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Biological activities of essential oils – A review

Hesham Hussein Rassem[1, 2], Abdurahman Hamid Nour[3], Rosli Mohammed Yunus[4]

[1]Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Pahang, Malaysia, hesham_rassem@yahoo.com
[2]Faculty of Science, University of Hodeidah, Hodeidah, Republic of Yemen
[3]Faculty of chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Pahang, Malaysia, nour2000_99@yahoo.com
[4]Faculty of chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Pahang, Malaysia, rmy@ump.edu.my

Address for Correspondence:
Hesham Hussein Rassem , Faculty of chemical and Natural Resources Engineering, Universiti Malaysia Pahang (UMP), Malaysia, 26300 Gambang, Pahang, Malaysia, hesham_rassem@yahoo.com

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ABSTRACT
Essential oils are liquid preparations, produced from plant materials. These essential oils have a very high commercial value due to its properties. Plants essential oils are widely used in the various fields of industries, such as perfumery industries and pharmaceuticals and have a very high commercial value due to its therapeutic properties. Although essential oils showed a promising bioactivity in vitro, they could interact in plants with some components (Terpenes, acids, hydrocarbons, esters) and pH, thus many authors have reported that a significant effect of essential oils toward spoiling and pathogenic microorganisms could be achieved in vivo by using small amounts of oils. Different methods can be used to assess the bioactivity of essential oils. This study show benefits the essential oils and how using this essential oils in biological activities. It can be used oils extracted from different plants, whether they have a biological effect such as antioxidant or anti-bacteria or fungi. This review is a focus on which methods can use to determine biological activity for essential oils. Roads have also been paved on how to know Cytotoxicity of essential oils and how to find out.

Keywords:
Essential oils, Antimicrobial activities, Antioxidant activities, Cytotoxicity

1. Introduction

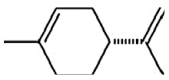
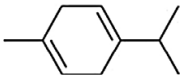
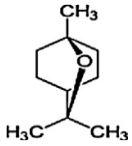
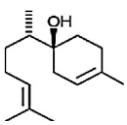
Infectious diseases and foodborne illnesses can cause severe health effects and can even lead to death among the residing population, especially in the developing regions of the world. The continual emergence of antibiotic-resistant microorganisms has prompted researchers' world over to search for new antimicrobial agents that are more effective against the resistant microbial pathogens. Structural modification of the antimicrobials (against which microbial resistance has been developed) is reported to improve the effectiveness of antimicrobial agents against bacteria, fungi, and viruses. However, of late, research efforts have been put forth to improve the effectiveness of antimicrobial drugs by developing novel and a new class of antimicrobial drugs that can effectively work on multi-targeted sites or organisms [1].

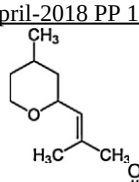
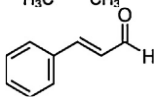
The effectiveness of these procedures has been attributed mainly to the presence of active phytochemicals or bioactive compounds in plants. Given the scope of searching new antimicrobial agents, antimicrobials derived from plant materials are often regarded as natural and safe compared to industrial chemicals. Of late, plant-based medicine has become more popular due to the increasing concern of consumers with regard to the use of synthetic chemical preparations and use of artificial antimicrobial preservatives. Several reports have been published concerning the composition together with the biological properties such as antimicrobial, antioxidant and anti-inflammatory of these plant extracts [2-5].

2. Chemical constituents of Essential Oils

Essential oils are factory the volatile oils with aromatic components that are made up of chemical compounds. For example esters, alcohols, aldehydes, ketones, hydrocarbons, and phenols, are some of the major components of essential oil [6]. Oils of plant species are available commercially in the market [7]. Numerous studies have demonstrated the efficiency of Essential Oils in low doses in the activity biological [8, 9]. The essential oil is so called because they were believed to represent the quintessence of odor and flavor from the flower kingdom – differ in composition properties from fatty or fixed oils, which consist for the most part of glycerides and from mineral or hydrocarbon oils. A scientific definition of the term essential or volatile oils is not possible, although several practical definitions exist. The most common one defines an essential oil as a more or less volatile material isolated from an odorous plant of a single botanical species by a physical process (see Table 1). They are oxygenated derivatives of hydrocarbon terpenes such as aldehydes, ketones, alcohols, phenols, acids, ethers and esters [10]. Some terpenes are potent drugs against diseases such as heart disease [11], malaria [12] and cancer [13].

Table 1: Some compounds of essential oils with biological applications.

EO components	Molecular structure	Chemical formula	Molecular Weight	Boiling point C^0	Refractive index (20 C^0)	Plant source	Some biological applications	References
Monoterpenes D-Limonène		$C_{10}H_{16}$	136.23	175.4	1.473	Citrus limon	Antifungal, antioxydant	[14]
g-Terpinène		$C_{10}H_{16}$	136.23	183	1.474	Origanum Vulgare	Antioxidant	[15]
Terpenic oxides 1,8-Cineole		$C_{10}H_{18}O$	154.25	176	1.457	Eucalyptus Polybractea	Antiinflammatory activity (asthma)	[16]
Oxygenated sesquiterpenes a-Bisabolol		$C_{15}H_{26}O$	222.37	153	1.496	Matricaria recutita	Anti-irritant, anti inflammatory, antimicrobial	[17]

Terpenic oxides Cis-Rose oxide 	$C_{10}H_{18}O$	154.25	70–71	1.454	RosaDamascene	Antiinflammatory, relaxant	[18]
Cinnamaldehyde 	C_9H_8O	132.16	248–250	1.621	Cinnamomum, Zeylanicum	Bactericide, fungicide, insecticide	[19]

3. Biological activities of Essential Oils

Essential oils from different plants as in flowers achieve much interest due to their uses in biological activities antibacterial, antioxidant, antifungal and insecticidal properties [20]. Some Essential Oils for plants and uses in biological activities see Table 2.

Table 2: Some Essential Oils for plants and uses in biological activities

Essential Oil	Biological Name	Properties	Uses
Lavender	*Lavendula Vera Officinalis*	antimicrobial, antidepressant, antirheumatic,	Useful for treating skin conditions such as acne, allergies, boils, dandruff, dermatitis, sunburn, eczema. Treatment of disorders such as rheumatism, throat infections, flu, bronchitis.
Ylang –Ylang	*Cananga Odorata ver Genuine*	Antidepressant, antiinfectives, relaxant, antiseptic, hypotensive, regulator, sedative	Depression, nervous tension, high blood pressure, digestive upsets. For nervous system disorders such as insomnia
Geranium	*Pelargonium Graveolens*	relaxing, antidepressant, astringent, antiseptic, antihaemorrhagic, deodorant, diuretic, fungicidal, anti-inflammatory	Anxiety, sore throat, tonsillitis, cellulitis, broken capillaries, eczema, ulcers, wounds.
Clory Sage	*Salvia Sclarea*	Antiseptic, astringent, antiphlogistic, deodorant, bactericidal, antidepressant.	Anxiety, depression, high blood pressure, acne boils, oily skin nd hair, cramp, migraine, the genitor-urinary system disorders.

3.1. Antioxidant activities

An antioxidant is a molecule that discourages the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may impair cells. In biological scope, the antioxidants have been defined as substances that when present in concentrations below than the oxidation substrate are capable of interception or prevent oxidative processes.

3.1.1. The gauge of antioxidant activity

Mechanisms of antioxidant Natural compounds: (1) Remove the chain by donation of hydrogen atoms or electrons that convert free radicals into more stable species, (2) Generation of reactive oxygen species by used chelating metal ions which are involved in that, (3) Change the lipid peroxides 43 into stable final products and (4) Inhibiting the harmful action of pro-oxidant enzymes[21-24].

3.1.2. In Vitro assays for antioxidant activities of essential oils

There are various methods to control and comparison the antioxidant activity of plants and the use of different methods is necessary for antioxidant activity assessment [25-27]. In a number of in-vitro studies has been known it the antioxidant potential of essential oils and extracts. Most commonly used methods for the determination of antioxidant activity of plant essential oils and extracts are by radical scavenging assay (2,2-di(4-tert-octaphenyl)-1-picrylhydrazyl) (DPPH), Inhibition of linoleic acid peroxidation and bleaching of β- carotene in linoleic acid system assays [14, 21, 26, 28-33]. The antioxidant activity measure of essential oils for the plant can give varying results depending on the specific free radical being used as a reactant.

In-vitro assays simply provide an idea of the protective efficacy of the test sample. Thus it is necessary to use at least two methods depending on the expected antioxidant possibility and/or on the source of the substance [34]. for this, [35] suggested the DPPH radical scavenging activity assay and β-carotene bleaching as methods of choice for standardizing assessment of the antioxidant capacity of essential oils.

3.1.3. Antioxidant potential of essential oils

These synthetic antioxidants are known to have carcinogenic and toxic effects on food systems and human lives [36-38]. Synthetic antioxidants may cause cerebrovascular diseases and liver bulge [39, 40]. There is a strong need for effective and safer antioxidants based on natural sources, as alternatives, to prevent the deterioration of foods and human lives. The appear many studies and reports of extracts (Essential Oils) from the natural sources that have demonstrated strong antioxidant activity [36, 37]. Many sources of antioxidants have been explored and still, studies are going on in this fields and to improve this objective.

Some publications show in Essential oils or extracts from flowers using to antioxidant activities varying degrees [36, 41-46] showed antioxidative activities of essential oils. Some of these essentials oils and extracts have been reported to be more effective than some synthetic antioxidants [26, 47].

3.2. Cytotoxicity of essential oils

Cytotoxicity is the quality of being toxic to cells. Examples of toxic agents are an immune cell or some types of venom. The cytotoxicity of essential oils is believed to be due to its action on cellular safety leading to disease and apoptosis. An attempt has been made to review medicinal plants and their essential oil components which have been proven for their in vitro cytotoxic

nature. Due to a number of chemical constituents, essential oils apparently have no specific cellular targets [48]. Cytoplasmic membrane is passed through easily by essential oils which disrupt its structure and make it permeabilized. Cytotoxicity can thus cause such damage to the cell membrane. Essential/volatile oils have the ability to harden the cytoplasm, hence damaging lipids and proteins [20, 49, 50]. Injury to the cell wall and cell membrane can lead to lysis and to the escape of macromolecules [8, 51, 52]. Essential oils are liable to stimulate depolarization in mitochondrial membranes of eukaryotic cells by decreasing the membrane potential, affect ionic Ca^{++} cycling and other ionic channels and reduce the pH gradient [10, 53-55].

3.2.1. In Vitro assays for Cytotoxicity of essential oils

Various assays are reported in the literature to evaluate the cytotoxic activities of essential oils or their main constituents using fluorescent dyes or specific cell staining including MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) test [56-61], Neutral Red Uptake test [62], Trypan Blue exclusion test [63, 64], resazurin test [65], or Hoechst 33342 and propidium iodide test [66]. MTT assay has been extensively reported because of its simplicity and reliability to measure cell viability for the screening of anti- proliferative agents [59, 67]. Viable cells rely on an intact mitochondrial membrane and an intact mitochondrial respiratory chain. Identification of bioactive constituents with regard to their toxicity can be carried out using mitochondrial dehydrogenases from viable cells.

3.3. Antimicrobial activities

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. The antimicrobial activity of plant extracts and phytochemicals was evaluated with antibiotic susceptible and resistant microorganisms [1]. The use of plant extracts both with known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency [68-74]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant.

3.3.1. Antimicrobial agents

Microbiologists differentiate two groups of antimicrobial agents used in the treatment of infectious diseases. 1) Antibiotics, that are natural substances produced by certain groups of microorganisms, and 2) chemotherapeutic agents, which are chemically synthesized [75]. The most important property of an antimicrobial agent is its selective toxicity. This implies that the biochemical processes in the bacteria are in some ways different from those in the animal cells and that the advantage of this difference can be taken in chemotherapy. Antibiotics may have a static (inhibitory) effect on a range of microorganisms. The range of bacteria or other microorganisms that are affected by a certain antibiotic is expressed as its spectrum of action [20].

3.3.2. In Vitro tests of antimicrobial activity

There are a number of methods used for evaluation of antibacterial activity of essential oils [76-78]. Different assays like microdilution assay, well diffusion assay, disc diffusion assay, measurement of minimum inhibitory concentration are often used for measuring the antimicrobial activity of essential oils and plants based constituents [10, 20, 52, 79, 80]. The

principles and chemistry behind these methods are explained in the references [81] but no standardized method is developed for assessing the antimicrobial activity of plant-based compounds against food-spoiling and pathogenic microorganisms [75]. The NCCLS method for antibacterial susceptibility testing has been modified for testing essential oils and extracts [82].

Screening of essential oils for antibacterial activity is often done by the disc diffusion assay, in which a paper disc soaked with a known concentration of essential oil is laid on top of a protected agar plate. This is generally used as a preliminary check for antibacterial activity prior to more detailed studies. This method is useful for screening between essential oils but direct comparison of published data is not feasible [83, 84]. In agar well diffusion assay, well are formed by cutting wells in agar and the essential oils are loaded into that wells. This method is mostly sued as a screening method when large numbers of essential oils and/or large numbers of bacterial isolates are to be screened [85, 86].

The most important method in the antimicrobial performance of essential oils is the measurement of minimum inhibitory concentration (MIC), which tells us the accurate, exact and reproducible results. In some cases, the minimum bactericidal concentration (MBC) or the bacteriostatic concentration is stated, both terms agreeing closely with the MIC. The strength of the antimicrobial activity can be determined by dilution of essential oils in agar or broth [87, 88].

3.3.3. Essential oils as a natural antimicrobial agents Essential

Essential oils and other naturally occurring antimicrobials are attractive to the food industry and pharmaceuticals. Some essential oils from flowers have shown promise as potential food safety interventions when added to processed and raw foods and also can use in pharmaceuticals.

4. Methods to assess the biological activity of essential oils

4.1. Evaluation of antioxidant activity of essential oils

4.1.1. DPPH assay on TLC

The assay was performed as described by [89]. The hydrogen atom- or electron donation ability of the Lamiaceae essential oils was measured from the bleaching of methanol solution of DPPH. In this assay, a stable DPPH (2, 2′-diphenyl-1-picrylhydrazyl) radical (purple in color), is reduced to yellow colored diphenyl picryl hydrazine (by the action of antioxidant components. Five microliters of a 1:10 dilution of the oils in hexane were applied as spots on the TLC plates (aluminum sheets covered with silica gel 60 F254, Merck). The plate was sprayed with DPPH reagent (0.2% in methanol) and left at room temperature for 30 min. Yellowish spots formed as the result of bleaching of the purple color of DPPH reagent were evaluated as positive antioxidant activity [90].

4.1.2. Spectrophotometric DPPH assay

The antioxidant activity of the Lamiaceae essential oils was assessed by measuring their ability to scavenging 2, 2′-diphenyl-1-picrylhydrazyl stable radicals (DPPH). The assay was carried out spectrophotometrically as described by [90]. The samples from 0.5 to 300.0 µg mL-1 of methanol were mixed with 1 mL of 90 µM DPPH solution followed by addition of 95% methanol up to final volume of 4 mL. After 1 h at room temperature, the absorbances of blank and resulting solutions were recorded. Synthetic antioxidant, Butylhydroxytoluene (BHT) was used as a positive control. The disappearance of DPPH was read spectrophotometrically at 515 nm using a spectrophotometer (U-2001, model 121-0032 Hitachi, Tokyo, Japan). Scavenging (%) of DPPH free radical by essential oil was calculated in following way:

Scavenging (%) = 100 x (A blank – A sample/A blank)

Where A blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. The concentration of essential oils providing 50% scavenging (IC_{50}) was calculated from the graph-plotted scavenging percentage against essential oils concentration.

4.2. Evaluation of cytotoxicity of essential oils

Essential oils were solubilized in DMSO and then diluted in culture media for use. The human breast cancer (MCF-7) and fibroblast (NIH-3T3) cell lines were maintained in Dulbecco's Minimum Essential Medium (DMEM) while hormone-dependent prostate carcinoma (LNCaP) cell line was cultured in RPMI 1640 medium. Both media were supplemented with 10% heat-inactivated fetal calf serum, 1% L-glutamine, 1% penicillin/streptomycin. Cells (104/well) were cultivated in 96 well plates for 24 h before the essential oils/test compounds were added.
Essential oils dilutions (10-500 µg mL-1) were added to triplicate wells and cells were incubated for further 24 h. DMSO, at the same concentration carried by the essential oils, was tested as a solvent control while Doxorubicin as a reference standard. Cell viability was evaluated by the MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyl- tetrazolium bromide] assay and the percent inhibition of cell viability was calculated using cells treated with DMSO as control (Mosmann et al., 1993)[91]. The IC_{50} values (concentration at which 50% of cells were killed) were calculated from the graph-plotted concentration against percent cell viability.

4.3. Evaluation of antimicrobial activities of essential oils

The essential oils were individually tested against a panel of microorganisms selected. Bacterial strains were cultured overnight at 37 ºC in nutrient agar (NA) while the fungal strains cultured overnight at 30 ºC using potato dextrose agar (PDA). Following antimicrobial assays were employed for the determination of the antimicrobial potential of essential oils.

4.3.1. Disc diffusion method

The antimicrobial activity of the selected essential oils was determined by disc diffusion method [92]. Briefly, 100 µL of a suspension containing 108 colony-forming units (CFU)/mL of bacteria cells and 104 spores/mL of fungi were spread on Petri plates containing NA and PDA medium (50 mL media/plate), respectively. The paper discs (6 mm in diameter) were separately impregnated with 15 µL of essential oils or main components of essential oils and placed on the agar which had previously been inoculated with the selected test microorganism. Amoxycillin and Ciprofloxacin were used as a positive reference for bacteria while Flumequine and Fluconazole for fungi. Discs without samples were used as a negative control. Plates were kept at 4 ºC for 1h. The plates were incubated at 37 ºC for 24 h for bacteria and at 30 °C for 48 h for fungal strains. Antimicrobial activity was assessed by measuring the diameter of the growth-inhibition zone in millimeters (including disc diameter of 6 mm) for the test organisms comparing to the controls.

4.3.2. Micro-dilution broth susceptibility assay

For minimum inhibitory concentration (MIC), a micro-dilution broth susceptibility assay was used, as reported in [92]. Essential oils were solubilized in dimethylsulfoxide (10% DMSO) then diluted in culture media for use. Dilutions series were prepared from 0.01 to 30.0 mg mL-1 of the

essential oils in a 96-well microtitre plate, including one growth control, solvent control and one sterility control. 160 µL of nutrient broth and sabouraud dextrose broth for bacteria and fungi, respectively were added onto microplates and 20 µL of test solution. Then, 20 µl of 5 x 105 cfu/ml (confirmed by viable count) of standard microorganism suspension were inoculated onto microplates. Plates were incubated at 37 ºC for 24 h for bacteria, and at 30 ºC for 48 h for fungi. Amoxycillin (1.0 mg/mL in 10% DMSO) was used as a positive control for bacteria while Flumequine (1.0 mg/mL in 10% DMSO) and Fluconazole (1.0 mg/mL in 10% DMSO) were for fungi. The growth was indicated by the presence of a white ‘‘pellet’’ on the well bottom. The MIC was calculated as the highest dilution showing complete inhibition of the test strains.

4.3.3. Resazurin microtitre-plate assay

Modified resazurin microtitre-plate assay was also used for the measurement of MIC of some essential oils as reported by [93]. Briefly, a volume of 100 µL of 5mg/mL (w/v) essential oil solutions in 10% DMSO (v/v) and standard antibiotic (1 mg/mL in 10% DMSO) was pipetted into the first row of the 96 well plates. To all other wells, 50 µL of nutrient broth was added. Two-fold serial dilutions were performed using a multichannel pipette such that each well had 5 µL of the test material in serially descending concentrations. 30 µL of 3.3x strength isosensitised broth and 10 µL of resazurin indicator solution (prepared by dissolving 270 mg tablet in 40 mL of sterile distilled water) were added to each well. Finally, 10 µL of bacterial suspension was added to each well to achieve a concentration of approx 5 × 105 cfu mL- 1. Each plate had a set of controls: a column with a ciprofloxacin as positive control, a column with all solutions with the exception of the test compound, a column with all solutions with the exception of the bacterial solution adding 10 µL of nutrient broth instead and a column with 10% DMSO (v/v) solution as a negative control. The plates were prepared in triplicate.

Plates were enfolded loosely with cling film and incubated at 37 °C for 24 h. The color change was then assessed visually. The growth was indicated by color changes from purple to pink or colorless. The lowest concentration at which color change occurred was taken as the MIC value.

4.3.4. Determination of minimum bactericidal concentration (MBC) of essential oils

The wells showing a complete absence of growth in the MIC assay were identified and 10 µL mixtures from each well were transferred to nutrient agar plates [77]. The agar plates were incubated at the same time and temperatures, as in disc diffusion assay. The complete absence of growth on agar plates was considered as the MBC.

5. Conclusion

The search for biological activities in essential oils has also increased during these last years. This work reports the biological activities of essential oils which may act by preventing lipid peroxidation, scavenging free radicals or, and in very few cases, chelating metal ions. Some works also showed that the constituents of essential oil act synergistically because their main components, when used as references, have less activity than the essential oil. According to the literature, it was possible to conclude that depending on the chemical composition of the oils, they can act as anti-inflammatory affecting the arachidonic metabolism or the cytokines production, or on the modulation of pro-inflammatory gene expression. The antioxidant and anti-inflammatory activities of the essential oils are well documented; nevertheless, their uses can be hampered due to the chemical variability of the oils. Several factors including harvesting time of the aromatic plant, climatic and agronomic conditions, vegetative development of the plant, the

plant part used, type of extraction used can be considered as responsible for fluctuations in their chemical compositions.

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