; obtuse or shortly acuminate at apex with stalks of leaflets of about 4 mm long [7]. The bark extract is used for treating jaundice, dermatoses, hookworm infection and malaria [8]. Considering the vast potential of plants as sources of antimicrobial drugs, this research work was designed to investigate the chemical composition, antimicrobial and free- radical scavenging activity of essential oils from *Khaya senegalensis* in order to validate its folkloric use.

# 2. Material and methods

#### 2.1. Collection and processing of plant materials

Fresh leaves and stem barks of *Khaya senegalensis (Desr)* A Juss were harvested from uncultivated farmlands located in Owo, Ondo State, Nigeria between October 2017 and May 2019. The plant materials were collected in clean plastic bags while the plants were still moist with the morning dew and properly labeled. The plants were then authenticated at the Herbarium of the Department of Botany, University of Lagos. Voucher specimens were deposited at the Department of Forestry and Wood Technology, Rufus Giwa Polytechnic, Owo. The authenticated plants were washed and cleaned thoroughly with tap water and then air-dried under shade for six weeks. The dried samples were then ground into coarse powder, stored in clean air- tight containers and kept in a cool, dry place until required for use.

## 2.2. Extraction of essential oils

Essential oils were separately extracted from the leaf and stem bark of the plant by steam distillation. A five hundred grams portion of the respective plant parts in 2000ml of distilled water was hydro distilled using an improvised Stovestill apparatus for 5hr to obtain the oil. The steam distillate was dried over anhydrous sodium sulphate and 10mg of it was diluted with 100ml 0f 0.01% Tween-20 to obtain a  $100\mu$ g/ml solution. Serial dilution of each 100ml stock solution was made with 0.01% Tween-20 to give test solutions 50, 25, 12.5 and  $6.3\mu$ g/ml respectively [9].

#### 2.3. Collections of test microorganisms

Fifteen clinical isolates (*Bacillus subtilis, Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus spp, Streptococcus pyogenes, Aspergillus flavus, Candida albicans, Candida glabrata, Cryptococcus neoformans* and Trichophyton rubrum) and five typed cultures (*Bacillus substilis* ATCC 6633, *Stapylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 6539 and *Candida albicans* ATCC 10231) obtained from Federal Medical Center, Owo and Federal Institute of Industrial Research, Oshodi, Lagos State respectively were used for this study.

#### 2.4. Purification of test organisms

The organisms were confirmed by sub-culturing into Muller Hinton broth and Sabouraud Dextrose broth and incubated at 37 °C for 18 hr (for bacteria) and 25 °C (for fungi). They were further streaked on Muller Hinton agar and Sabouraud Dextrose agar and incubated at 37 °C for 18hr and 25 °C for 5 days for bacteria and fungi respectively. Biochemical tests were used to confirm the bacterial species and kept on agar slant at refrigeration temperature until needed [10].

#### 2.5. Standardization of microbial culture

A loopful of bacterium test organism was aseptically inoculated into Mueller Hinton broth (MHB) and incubated for 24 h at 37 °C. A portion of 0.2 ml from the 24 h culture of the organism was dispensed into 20ml sterile Muller Hinton broth and incubated for 3-5h to standardize the culture to 0.5 McFarland standards (1.0  $\times 10^6 cfu/ml$ ). Molds inoculum suspensions were obtained by fine colonies (>1mm diameter) from 24h-old cultures grown on Saboraud dextrose agar and lawn prepared were suspended in 5ml 0f sterile saline (0.85% NaCl). The inoculum suspensions were shaken for 15s and the inoculum density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to  $1\times 10^6 sfu/ml$ ) with sterile saline according to the method of [11].

#### 2.6. In vitro antibacterial susceptibility test

The essential oils obtained from the test plant materials were screened against the test bacteria by agar well diffusion method [12]. A 25ml aliquot of Mueller Hinton agar (MHA, Lab Oratorios Britania, Argentina) was poured into each Petri plate. When the agar solidified, the pathogenic test organisms were inoculated on the surface of the plates  $(1 \times 10^6 cfu/ml)$  using a sterile glass spreader and allowed to sink properly. Subsequently, the surface of the agar was punched with 6mm diameter cork borer into wells and a portion of 50 µl of each of the oil concentrations was filled into the wells. Control wells containing the same volume of 30% dimethyl sulphoside (DMSO) served as negative control, while

Chloramphenicol (50  $\mu$ g) was used as positive control for the plates respectively and the plates were incubated at 37 °C for 24 h. Each experiment was carried out in triplicate and the diameter of the zones of inhibition was then measured in millimeters.

## 2.7. In vitro antifungal susceptibility test

A modified method of [12] was adopted. The essential oils obtained from the test plants were screened against test fungi by agar diffusion plate method using Saboraud Dextrose agar (SDA). An aliquot of 100 $\mu$ l of the respective spore suspension (2×10<sup>6</sup> *sfu*/ml) was incorporated into the 25ml SDA medium and poured into sterile Petri-dish. This was allowed to set and punched with 6mm cork borer. A portion of 50 $\mu$ l of each of the oil concentrations was introduced into the wells. Control wells containing the same volume of 30% dimethyl sulphoxide (DMSO) served as negative control while Miconazole

 $(50 \ \mu g)$  was used as positive control. The test was carried out in triplicates and incubated at 25 °C for 72 h. The zones of inhibition were then measured in millimeters.

## 2.8. Minimum inhibitory concentration (MIC)

The MIC of the plant essential oils was determined by double dilution broth methods of [13]. Twofold serial dilutions of the oils were prepared in Mueller Hilton broth and Saboraud broth for bacteria and fungi respectively to achieve a decreasing concentrations ranging from the least concentration that produced clear zone of inhibition (10 mg/ml to 0.156 mg/ml). All tubes with the controls were labeled accordingly. Each dilution was seeded with 1ml of standardized inoculums ( $1.0 \times 106$  cfu/ml for bacteria and  $1.0 \times 106$  sfu/ml for fungi) incubated at 37 °C for 24 hr and 25 °C for 72 hr for bacteria and fungi respectively. A tube containing only seeded broth (i.e. without plant extract) was used as the positive control while the un-inoculated tube was used as negative control. The lowest concentration of each oil sample that showed a clear zone of inhibition was when compared with the controls was considered as the MIC.

#### 2.9. Determination of antioxidant activity of plant essential oils

A 3ml portion of the oil concentrations (0.00 to 2.0 mg/ml) was added to 1ml of  $100\mu$ M methanol solution of DPPH. The mixture was shaken vigorously and incubated in the dark at room temperature for 30min. The absorbance at 517 nm was measured against the blank (methanol) and ascorbic acid as positive control using a Spectrophometer. The DPPH radical scavenging activity (%) was determined by the following equation:

DPPH radical scavenging: Activity (%) = (Ao –As) /Ao × 100 where Ao = absorbance of DPPH without sample; As = absorbance of mixture of sample and DPPH. The radical scavenging activity of the samples was expressed as IC<sub>50</sub> value which is the effective concentration at which DPPH radicals were scavenged by 50% [14].

#### 2.10. Gas chromatography/mass spectrometer (GC/MS) analysis

The GC/MS was performed using an Agilent Technologies 6890N gas chromatography connected to a 5973 INERT selected mass spectrophotometer HP5MS following the method of [15]. The column dimensions are  $30m \ge 0.25mm$  (i.d)  $\ge 0.25 \mu m$  (film thickness). The ionization source temperature was held at 350C for 9mins, increased by 3 °C/min to 150 °C and held for 10 mins, increased by 10 °C/min to 250 °C and increased by 3 °C/min to 270 °C and held for 10 mins. The flow rate of the helium carrier gas (99.99% purity) was 0.5 ml/min, and the ionization energy was 70eV. The samples were injected in split less mode. The injection volume for each sample was 2  $\mu$ l (2 mg/ml) in methylene chloride and the MS spectra were acquired in the scan mode. The percentage of each compound in the sample was calculated by dividing its peak area by the total peak area of the sample and multiplying by 100. It was assumed that the detector (Flame ionization detector, Perkin Elmer) response was the same for all the compounds [16]. The oil components were then identified by comparison of their retention indices to C5 – C20 n-alkanes (Sigma -Aldrich) and comparison of their MS spectra with those of the US National Institute of Standards and Technology (NIST) library and reference data.

#### 2.11. Data analysis

Data were presented as mean standard error (SE). Significance difference between different groups was tested using two-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range Test (DNMRT) using SSPS window 7 version 17.0 software. The significance was determined at the level of p< 0.05.

## 3. Results and discussion

The percentage extraction yields of the essential oils obtained from the leaf and stem bark of *K. senegalensis* were  $0.70\pm0.01\%$  and  $0.50\pm0.11\%$  respectively. The results revealed that the leaf material contained a higher amount of essential oil. The choice of plant organs used is justified by the fact that the leaf and stem bark are the major sites of choice for biosynthesis and storage of secondary metabolites responsible for the biological properties of plants [17, 18]. However, the yields were significantly low which may be attributed to the solubility and antioxidant capacities of the plant materials. This finding agrees with the investigations by [19] who worked on similar medicinal plants.

The results of the antimicrobial susceptibility test of the essential oils of the plant materials against some pathogenic microorganisms as presented in Tables 1 and 2 indicated zones of inhibition ranging from  $8.33 \pm 0.33$  to  $14.33 \pm 0.33$  and  $7.67 \pm 0.33$  to  $16.33 \pm 0.33$  for the leaf and stem back oils respectively. The stem bark oil showed the most significant activity against *Escherichia coli* ( $16.33 \pm 0.33$ ) followed by the leaf oil against *Candida albicans* ATCC10231 ( $14.33 \pm 0.33$ ) while *P. aeruginosa* recorded the least sensitivity of  $7.67 \pm 0.33$  against the stem bark oil at  $50 \mu g/m$ l. It was also noted that the antimicrobial activities of the plant oils were concentrations dependent against the test pathogens. The potent activity of the plant oils might be due to the phytocompounds of various metabolites present in the essential oils as well as the pathogen strains. This observation was in agreement with the antimicrobial investigations carried out by [20, 21].

Conc. (µg/ml)/	Zones of inhibition (mm)								
Organisms	6.3	12.5	25	50	DMSO (30%)	Chl	Myz		
<i>B. S</i>	NI	4.33 ±0.33 <sup>a</sup>	9.67 ±0.67 <sup>b</sup>	12.67±0.67°	NI	11.00 ±0.00 <sup>b</sup>	N.A		
B. S ATCC6633	NI	5.33 ±0.33 <sup>a</sup>	10.00±0.58 <sup>b</sup>	13.33 ±0.33 <sup>c</sup>	NI	13.33 ±0.33c	N.A		
<i>S. A</i>	NI	$3.33 \pm 0.33^{a}$	7.33 ±0.33 <sup>b</sup>	11.33 ±0.33°	NI	18.00 ±0.00 <sup>d</sup>	N.A		
<i>S. A</i> ATCC25923	NI	$4.33 \pm 0.33^{a}$	7.33 ±0.33 <sup>b</sup>	12.67 ±0.67°	NI	20.33 ±0.88 <sup>d</sup>	N.A		
Е. С	NI	NI	6.33 ±0.33 <sup>a</sup>	9.33 ±0.88 <sup>b</sup>	NI	14.33 ±0.00°	N.A		
E. C ATCC25922	NI	NI	$7.33 \pm 0.33^{a}$	10.33 ±0.33 <sup>b</sup>	NI	16.33 ±0.33 <sup>c</sup>	N.A		
К. Р	2.33±0.33ª	5.33 ±0.33 <sup>b</sup>	8.33 ±0.33 <sup>c</sup>	12.33 ±0.33 <sup>d</sup>	NI	15.00 ±0.58 <sup>e</sup>	N.A		
Ps. A	NI	4.33 ±0.33 <sup>a</sup>	6.33 ±0.33 <sup>b</sup>	8.33 ±0.33 <sup>c</sup>	NI	12.67 ±0.00 <sup>d</sup>	N.A		
F	NI	5.33 ±0.33ª	7.33 ±0.33 <sup>b</sup>	10.00 ±0.58 <sup>c</sup>	NI	N.A	12.00 ±0.00 <sup>c</sup>		
С. А	3.33 ±0.33ª	$6.00 \pm 0.58^{b}$	8.00 ±0.58 <sup>c</sup>	13.33 ±0.33 <sup>e</sup>	NI	N.A	12.00 ±0.00 <sup>d</sup>		
С. А АТСС10231	$4.00 \pm 0.58^{a}$	7.33 ±0.33 <sup>b</sup>	10.00 ±0.58 <sup>c</sup>	14.33 ±0.33 <sup>d</sup>	NI	N.A	14.67 ±0.33 <sup>d</sup>		
С. G	NI	NI	6.33±0.33ª	$10.00 \pm 0.58^{b}$	NI	N.A	16.33		

Table 1 Antimicrobial activity of essential oil of K. senegalensis (DESR) A. Juss leaf on selected pathogens

Legend; Values are Mean±S.E.M (mm), Values followed by different alphabet along the rows are significantly different at p≤0.05, NI= no inhibition, N.A= not applicable, Chl=Chloramphenicol, Myz=Miconazole, B.S= Bacillus subtilis, S.A= Staphylococcus aureus, E.C= Escherichia coli, K. P= Klebsiella pneumoniae, Ps.A= Pseudomonas aeruginosa, A.F= Aspergillus flavus, C.A= Candida albicans, C.G= Candida glabrata

Conc. (µg/ml)/	Zones of inhi	nes of inhibition (mm)						
Organisms	6.3	12.5	25	50	DMSO(30%)	Chl		
<i>B. S</i>	NI	4.33 ±0.33 <sup>a</sup>	6.33 ±0.33 <sup>b</sup>	9.67 ±0.67°	NI	$11.00 \pm 0.00^{d}$		
<i>B. S</i> ATCC6633	NI	5.67 ±0.67 <sup>a</sup>	6.33 ±0.33 <sup>a</sup>	$10.00 \pm 0.58^{b}$	NI	13.33 ±0.33 <sup>c</sup>		
<i>S. A</i>	3.33 ±0.33 <sup>a</sup>	5.33 ±0.33 <sup>b</sup>	7.67 ±0.67 <sup>c</sup>	$11.67 \pm 0.33^{d}$	NI	$13.00 \pm 0.00^{e}$		
<i>S. A</i> ATCC25923	$4.67 \pm 0.67^{a}$	$6.67 \pm 0.33^{b}$	$7.67 \pm 0.33^{b}$	11.33 ±0.33 <sup>c</sup>	NI	$14.67 \pm 0.33^{d}$		
Е. С	6.33 ±0.33 <sup>a</sup>	10.33 ±0.33 <sup>b</sup>	13.33 ±0.33 <sup>c</sup>	16.33 ±0.33 <sup>e</sup>	NI	$14.00 \pm 0.00^{d}$		
<i>E. C</i> ATCC25922	$7.33 \pm 0.33^{a}$	$10.00 \pm 0.58^{b}$	12.67 ±0.33 <sup>c</sup>	$16.33 \pm 0.33^{d}$	NI	15.67 ±0.33 <sup>d</sup>		
К. Р	NI	NI	$7.00 \pm 0.58^{a}$	$11.00 \pm 1.15^{b}$	NI	$12.33 \pm 0.00^{b}$		
Ps. A	NI	NI	4.67 ±0.67 <sup>a</sup>	7.67 ±0.33 <sup>b</sup>	NI	13.33 ±0.33°		

Table 2 Antimicrobial activity of essential oil of K. senegalensis stem bark on selected pathogens

Legend: Values are Mean±S.E.M (mm), Values followed by different alphabet along the rows are significantly different at p≤0.05, NI= no inhibition, N.A= not applicable, Chl=Chloramphenicol, Myz=Miconazole, B.S= Bacillus subtilis, S.A= Staphylococcus aureus, E.C= Escherichia coli, K. P= Klebsiella pneumoniae, Ps.A= Pseudomonas aeruginosa

The values obtained for the MIC determination ranged from 5-40  $\mu$ g/ml and 6.3 to 20  $\mu$ g/ml for the leaf and stem bark oils respectively (Table 3). The leaf oil was more active on *S. aureus* ATCC25933, *S. pyogenes*, *P. aeruginosa*, *A. flavus*, *C. albicans* while the stem bark oil showed more activity on *B. subtilis*, *B. subtilis* ATTCC6653, *S. aureus*, *E. coli* and *E. coli* ATCC25922 with MIC values ranging from 5 to 10 ug/ml,. The inhibitory effect obtained on these microorganisms is significant for the plant materials as the previous epidemiological study by [22] on these microorganisms indicated that they are highly resistant to ampicillin, chloramphenicol and augmentin. The values obtained in the present study were in agreement with those reported by [23, 24] who assessed the antimicrobial potential of the essential oils obtained from some members of the family Meliaceae. The results revealed that *K. senegalensis* may be a good source of antimicrobial agent with interesting activity on multi resistant strains and may therefore be exploited for the development of new therapeutic agents.

Microorganisms	Leaf	Stem bark	
Bacillus subtilis	40	10	
B. subtilis	20	10	
Escherichia coli	15	5.0	
E. coli ATCC25922	15	5.0	
Enterococcus faecalis	ND	ND	
Staphylococcus aureus	6.3	6.0	
S. aureus ATCC25923	5.0	5.0	
Streptococcus pyogenes	ND	ND	
S. epidermidis	ND	ND	
S. typhi	ND	ND	
S. typhi ATCC6539	ND	ND	
Klebsiella pneumoniae	ND	20	
Pseudomonas aeruginosa	10	ND	
Aspergillus flavus	10	ND	
Candida albicans	5.0	ND	
C. albicans ATCC10231	5.0	ND	
Trichophyton rubrum	ND	ND	

Table 3 MIC of the essential oils of *K. senegalensis* leaf and stem bark against selected pathogens (µg/ml)

The results obtained on the scavenging effect of the plant oils on 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) are presented in Figure 1. The inhibition percentage of the different concentrations of the tested oils increased with increased in oil concentration. At the highest concentration of 2mg/ml, the percentages of inhibition were 79.64 ± 0.29% and 83.73 ± 0.02% as compared with the reference ascorbic acid (93.81 ±0.05% and 86.26 ± 0.02%) for the leaf and stem bark oils respectively. This observation agrees with the work of [25, 26] on antioxidant properties of some plant oils. The Ic<sub>50</sub> values for the leaf and stem bark oils were 1.40 ± 0.29 and 1.00 ±0.02 respectively. The leaf oil exhibited better antioxidant activity and broader antimicrobial activity against the test pathogens. The identified fractions exhibited significant antioxidant activity when compared with ascorbic acid standard (0.40 ±0.15). These results however differ from the work of [27] who obtained a higher concentration with *K. senegalensis* but agree with [28] who obtained closer results from the same plant. The study indicated that the plant oils have good antioxidant potential comparable to the ascorbic acid used as positive control and could therefore have application in cosmetic and pharmaceutical industry.



Figure 1 Antioxidant activity of essential oils from K. senegalensis (%DPPH scavenging inhibition)



Figure 2 Chemical composition of Khaya senegalensis leaf oil

The chemical composition of the plant materials as presented in Figures 2 and 3 revealed that the plant materials contained a complex mixture of compounds. The leaf oil was mostly khayasin (39.11%), followed by 7-diacetyl-7-oxoguanine (27.09%) while 3-destigloyl-dioxy3B-12B diacetoxyswietenine constituted the least (2.17%). The stem bark oil compounds were mostly ivorenolide (28.27%), followed by khivorine (16.33%) and lowest amount of limonene (6.59%). Secondary metabolites such as alkaloids, terpenoids, saponins, tannins, phenolic and flavonoids

from *K. senegalensis* have also been implicated in antimicrobial activity against selected pathogens [29, 30, and 31]. Several earlier studies have suggested that the stem bark of *K. senegalensis* mainly contain limonoids [32]. However, the present discovery of 3-diaacetyl khivorine and khivorine in the stem bark oil agreed with the works of [33] who isolated the same compounds from the seed while some of the compounds had been reported to possess antimicrobial, antioxidant and anticancer activity[34, 35, 36,37] which supports the present investigation.

However, it is significant to note that all the compounds isolated in this study were different from the eight compounds isolated from the bark of *K. senegalensis* by [38]. The variation of the compounds present in the essential oils can be attributed to different factors such as seasonality, circadian rhythm, age, temperature, water availability, ultra violet radiation, nutrient content, atmospheric pollution and attack by pathogens [39]. The presence of these compounds may be attributed to the strong antimicrobial activity of the oils and its wide spread folkloric use in treatment of diseases caused by some of the test pathogens.



Figure 3 Chemical composition of Khaya senegalensis stems bark oil

## 4. Conclusion

The demonstration of broad antimicrobial spectrum, high free radical scavenging activity and the presence of bioactive compounds in the plant materials could explain the basis for the use of *K. senegalensis* in the traditional treatment of diseases caused by the test microorganisms. However, efforts should be geared toward the isolation of active ingredients in the identified compounds in order to establish the possible use of the essential oils as sources of alternative antibiotics.

## **Compliance with ethical standards**

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#### Disclosure of conflict of interest

The authors declare no conflict of interest in any form.

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