Chemical Composition, Antimicrobial and Antioxidant Activities of Different Extracts from Laurel Leaves and Frankincense Resin: A Comparative Study with In vitro Cytotoxicity Evaluation

Noha M. Mohamed, Fatma A.A. Mostafa and Adel S. Abdel Rahman

Regional Centre for Food and Feed (RCFF), Agricultural Research Centre, P.O. Box. 588 Orman, Giza, Egypt

Received: 07 Nov. 2021  Accepted: 10 Dec. 2021  Published: 15 Dec. 2021

ABSTRACT
The aim of the present study is to compare the antimicrobial and antioxidant activities of different extracts of two medicinal and aromatic plant species that are widely used in middle east and Egypt, laurel leaves (Laurus nobilis L.) and Frankincense resin. Three different extracts were prepared (aqueous, alcohol and essential oil) and individually tested for evaluation of their antimicrobial activity and determination of their antioxidant capacity using DPPH-free radical scavenging technique. Bioactive components in these extracts were identified by analysis based on GC-MS. Also, in vitro cytotoxicity of the aqueous extracts were tested against two cancer cell lines, liver HepG2 and colon HCT116, to evaluate their anticancer potential using MTT assay. The results revealed that both Laurel leaves and Frankincense extracts have an efficient and comparable antimicrobial activity against most tested organisms at 10 and 20 mg/mL. Laurel extracts showed higher inhibitory effect against tested Gram-negative bacteria while Frankincense extracts exerts highest inhibitory effect against Gram-positive bacteria. Laurel leaves essential oil (EO) extract reached 100% antioxidant activity with IC_{50} value 3.9 mg/mL. For Frankincense, alcoholic extract showed the highest antioxidant activity 77% with IC_{50} value 12.6 mg/mL. The chemical composition showed that the major components for all tested extracts were terpenes (mono, sesqui and oxygenated derivatives), flavonoids, and polyphenols. The investigated Frankincense extract exhibits high cytotoxic activity against both cancer cell lines with IC_{50} value 3.47 µg/mL for HepG2 and 10.7 µg/mL for HCT116. The laurel extract showed activity against HepG2 cell line only with IC_{50} 8.61 µg/mL.

Keywords: Laurel leaves (Laurus nobilis L.), Frankincense, plant extracts, antimicrobial activity, antioxidant activity, cytotoxicity

1. Introduction
For many thousands of years plant oils and extracts have been used worldwide for a variety of purposes (Jones, 1996) including traditional medicines, pharmaceuticals, flavoring and food preservation (Mishra and Dubey, 1994). Recently medicinal and aromatic plants have been developed extensively and have gained great economic importance (Schippmann et al., 2002), due to the increased resistance of pathogens to conventional synthetic drugs as a consequence of the wide use of antibiotics and their common side effects (Chmit et al., 2014), which encouraged the search for novel therapeutic alternatives. These plants are used in novel pharmaceutical industries as natural product rich in bioactive compounds (Patrakar et al., 2012). Laurus nobilis L., commonly known as bay leaves, laurel leaves or sweet bay, belongs to Laureaceae family which comprises several aromatic and medicinal plants (Fernández et al., 2018), is an evergreen shrub cultivated in warm regions as southern Europe and Mediterranean sea countries (Maatallah et al., 2016). They are widely used as a spicy fragrance and flavor in traditional dishes (Ouchikhi et al., 2011). Its leaves and extracts are used as medicinal remedy

Corresponding Author: Noha Mahmoud Mohamed, Regional Centre for Food and Feed (RCFF), Agricultural Research Centre, P.O. Box. 588 Orman, Giza, Egypt, Plant Protection Research Institute, Agriculture Research Center, Dokki, Cairo, Egypt.
E-mail: noha_mahmoud31@yahoo.com
to suppress high blood sugar, fungal and bacterial infections, to treat eructation, flatulence and gastrointestinal problems (Dias et al., 2014). *Laurus nobilis* essential oil (EO) is used in cosmetics, as a preservative, culinary spice and as flavoring agent (Taban et al., 2018) beside its medicinal purposes as treating rheumatic pain (daSilveira et al., 2014; PilarSantamarina et al., 2016; Sellami et al., 2011). Antioxidant activity of *L. nobilis* EO extract was reported by several authors (Cherrat et al., 2013; Conforti et al., 2006; Ramos et al., 2012). According to the extraction process applied for the laurel leaves, a complex mixture can be obtained including mono- and sesquiterpenes in EO extract (Di Leo et al., 2009; Ortiz et al., 2009), while in alcoholic or hydroalcoholic extracts, compounds as flavonoids, saponins, alkaloids and polyphenols are usually obtained (Škerget et al., 2005).

Frankincense is an aromatic gum resin (Luban in Arabic) obtained from trees of the genus *Boswellia* (Han et al., 2017) of the family Burseraceae that grow in the East Africa, Arabian peninsula, China and India (Frank et al., 2009). Since ancient times, Frankincense resin has been considered as a precious commercial product and incense material (Hasson et al., 2011) in many countries as India, China and Middle East which exhibit numerous health supporting properties in folk medicine for prevention and treatment of various diseases, including the treatment of rheumatoid arthritis (Banno et al., 2006) and as anti-inflammatory, antibacterial, antifungal and anticancer agent (Weckesser et al., 2007; Huang et al., 2000). Recently, the use of the Frankincense resin has become more popular in the European countries for the treatment of chronic inflammatory problems as arthritis, chronic bowel diseases, peritumoral brain edema, alzheimer, asthma and other diseases (Ammon, 2006; Mishra et al., 2020). Frankincense EO has become widely used in promoting skin health (Han et al., 2017) and possess anti-tumor activity (Frank et al., 2009). The penta-cyclic triterpenes have been reported as the active medicinal ingredient of Frankincense extracts that modulate important biological activities (Nusier et al., 2007) and closely resemble the chemical structure of steroids but have different mode of actions (Ammon, 2006).

The aim of the present study is to compare and evaluate the antimicrobial and antioxidant activity of laurel leaves and frankincense gum extracts obtained by different traditional extraction methods in order to obtain natural food preservatives that possess no or very low toxic effect in contrast to synthetic ones. Also the extracts were investigated using GC-MS to identify the major bioactive ingredients where total peak areas gave good approximations to relative concentrations. Moreover, the aqueous extracts were tested for their cytotoxicity against two cancer cell lines to evaluate their anticancer potential.

2. Materials and Methods

2.1. Plant Samples

Laurel leaves were obtained and collected from the Egyptian local markets and herbal shops as air-dried leaves (not fresh leaves). The frankincense gum (imported from Somalia) were purchased from Harraz herbs company – identified as *Boswellia carterii*. The samples were washed with deionized water and air dried at room temperature then ground to fine powder (20 mesh) and stored in tightly sealed plastic bags at room temperature.

2.2. Reagents and Chemicals

All solvents used in extraction process were HPLC grade, Ethanol 99 %, Methanol 99.8 % and Chloroform 98.9 % were obtained from Fisher Scientific. 2.2-Diphenyl-1-picrylhydrazyl (DPPH) and DMSO were purchased from Sigma-Aldrich. A Milli-Q water purification system (Millipore Corp., USA) was used for obtaining distilled and deionized water.

2.3. Preparation of Extracts

Essential oil extract (EO): 20 gm of powdered dried sample were added to 400 mL distilled water and extraction was carried out by steam distillation using method of (Aqel and Shaheen, 1996). The process continued until about 200 mL of distillate were collected. The distillate was extracted 3 times with chloroform. After removing moisture by using anhydrous sodium sulphate, the extract was evaporated on a water bath at 40°C.

The alcoholic extract (EE): was obtained by mixing 100 g of sample individually with 500 ml of 80% (v/v) ethanol. The mixture was kept for 5 days in tightly sealed vessels at room temperature,
protected from sunlight, and shaken several times daily. This blend was vacuum filtered and further extractions of the leaf residues were repeated until a clear supernatant was obtained. The extracted liquid was subjected to evaporation in a stove (40°C) to remove the ethanol until getting a semi solid extract (Porrini et al., 2011). Both EO and EE extracts were stored in screw-capped dark glass vials at 4°C until further testing.

Aqueous extracts (WE): 10 g dried samples were mixed individually with 100 mL distilled water and maintained at 100 °C using water bath with constant stirring (300 rpm) for 2 hours as reported by (Ghita et al., 2009). The mixture temperature was lowered to room temperature, stored over night at 4°C and then filtered through 40 mesh screen. The filtrate was dried at 50°C using rotary vacuum evaporator and stored at -18°C until use.

2.4. Chemical composition determination using GC-MS analysis

The chemical compounds present in Laurus nobilis L. and Frankincense resin extracts were identified using Agilent Technologies 7890A gas chromatography coupled to mass spectrometer interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane as a stationary phase) capillary column (30 m length x 0.25 mm internal diameter and 0.25 µm film thickness). The carrier gas (mobile phase) was helium with linear velocity of 1 mL/min. The temperature of the injector and detector were 200°C and 250°C respectively. A volume of 1 µL of the sample was injected and the operating parameters of the MS were as follow: ionization potential 70 eV, interface temperature 250°C and acquisition mass range 50-800 amu.

The identification of the components was based on the comparison of their obtained mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY GC-MS library as well as by comparison of the fragmentation pattern of the mass spectral with those reported in the literature (Patricia et al., 2013). The compounds were expressed in percent peak area (%), calculated without any correction factor.

2.5. Antimicrobial activity evaluation

Reference strains and microorganisms for antimicrobial activity evaluation were obtained from the culture of the Regional Center for Mycology and Biotechnology (RCMB), faculty of science, Al-Azhar University in Cairo. Pseudomonas argenosa and Escherichia coli are tested as an example for Gram-negative bacteria while Staphylococcus aureus and Bacillus subtilis as Gram-positive bacteria. Four fungal strains were tested. The diffusion agar technique was used to obtain the in vitro antimicrobial screening (Atlas et al., 1995). 100 µL of each extract concentration was tested (2.5, 5, 10, 20 mg/mL); well diameter =0.6 cm. The antimicrobial activities were expressed as the diameter of the inhibition zones.

2.6. Antioxidant activity determination

For the determination of antioxidant activity, the DPPH free radical scavenging assay was used. The obtained extracts were redissolved in methanol, assisted by ultrasonic bath (model Labocon digital pro, operated at frequency of 40 kHz at room temperature), to obtain 20 mg/mL as a final concentration. Further dilutions were carried out to obtain four different concentrations for each extract (2.5, 5, 10, 20 mg/mL). DPPH radical - scavenging ability was determined for each prepared concentration according to method used by (Cherrat et al., 2013) to estimate the degradation of DPPH, by adding 150 µL of each extract concentration to 50 µL of 1 mmol/L DPPH. The mixtures were then left for 30 min in darkness. The absorbance of the resulting solutions was measured using (Analytik Jena spectrometer -Specord 200) at 517 nm and the DPPH free radical scavenging activity percentage was calculated for each concentration by using the formula:

\[
\% \text{ Scavenging capacity} = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100
\]

Where \(A_{\text{blank}}\) is the control absorbance which include all reagents except the test sample and \(A_{\text{sample}}\) is the absorbance of the mixture including the sample.

All tests were performed in triplicate. The inhibition percentages were plotted against the extracts concentrations in order to determine the extract concentration that can provide 50 % inhibition (IC\textsubscript{50}).
2.7 In vitro cytotoxicity study

The in vitro cytotoxicity study was carried out at the confirmatory diagnostic unit VACSERA-EGYPT using the MTT reduction assay as described by (Mosmann, 1983). This test is based on the ability of Mitochondrial dehydrogenases of viable, metabolically active cells to reduce the water soluble compound 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), causing the cleavage of the tetrazolium ring and converting the yellowish MTT in to purple formazan crystals which are insoluble in aqueous solutions. The crystals were dissolved in acidified isopropanol and the resulting purple solution is spectrophotometrically measured. MTT and MTT solubilization solution were obtained from Sigma. HepG2 and HCT116 cell lines were obtained from American Type Culture Collection. Cells were cultured using DMEM (Invitrogen/Life Technologies) supplemented with 10% FBS (Hyclone), 10 μg/mL of insulin (Sigma), and 1% penicillin-streptomycin. Cells culture was done in 5% CO2 incubator at 37°C to produce a monolayer. Cells (1.5x10^4 cells/well) were plated in 96-well plates with 100 μL complete growth medium and incubated for 24h under CO2 at 37°C, after that the cells were treated with 100 μL of serial concentrations of the tested extracts dissolved in 0.1% DMSO. Further incubation is carried out for 48 h at 37°C. Control cells were only supplemented with 2% FBS. Each test included a blank containing complete medium without cells. DMSO cytotoxicity was also evaluated at the concentration of stock solution after dilution. MTT prepared solution was added to each well (in an amount equal to 10% of the culture medium volume) and incubated for 4h. After the incubation period, the resulting purple formazan crystals were then dissolved by adding an amount of MTT solubilization solution equal to the original culture medium volume.

Viability of the cells were determined by measuring the absorbance at 750 nm using BIOLINE ELIZA plate reader where:

\[
\text{Cell viability (\%) = } \frac{A_{570} \text{ nm of treated cells}}{A_{570} \text{ nm of control cells}} \times 100
\]

The cytotoxicity was expressed as IC50 (concentration required to inhibit 50% of cell proliferation)

3. Results and discussion

3.1. Identification of chemical composition of extracts:

Data in Table (1) for the EO extracts showed that flavones and large number of phenolic compounds are present as major and minor constituents. Six major components are contributing by 64.9% of total peak area for Laurel EO extract including 1, 8-Cineole 29%, 3-Carene 8.18%, cis-Sabinen hydrate 7.56%, 4-Terpinenyl acetate 7.48%, Camphene 6.9%, and Methyleugenol 5.71%. Other compounds were present in minor amounts including Quercetintetramethyl ether (a plant flavonol from the flavonoid sub group of polyphenols) 4.48%, α-Terpineol 3.84%, Caryophyllene oxide 2.85% and Linalool 2.2%. For Frankincence EO, five components representing 57.7% of the EO extract including α-Pinene 32.15%, Linalool 7.03%, Delphinidin cation 7.85%, α-Santalol 6.57% and Nerolidol 4.11%. As illustrated in Table (2) for the alcoholic extracts, five major components in the Laurel EE were representing 56% of total peak area including 4-Methylecatechol 22.86%, followed by Phytol 10.27%, cis-Sabinen hydrate 8.3%, Quercetintetramethyl ether 7.51% and Dimethoxy-3-hydroxyflavone 7.11%. Farnesol 2.46% and Bornyl acetate 2.8% are minor constituents. Frankincence EE components, listed in Table (2), were rich in methoxylated flavones, a subdivision of the flavonoids group, where Ten compounds were identified in the extract as derivatives of O-methylated flavone with 43 % total peak area. Whereas five components represent 57.4% of the total peak area constituents including Dihydroxy-7-methoxyisoflavone 22.3%, Squalene 14.8%, Quercetin tetramethyl ether 9.05%, Dimethoxy flavone 5.77% and Pentamethoxyflavone 5.48%. Several minor components were present in the investigated sample extract including Thunbergol 4.4 % and Phytol 4.98 %. In Laurel WE, Table (3), Caryophyllene oxide 11.67% and Trimethoxycalcone (a flavononol, flavonoid subderivative) 6.19% are major constituents and variety of minor compounds including 1,8-cineole, linalool, Geranyl acetate, Hexa-hydro-farnesol, flavones, terpenes and sesquieterpene hydrocarbons. Composition of Frankincense WE shown in Table (3), was comparable to the EE in its richness with flavonoids derivatives, where five hydroxyl flavone compounds with total peak area 33.3% were detected. Pentahydroxyflavone 22.52%, Dihydroquercetin 15.44%, Farnesol 13.06%,

994
Tetramethoxychalcone 5.62% and Dimethoxy-3-hydroxyflavone 4.57% are five major compounds with total peak area 61.2% of the Frankincense WE. Several researchers have studied the chemical composition for laurel leaves from different origins (Sellami et al., 2011; Guedri et al., 2020). Several studies showed that the major compounds of *L. nobilis* EO are mono- and sesquiterpenes hydrocarbons (Peixoto et al., 2017; Cherrat et al., 2013; Goudjil et al., 2015) and that Eugenol and Chavicol are minor compounds (Yalçın et al., 2007).

Table 1: Identification of laurel leaves and Frankencense essential oil extract compounds and their area percentage in the mass spectra

| **Laurel leaves essential oil** | **Compound** | **Area (%)** | **Frankencence essential oil** | **Compound** | **Area (%)** |
|--------------------------------|-------------|--------------|-------------------------------|-------------|--------------|
| Sabinene                       | 0.25        | 4(10)-Thujen-3-ol | 0.89                          | Sabinene    | 2.48         |
| α-Pinene                       | 1.41        | 1,8-Cineole   | 0.58                          | α-Pinene    | 32.15        |
| 1,8-Cineole                    | 29.1        | 4-Terpinenyl acetate | 0.58                      | 1,8-Cineole | 5.81         |
| Linalool                       | 2.20        | α-Terpinyl acetate | 1.35                         | Linalool    | 7.03         |
| Myrtenol                       | 0.24        | α-Pinene      | 32.15                         | 6-Methoxyluteolin | 0.53 |
| Dihydrocarveol                 | 0.28        | α-Terpineol   | 0.59                          | Sabinene    | 2.48         |
| cis-Sabinenhydrate             | 7.56        | β-Selinene    | 3.84                          | Sabinene    | 2.48         |
| α-Terpineol                    | 2.37        | 1,8-Cineole   | 0.58                          | α-Terpineol | 32.15        |
| Sesquisabinene hydrate         | 0.24        | Geraniol      | 0.84                          | Sesquisabinene hydrate | 2.3 |
| Bornyl acetate                 | 0.67        | cis-Sesquisabinene hydrate | 2.3              | Bornyl acetate | 0.67         |
| 9-cis- Retinoic acid           | 0.38        | Oleic Acid    | 0.21                          | 9-cis- Retinoic acid | 1.48 |
| 3-Carene                       | 8.18        | Ledol         | 1.78                          | 3-Carene    | 1.78         |
| Camphene                       | 6.9         | cis-Sesquisabinene hydrate | 2.3              | Camphene    | 1.78         |
| Methyleugenol                  | 5.71        | Geranylisovalerate | 0.39                         | Methyleugenol | 5.71        |
| Caryophyllene                  | 0.28        | 6,7-Dihydroxy-4-phenylcoumarin | 0.11          | Caryophyllene | 0.28         |
| β-Gurjunene                    | 0.57        | Oleic Acid    | 0.21                          | β-Gurjunene | 0.57         |
| Cubebol                        | 0.3         | 9-cis- Retinal | 0.14                          | Cubebol    | 0.3         |
| Nerolidol                      | 1.21        | Isopinocampheol | 0.79                        | Nerolidol  | 4.11         |
| Farnesol                       | 2.44        | Cembrene      | 0.45                          | Farnesol   | 2.44         |
| Thunbergol                     | 1.56        | (-)-β-Elemene | 3.16                          | Thunbergol | 1.56         |
| Leden                          | 0.39        | (-)-Kaurene   | 3.25                          | Leden      | 0.39         |
| Cedrenol                       | 1.83        | 2,6-Di-tet-butyl-4-hydroxymethylphenol | 0.89          | Cedrenol   | 1.83         |
| Kaur-16-ene, (8β,13β)           | 1.21        | Farnesol      | 1.29                          | Kaur-16-ene, (8β,13β) | 1.21 |
| Longifolene                    | 2.11        | 5β,7βH,10α-Eudesm-11-en-1α-ol | 3.76          | Longifolene | 2.11         |
| Cembrene                       | 1.05        | Delphinidin cation | 7.85                         | Cembrene   | 1.05         |
| Squalene                       | 0.57        | α-Santalol    | 6.57                          | Squalene   | 0.57         |
| Quercetin-3,7,3',4'-tetramethyl ether | 4.48 | β-Santalol | 0.25                          | Quercetin-3,7,3',4'-tetramethyl ether | 4.48 |
| Caryophyllene oxide            | 2.85        | Isolongifolol | 1.11                          | Caryophyllene oxide | 2.85 |
| Hexa-hydro-farnesol            | 2.34        | Astilbin      | 2.97                          | Hexa-hydro-farnesol | 2.34 |
| Dehydrodieugenol              | 0.34        | Longiborneol  | 1.27                          | Dehydrodieugenol | 0.34 |
| 5,7,2'-Trimethoxyflavone       | 0.26        | Hexa-hydro-farnesol | 3.42                        | 5,7,2'-Trimethoxyflavone | 0.26 |
| 3,7,8,2'-Tetramethoxyflavone   | 0.54        | Phytol        | 1.1                           | 3,7,8,2'-Tetramethoxyflavone | 0.54 |
| 3-(3,4-Dimethoxyphenyl)-6-ethoxy-4-methylcoumarin | 0.3 | Heptacosane | 0.6                           | 3-(3,4-Dimethoxyphenyl)-6-ethoxy-4-methylcoumarin | 0.3 |
| Astilbin                       | 0.25        | Vitexin       | 0.71                          | Astilbin    | 0.25         |
|                                |             | Flavone, 5-hydroxy-3,3',4',7-tetramethoxy | 0.83          | Flavone, 5-hydroxy-3,3',4',7-tetramethoxy | 0.83 |
|                                |             | Octacosane    | 0.63                          | Octacosane  | 0.63         |
|                                |             | Salicylic acid β-D-O-glucuronide | 0.32          | Salicylic acid β-D-O-glucuronide | 0.32 |
|                                |             | Nonacosane    | 0.47                          | Nonacosane  | 0.47         |
Table 2: Identification of laurel leaves and Frankencense alcoholic extract compounds and their area percentage in the mass spectra

| Laurel leaves alcoholic extract | Area (%) | Frankencense alcoholic extract | Area (%) |
|---------------------------------|----------|---------------------------------|----------|
| 4-Methylcatechol                 | 22.86    | Sabinene                        | 0.27     |
| 3-Hydroxy-7,2',3'-trimethoxyflavone | 0.96 | α-Pinene                        | 0.28     |
| 5-Hydroxyisovanillic acid        | 1.36     | Sabinol                         | 0.24     |
| Terpinen-4-ol                    | 1.09     | Isothujol                       | 0.25     |
| L-α-Terpineol                    | 1.19     | 1-Decene                        | 0.27     |
| α-Terpinyl acetate               | 0.6      | 1-Octanol                       | 1.18     |
| Bornyl acetate                   | 2.8      | Hexa-hydro-farnesol             | 0.45     |
| δ 3-carene                       | 1.39     | Isopinocampehol                 | 0.3      |
| Eugenol                          | 0.94     | 6-Methoxyluteolin               | 0.35     |
| Methyluteugenol                  | 0.4      | (S)-(−)-Citronellic acid        | 0.38     |
| cis-Cardene                      | 0.69     | γ-Selinene                      | 0.22     |
| 3,4,5-Trimethoxyxycinnamic acid  | 0.61     | Retinyl propionate              | 0.52     |
| Nerolidol                        | 0.66     | cis-Sesquisabinene hydrate      | 0.78     |
| Cedrenol                         | 0.98     | γ-Elemene                       | 1.72     |
| Caryophyllene oxide              | 0.81     | Thunbergol                      | 4.4      |
| Elemol                           | 0.23     | 5,3'-Dihydroxy-6,7,4'-trimethoxyflavone | 4.03 |
| Corymbolone                      | 0.63     | Cembrene                        | 1.12     |
| Hexa-hydro-farnesol              | 3.41     | Caryophyllene oxide             | 1.79     |
| β-Eudesmol                       | 0.91     | cis-Cis-Sesquisabinene hydrate  | 2.23     |
| Citronellyltiglate               | 2.49     | 4',5-Dihydroxy-7-methoxysisoflavone | 22.3 |
| Epicubebol                       | 0.82     | Squalene                        | 14.8     |
| Germacrene D-4-ol                | 1.09     | Phytol                          | 4.98     |
| cis-Sesquisabinene hydrate       | 8.3      | 3,5,7-Trihydroxy-3',4',5'-trimethoxyflavone | 1.2 |
| 6,7-Dimethoxy-3-hydroxyflavone   | 7.11     | cis-Thujopsene                  | 2.73     |
| Spathulenol                      | 4.39     | 3,6,2',4',5'-Pentahydroxyflavone | 1.83 |
| β-Santalol                       | 0.64     | Farnesol                        | 2.29     |
| 2,6-Di-tet-butyl-4-              | 0.66     | 7,8-Dimethoxyflavone            | 5.77     |
| hydroxyethylphenol               |          |                                 |          |
| Retinol                          | 1.66     | Quercetin-3,7,3',4'-tetramethyl ether | 9.05 |
| Phyrol                           | 10.27    | 3,6,2'-Trimethoxyflavone        | 0.75     |
| Isolongifolol                    | 1.04     | 7,3',4',5'-Tetramethoxyflavanone | 1.49 |
| Farnesol                         | 2.46     | Isolongifolol                   | 1.29     |
| Quercetin-3,7,3',4'-tetramethyl ether | 7.51 | α-Amyrin                        | 0.46     |
| Kaur-16-ene, (8β,13β)            | 0.93     | Resveratrol                     | 1.64     |
| 4,4'-Thiobis(2-methylphenol)     | 3.31     | Himbacco                        | 0.29     |
| β-Gurjunene                      | 0.36     | 3,7,3',4',5'-Pentahydroxyflavone | 0.33 |
| Guaiol                           | 0.29     | p-Cresol, 2,2'-methylenebis[6-tet-buty] | 0.52 |
| Apigenin 8-C-glucoside           | 0.46     | Beta-santalol                   | 0.48     |
| β-Carotene                       | 0.58     | 2'-Hydroxy-2,4,5,5'-tetramethoxychalcone | 0.35 |
| Probucol                         | 0.41     | Vitexin                         | 0.42     |
| Genistin                         | 0.34     | 3,3',7,8-Tetramethoxyflavone    | 0.23     |
| 2',4'-Dimethoxy-3-hydroxy-6-methylflavone | 0.24 | Baicalintrimethyl ether        | 0.29     |
| 3,7,3',4'-Tetrahydroxyflavone    | 0.73     | 9-Octadecenoic acid             | 0.26     |
|                                  |          | Ethyl Oleate                    | 0.28     |
Flavonoids are sub
sesquiterpenes with
2007). In our study, phenolic compounds including methoxylated flavones, monoterpenes and
tri
mixture of polysaccharides (gums) and 5

| Compound                     | Area (%) | Compound                     | Area (%) |
|------------------------------|----------|------------------------------|----------|
| 2,3-Dehydro-1,8-cineole      | 4.11     | 4,4'-Dimethoxychalcone       | 0.52     |
| Isobornyl acetate            | 0.51     | D-Limonene                   | 0.31     |
| 8-Hydroxyinalool             | 0.6      | Isopinocampheol              | 0.39     |
| Geranyl acetate              | 3.96     | 1-Octene                     | 1.72     |
| Linalool oxide               | 1.27     | Nerolid                      | 1.12     |
| 2-Hexadecanol                | 1.17     | Ledol                        | 1.63     |
| Hexa-hydro-farnesol          | 3.87     | 3',7-Dimethoxyflavonol       | 1.58     |
| (S)-( )-Citronelic acid      | 1.56     | Cembrene                     | 0.64     |
| cis-p-Mentha-2,8-dien-1-ol   | 0.4      | Caryophyllene oxide          | 0.64     |
| Dihydrocarveol               | 2.65     | 6,3'-Dimethoxy-3-hydroxyflavone | 3.68 |
| Herbacetin                   | 4.2      | 7,4'-Dimethoxy-3-hydroxyflavone | 4.57 |
| Citronellal                  | 2.49     | Isovitexin                   | 0.44     |
| Isovitexin                   | 0.95     | 3',5'-Dimethoxy-3,5,7,4'-tetrahydroxyflavone | 0.94 |
| 5-Hydroxyisovanillic acid    | 3.03     | 2',4'-Dihydroxy-3,4-dimethoxychalcone | 1 |
| 2'-Hydroxy-2,4,5,5'-tetramethoxychalcone | 2.34     | Farnesol                     | 13.06    |
| Nerolid                      | 0.8      | 3,6,2',4',5'-Pentahydroxyflavone | 22.52 |
| Ledol                        | 1.92     | 2,3-Dihydroquercetin         | 15.44    |
| Thunbergol                   | 2.5      | Longiborneol                 | 2.69     |
| Ascaridole                   | 2.39     | Isolongifolol                | 2.56     |
| Rhammetin                    | 2.64     | 2'-Hydroxy-2,4',5'-tetramethoxychalcone | 5.62 |
| Gardenin                     | 2.37     | Lutein                       | 2.52     |
| Terpineol                    | 3.36     | Astilbin                     | 1.25     |
| Farnesol                     | 1.96     | 6,7-Dimethoxy-3-isochromanone | 0.75 |
| 5,7-Dimethoxyflavone         | 3.35     | 2',3',4'-Trihydroxychalcone  | 2.7      |
| cis-Sesquibisinene hydrate   | 1.07     | Himbaccol                    | 1.62     |
| Kaur-16-ene, (8β,13β)-       | 2.18     | Cedrol                       | 1.07     |
| Geranylisovalerate           | 0.69     | ( )-Globulol                 | 1.25     |
| 4'-Acetoxy-7-hydroxy-6-methoxyisoflavone | 5.07     | β Carotene                   | 1.98     |
| Epicubebol                   | 1.89     | Clovane                      | 0.55     |
| 1-Tricosanol                 | 0.97     | Shyobunol                    | 1.24     |
| Phytol                       | 0.64     | Apigenin 8-C-glucoside       | 1.3      |
| Isolongifolol                | 5.17     | Juniper camphor              | 1.34     |
| Caryophyllene oxide          | 11.67    | Heptacosane                  | 1.08     |
| 9-cis-Retinoic acid          | 1.65     |                             |          |
| 2'-Hydroxy-3,4,5-trimethoxychalcone | 6.19     |                             |          |
| 5,7-Dimethoxy-4-methylcoumarin | 1.58     |                             |          |
| β Carotene                   | 1.12     |                             |          |
| 3-(3,4-Dimethoxyphenyl)-4- methylcoumarin | 0.68     |                             |          |
| Vitexin                      | 1.54     |                             |          |
| 3,4-Dihydrocoumarin          | 0.34     |                             |          |
| 3,2',4',5'-Tetramethoxyflavone | 0.48     |                             |          |
| 3-Hydroxy-7,8,2'-trimethoxyflavone | 1.42     |                             |          |
| 4-Hydroxy-2',4',6'-trimethoxychalcone | 0.64     |                             |          |

Frankincense is reported in literature to contain 60-85% mixture of terpenes (resins), 6-30% mixture of polysaccharides (gums) and 5-9% essential oil (Hamidpour et al., 2013), and the penta cyclic tri-terpenes are reported as the bioactive component in the Frankincense EO extract (Nusier et al., 2007). In our study, phenolic compounds including methoxylated flavones, monoterpenes and sesquiterpenes with their oxygen derivatives are the major bioactive ingredients that can be the reason for the activity of the studied extracts. Flavonoids are sub-group of phenolic compounds and possess various biological bioactivities including antioxidant and antimutagenic activity (Priyanka and Daljit, 2017).
The chemical composition and the compounds percentage for plant extracts can vary according to several parameters including plant age, climate conditions during planting, soil composition, extraction method, time and the plant organ from which the extract is obtained (Kamatou et al., 2012; Fidan et al., 2019).

3.2. Antimicrobial activity

The results of the antimicrobial study for laurel leaves extracts presented in Table (4) revealed that the highest antifungal activity was obtained by the concentration 10 mg/ml for all extracts. The WE (10, 20 mg/mL) and the (10 mg/mL) for EO and EE showed the highest inhibitory effect against A. flavus. The EE was the most efficient against C. albicans (10 mg/mL) and P. expansum (20 mg/mL). Similar good inhibitory action for all extracts against S. racemusom at 10 mg/mL. Concerning the antibacterial activity, The EO and EE showed high efficiency against S. aureus at (10 mg/mL) and WE against B. subtilus at (20 mg/mL). EO showed the highest inhibitory effect against E. coli and P. argenosa at (20 mg/mL). All WE concentrations showed no efficiency against P. argenosa.

Table 4: Antimicrobial activity of Laurel leaves extracts compared to standard antimicrobial agents.

| Test Organisms | Water extract mg/mL | Alcoholic extract mg/mL | Essential oil mg/mL | Std mg/mL |
|----------------|----------------------|-------------------------|---------------------|-----------|
|                | 20 10 5 2.5          | 20 10 5 2.5             | 20 10 5 2.5         | 5 2.5     |
| Fungal isolates |                      |                         |                     |           |
| Aspergillusflavus | ++ ++ + + + + + + + + ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ + |
The *P. expansum* showed high resistant to EO. An observed efficient antibacterial activities for the Frankincense extracts against all tested strains except *P. argenose* which showed high resistance to EO followed by EE then WE. The EE exhibited the highest inhibitory effect against *S. aureas* followed by EO and WE. WE showed higher efficiency against *B. subtilus* (10 mg/mL). All extracts have comparable antimicrobial activity against *E. coli*. It was noticed that laurel leaves extracts, mainly the EO, have the highest inhibitory effect against Gram-negative bacteria while the Frankincense extracts (WE and EE) have highest inhibitory effect against Gram-positive bacteria.

The antimicrobial activity can be attributed to the presence of flavonoids and phenolic compounds as major components in these extracts which affect the microbial growth on most tested strains. Phenolic compounds are reported to inhibit the peptidoglycan synthesis (Ogunlana et al., 1987), damage the structure of the microbial membrane (Cox et al., 2000) and modify the bacterial membrane surface hydrophobicity (Türi et al., 1997).

### 3.3. Antioxidant activity

Fig.1 and Table (6) showed a strong DPPH radical scavenging activity among the three laurel extracts which revealed that the EO reached 100% activity with IC\textsubscript{50} value = 3.9 mg/mL followed by EE 93.6% with IC\textsubscript{50} value =1.38 mg/mL then the WE 87.5 % with IC\textsubscript{50} value = 4.6 mg/mL . The scavenging activity increased by increasing the antioxidant substances concentration representing a measure for their hydrogen donating ability to the DPPH stable radical compound. As shown in Fig. 1 and Table (6), the EE possessed higher antioxidant activity among the Frankincense-resin extracts with 77.04 % and IC\textsubscript{50} value=12.6 mg/mL followed by WE 40.68% and IC\textsubscript{50} value=25.4 mg/mL. EO has relatively lower activity compared to other extracts 20.35% with high IC\textsubscript{50} value= 53.7 mg/mL.

![Fig. 1: DPPH radical scavenging activity for laurel leaves and Frankincense extracts](image)

**Table 6**: The inhibitory concentration (IC\textsubscript{50}) of laurel leaves and Frankincense extracts tested by DPPH method

| Extract | Laurel leaves | Frankincense |
|---------|---------------|--------------|
| EO      | 3.9 ± 0.59    | 53.7 ± 5.2   |
| EE      | 1.38 ± 0.46   | 12.6 ± 0.09  |
| WE      | 4.6± 0.15     | 25.4±1.1     |

*Results are average of three independent determinations and expressed as mean ± SD*

Fig. 2 revealed that the laurel leaves extracts have higher antioxidant activity compared to the Frankincense extracts specially for the EO. Mono- and sesquiterpenes hydrocarbons and the great amount of phenolic compounds presented in the chemical composition profile of the laurel leaves EO extract have significant role as free radical scavengers and act as hydrogen donors, reducing agent and singlet oxygen quenchers (Akter et al., 2019). The Frankincense EE antioxidant activity can be related
to the high percentage of phenolic compounds including flavonoids and methoxyflavones in its chemical composition.

The human body produces continuously free radicals such as superoxide, hydroxyl and singlet oxygen radicals which are produced by complicated metabolic redox reactions. When the level of these free radicals exceeds the endogenous enzymes responsible for overcoming them (Gülçin et al., 2002), an oxidative stress occurred leading to the development of several chronic diseases as cancer, cardiovascular diseases, aging, diabetes and neurodegenerative diseases (Pham-Huy et al., 2008). The antioxidant substances prevent oxidative cell damage by acting as free radicals scavengers (Akter et al., 2019).

![Fig. 2: Antioxidant activity of laurel leaves and Frankincense different extracts](image)

### 3.4. In vitro cytotoxicity study

The in vitro determination of the cytotoxicity activity for the aqueous extracts was tested against human liver cancer cell line HepG2 and human colon cancer cell line HCT116 using MTT-Cytotoxicity assay. Serial concentrations (0.4, 1.6, 6.3, 25, 100 µg/mL) for each extract were used to obtain the IC$_{50}$ values.

The results shown in Fig. 3 and Table (7) demonstrate that Frankincense extract exhibits a better anticancer potential against both cell lines with the IC$_{50}$ values for HepG2 and HCT116 were 3.47 (±0.19) µg/mL and 10.7 (±0.62) µg/mL respectively compared to laurel extract which showed activity only against HepG2 cell line with IC$_{50}$ value 8.61(±0.64) µg/mL. Laurel extract exhibits higher IC$_{50}$ value against HCT116 cell line 85 (±4.94) µg/mL which indicates lower toxicity to the colon cancer cell. Also, it was observed that cell viability is concentration dependent, so by increasing the extract concentration, the cell growth inhibition increased and percentage of cell viability decreased. The reason that Frankincense extract is therapeutically more effective against HCT116 cell line than laurel extract can be attributed to the huge phenolic constituents in its water extract.

![Fig. 3: In vitro cytotoxicity activity of laurel leaves and Frankincense water extracts](image)
Table 7: *In vitro* cytotoxicity activity of laurel leaves and Frankincense extracts.

| Sample               | HepG2     | HCT116    |
|----------------------|-----------|-----------|
| Frankincense extract | 3.47 ± 0.19 | 10.7 ± 0.62 |
| Laurel leaves extract | 8.61 ± 0.64 | 85 ± 4.94 |

*Results are average of three independent determinations and expressed as mean ± SD

Phenolic compounds including, flavonoids, flavones, flavonones and isoflavones which are found at high ratios in Frankincense WE have been reported to have antiviral, anti-allergic, anti-platelet, anti-inflammatory, anti-tumor and antioxidant activities (Priyanka and Daljit, 2017; Schmiech, 2021). Also our findings are in agreement with (Walle, 2007) who reported that methoxylated flavones (a flavonoid subclass) are considered as cancer chemopreventive agents.

4. Conclusion

The studied extracts possess high content of mono- and sesquiterpene hydrocarbons beside other phenolic compounds including methoxylated flavones. The antimicrobial efficiency towards most tested strains and the high antioxidant activity for all laurel leaves extracts and for Frankincense EE and WE extracts showed that they have organoleptic properties with potential beneficial effects on human health, supporting the traditional application of these extracts in treating various diseases. Also, the anticancer activity for Frankincense extract against HepG2 and HCT116 cell lines and laurel extract activity against HepG2 cell line are leading them to be health-promoting natural substances with a promising medical values. The obtained results confirm the fact that Spices and herbs are rich in bioactive compounds including antioxidants with very low or limited toxic effect in contrast to synthetic antioxidants. An increasing interest in using phenolics in the food industry to improve the quality and nutritional value of food because they retard oxidative degradation of lipids. Therefore, using these plant extracts in food preservation is recommended and will be of great value. Further studies are recommended to isolate the bioactive compounds and to determine their safe dose.

References

Akter, J., A. Hossain, K. Takara, Z. Islam, and D. Hou, 2019. Antioxidant activity of different species and varieties of turmeric (*Curcuma* spp): Isolation of active compounds. Comparative Biochemistry and Physiology, Part C, 215: 9-17.

Ammon, H.P., 2006. Boswellic acids in chronic inflammatory diseases. Planta Med., 72: 1100-1116.

Aqel, M. and R. Shaheen, 1996. Effects of volatile oil of *Nigella sativa* seeds on the ulerine smooth muscle of rat and guinea pig. Journal of Ethanopharmacol., 52:23-26

Atlas, M., AE Brown, and LC. Parks, 1995. Laboratory Manual of experimental microbiology, New York, NY: Mosby-Year Book, Inc.

Banno, N., T. Akihisa, K. Yasukawa, H. Tokuda, K. Tabata, Y. Nakamura, R. Nishimura, Y. Kimura, and T. Suzuki, 2006. Anti-inflammatory activities of the triterpene acids from the resin of *Boswellia carteri*. Journal of Ethnopharmacol, 107: 249-253.

Cherrat, C., L. Espina, M. Bakkali, D. Garcia-Gonzalo, R. Pagan, and A. Laglaoui, 2013. Chemical composition and antioxidant properties of *Laurus nobilis* L. and *Myrtus communis* L. essential oil from Morocco and evaluation of their antimicrobial activity acting alone or in combined processes for food preservation. J. Sci. Food Agric., 94: 1197-1204.

Chmit, M., H. Kanaan, J. Habib, M. Abbas, A. Mcheik, and A. Chokr, 2014. Antibacterial and antibiofilm activities of polysaccharides, essential oil and fatty oil extracted from *Laurus nobilis* growing in Lebanon. Asian Pacific Journal of Tropical Medicine, 7 (1): S546-S552.

Conforti, F., G. Statti, D. Uzunov, and F. Menichini, 2006. Comparative chemical composition and antioxidant activities of wild and cultivated *Laurus nobilis* L. leaves and *Foeniculum vulgare* subsp. *Piperitum* (Ucria) Coutinho seeds. Biol. Pharm. Bull., 29: 2056-2064.

Cox, S.D., J.L. Mann, H.C. Markham, J.E. Bell, J.R. Gustafson, and S.C. Warlington, 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). Appl. Microbiol., 88: 170-175.
Dasilveira, S.M., F.B. Luciano, N. Fronza, Jr.A. Cunha, G.N. Scheuermann, and C.R. Werneck Vieira, 2014. Chemical composition and antibacterial activity of Laurus nobilis essential oil towards food borne pathogens and its application in fresh Tuscan sausage stored at 7 °C. LWT – Food Sci. Technol., 59: 86-93.

Di Leo Lira, P., D. Retta, E. Tkacik, J. Ringuelet, J.D. Coussio, C. van Baren, and A.L. Bandoni, 2009. Essential oil and by-products of distillation of bay leaves (Laurus nobilis L.) from Argentina. Ind. Crop. Prod., 30: 259-264.

Dias, M.I., L. Barros, M. Dueñas, R.C. Alves, M.B.P.P. Oliveira, C. Santos-Buelga, and I.C.F.R. Ferreira, 2014. Nutritional and antioxidant contributions of Laurus nobilis L. leaves: Would be more suitable a wild or a cultivated sample. Food Chemistry, 156: 339–346. http://dx.doi.org/10.1016/j.foodchem.2014.01.122.

Fernández, N.J., N. Damiani, E.A. Podaza, J.F. Martucci, D. Fasce , F. Quiroz, P.E. Meretta, S. Quintana, M.J. Eguaras, and L.B. Gende, 2018. Laurus nobilis L. Extracts against Paenibacillus larvae: Antimicrobial activity, antioxidant capacity, hygienic behavior and colony strength. Saudi Journal of Biological Sciences, https://doi.org/10.1016/j.sjbs.2018.04.008.

Fidan, H., G. Stefanova, I. Kostova, S. Damyanova, A. Stoyanova, and V.D. Zheljazkov, 2019. Chemical composition and antimicrobial activity of Laurus nobilis L. essential oils from Bulgaria. Molecules, 24, 804; doi:10.3390/molecules24040804

Frank, M.B., Q. Yang, J. Osban, J.T. Azzarello, M.R. Saban, R. Saban, R.A. Ashley, et al., 2009. Frankincense oil derived from Boswellia Carteri induces tumor cell specific cytotoxicity. BMC Complement. Altern. Med., 9(6). DOI: 10.1186/1472-6882-9-6.

Ghita, E.I., H.M.F. EL-Din, A.S. Gad, S.M.A. Badran and T.M. EL-Messery, 2009. Supplementation of milk and light yoghurt with Rosemary and green tea as natural antioxidant. Egypt. J. Nutri., 4: 1-28.

Goujdil, M.B., L. Segni, S.E. Bencheikh, S. Zighmi, and D. Hamada, 2015. Study of the chemical composition: Antibacterial and antioxidant activities of essential oil extracted from the leaves of Algerian Laurus nobilis Lauraceae. Journal of Chemical and Pharmaceutical Research, 7(1): 379-385.

Guedri, M.M., M. Romdhane, A. Lebrihi, F. Mathieu, and J. Bouajila, 2020. Chemical composition and antimicrobial and antioxidant activities ofTunisian, France and Austrian Laurus nobilis (Lauraceae) essential oils. Not. Bot. Horti. Agrobo. 48(4):1929-1940DOI:10.15835/48412145

Gülçin, I., M. Oktay, O.I. Kűférence, and A. Aslan, 2002. Determination of antioxidant activity of lichen Cetraria islandica (L). J. Ethnopharmacol., 79: 325-329.

Hamidpour, R., S. Hamidpour, M. Hamidpour, and M. Shahlari, 2013. Frankincense: From the selection of traditional applications to the novel phytotherapy for the prevention and treatment of serious diseases. Journal of Traditional and Complementary Medicine, 3 (4): 221-226.

Han, X., D. Rodriguez, and T.L. Parker, 2017. Biological activities of frankincense essential oil in human dermal fibroblasts. Biochimie Open, 4: 31-35.

Hasson, S.S., M.S. Al-Baloushi, T.A. Sallam, M.A. Idris, O. Habbal, and A.A. Al-Jabri, 2011. In vitro antibacterial activity of three medicinal plants - Boswellia (Luban) species. Asian Pacific Journal of Tropical Biomedicine, 1: S178-S182.

Huang, M.T., V. Badmaev, Y. Ding, Y. Liu, J.G. Xie, and C.T. Ho, 2000. Anti-tumor and anti-carcinogenic activities of triterpenoid, β-boswellic acid. BioFactors, 13: 225-250.

Jones, F.A., 1996. Herbs-useful plants. Their role in history and today. European Journal of Gastroenterology and Hepatology, 8: 1227-1231.

Kamatou, G.P., I. Vermaak, and A.M. Viljoen, 2012. Eugenol- From the remote Maluku Islands to the international market place: A review of a remarkable and versatile molecule. Molecules, 17: 6953-6981.

Maatallah, S., N. Nasri, H. Hajlaoui, A. Albouchi, and A. Elaissi, 2016. Evaluation changing of essential oil of laurel (Laurus nobilis L.) under water deficit stress conditions. Industrial Crops and Products, 91: 170-178.

Mishra, A.K. and N.K. Dubey, 1994. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. Applied and Environmental Microbiology 60: 1101-1105.
Mishra, S., R. Bishnoi, R. Maurya, and D. Jain, 2020. *Boswellia serrata* ROXB. A bioactive herb with various pharmacological activities. Asian J. Pharm. Clin. Res., 13(11): 33-39.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay, Journal of Immunological Methods, 65: 55-63.

Nusier, M.K., H.N. Bataneih, Z.M. Bataneih, and H.M. Daradka, 2007. Effect of frankincense (*Boswellia thurifera*) on reproductive system in adult male rat. Journal of Health and Science, 53: 365-370.

Ogunlana, E.O., Hoeglund, S., Onawunmi, G. and O. Skoeld, (1987). Effect of lemongrass oil on the morphological characteristics and peptido-glycan synthesis of *Escherichia coli* cells. Microbios, 50: 43-59.

Ortiz, H.F., W.F. Sánchez, J.A. Mèndez, and Murillo, E.P., 2009. Potencial antioxidante de hojas y corteza de *Bauhinia kalbreyei* Harms: Contribución de sus flavonoides en estaactividad. Rev. Acad. Colomb. Cienc., 53 (127): 183-191.

Ouchikh, O., T. Chahed, R. Ksouri, M.B. Taarit, H. Faleh, and C. Abdelly, 2011. The effects of extraction method on the measured tocopherol level and antioxidant activity of *L. nobilis* vegetative organs. Journal of Food Composition and Analysis, 24: 103-110.

Patrakar, R., M. Mansuriya, and P. Patil, 2012. Phytochemical and Pharmacological Review on *Laurus nobilis*. International Journal of Chemistry and Pharmaceutical Sciences, 1: 595-602.

Patricia, M.S., M. Migdalia, A.P. Juan, S. Mario, H. Victor and P. Esther, 2013. Gas Chromatography-Mass Spectrometry Study from the Leaves Fractions Obtained of *Vernonanthura patens* (Kunth) H. Rob. International Journal of Organic Chemistry, 3: 105-109.

Peixoto, L.R., P.L. Rosalen, G.L.S. Ferreira, and I.A. Freires, 2017. Antifungal activity, mode of action and anti-biofilm effects of *Laurus nobilis Linnaeus* essential oil against *Candida spp*. Archives of Oral Biology, 73:179-185.

Pham-Huy, L.A., H. He, and C. Pham-Huyc, 2008. Free radicals, antioxidants in disease and health. International Journal of Biomed. Sci., 4: 89-96.

Pilar Santamarina, M., J. Rosello, S. Gimenez, and B.M. Amparo, 2016. *Commercial Laurus nobilis L.* and *Syzygiumaromaticum L.* Merr.and Perry essential oils against post-harvest phytopathogenic fungi on rice. LWT – Food Sci. Technol., 65: 325-332.

Porrini, M., N. Fernández, P. Garrido, L. Gende, S. Medici, and M. Egurra, 2011. *In vivo* evaluation of anti parasitic activity of plant extracts on Nosemaceranae (Micosporidia). Apidologie, 42 (6): 700–707.

Priyanka, C. and S.A. Daljit, 2017. Management Antioxidant Compounds Derived from Plants, Description and Mechanism of Phytochemicals. Journal of Agroecology and Natural Resource, 4(1): 55-59.

Ramos, C., B. Teixeira, I. Batista, O. Matos, C. Serrano, and N.R. Neng, 2012. Antioxidant and antibacterial activity of essential oil extracts of bay leave *Laurus nobilis Linnaeus* (Lauraceae) from Portugal. Natural Product Research, 26 (6): 518-529.

Schippmann, U., D.J. Leaman, and A.B. Cunningham, 2002. Impact of Cultivation and Gathering of Medicinal plants on Biodiversity: Global Trends and Issues. FAO – Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries.Satellite event on the occasion of the Ninth Regular Session of the Commission on Genetic Resources for Food and Agriculture.

Schmiech, M., J. Ulrich, S.J. Lang, B. Büchele, C. Paetz, A. St-Gelais, T. Syrovets, and T. Simmet, 2021. 11-Keto-α-Boswellic Acid, a Novel Triterpenoid from *Boswellia spp* with Chemotaxonomic Potential and AntitumorActivity against Triple-Negative Breast Cancer Cells. Molecules, 26, 366.https://doi.org/10.3390/molecules26023666

Sellami, H.I., W.A. Wannes, I. Bettaieb, S. Berrima, T. Chahed, B. Marzouk, and F. Limam, 2011. Qualitative and quantitative changes in the essential oil of *Laurus nobilis* L. leaves as affected by different drying methods. Food Chemistry, 126: 691-697.

Škerget, M., P. Kotnik, M. Hadolin, A. Hraš, M. Simončič, and Ž. Knez, 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chemistry, 89: 191-198.
Taban, A., M.J. Saharkhiz, and M. Niakousari, 2018. Sweet bay (*Laurus nobilis* L.) essential oil and its chemical composition, antioxidant activity and leaf micromorphology under different extraction methods. Sustainable Chemistry and Pharmacy, 9: 12–18. https://doi.org/10.1016/j.scp.2018.05.001

Türi, M., E. Türi, S. Köljalg, and M. Mikelsaar, 1997. Influence of aqueous extracts of medicinal plants on surface hydrophobicity of *Escherichia coli* strains of different origin. APMIS, 105: 956-962.

Walle, T., 2007. Methoxylated flavones, a superior cancer chemopreventive flavonoid subclass. Seminars in Cancer Biology, 17(5):354-62 DOI:10.1016/j.semcancer.2007.05.002

Weckesser, S., K. Engel, B. Simon-Haarhaus, A. Wittmer, K. Pelz, and C.M. Schempp, 2007. Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. Phytomedicine, 14: 508-516.

Yalçın, H., M. Anik, M.A. Sanda, and A. Cakir, 2007. Gas chromatography / mass spectrometry analysis of *Laurus nobilis* essential oil composition of northern Cyprus. J. Med. Food, 10: 715-719.