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ABSTRACT

Traditional local beer "dolo" sold in marketplaces in Burkina Faso are often assumed to be safe. This study investigated the conservation and quality of "dolo" using essential oils extracted from five plants. Twenty five samples of "dolo" were collected from traditional producers in different areas of Ouagadougou (Burkina Faso).

The chemical composition of the essential oils from the barks of Boswellia papyrifera, the flowers of Jasminum sambac, leaves of Pandanus odoratissimus and Matricaria chamomilla obtained by hydrodistillation were analyzed using GC–MS.

The chemical composition of the essential oil obtained from Boswellia sacra was composed of sesquiterpene hydrocarbons (57.60%) and oxygenated sesquiterpenes (20.02%). The major compounds were incensole acetate (43.76%), isoincensole plus isoincensole acetate (18.42%) and incensole (5.48%) for B. papyrifera. The essential oil obtained from Matricaria chamomilla contained 95.21% of oxygenated monoterpenes and 89.98% of piperitone. Ten components, representing 94.93% of the total essential oil from the leaves of Pandanus odoratissimus, were found.

The ability of the essential oils to preserve and stabilize "dolo" samples was revealed by μ_{max} of bacteria strains tested. Among the plants tested B. sacra presented the more capacity of inhibition on the strains with μ_{max} value as 0.0041±0.00038, 0.0061±0.00011, 0.0099±0.00073, 0.0176±0.0011, 0.0160±0.0010 respectively on E. coli, S. typhi, S. Nigeria, Y. enterocolitica, L. monocytogenes. The low values of μ_{max} showed the potential of essential oils to inhibit the microorganisms found in the "dolo" beer and to preserve drink, keeping it safe. The essential oils used, did not change organoleptic and nourishing quality of "dolo".

Keywords: *Sorghum beer, microorganisms, plants, essential oils, preservation.*

INTRODUCTION

"Dolo" is a local beer, brewed from malted red sorghum grains. It is a popular alcoholic drink in West Africa, particularly in Burkina Faso ([1], [2], [3]). However, it undergoes some deterioration caused by microbial alteration resulting in preservation problems [4]. The maximum shelf life is 3 days. Aside from the deterioration of "dolo" beers, microorganisms could be responsible of many diseases. Dolo is constantly exposed to and threatened by a variety of pathogenic microorganisms found in the environment. Deterioration caused by bacteria significantly contributes to the overall loss in "dolo" production yield. In order to reduce the contamination, various mechanisms have devised to fend off microbial invaders. Despite the existence of defense mechanisms, a major difficulty encountered is the lack of effective control against some microorganisms. Several preservative technics have been developed, but they remain inadequate; hence the need to focus on the way of the bioconservation through the use of bioactive molecules.

The presence of antioxidants in the diet has become essential for the quality and safety of the food. The negative effects of synthetic antioxidants should encourage their substitution by natural agents. Several aromatic plants, characterized by the biosynthesis of fragrant molecules which are referred to as essential oils for antiseptic and therapeutic activity are known in traditional medicine [5]. Medicinal and aromatic plants have been used for a long time oxidative stress as well as the fight against infectious diseases.

Essential oils currently used as flavouring ingredients have antibacterial activities and could therefore serve as food preservatives [6]. Aromatic plants are promising and good sources of antioxidants as well as natural antibacterial for the agri-food industry ([7], [8], [9]).

In recent years, renewed interest in the use of plant extracts including essential oils for the conservation of food has been grown ([10], [11], [12], [13], [14]).

Essential oils from *Boswellia papyrifera* (Del.) Hochst. and *Boswellia sacra* Flueck. (family Burseraceae), *Jasminum sambac* (L.) Aiton., *Matricaria chamomilla* (L.) Rydb. and *Pandanus odoratissimus* L. syn. *P. fascicularis* Lam. (family Oleaceae, Asteraceae and Pandanaceae respectively) were extracted. Both species grow mainly in Arabia, on the eastern coast of Africa and in India ([15], [16]). Both plants are used for a variety of purposes from traditional medicine to industries such as pharmacology, perfume and food industries ([17], [18], [19], [20]).

In the present study, the chemical composition of the essential oils of the previous plants is analyzed and their capacity to preserve safety and hygienic quality of local drink "dolo" has been investigated.

MATERIAL AND METHODS

Plant source and essential oils extraction

Barks from *B. papyrifera* and *B. sacra* were collected at Arta (11.52'N 42.84'E) in Djibouti. Flowers from *J. sambac*, leaves from *M. chamomilla* and *P. odoratissimus* were collected at the garden of Ambouli (11.33'N 43.08'E) in Djibouti. These five plants were collected in March 2011. Reference specimens were deposited in the Herbarium of the Centre d'Etude et de Recherches de Djibouti under the numbers 40, 47, 41, 48 and 32 respectively for *B. papyrifera, B. sacra, J. sambac*, *M. chamomilla* and *P. odoratissimus*.

Three parts (100 g/portion) of the crushed plant material were individually used to hydrodistillation for 4 h using a Clevenger-type apparatus. The collected oil was dehydrated using Na_2SO_4 (Sigma-Aldrich, Germany) and stored at +4°C until use.

GC-FID and GC-MS analyses

Gas chromatography system with a flame ionisation detector (GC-FID) consisted of a Varian 3900 gas chromatograph (Palo Alto, California, USA) equipped with an automatic injector and non-polar fused silica capillary column DB-5 (30 m × 0.25 mm × 0.25 µm). The oven temperature was programmed from 120 °C to 250 °C (5.0 min hold) at 2°C/min for *P. odoratissimus*. Injection volume was 0.5 µL at 1:100 split; injector and detector temperature was 260 °C. Oven temperature was programmed from 40°C to 300°C at 4°C/min then hold 30 min at 300°C for others essential oils. Injection volume was 0.1µL at 1:100 split. Injector and detector temperatures were 250°C and 300°C respectively.

A Varian 3900 gas chromatograph equipped with a Clarus 500 mass selective detector (Perkin Elmer, USA) and

a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm) was used. Mass spectra was acquired over 40–500 amu ranged at 1scan/s with ionizing electron energy 70eV, ion source 200 °C. Carrier gas was He at 1.0 mL/min. Oven temperature was programmed from 120 °C to 250 °C (5.0 min hold) at 2°C/min for *P. odoratissimus*.

Injector and detector temperatures were 250°C. Oven temperature was programmed from 40°C to 250°C for others essential oils. Injector and detector temperatures were 250°C and 260°C respectively.

The identification of the oil components was performed by their retention indices (RI), authentic reference compounds, peak matching library search as well as published mass spectra ([21], [22], [23]). Retention indices were calculated using an n-alkane series (C_6–C_{24}) under the same GC conditions as for the samples. The relative amount (%) of individual components of the oil is expressed as percent peak area relative to total peak area from the GC/FID analysis of the whole extracts.

Strains collection

Five indicator bacteria strains were investigated (Table 1). They were kindly supplied by the Department of Food Science, Food Microbiology in Copenhagen University, Denmark.

Sampling of “dolo” beer

Twenty five samples of “dolo” were collected in May 2011 at different sources in Ouagadougou (12.4° N 1.5° W) in Burkina Faso. Samples were transported in a cooling box (+4°C) to the laboratory. One liter was collected from each drinkable sample produced at least two days ago. Samples were stored at -20°C until use.

Preservation test of “dolo” contained essential oils

Essential oils have been used for the preservation of “dolo”. Microbial growth in “dolo” was assessed by microdilution method. For each test, 100 μL of dolo containing the effective concentration of essential oil was introduced in three wells. A volume of 10 μL of bacterial inoculum cultured for 18 to 24 hours has been added per strain. The positive control contains dolo and bacterial strain. Negative control contains “dolo” and essential oil.

Bacterial strains as *Listeria monocytogenes* 057, *Salmonella nigeria* SKN 1160, *Salmonella typhimurium* SKN 533, *Escherichia coli* 81 nr. 149 SKN 541 and *Yersinia enterocolitica* 6A28 SKN 599 (Table 1) were subcultured in Mueller Hinton broth and incubated for 18 hours at 37°C.

A volume of 100 mL of “dolo” was centrifuged at 6000 rpm for 30 min. The supernatant obtained was sterilized at 121°C for 15 min and then used for the preservation test. The plates were incubated at 37°C and the optical densities (OD) read at 600 nm hourly for 24 hours. The results were treated using GraphPad Prism v.4 software in order to obtain curves and determine the rate of growth or degeneration of the strains.

Tasting test of “dolo” containing essential oils

“Dolo” samples incorporated of up to 0.02% (v/v) essential oils were subjected to taste test using the triangular method. The panel was composed of 30 qualified persons.

Each taster was relished 3 samples (2 identical and 1 abnormal samples). It must refer to the non-repeated product (different from 2 other products) and give a general appreciation by showing the difference between the products. The results of the test were extracted by a process of compilation and synthesis of individual results made by the subjects for an overall evaluation [24].

Statistical analysis

All the experiments were performed in triplicate on three separate occasions. Data obtained were expressed as means ± standard deviation (SD). Maximal growth rate of bacteria were established using Prism GraphPad v.4 software by plotting the logarithm of the optical density of bacteria cells. The statistical significance of differences between the treatments was assessed using the student test from SPSS 10.0 software. The differences of degeneration cell among the bacteria have been tested on SAS (Statistical Analysis Systems Inc., Cary, USA) after the transformations in an object of normalization of the results distribution. The degree of significance has been fixed to $p = 0.05$.

RESULTS AND DISCUSSION

Essential oils extracted

The content of essential oils obtained were 0.14±0.010%, 0.37±0.013%, 0.11±0.010%, 0.16±0.017% and 0.22±0.016% (w/w) for *B. papyrifera*, *B. sacra*, *J. sambac*, *M. chamomilla* and *P. odoratissimus* plants respectively.

Chemical composition of essential oils

B. papyrifera and *J. sambac*

The essential oil components identified in *B. papyrifera* and *J. sambac* are shown in Table 2. GC-FID and GC-MS analysis led to the identification of fourty three and fourty eight components representing 99.86% and 87.95% of the total oil composition of *B. papyrifera* and *J. sambac* respectively. The essential oils were dominated by the diterpenes (71.75% and 47.85%) followed by the sesquiterpenes (18.98% and 20.70%) and the monoterpenes which represent 9.13% and 16.54% of the total oil composition of *B. papyrifera* and *J. sambac* respectively. The lipidic derivatives were found at 2.87% in the essential oil of *J. sambac*. *B. papyrifera* exhibited three major components: incensole acetate (43.76%), isoincensole+isoincensole acetate (18.42%) and incensole (5.48%). The others minor compounds were found to be n-hexyl hexanoate (3.26%), 1-hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene (3.1%), δ-cadinol (2.92%), neryl acetate (2.84%), n-decyl acetate (1.94%), isocaryophyllene (1.80%), 2-isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphtalene (1.70%), incensole oxide (1.40%) and cembrene A (1.29%). The essential oil *from J. sambac* were composed of geranylgeraniol (20.72%), trans-totarol (12.66%), linalool (11.67%), *cis*-totarol (6.91%), (E,E)-α-farnesene (3.72%), isoincensole+isoincensole acetate (3.31%), α-cadinol (2.81%), octadecanoic acid, methyl ester (2.33%), *α*-muurolene (2.26%), cembrene A (1.79%), dibutyl phthalate (1.69%), linalyl acetate (1.57%), α-terpineol (1.42%), *epi-α*-muurolol (1.35%) and *γ*-curcumene (1.08%).

Both essential oils were characterized by high contents of oxygenated components 89.57% and 71.37% for *B. papyrifera* and *J. sambac*.

B. sacra and *M. chamomilla*

Table 2 shows the essential oil identified in *B. sacra* and *M. chamomilla*. GC-FID and GC-MS analysis led to the identification of sixty and fourty two components representing 98.98% and 99.28% of the total oil composition of *B. sacra* and *M. chamomilla* respectively.

The essential oils from *B. sacra* were dominated by the presence of sesquiterpenes (77.62%) followed by diterpenes (13.29%) and monoterpenes which represent 8.07% of the total oil. The essential oils obtained from *M. chamomilla* leaves were composed of monoterpenes (95.30%), sesquiterpenes (3.84%) and traces of diterpenes (0.14%).

The essential oils from *B. sacra* essential oil were composed of germacrene D (15.23%), β-eudesmene (11.78%), β-eudesmol (8.21%), γ-cadinene (7.78%), δ-cadinene (7.57%) and α-copaene (5.78%). Others minor compounds were isoincensole+isoincensole acetate (3.65%), α-pinene (3.59%), γ-muurolene (3.00%), α-bisabolol (2.73%), dehydroab ietadiene (2.59%), α-humulene (1.68%), τ-cadinol (1.59%), *p*-cymene (1.50%), *ent*-pimara-8(14),15-diene (1.44%), β-caryophyllene (1.40%), caryophyllene oxide (1.34%), α-cadinol (1.33%), manool (1.25%), β-elemene (1.12%), *p*-cymen-8-ol (1.06%), 1-hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene (1.06%) and abietatriene (1.01%).

Piperitone (89.98%) was found to be the major constituent of *M. chamomilla* essential oil. Its minor components were menthyl acetate (1.05%), β-bisabolene (0.82%), (Z)-caryophyllene (0.74%) and isobornyl acetate (0.53%). *M. chamomilla* essential oil was characterized by high contents of oxygenated components 96.40%.

P. odoratissimus

Ten compounds, representing 94.93% of the essential oil of *P. odoratissimus*, were identified by GC and GC/MS analysis (Table 2).

The essential oils was mostly composed of oxygenated diterpenes and oxygenated sesquiterpenes.

Incensole oxide (31.94%), *cis*-totarol (23.04%), *α*-bisabolol (12.85%) and *epi*-13-manoyl oxide (11.07%) were found to be the major components, representing 78.90% of the essential oil. Minor components included phytol (4.62 %) incensole oxide acetate (4.14%), nezukol (2.98%), α-eudesmol (2.53%) and piperitone (1.21%). The chemical class distribution of the essential oil is shown in Table 1. Compounds were separated into three main classes: oxygenated diterpenes (77.79%), oxygenated sesquiterpenes (15.38%) and oxygenated monoterpenes (1.21%).

Preservation capacity of essential oils on "dolo" beer

The origin of strains is shown in the Table 1. Five different microorganisms were used to test the efficacy of the extracted essential oils. The conservation ability of "dolo" is revealed by the growth curves as well as the kinetic parameters (μ_{max}) of bacteria strains evolving under the action of essential oils and the main results were mentioned in Table 3.

The values of maximal growth rate in the standard medium (without essential oils) for the strains *E. coli, S. typhi, S. nigeria, L. monocytogenes* and *Y. enterocolitica* were **0.31±0.0077**, **0.29±0.0012**, **0.24±0.0066**, **0.22±0.0029** and **0.20±0.0013** respectively. The analysis of values of bacteria maximal growth rate, showed no significant difference ($p>0.05$) between the strains. These results revealed a normal tendency of bacterial growth without influence of essential oils.

The active phase of growth was characterized by a proliferation of biomass whereas in stationary phase, the rate of multiplication becomes low. Starting nearly with an identical initial inoculum (OD = 0.01), five strains have reached different final populations and have presented different rates of growth.

The growth levels obtained are comparable to those of the strains coming from the same bacterial group which has been developed under similar conditions of culture.

Begot *et al.* [4] showed that with the inoculum (OD = 0.01); the strains *L. monocytogenes, Y. enterocolitica, S. nigeria* reach average optima rate of growth at 0.22.

However, in presence of essential oils, changes in the growth of the strains were observed. The optical density of the cells decreased according to the properties of the essential oil studied and the contact time. The essential oils extracted from *B. sacra, M. chamomilla, B. papyrifera, J. sambac P. odoratissimus* showed an inhibition on *E. coli* expressed by maximal growth rate low values with significant difference comparing the standard culture ($p<0.05$) in the following order 0.0041±0.00056, 0.0072±0.00038, 0.01±0.0025, 0.013±0.0019, 0.0215±0.0012 (Table 3). The most inhibitory effect of the essential oil on *E.coli* was obtained with *B. sacra*.

The highest inhibition on *S. typhi* was obtained from essential oil extracted from *B. sacra* with a value as 0.0061±0.00011. It was followed by *J. sambac, B. papyrifera, M. chamomilla* and *P. odoratissimus* with respective values as: 0.0084±0.00015, 0.0128±0.0018, 0.0152±0.0014 and 0.0179±0.0015. The values were significantly different ($p<0.05$).

The regression values were significantly different ($p<0.05$), and in the inhibition order of 0.0099±0.00073, 0.0137±0.0076, 0.0164±0.0033, 0.0188±0.0007 and 0.0234 ±0.0059 were obtained respectively under effect of *B. sacra* (more inhibitor), *P. odoratissimus, J. sambac, M. chamomilla, B. papyrifera* and on *S. nigeria* .

The inhibition on *L. monocytogenes* was ranged with values as 0.0160±0.0010, 0.0206±0.0004, 0.023±0.0099, 0.0239±0.0013 and 0.0256±0.001under the effect of *B. sacra* (more active), *P. odoratissimus, J. sambac, M. chamomilla* and *B. papyrifera*. The regression values were significantly different with standard culture ($p<0.05$).

The inhibition effect of the essential oils from the plants on *Y. enterocolitica* is shown in Table 3. It was more pronounced ($p<0.05$ compare to standard culture) with *B. sacra* (0.0176±0.0011), followed by *M. chamomilla* (0.0209±0.0052), *P. odoratissimus* (0.0229±0.0024), *B. papyrifera* (0.0281±0.0076), *J. sambac* (0.0309±0.0050). Furthermore a significant difference ($p<0.05$) was found with regard to the inhibition of cell growth in presence or absence of essential oils.

The analysis of bacteria rate of growth under the influence of the essential oils showed the heterogeneity of

inhibitory effect of the five plants on microbial strains. These essential oils were more active on *E. coli* and respectively *S. typhi, S. nigeria, Y. enterocolitica* and *L. monocytogenes*. *B. sacra* has presented the more capacity of inhibition on the strains. The variability of inhibition may be due to the capacity of resistance related to bacterial groups or the nature of the compounds found in the essential oils. It is possible that chromatographic profiles of essential oils of these five plants may contain polyphenolic, aromatic and terpenic compounds that are responsible of the antibacterial effect.

These compounds have been shown to inhibit the synthesis of the peptidoglycan by binding on certain bacterial proteins and are also active on Gram positive and negative bacteria, aerobic or anaerobic. These hypotheses are confirmed by Carson and Riley [5] who showed that the activity of essential oils is often assimilated to a bacteriostatic activity. However, they underlined that some chemical constituents of essential oils have bactericidal properties that can alter or even eliminate bacteria. This inhibition depends on the mechanisms of resistance setting up by bacterial groups.

The biological activity of an essential oil is related to its chemical composition. The important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable [25]. Leakage of ions and other cell contents can then occur [26]. Although a certain amount of leakage from bacterial cells may be tolerated without loss of viability, extensive loss of cell contents or the exit of critical molecules and ions will lead to death [27].

The biological activity of an essential oil is in relation to its chemical composition, the majority compounds functional groups (alcohols, phenols, terpene and ketone compounds) and the possible synergistic effects between components. Thus, the nature of chemical structures and proportions play a decisive role. The activity of essential oils is often reduced to the activity of its majority compounds or those likely to be active. These assumptions are confirmed by the work of several authors.

Generally, the essential oils possessing the strongest antibacterial properties contain a high percentage of phenolic compounds such as carvacrol, eugenol (2-methoxy-4-(2- propenyl) phenol) and thymol ([26], [28]). It seems reasonable that their mechanism of action would therefore be similar to other phenolic; this is generally considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport and coagulation of cell contents [25]. The chemical structure of the individual essential oil components affects their precise mode of action and antibacterial activity [29].

Lahlou [30] proved that the value of an essential oil is in the entirety of its components, not only to its majority compounds. Some authors suggest that deemed active molecules are terpenoids because the saturated hydrocarbons and ionic acetates were inactive by the nature of hydrogen bonds and their low solubility [31].

The effect of terpenoids isolated bacterial membranes suggests that their activity is a function of the lipophilic properties of terpene constituents, the nature of functional groups, their solubility in aqueous phase and the stereochemistry of molecule [29]. The alcohols are generally known for their bacteriostatic lethal activity in vegetative cells. They act in denaturing proteins, such as solvents or as agents of dehydration [29].

Aldehydes are potent antibacterial agents. An aldehyde group conjugated to a double bond is strongly electronegative. Electronegative compounds can induce electron transfer reactions and react with vital for the bacterium nitro compounds: proteins and acids nucleic ([29], [32]).

Phenols are responsible of irreversible damage of the membrane. They are responsible of fungicide activity [33] and bactericidal essential oils that contain [34]. Phenols have inhibitory and lethal effect on various strains, including *E. coli* which cause leak of potassium K^+ ions. Levels of phenols are related to the activity of essential oils. However, phenols are not only responsible of full activity; all of the chemical must be taken into account [35].

Taste test of the preservation of dolo by essential oils

All dolo containing essential oils have been differentiated from natural dolo during different tests.

• Dolo containing a dose of 0.02% essential oil from *B. papyrifera* vs. natural dolo: tasters found the unique sample 30 times on 30 tests. The proportion of correct responses was 100%. Tasters found this sample nice.

• Dolo containing a dose of 0.02% essential oil from *B. sacra* vs. natural dolo: tasters found the unique sample 19 times on 30 tests. The proportion of correct responses was 63%. Tasters found this sample very nice.

• Dolo containing a dose of 0.02% essential oil from *J. sambac* vs. natural dolo: tasters found the unique sample 23 times on 30 tests. The proportion of correct responses was 77%. Tasters found this sample quite nice.

• Dolo containing a dose of 0.02% essential oil from *M. chamomilla* vs. natural dolo: tasters found the unique sample 28 times on 30 tests. The proportion of correct responses was 93%. Tasters found this sample nice.

• Dolo containing a dose of 0.02% essential oil from *P. odoratissimus* vs. natural dolo: tasters found the unique sample 25 times on 30 tests. The proportion of correct response was 83%. Tasters found this sample quite nice.

The difference has been well discerned by the panelists. At least, 60% panelists have discerned a difference between drinks. Appreciation given to the aroma varies according to essential oils tested. The recorded color is brown for all tested samples. The smell was wooded for *B. papyrifera*, balsamic for *B. sacra*, floral for *J. sambac*, herbal for *M. chamomilla* and pink for *P. odoratissimus*. The taste was slightly acid and slightly bitter. The absence of gas release was noticed. However the tested essential oils stabilized yeast of dolo.

CONCLUSION

The capacity of “dolo” preservation by essential oils was revealed by maximal rate of growth decrease of microbial strains under the influence of these oils. The effect of the essential oils on the organoleptic quality of “dolo” in preservation showed that it was acceptable. The chemical properties of these essential oils are those permitted for use in food and thus helped to keep “dolo” organoleptic, nourishing and microbiological quality intact.

The findings of the present study showed that several essential oils exhibited antibacterial activity against drinking beer pathogenic bacteria *in vitro* system. So the development of natural antibacterial would help to decrease the negative impact of synthetic agents, such as residues, resistance and environmental pollution. In this respect, essential oils as natural biological curator may be effective, selective to food and drinking beer industries.

Table 1. Indicator bacteria strains and their origins (references)

Indicator bacteria strains	Codes	Origin (reference)
Listeria monocytogenes 057	Lm	Culture Collection of
Salmonella nigeria SKN 1160	Sn	Department of Food
Salmonella typhimurium SKN 533	St	Science, Food Microbiology in
Escherichia coli 81 nr. 149 SKN 541	Ec	Copenhagen University, Denmark
Yersinia enterocolitica 6A28 SKN 599	Ye	

Table 2. Essential oils of *B. papyrifera, B. sacra, J. sambac, M. chamomilla* and *P. odoratissimus* obtained by hydrodistillation

Peak N°	RI	Compounds	*B. papyrifera* (%)	*B. sacra* (%)	*J. sambac* (%)	*M. chamomilla* (%)	*P. odoratissimus* (%)
1	916	α-Thujene	-	0.07	-	-	-
2	942	α-Pinene	0.24	3.59	-	0.03	-
3	963	Sabinene	-	-	0.52	0.02	-
4	966	β-Pinene	0.12	-	-	0.04	-
5	1003	α-Phellandrene	-	0.20	-	-	-
6	1014	*p*-Cymene	-	1.50	-	-	-
7	1020	Limonene	0.07	0.30	-	-	-
8	1022	Eucalyptol	0.24	0.44	-	0.02	-
9	1063	n-Octanol	0.13	-	-	-	-
10	1101	Linalool	-	0.17	11.67	0.47	-
11	1111	Thujone	-	0.10	-	-	-
12	1123	*trans-p*-2,8-Menthadien-1-ol	-	-	-	0.10	-
13	1146	Camphor	-	-	-	0.20	-
14	1170	*cis*-Sabinol	-	0.04	-	0.53	-
15	1182	4-Terpineol	-	0.03	-	-	-
16	1187	*p*-Cymen-8-ol	-	1.06	-	0.22	-
17	1193	α-Terpineol	-	0.24	1.42	0.24	-
18	1198	Myrtenol	-	-	-	0.12	-
19	1206	*trans*-Piperitol	-	-	-	0.33	-

20	1221	Verbenone	-	-	0.27	-	-
21	1225	*trans*-Carveol	-	-	-	0.26	-
22	1230	*cis*-Carveol	-	-	-	0.40	-
23	1235	Cumaldehyde	-	-	-	0.12	-
24	1242	*p*-Anisaldehyde	-	-	-	0.04	-
25	1249	Linalyl acetate	-	-	1.57	-	-
26	1250	Piperitone	-	-	0.39	89.98	1.21
27	1275	Bornyl acetate	-	0.03	-	0.30	-
28	1281	Carvacrol	-	0.27	-	-	-
29	1286	Isobornyl acetate	-	-	-	0.53	-
30	1291	Menthyl acetate	-	-	-	1.05	-
31	1293	Thymol	-	-	-	0.15	-
32	1301	Carvacrol	-	-	0.22	-	-
33	1306	δ-Octalactone	-	-	-	0.15	-
34	1321	1-Methyl-4-(1-methyl-ethenyl)-	-	0.03	-	-	-
35	1344	Terpinyl acetate	0.29	-	-	-	-
36	1347	α-Cubebene	-	0.23	-	-	-
37	1375	Neryl acetate	2.84	-	0.47	-	-
38	1383	α-Copaene	-	5.78	0.41	0.01	-
39	1387	n-Hexyl hexanoate	3.26	-	-	-	-
40	1388	β-Bourbonene	-	0.41	-	-	-
41	1390	β-Cubebene	-	0.02	-	-	-
42	1393	β-Elemene	-	1.12	-	-	-
43				-	-	-	-

	1406	n-Decyl acetate	1.94				
44	1418	(Z)-Caryophyllene	0.78	-	-	0.74	-
45	1426	β-Caryophyllene	-	1.40	-	0.16	-
46	1427	β-Gurjunene	-	-	-	0.21	-
47	1444	Aromadendrene	-	-	0.12	-	-
48	1454	α-Humulene	0.27	1.68	-	-	-
49	1456	allo-Aromadendrene	0.75	0.31	-	-	-
50	1475	γ-Muurolene	0.67	3.00	0.28	0.39	-
51	1479	Germacrene D	0.75	15.23	-	0.02	-
52	1480	Isocaryophyllene	1.80	0.28	-	-	-
53	1481	β-Bisabolene	-	-	-	0.82	-
54	1483	β-Eudesmene	0.15	11.78	-	-	-
55	1485	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphtalene	1.70	0.41	-	-	-
56	1490	β-Selinene	-	-	0.99	0.03	-
57	1495	Cadina-1,4-diene	-	-	0.44	0.08	-
58	1501	α-Muurolene	-	0.60	2.26	-	-
59	1502	(E,E)-α-Farnesene	-	-	3.72	-	-
60	1503	δ-Guaiene	-	-	0.32	-	-
61	1509	γ-Cadinene		7.78		0.01	-

			0.79		0.69		
62	1511	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	3.10	1.06	-	-	-
63	1515	γ-Curcumene	-	-	1.08	0.16	-
64	1518	α-Amorphene	-	-	0.18	-	-
65	1524	δ-Cadinene	0.17	7.57	-	-	-
66	1528	1,2,4a,5,6,8a-Hexahydro-1-isopropyl-4,7-dimethylnaphtalene	0.12	-	-	-	-
67	1533	Elemicin	0.27	-	0.08	-	-
68	1540	α-Cadienene	-	-	0.18	-	-
69	1558	(E)-Nerolidol	-	-	0.59	-	-
70	1568	Isogenol acetate	-	-	0.47	-	-
71	1572	Lauric acid	0.25	-	-	-	-
72	1578	Spathulenol	-	-	0.20	0.45	-
73	1579	Hexyl caprylate	0.87	-	-	-	-
74	1582	Caryophyllene oxide	-	1.34	-	-	-

75	1587	Ledol	0.47	0.69	-	-	-
76	1604	Cedrenol	0.14	0.44	0.38	-	-
77	1607	1,5,5,8-Tetramethyl-12-oxabicyclo-[9.1.0]dodeca-3,7-diene	0.56	0.62	-	-	-
78	1609	γ-Eudesmol	0.47	0.57	0.50	-	-
79	1634	τ-Cadinol	0.23	1.59	-	-	-
80	1636	δ-Cadinol	2.92	-	-	-	-
81	1638	α-Muurolol	-	-	0.83	-	-
82	1642	*epi*-α-Muurolol	-	-	1.35	0.04	-
83	1649	α-Eudesmol	0.23	-	-	-	2.53
84	1651	α-Cadinol	0.46	1.33	2.81	-	-
85	1654	β-Eudesmol	0.48	8.21	-	-	-
86	1655	Bisabolol oxide B	-	-	-	0.22	-
87	1662	α-Bisabolol	0.34	2.73	0.17	-	12.85
88	1666	β-Bisabolol	0.24	0.94	-	-	-
89	1667	*epi*-β-Bisabolol	-	-	-	0.41	-
90	1722	(E)-farnesol	-	-	0.14	-	-
91	1736	Curcumenol	-	0.50	-	-	-
92	1761	Benzyl benzoate	-	-	0.20	-	-
93	1840	Guaiazulene	-	-	0.62	0.04	-
94	1860	Dibutyl Phthalate	-	-	1.69	0.05	0.55

95	1959	Cembrene A	1.29	0.36	1.79	0.02	-
96	1963	*ent*-Pimara-8(14),15-diene	-	1.44	0.17	-	-
97	1975	Kaur-15-ene	-	0.18	-	-	-
98	1986	1,3-Epimanoyl oxide	-	0.66	-	-	-
99	1990	Manoyl oxide	-	0.16	-	-	-
100	2004	Verticilla-4(20),7,11-triene	0.62	0.19	-	-	-
101	2010	*epi*-13-Manoyl oxide	-	0.04	-	-	11.07
102	2011	Phyllocladene	-	0.05	-	-	-
103	2025	Geranylgeraniol	-	-	20.72	-	-
104	2037	Dehydroab ietadiene	-	2.59	-	-	-
105	2054	Abietatriene	-	1.01	0.48	-	-
106	2062	Manool	-	1.25	-	-	-
107	2077	9,12,15-Octadecatrienoic acid (Z,Z,Z), methyl ester	-	-	0.31	-	-
108	2100	Octadecanoic acid, methyl ester	-	-	2.33	-	-
109	2104	Phytol	-	-	0.17	-	4.62
110	2111	8.β-Hydroxysandaracopimarane	-	0.86	-	-	-
111	2126	Nezukol	0.11	0.48	0.36	-	2.98
112	2134	Ethyl linoleolate	-	-	0.23	-	-
113	2150	Incensole	5.58	0.11	-	0.02	-
114	2152	Isoincensole+Isoincen	18.42	3.65	3.31	-	-

		sole acetate					
115	2180	Neophytadiene	-	-	-	0.10	-
116	2189	Incensole acetate	43.76	0.12	0.87	-	-
117	2260	Incensole oxide	1.40	0.14	-	-	31.94
118	2278	*cis*-Totarol	-	-	6.91	-	23.04
119	2303	*trans*-Totarol	-	-	12.66	-	-
120	2329	Incensole oxide acetate	0.57	-	0.41	-	4.14
		Total of identified compounds	**99.86**	**98.98**	**87.95**	**99.28**	**94.93**
		Monoterpene hydrocarbons	0.43	5.69	0.52	0.09	-
		Oxygenated monoterpenes	8.70	2.38	16.01	95.21	1.21
		Sesquiterpene hydrocarbons	7.95	57.60	10.75	2.67	-
		Oxygenated sesquiterpenes	11.03	20.02	9.95	1.17	15.38
		Diterpene hydrocarbons	1.91	5.82	2.44	0.12	-
		Oxygenated diterpenes	69.84	7.47	45.41	0.02	77.79
		Plasticizer compounds	-	-	-	0.05	0.55
		Lipidic derivatives	-	-	2.87	-	-

Table 3. Maximal rate of growth (μ_{max}) of 5 bacterial strains

Bacterial strains ± essential oils	μ_{max}	SD
E. coli	**0.31**	**±0.0077**
E. coli / *B. sacra*	0.0041	±0.00038
E. coli / *B. papyrifera*	0.01	±0.0025
E. coli / *M. chamomilla*	0.0072	±0.00056
E. coli / *P. odoratissimus*	0.0215	±0.0012
E. coli / *J. sambac*	0.013	±0.0019
S. typhi	**0.29**	**±0.0012**
S. typhi / *J. sambac*	0.0061	±0.00015
S. typhi / *B. sacra*	0.0084	±0.00011
S. typhi / *B. papyrifera*	0.0128	±0.0018
S. typhi / *P. odoratissimus*	0.0179	±0.0015
S. typhi / *M. chamomilla*	0.0152	±0.0014
S. nigeria	**0.24**	**±0.0066**
S. nigeria / *P. odoratissimus*	0.0137	±0.0076
S. nigeria / *B. sacra*	0.0099	±0.00073
S. nigeria / *B. papyrifera*	0.0234	±0.0059
S. nigeria / *M. chamomilla*	0.0188	±0.00070
S. nigeria / *J. sambac*	0.0164	±0.0033
L. monocytogenes	**0.22**	**±0.0029**
L. monocytogenes / *P. odoratissimus*	0.0206	±0.0004
L. monocytogenes / *M. chamomilla*	0.0239	±0.0013
L. monocytogenes / *B. sacra*	0.0160	±0.0010
L. monocytogenes / *B. papyrifera*	0.0256	±0.001
L. monocytogenes / *J. sambac*	0.023	±0.0099

Y. enterocolitica	**0.20**	**±0.0013**
Y. enterocolitica / *B. sacra*	0.0176	±0.0011
Y. enterocolitica / *B. papyrifera*	0.0281	±0.0076
Y. enterocolitica / *P. odoratissimus*	0.0229	±0.0024
Y. enterocolitica / *J. sambac*	0.0309	±0.0050
Y. enterocolitica / *M. chamomilla*	0.0209	±0.0052

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