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Biopreservation of meat using the essential oil from *Hyptis suaveolens* Poit. (Lamiaceae) in Burkina Faso

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Essential oils are natural substances which can be used as natural conservators for foods. The present work aimed to evaluate biological properties of essential oil of *Hyptis suaveolens* and its effect on shelf-life of beef. The extraction was carried out by hydro-distillation. Antioxidant activity was determined by method of DPPH and FRAP. Antimicrobial activity was determined by the microdilution and agar diffusion methods. The sensitivity of eleven pathogen microbial strains was tested. The conservation test of ground meat using essential oil was carried out by monitoring the evolution of the microbial groups. Result showed that extraction yield of essential oil was 0.21% (m/v). The DPPH assay gave IC_{50} of 16.367 ± 0.0739 µL and was confirmed by the FRAP assay. The essential oil from *H. suaveolens* showed inhibitory activity on microbial strains. Biopreservation assay revealed a significant decrease of microbial charge during conservation time. Thus essential oil was successful *in vitro* and its activity on prolonging shelf-life of beef was found to be 7 days. The results of the present study demonstrated the possibility of essential oil from *H. suaveolens* to be used as natural conservator for food industry.

Key words: *Hyptis suaveolens*, essential oil, biopreservation, biological properties, meat.

INTRODUCTION

The sanitary quality of a food is one of essential bases of its ability to satisfy the safety of the consumers. A food

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exposed to microbial spoilage could lose its organoleptic, nutritional and sanitary qualities (Guiraud, 2003). Despite the improved techniques for preserving food, the kind of preservatives remains as one of the most important issues for public health (Burt, 2004). One of the main problems in food industry is to ensure safe food preservation for consumers (Nessrien and Mohamed, 2007). To cope with the problems of oxidation and contamination of foodstuffs, new chemical compounds have often been used to prevent the deterioration of food (Nakahara et al., 2003a). Preservatives such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-Butylhydroquinone (TBHQ) used in food preservation have been limited in several countries due to their undesirable toxicological and carcinogenic effects at short or long time (Rachid et al., 2010). Also, the current trend of consumers to seek a more natural diet has increased. The demand by customers for natural preservative has directed researchers' interest to develop protective methods for reliable foods having better nutritional and organoleptic properties and with high microbial quality (Goni et al., 2009). Aromatic plants have interesting properties due to metabolic substances during their secondary metabolism (Rashid et al., 2010). These substances (the essential oils) are getting more interest for industries and scientific research due to their antioxidant, antibacterial and antifungal activities (Dung et al., 2008). They are also useful as natural preservatives in agro-food industries (Rasooli et al., 2008). In the developing countries where the demographic growth rate is raised, the meat consumption progresses quickly. It is in the case in sub-Saharan Africa, where the beef represent the main of the additional consumption of the area. If the majority of the beef consumed in sub-Saharan Africa is produced in the area, half of additional consumption will come from the importation. The beef consumption will increase gradually during ten next years. It should increase approximately 17% in the areas under development (OCDE/FAO, 2017).

Essential oils are a source of natural preservatives for perishable dairy products in Sub-Saharan Africa where the capacity of food preservation by cold chain is very limited. Among commodities, meat products are very susceptible to deterioration. Poorly preserved meat can lead to economic losses and health consequences in most of developing countries. In Burkina Faso, beef is a food of choice because of its importance as a source of protein. However, rapid deterioration can be observed due to microbial contamination. Some microorganisms can be pathogenic and harmful for consumers' health. To overcome or stop meat spoiling, it is important to use natural preservatives such as essential oils. Although the antimicrobial activity of essential oils derived from plants has been proved by in-vitro tests, more research is required in order to investigate their activities on food. The present study aimed to investigate the biological properties of essential oils from *H. suaveolens* as well as it capacity to prolong shelf-life of meat.

MATERIALS AND METHODS

Plant material

The leaves from *H. suaveolens* were collected in different areas of Ouagadougou. The identification of the plant (*H. suaveolens*) was carried out by the Laboratory of Plant Ecology Biology in University Joseph Ki-Zerbo. Fresh plant material was dried about 25°C for 72 h.

Indicator microorganisms

The antimicrobial activity study focused on eleven (11) microbial strains used as indicator microorganisms:

1. Nine (09) bacterial strains including 5 Gram-negative (*Escherichia coli* ATCC8739, *Salmonella typhi*, *Salmonella paratyphi*, *Shigella dysenteria*, and *Pseudomonas aeruginosa* ATCC9027) and 4 Gram-positive strains (*Staphylococcus aureus* ATCC25923, *Listeria monocytogenes*, *Clostridium perfringens* and *Bacillus cereus*), maintained as stock cultures at -80°C in Mueller-Hinton broth medium (Liofilchem, Italy) with 20% (v/v) glycerol as cryoprotectant.
2. Two (02) fungal strains (*Candida albicans* and *Aspergillus niger*), maintained as stock cultures at -80°C in Sabouraud broth medium (Liofilchem, Italy) with 20 % (v/v) glycerol as cryoprotectant.

Food matrix: Ground beef

Ground beef was purchased at Zogona market (Ouagadougou). The meat samples were placed in icebox to maintain refrigerated conditions and transported to the laboratory.

Essential oil extraction

The essential oil was extracted from the plant material by hydro-distillation according to the method described by Baser and Buchbauer (2010). 500 g of dry matter was immersed in a Clevenger type apparatus containing about 5 L of distilled water. The mixture was boiled for three (03) hours. The obtained essential oil was collected in a sterile amber glass bottle for preservation. Yield, expressed as a percentage (% weight/weight), and was calculated using the following equation:

$$\text{Yield} = (\text{Mass of essential oil (g)}) / (\text{Mass of dry matter (g)}) \times 100$$

Analysis of the organoleptic properties of essential oil

Organoleptic properties such as color, appearance and odor have been determined directly by the sense organs. The essential oil was first transferred to a transparent bottle and analysis by using of standard procedures.

Determination of biological activities of essential oil

Antioxidant activity

DPPH radical scavenging assay: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) test was performed according to the method described by Joshi et al., (2010). Different volumes of essential oil (5, 10, 15, 20 and 25 µL) were mixed with 5 mL of ethanolic solution of DPPH (0.004 % weight/volume). The mixture obtained was incubated in dark space for 30 min and absorbance was read at

517 nm using a spectrophotometer (Jasco, Japan). Butylhydroxytoluene (BHT) (0.05 M), ascorbic acid (0.005 M) and quercetin (0.05 M) (5, 10, 15, 20 and 25 µL) were used as standard antioxidants and a negative control was also prepared in the same conditions like the samples. The tests were carried out in triplicate.

The percentage of inhibition (% I) was calculated according to the following equation:

% Inhibition = $[(A_{blank}–A_{sample}) / A_{blank}] \times 100$; where A_{blank} is the absorbance of the negative control and A_{sample} is the absorbance of the essential oil.

The antioxidant activity of the essential oil was expressed by the 50% inhibitory concentration (IC50) which is the amount of essential oil needed to reduce the initial concentration of DPPH by 50%. The 50% inhibitory concentration was calculated using a regression line (% inhibition = f (concentrations)). The tests were carried out in triplicate.

Ferric reduction antioxidant power (FRAP): The reducing power of essential oil of *H. suaveolens* was determined according to the method of Singh et al. (2006). Different volumes of essential oil (5, 10, 15, 20 and 25 µL) were mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of potassium ferricyanide ($K_3Fe(CN)_6$) at 1%. The mixture was incubated at 50°C for 30 min then 2.5 mL of trichloroacetic acid (10%) were added to the mixture followed by centrifugation at 650 G for 10 min. The supernatant was collected (5 mL) and mixed with 5 mL of distilled water and 1 mL of 0.1% iron ($FeCl_3$) chloride. Absorbance was then measured at 700 nm using a spectrophotometer. Ascorbic acid was used as a standard at 5 mg/Ml. A negative control (blank) was also included in each test. The standard and blank were prepared according to the process of essential oil. An increase in absorbance indicates an increase in reducing power. A standard curve of essential oil absorbance was developed. The tests were carried out in triplicate.

Screening of essential oil for antimicrobial activity

Preparation of the inocula of bacterial and fungal strains

The inocula of bacterial and fungal strains used as indicators were prepared before inoculating during the assays.

A suspension of each bacterial strain was prepared in 10 mL of Mueller-Hinton Broth for 18 to 24 h at 37°C. Using the sterile diluent (physiological saline) (Liofilchem, Italy), the concentration was adjusted in each tube to about 1.0×10^8 CFU/mL comparable to that of the McFarland 0.5 standard according to Lennette et al. (1987). For the fungal suspension, each strain was prepared in Sabouraud broth (Liofilchem, Italy) for 18 to 48 h at 30°C. With a sterile diluent, the concentration was adjusted to about 1.0×10^8 CFU/mL comparable to that of the McFarland 0.5 standard (Lennette et al., 1987).

Agar diffusion method

Petri dishes containing Sabouraud Chloramphenicol medium (for fungal strains) and Mueller-Hinton agar (for bacterial strains) were inoculated aseptically with the inocula. Seeding was done by flooding the Petri dish and the excess was aspirated. After drying the dishes, wells were cut with a sterile cork borer (diameter: 6 mm) in the agar and 10 µL of *H. suaveolens* essential oil was added into the different wells. The dishes were exposed at room temperature for one (1) hour before incubation to promote diffusion of essential oil on agar plate. The dishes were incubated at 37 °C for 24 hours for bacteria and at 30°C for 48 h for fungal strains. The presence of a clear zone around the well indicates the inhibition. The results were read by measuring the diameters of inhibition zones in mm (Rhayour, 2002).

The activity of essential oil was assessed according to the diameter of inhibition values as described by Negreiros et al., (2016). The microbial strains were classified as non-sensitive when a diameter is less than 8 mm, sensitive from 9 to 14 mm, highly sensitive from 15 to 19 mm and extremely sensitive for more than 20 mm.

Determination of minimum inhibitory concentration (MIC)

Determination of the minimum inhibitory concentration (MIC) was performed in a sterile 96-well microplate.

For bacterial strains, 190 µL of Muller-Hinton broth supplemented with tween 80 (0.5%) were introduced into the wells of line 1 and 100 µL into the other wells of the microplate from line 2 to line 12, then 10 µL of essential oil were added to the wells of line 1. The contents of the wells of line 1 were well mixed. 100 µL were collected from these wells (line 1) for cascade dilutions in the other wells (line 1) to the 0.004 % concentration.

The inoculum density was adjusted with sterile saline solution (NaCl 0.9 % w/v) to McFarland 0.5 corresponding to 10^8 CFU/mL. 100 µL of bacterial inoculum were added to all wells except the wells in line 11 which contained only the essential oil and Muller-Hinton broth. Line 11 (without inoculum) served as a negative control. Line 12 containing Muller-Hinton broth and bacterial suspension served as a positive control. The microplates were closed and incubated for 18 to 24 h at 37°C (Yu et al., 2004; Obame, 2009).

For fungal strains, Sabouraud broth supplemented with tween 80 (0.5%) was used. The same procedure as described previously was performed for experiment. The microplates were incubated at 30°C for 48 to 72 h.

Microbial growth is indicated by reading the optical density of culture (Yu et al., 2004). The lowest essential oil concentration inhibiting the microbial growth after incubation period was identified as minimum inhibitory concentration (MIC). The treatments were repeated three times and mean values calculated.

Determination of minimum bactericidal concentration (MBC)

One hundred (100) microliter were taken from wells without detectable growth after 24 h of incubation at 37°C and seeded by spreading on Mueller-Hinton agar (Ouedraogo, 2012). The lowest concentration at which 99.99% of bacteria cells were inhibited after 24 h of incubation was identified as minimum bactericidal concentration (MBC). Experiments were done in triplicate.

Determination of minimum fungicidal concentration (MFC)

A volume of one hundred (100) µL was collected from wells that did not present detectable growth after incubation at 30°C for 72 h. It was seeded on Sabouraud Chloramphenicol agar (Ouedraogo, 2012). The lowest concentration at which 99.99% of fungal cells were inhibited after 72 h of incubation was identified as minimum fungicidal concentration (MFC). Experiments were done in triplicate.

Determination of ratio MBC/MIC and MFC/MIC

The ratio MBC/MIC and MFC/MIC allowed the determination of the bactericidal or bacteriostatic and fungicidal or fungistatic capacities of essential oil on the strains tested according to Canillac and Mourey (2001) and Derwich et al., (2010):

Table 1. Organoleptic characteristics of the essential oil of *Hyptis suaveolens.*

Color	Light yellow
Appearance	Fluid, limpid, very transparent, very bright
Odor	Very strong, aromatic, minty, characteristic of the plant

Essential oil is bacteriostatic when MBC/MIC>4;
Essential oil has bactericidal property when MBC/MIC≤4;
Essential oil has fungicidal effect when MFC/MIC<4;
Essential oil has fungistatic effect when MFC/MIC≥4.

Preservation of ground meat by essential oil

The essential oil of *H. suaveolens* (20 µL) was incorporated in a Stomacher bag containing 50 g of fresh ground meat; a second bag containing only ground meat was used as control. The samples were stored in the refrigerator at 4°C for 7 days. Microbiological analysis was carried out on the 1st, 4th and 7th day on ground meat treated with the essential oil and on the control. Enumeration included total mesophilic aerobic bacteria (TMAB), total coliforms (TC) and thermotolerant coliforms (TTC).

For all the determinations, 10 g of the samples were homogenized in a stomacher (Stomacher 400) with 90 mL of sterile peptone buffered water (Liofilchem, Italy). Tenfold serial dilution was prepared and spread-plated for microorganisms count. 1 mL of each dilution was used for spreading.

Aerobic mesophilic bacteria (AMB) were enumerated on pour plates of Plate Count Agar (Liofilchem, Italy) incubated at 30°C for 72 h (ISO 4833, 2003). Coliforms were enumerated on Eosin Blue Methylene Agar (Liofilchem, Italy), incubated at 37°C (Total coliforms) or 44°C (thermotolerant coliforms) for 24 h according to ISO 4832 (2006) and AFNOR NF V 08-060 (2009) respectively.

Statistical analysis

The data were collected on Excel and analyzed using XLSTAT software version 7.5.2. Variance analysis (ANOVA) was used and Fisher's Least Significant Difference (LSD) was used for comparisons of means in case of significant difference. The difference between means was significant when p value < 0.05. The values were estimated with a 95% confidence interval.

RESULTS AND DISCUSSION

Characteristics of essential oil

Yield and organoleptic characteristics of essential oil from H. suaveolens

The essential oil yield from *Hyptis suaveolens* leaves was 0.215 ± 0.002%. This yield corroborates with that obtained by Ngom et al., (2014) in Senegal which was 0.22%. In Benin, Adjou and Soumanou (2013) obtained similar results (0.23 ± 0.02%). On the other hand, the yield obtained is lower than those obtained by Ilboudo (2009) and Goly et al., (2015) which were respectively 0.23 and 0.34 ± 0.02%. Differences in yield could be explained by several factors such as geographical origin, ecological climatic factors such as temperature and humidity, soil type, plant organ, stage of growth, period of harvesting, conservation of plant material, extraction time and drying time (Marzoukia et al., 2009, Aprotosoaie et al., 2010).

The organoleptic characteristics of the essential oil have been presented in Table 1. The essential oil of *Hyptis suaveolens* was light yellow, its appearance was fluid, limpid, very transparent and very bright with a very strong, aromatic, minty and characteristic smell of the plant. The color of essential oil is different of that obtained by Ngom et al., (2014) which was light green.

Biological properties of essential oil from *H. suaveolens*

Antioxidant activity

Capacity of inhibition of the radical DPPH: Antioxidant activity was determined and compared to that of the reference antioxidants (ascorbic acid, BHT and quercetin). Figure 1 shows the variation of the percentage of inhibition as a function of the quantity of essential oil and standards.

The increase in the percentage of inhibition of the free radical was directly proportional to the increase in the quantity of essential oil and standards. Statistical analysis showed a significant difference ($p < 0.0001$) between percent inhibition of essential oil and standards. For each of the essential oil and the standards, the concentration needed to reduce the DPPH free radical by 50% was determined from the linear regression equations. The 50% inhibitory concentrations (IC_{50}) were presented in Table 2.

For the essential oil from *H. suaveolens*, its antioxidant activity was higher than that of ascorbic acid (0.005 M), but was lower than that of BHT (0.05 M) and quercetin (0.05 M). Indeed, the IC_{50} of *H. suaveolens* was 16.367 ± 0.074 µL whereas with ascorbic acid, BHT and quercetin, the IC_{50} was respectively 22.098 ± 0.128, 13.013 ± 0.053 and 12.142 ± 0.234 µL.

Reducing power of essential oil of *H. suaveolens:* The reducing power of essential oils was evaluated using the FRAP method. The diagram showing the variation of the reducing power as a function of the quantity of essential oils and the standard (Ascorbic acid) are recorded in Figure 2.

The results obtained by the FRAP test show that the reduction of ferric ions was related to the amount of

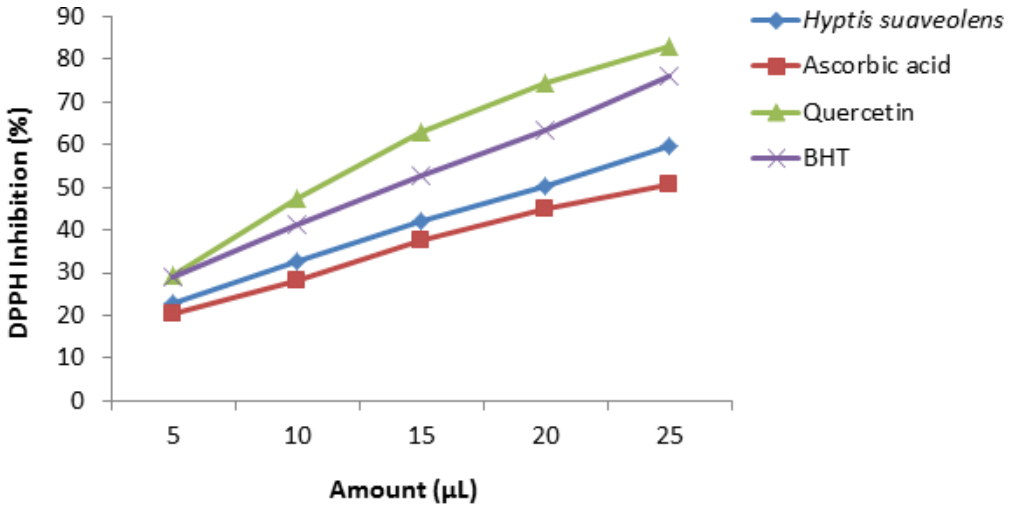

Figure 1. DPPH radical scavenging power.

Table 2. Inhibitory concentrations at 50% of the essential oil of *H. suaveolens.*

Essential oil and standards	Regression equation	R^2	IC_{50} (µL)
H. suaveolens	Y = 2.2789x + 12.698	0.9894	16.367 ± 0.074
Ascorbic acid (0.005 M)	Y = 1.7179x + 12.080	0.9894	22.098 ± 0.128
Quercetin (0.05 M)	Y = 2.9372x + 14.316	0.9967	12.142 ± 0.234
BHT (0.05 M)	Y = 2.083x + 22.891	0.9908	13.013 ± 0.053

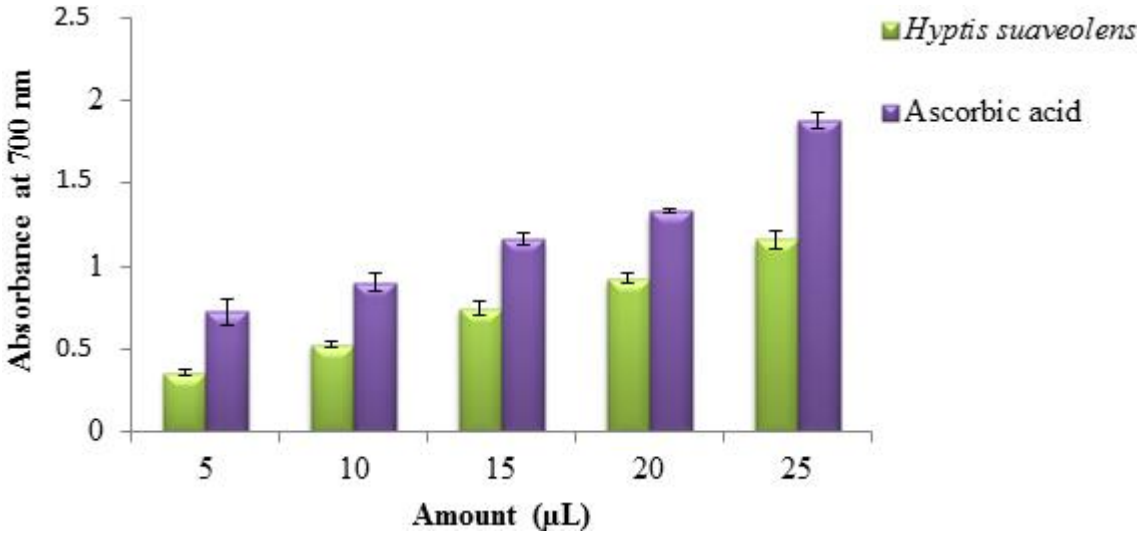

Figure 2. Reducing power of essential oil and ascorbic acid.

essential oils and that the ability of *H. suaveolens* essential oils to reduce iron was lower than that of ascorbic acid.

The evaluation of the antioxidant activity by the DPPH technique and that of FRAP revealed that the essential oil of *H. suaveolens* has antioxidant properties. Previous studies have shown that oxygenated monoterpenes such as thymol, carvacrol and α-terpineol are mainly responsible for the antioxidant potential of their essential oils (Bicas et al., 2011). Monoterpenes such as β-

Table 3. Average diameters of inhibition of the essential oil of *H. suaveolens.*

Bacterial strain	Diameters of inhibition (mm)	Sensitivity of strains
Shigella dysenteria	43.667 ± 1.732[a]	Extremely sensitive
Staphylococcus aureus ATCC25923	36.167 ± 2.055[b]	Extremely sensitive
Salmonella paratyphi	18.833 ± 1.434[c]	Very sensitive
Listeria monocytogenes	18.167 ± 2.718[cd]	Very sensitive
Bacillus cereus	18.000 ± 1.780[cde]	Very sensitive
Escherichia coli ATCC8739	14.333 ± 1.247[def]	Sensitive
Clostridium perfringens	14.167 ± 1.027[def]	Sensitive
Salmonella typhi	13.500 ± 2.273[f]	Sensitive
Pseudomonas aeruginosa ATCC9027	12.833 ± 1.546[f]	Sensitive
Fungal strains		
Aspergillus niger	13.667 ± 1.434[ef]	Sensitive
Candida albicans	10.667 ± 0.471[f]	Sensitive

Values with the same superscript letters are not significantly different ($p<0.05$).

caryophyllene, also has free radical scavenging activity using the DPPH test (Dar et al., 2011). Oxygen-rich essential oils have a stronger anti-radical activity compared to hydrocarbon terpenes. This could explain the moderate antioxidant activity of the essential oil of *H. suaveolens* (Miladi et al., 2013).

Antimicrobial activity

Inhibiting capacity of essential oil of *H. suaveolens*: The well diffusion method highlights the antimicrobial capacity of the essential oils against nine bacterial strains and two fungal strains. The average inhibition diameters obtained are mentioned in Table 3.

Table 3 shows that the essential oils studied have antimicrobial activity. The importance of the action of each essential oil varied according to the microorganism tested.

The essential oil of *H. suaveolens* showed antimicrobial activity on the strains tested. The largest diameter was obtained with *Shigella dysenteria* (43.667 mm) followed by *Staphylococcus aureus* (36.167 mm) and the smallest diameter with *Candida albicans* (10.667 mm). The sensitivity of *H. suaveolens* essential oil was not related to bacteria nature. *S. aureus* (Gram positive) and *S. dysenteria* (Gram negative) were more sensitive to the essential oil. This activity of the essential oil of *H. suaveolens* could be explained by its chemical advantage (Ngom et al., 2014).

The antimicrobial activity could be explained by the presence of phenolic hydroxyl compounds by forming hydrogen bonds with the active sites of the targeted cell enzymes (Derwich et al., 2010). The factors determining the activity of essential oils are the chemical composition, the functional groups present in active components and their synergistic interactions (Chouhan et al., 2015).

Minimum inhibitory concentrations of essential oil of *H. suaveolens:* The results of the determination of minimum inhibitory (MIC), bactericidal (MBC) and fungicidal (MFC) concentrations are shown in Table 4.

Minimum inhibitory concentrations (MIC) ranged from 0.203 to 2.083%. The highest MIC was obtained with *E. coli* and the lowest MIC with *S. paratyphi*. Minimum bactericidal concentrations (MBCs) ranged from 0.625 to 5%. The largest MBC was obtained with *Staphylococcus aureus* (5.000±0.049%) and *C. albicans* (5.000±0.045 %) and the lowest with *C. perfringens* (0.625±0.021%).

Ratio MBC/MIC and MFC/MIC of essential oil from *H. suaveolens*

The results of the MBC/MIC and MFC/MIC report are presented in Table 5.The essential oil of *Hyptis suaveolens* had bactericidal activity on *E. coli*, *S. typhi*, *S. dysenteria*, *B. cereus*, *P. aeruginosa*, *L. monocytogenes* and *C. perfringens*. It had Bacteriostatic on *S. paratyphi* and *S. aureus*. Its effect was fungicidal on *A. niger* and fungistatic on *C. albicans*.

Effect of essential oil on prolonging shelf-life of food matrix

Initial microbial load of ground meat

Prior to the incorporation of the essential oil (EO) on ground meat (GM) for conservation testing, the initial microbial load of this meat was assessed by counting the total aerobic mesophilic flora, total and thermotolerant

Table 4. MIC, MBC and MFC of the essential oil of *H. suaveolens.*

Bacterial strains	MIC	MBC
Escherichia coli ATCC8739	2.083±0.189^{a}	2.167 ±0.302^{b}
Salmonella paratyphi	0.203±0.074^{e}	2.500 ±0.224^{b}
Salmonella typhi	0.625±0.000^{cde}	2.500 ± 0.072 ^{b}
Shigella dysenteria	0.521±0.048^{de}	1.250±0.044^{cd}
Bacillus cereus	1.042±0.012^{bc}	4.167±0.039^{a}
Pseudomona aeruginosa ATCC9027	0.625±0.189^{cde}	1.042±0.081^{cd}
Staphylococcus aureus ATCC25923	0.833±0.121^{bcd}	5.000±0.049^{a}
Listeria monocytogenes	0.521±0.140^{de}	1.250±0.053^{cd}
Clostridium perfringens	0.625±0.032^{cde}	0.625±0.021^{d}
Fungal strains	**MIC**	**MFC**
Candida albicans	1.250±0.000^{b}	5.000±0.045^{a}
Aspergillus niger	1.042±0.113^{bc}	1.667±0.062^{bc}

Values in the same column with the same superscript letters are not significantly different ($p < 0.05$).

Table 5. MBC/MIC and CMF/MIC ratio of essential oil *H. suaveolens.*

Bacterial strains	MBC/MIC	Sensitivity of strains
Escherichia coli ATCC8739	1	Bactericidal
Salmonella paratyphi	12	Bacteriostatic
Salmonella typhi	4	Bactericidal
Shigella dysenteria	2	Bactericidal
Pseudomonas aeruginosa ATCC9027	1	Bactericidal
Bacillus cereus	4	Bactericidal
Staphylococcus aureus ATCC25923	6	Bacteriostatic
Listeria monocytogenes	2	Bactericidal
Clostridium perfringens	1	Bactericidal
Fungal strains	**MFC/MIC**	
Candida albicans	6	fungistatic
Aspergillus niger	1	Fungicidal

coliforms. Figure 3 shows the initial microbial flora of minced meat before storage.

The Initial microbial load of ground meat was found to be 7.0×10^{4} CFU/g for total aerobic mesophilic bacteria, 1.0×10^{4} CFU/g for total coliforms and 5.0×10^{2} CFU/g for thermotolerant coliforms.

The high number of total initial aerobic mesophilic bacteria (TMAB) indicates that the meat was of poor microbiological quality, which could influence its shelf life. The presence of coliforms indicates recent fecal contamination that may be explained by the lack of good hygiene practices throughout the meat production chain.

Effect of essential oil on total mesophilic aerobic bacteria

The evolution of total mesophilic aerobic bacteria (TMAB) was followed in untreated minced meat (control) as well as in that incorporated with essential oils. The results obtained were recorded in Figure 4.

Figure 4 shows the evolution of TMAB during conservation. Thus, on Day 1, the microbial load was 8.3×10^{4} CFU/g for untreated ground meat, 3×10^{4} CFU/g for the ground meat (GM) + essential oil (EO) of *H. suaveolens*. On day 7, the microbial load was 2×10^{5} CFU/g for untreated ground meat and 7.3×10^{4}UFC/g for the GM + EO of *H. suaveolens*. Statistical analysis showed a significant difference with p value<0.0001. An increase in microbial load was noted during storage. This increase was much greater in untreated minced meat than in that incorporated with the essential oil. The reduction of microorganisms concentration (42%) in ground meat containing the essential oil could be explained by the inhibitory effect of this essential oil. The increase of microbial load in meat incorporated with

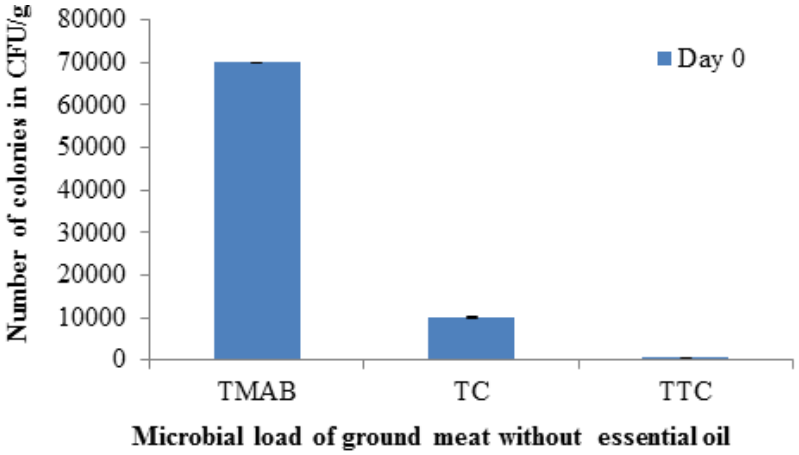

Figure 3. Initial microbial flora of bovine ground meat. TMAB: Total mesophilic aerobic bacteria; TC: Total coliforms; TTC: Thermotolerant coliforms.

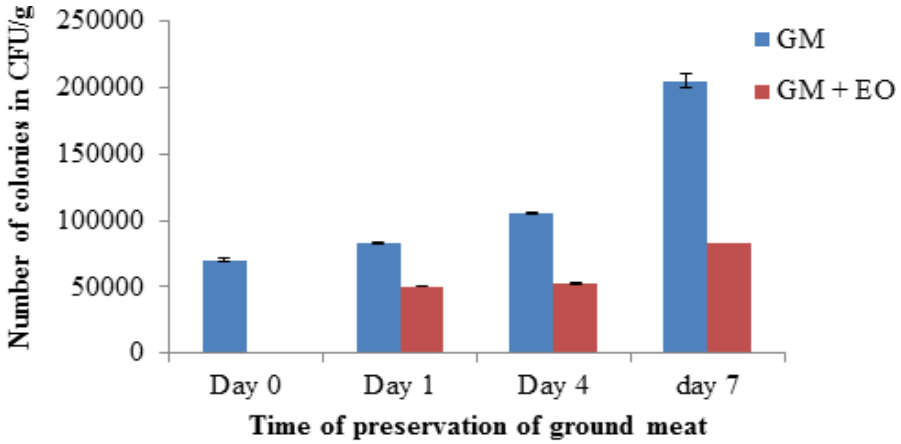

Figure 4. Evolution of TMAB loads during conservation. GM: Ground meat; GM+EO: Ground meat + essential oil.

essential oils could be related to the bacteriostatic effect of these essential oils on certain bacterial strains and the loss of bioactive molecules properties during storage. Meat and meat products can have a high fat content and rich source of protein, minerals, and vitamins. This structure reduces the effect of essential oils against bacteria (Hernandez et al., 2011; Seda and Nukhet, 2016).

Effect of essential oil on total coliform

The evolution of total coliforms concentration in untreated ground meat and in that incorporated with this essential oil is recorded in Figure 5.

The total coliform count at Day1 was 1.5×10^4UFC/g for untreated minced meat and 1.3×10^4 CFU/g for the GM + EO of *H. suaveolens*. After seven days of storage (Day 7), the numbers were 7.4×10^4 CFU/g for untreated minced meat and 8.0×10^3 CFU/g for GM + EO from *H. suaveolens*. Statistical analysis showed a significant difference ($p < 0.0001$).

An increase in total coliforms during storage was observed in untreated ground meat. In the meat supplemented with essential oil, there was a regression (80%) of the bacterial load, which reflects an important antibacterial activity of the essential oil of *H. suaveolens*.

Effect of the essential oil on the evolution of the thermotolerant coliforms load

The evolution of the number of thermotolerant coliforms during refrigeration is presented in Figure 6.

For the evolution of fecal coliforms, the microbial count varied from 7.3×10^3 CFU/g (Day 1) to 1.24×10^4 CFU/g

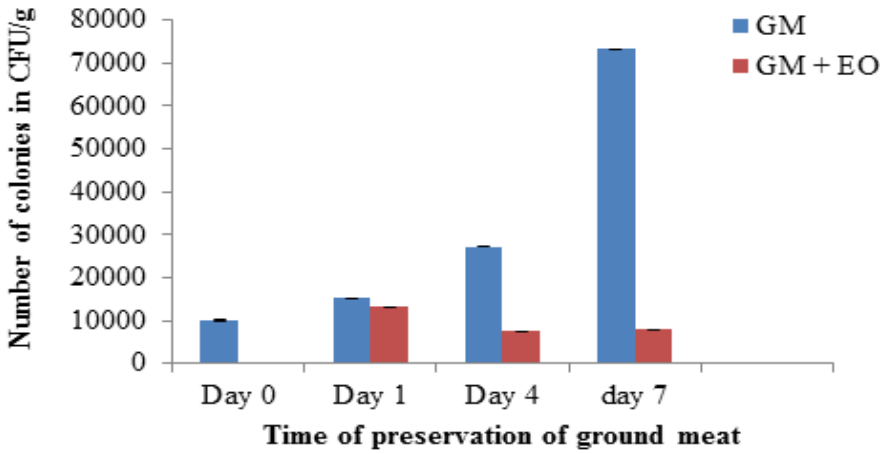

Figure 5. Evolution of total coliform loads during conservation. GM: Ground meat; GM+EO: Ground meat + essential oil.

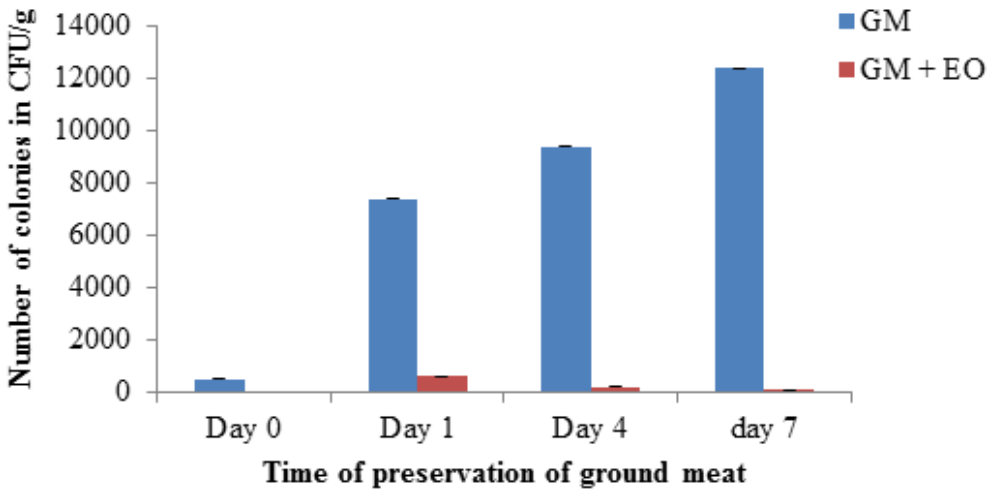

Figure 6. Evolution of thermo tolerant coliform loads during conservation. GM: Ground meat; GM+EO: Ground meat + essential oil.

(Day 7) for untreated ground meat. This number varied from 6.0×10^2UFC/g (Day 1) to 1.0×10^2 CFU/g (Day 7) for the GM+ EO of *H. suaveolens*. Statistical analysis showed a significant difference with p value < 0.0001.

Unlike untreated minced meat, there was a considerable reduction (84%) in the number of thermotolerant coliforms in minced meat incorporated with essential oils during storage. The almost complete inhibition (98%) of the number of thermotolerant coliforms could be explained by the bactericidal effect of these essential oils on *E. coli*. The presence of germs in the meat incorporated with essential oil could be explained by the presence of total coliforms. The results of the conservation tests confirm those of the antimicrobial activity observed previously by this essential oil. The results corroborate with several works that have dealt with the application of essential oils to foods in order to reduce the microbial count as compared to control (Smaoui et al., 2016). Thus, the studies of Caillet and Lacroix (2007) showed that the incorporation of the essential oil in ground beef contributed to the improvement of microbiological quality and the reduction of fat oxidation beyond its normal storage life.

Conclusion

The present work aimed to evaluate biological properties of essential oil of *H. suaveolens* and its effect on shelf-life of bovine ground meat. The essential oil from *H. suaveolens* showed inhibitory activity on microbial strains. Biopreservation assay revealed a significant

decrease of microbial charge during conservation time.

The results obtained in the present study show that the essential oil from *H. suaveolens* could be used as a promising bioconservative agent for the food industry that can prevent oxidation and reduce microbial spoilage.

CONFLICT OF INTERESTS

Authors have declared no conflict of interest.

REFERENCES

Adjou ES, Soumanou MM (2013). Efficacité des extraits de plantes dans la lutte contre les moisissures toxinogènes isolées de l'arachide en post-récolte au Bénin. Journal of Applied Biosciences 70:5555-5566. DOI: 10.4314/jab.v70i1.98755. ISSN 1997–5902.

Aprotosoaie AC, Spac AD, Hancianu M, Miron A, Tanasescu VF, Dorneanu V, Stanescu U (2010). The chemical profile of essential oils obtained from fennel fruits (*Foeniculum vulgare* Mill.). FARMACIA 58(1):46-54. Reference ID: 1433431.

Baser KHC, Buchbauer G (2010). Handbook of essential oils: Science, Technology, and Applications. Ed. Taylor and Francis Group, LLC. United States of America 994 p.

Bicas JL, Neri-Numa IA, Ruiz ALTG, De Carvalho JE, Pastore GM (2011). Evaluation of the antioxidant and antiproliferative potential of bioflavors. Food and Chemical Toxicology 49:1610-1615. https://doi.org/10.1016/j.fct.2011.04.012.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods. *A Review.* International Journal of Food Microbiology 94:223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022.

Caillet S, Lacroix M (2007). Les huiles essentielles: leurs propriétés antimicrobienne et leurs applications potentielles en alimentaires laboratoire de recherche en science appliquée à l'alimentation. INRS-Institues Armand- Frappier, (RESALA), 8p. https://e-dition.net/des-huiles-essentielles-aux-proprietes-antimicrobiennes.

Canillac N, Mourey A (2001). Antibacterial activity of the essential oil of *Picea excelsa* on *Listeria*, *Staphylococcus aureus* and coliform bacteria. Journal of Food Microbiology 18:261-268. https://doi.org/10.1006/fmic.2000.0397.

Chouhan S, Sharma K, Guleria L (2017). Antimicrobial Activity of Some Essential OilS-Present Status and Future Perspectives. Medicines 4(58):1-21. DOI:10.3390/medicines4030058.

Dar MY, Shah WA, Rather MA, Qurishi Y, Hamid A, Qurishi MCA (2011). Chemical composition, *in vitro* cytotoxic and antioxidant activities of the essential oil and major constituents of *Cymbopogon jawarancusa* (Kashmir). Food Chemistry 129(4):1606-1611. DOI: 10.1016/j.foodchem.2011.06.016.

Derwich E, Benziane Z, Boukir A (2010). GC/MS Analysis and antibacterial activity of the essential oil of *Mentha pulegium* grown in Morocco. Reseach Journal of Agriculture and Biological Sciences 6(3):191-198. Record Number: 20103179519.

Dung NT, Kim JM, Kang SC (2008). Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merr and Perry buds. Food and Chemical Toxicology 46(12):3632-3639. DOI:10.1016/j.fct.2008.09.013.

Hernandez Ochoa L, Aguirre Prieto YB, Nevarez Moorilon GV, Gutierrez Mendez N, Salas Munoz E (2011). Use of essential oils and extracts from spices in meat protection. Journal of Food Science Technology 51(5):957-963. DOI 10.1007/s13197-011-0598-3.

Goly KRC, Soro Y, Dadié A, Kassi ABB, Dje M (2015). Antibacterial activity of essential oils and extracts from the leaves of *Hyptis suaveolens* and *lippia multiflora* on multi-resistant bacteria. Rasayan Journal of Chemistry 8(4):396-403. ISSN: 0974-1496. http://www.rasayanjournal.com.

Goni P, Lopez P, Sanchez-Jarabo C, Nerin C (2009). Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chemistry 116(4):982-989. DOI: 10.1016/j.foodchem.2009.03.058.

Guiraud JP (2003). Microbiologie alimentaire. Ed. DUNOD, Paris.110 - 112.https://www.librairiedialogues.fr/livre/107946-microbiologie-alimentaire-joseph-pierre-guiraud-dunod

Ilboudo Z (2009). Activité Biologique de quatre huiles essentielles contre *Callosobruchus maculatus* Fab. *(Coleoptera : Bruchidae),* insecte ravageur des stocks de niébé au Burkina Faso. Thèse de doctorat. Université de Ouagadougou 150 p.

Joshi L, Gupta B, Prakash R (2010). Chemical synthesis of poly(5-carboxyindole) and poly (5- carboxyindole)/carboxylated multiwall carbon nanotube nanocomposite. Thin Solid Films 519(1):218-222. DOI: 10.1016/j.tsf.2010.07.123.

Lennette HE, Bellows A, Hausler JW, shadomy HJ (1987). Manual of Clinical microbiology, 4th Ed. pp. 336-359. Number ID: 971432431. ISBN-13: 978-0914826699.

Marzoukia H, Elaissib A, Khaldic A, Bouzidd S, Falconierie D, Marongiu B, Pirasa A, Porcedda S (2009). Seasonal and geographical variation of *Laurus nobilis* L. essential oil from Tunisia. The Open Natural Products Journal 8(2):86–91. DOI: 10.2174/1874848100902010086.

Miladi H, Ben Slama R, Mili D, Zouari S, Bakhrouf A, AE (2013). Chemical composition and cytotoxic and antioxidant activities of S*atureja montana* L. essential oil and its antibacterial potential against *Salmonella Spp.* strains. Journal of Chemistry 9–18. https://www.hindawi.com/journals/jchem/2013/275698/

Nakahara K, Alzoreky NS, Yoshihashi T, Nguyen HTT (2003a). Chemical composition and antifungal activity of essential oil from *Cymbopogon nardus* (Citronella Grass). Japan Agricultural Research Quarterly 37(4):249-252. Record ID:JP2005000711.

Negreiros MO, Pawlowski A, Soares GLG, Motta AS, Frazzon AP (2016). *In vitro* antimicrobial activity of essential oils from Heterothalamus Less. (*Asteraceae*) against clinically relevant bacterial and fungal species. Brazilian Journal of Biosciences 14(1):26-31. PUBMED : 27590861.

Nessrien MNY, Mohamed AT (2007). Antioxidant and antimicrobial effects of marjoram and thyme in coated refrigerated semi fried mullet fish fillets. World Journal Dairy and Food Science 2(1):01-09. ISSN 1817-308X.

Ngom S, Diop M, Mbengue M, Faye F, Kornprobst JM, Samb A (2014). Composition chimique et propriétés antibactériennes des huiles essentielles d'*Ocimum basilicum* et d'*Hyptis suaveolens* (L.) Poit récoltés dans la région de Dakar au Sénégal. Afrique Science 10(4):109-117.

Norme AFNOR NF V 08-060 (2009). Numeration of fecal coliforms by cell counting at 44°C, 10 p.

Norme Internationale ISO 4832 (2006). Food Microbiology-Flat Method horizontale for coliforms counting 6 p.

Norme Internationale ISO 4833 (2003). Food Microbiology-Flat Method horizontale for microorganisms counting. 6 p. Technic of colonies counting at 30°C. 9 p.

Obame ELC (2009). Study of phytochemical antimicrobial and activities of some medicinales aromatiques African plants. Thesis of PhD. University of Ouagadougou. 227p. http://www.beep.ird.fr/collect/uouaga/index/assoc/M11006.dir/M11006.pdf.

OCDE/FAO (2017). OCDE/FAO (2017). «Perspectives agricoles de l'Organisation de Coopération et de Développement Économiques (OCDE) et de l'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO)». Statistiques agricoles. http://dx.doi.org/10.1787/agr-outl-data-fr.

Ouedraogo I (2012). Anti-oxydant et antimicrobial activité of essentials oils of *Cymbopogon citratus* (DC) Stapf, *Citrus sinensis* (L.) Obsbeck and *Zingiber officinale* Roscoe for food preservation. Master Degree. University of Ouagadougou 94 p.

Rashid A, Qureshi MZ, Raza SA, William J, Arshad M (2010). Quantitative determination of antioxidant potential of *Artemisia persica*. Bucuresti–Chimie (serie Noua) 19(1):23-30. search.ebscohost.com.

Rhayour K (2002). Etude du mécanisme de l'action bactéricide des huiles essentielles sur Esherichia coli, Bacillus subtilis et sur Mycobacterium phlei et Mycobacterium fortuitum. Thèse de doctorat.

Université Sidi Mohamed Ben Abdellah, Maroc. 170p
Seda O, Nukhet NDZ (2016). Evaluation of usage of essential oils instead of spices in meat ball formulation for controlling *Salmonella* spp. Food Science and Technology International 22(2):93-101. DOI: 10.1177/1082013215571118.
Smaoui S, Hsouna AB, Lahmar A, Ennouri K, Mtibaa-Chakchouk A, Sellem I (2016). Bio-preservative effect of the essential oil of the endemic *Mentha piperita* used alone and in combination with BacTN635 in stored minced beef meat. Meat Science 117:196-204. DOI: 10.1016/j.meatsci.2016.03.006.
Yu J, Lei J, Yu H, Cai X, Zou G (2004). Chemical composition and antimicrobial activity of the essential oil of *Scutellaria barbata*. Phytochemistry 65(7):881-884. DOI:10.1016/j.phytochem.2004.02.005.