

Antibacterial Activity of Eucalyptus Essential Oil and Extraction

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ABSTRACT

Present study was included the assessment of biological activity of Eucalyptus as Antibacterial Activity of Eucalyptus Essential Oil (EO) and Extraction leaves. Plants extract tested toward pathogenic bacteria (two gram negative Klebsiella and Enterobacter and one gram positive Staphylococcus) by testing hot Aquacus extracts. The results showed that Plants extracts have the ability to inhibit the bacterial growth of both gram positive and gram negative, but the gram negative more sensitive than gram positive. The inhibition zone (in millimeter) was greater in gram positive (33mm) than in gram negative(25), the type of plant parts were effect upon bacteria as a result EO 25mm in Klebsiella, Extraction leaves give us 33mm at Staphylococcus then Bark was 29mm Staphylococcus while the extract gave high biological activity in all type of bacteria in concentrations 200 and 300 mg/ml respectively, the Conc. 300mg/ml more effect than 200mg/ml. The Virulence Factors of studied pathogenic bacteria. The Antibiotic resistance (zero) at Oxacillin. Hemolysis production in was detected phenotypically using blood agar supplied with 5% fresh human blood, Klebsiella non lysis but Enterobacter and Staphylococcus were blood lysis also the Virulence Factors of studied pathogenic bacteria the biofilm in Staphylococcus non-slime production but Klebsiella and Enterobacter slime producer. The percentage of free radical scavenging, EO was the best antioxidant.

1. INTRODUCTION

The herb supplement industry is growing at a rapid rate and until recent years a vast majority of medicinal plant produce in global trade was of unknown origin. There are some very real and urgent issues surrounding sustainability in the herb industry. These include environmental factors that affect the medicinal viability of herbs, the safety of the habitats that they are taken from, as well as the welfare of workers in the trade. One or more of medicinal herb is any herbal organs which contains elements its can be used aimed at beneficial resolves or originators for the production of suitable gel or cream. This report makes it imaginable to separate between ingredients have been well-known scientifically medicinal florae whose therapeutic beneficial resolves and, shrubberies that are observed as healing but which have not yet been exposed to a thorough scientific study (Al- Saati and Madhloom, 2023). A numeral of plants ensures been used in customary medicine for several years. Certain do appear to work while there may not be enough scientific facts to approve their value. Such plants should qualify as therapeutic plants. The term 'crude drugs of natural or biological origin' is mean by pharmacists to define whole plants or parts of plants which have pharmaceutical properties. A definition of therapeutic plants for the resolve of this presentation should contain the subsequent, all Plant or its

parts extraction as active gel therapeutically preparations direct medicinal used or hemi-synthesis(Al-Hadad, 2017).

This research aims to achieve the following objective:

- 1-Analyze the chemical composition of the volatile oil and extraction
- 2-Identify their most important active compounds.
- 3- Evaluating the biological activity of volatile oil and extraction by Determination of antibacterial activity G- as Enterobacter and Klebsiella . also G+ve as Staphylococcus.
- 4-Antioxidant Activity for EO. leaves and bark.

2. LITERATURE REVIEW

Eucalyptus leaves and bark are high in essential oils (1) Eucalyptus can be taken internally as an infusion or tincture of the leaf, but is more commonly used externally in the form of the essential oil (EO)(1). An infusion of the leaves is therapeutic in the early stages of a cold or flu to help with nasal and respiratory congestion, to support the immune system, soothe a sore throat and decongest the sinuses (2). The EO contains only the volatile oils, and is manufactured by steam distillation of large quantities of the leaf material (3). steam inhalation to apply the antimicrobial action directly to the mucous membranes of the respiratory tract. In the case of inhalation (from steam inhalation or a balm applied to the chest) .

Eucalyptus oil to clear airborne pathogens and acts as an insect repellent (2,4). A steam inhalation of the essential oil added to hot water is immediately clearing for the sinuses, being both uplifting and invigorating.

Eucalyptus plant

1-Skin health

as an ointment or cream can be used for skin infections, wounds, cold sores, herpes sores, shingles, chicken pox, measles, and fungal infections (2,6,11,16). This has been demonstrated in clinical research for the fungal infections tinea pedis (athlete's foot), tinea corporis (ringworm) and tinea cruris (jock itch) (25). Eucalyptus EO has significant wound healing activity via mechanisms of angiogenesis (formation of new blood vessels), collagen deposition, granulation tissue formation, epithelialization, and wound contraction at the proliferative stage (3). Leaf decoction can be used as a douche for thrush, vaginal discharges, leukorrhea, haemorrhoids (6).

2-Musculoskeletal health

EO or volatile oils in a balm can be applied topically for inflamed joints, arthritis, muscular aches and pains, gout and neuralgia (6,7). Antioxidant herbs help to protect the body from oxidative stress caused by free radicals which can damage cells, proteins, and DNA and can contribute to the development of disease.

3- Antitussive : suppress or reduce the urge to cough,. Antitussives act by soothing inflamed mucous membranes or relaxing the bronchial muscles.

4-Antipyretic reduce fevers by modulating the body's natural temperature regulation processes. They work to either cool or warm the body by increasing perspiration or circulation.

5-Antirheumatic :relieve and prevent rheumatic symptoms such as joint pain, stiffness, swelling, and impaired mobility.

6-Antispasmodic (spasmolytics) help to reduce or relieve smooth tissue muscle contractions in the digestive, urinary and reproductive systems. They are often applied to ease conditions including dysmenorrhoea, colic, muscle tension and gastrointestinal discomfort.

7- Bronchodilator: the relaxation and widening of the bronchi, the major air passages leading to the lungs, thereby improving airflow and easing breathing.

8-Carminative : Aid digestion by supporting the gastrointestinal tract and reducing discomfort by stimulating gastric secretions and increasing absorption

9-Diaphoretic :promote sweating by increasing circulation to the surface of the skin, often through dilating surface capillaries. Diaphoretics help to relieve fevers, support detoxification and improve blood flow. They work by stimulating peripheral blood flow, facilitating perspiration and elimination of toxins through the skin.

10-Expectorant: promote the secretion of mucus from the upper respiratory tract, helping to clear congestion.

11-Febrifuge: reduce a fever by acting on the body's thermoregulatory

12-Immunomodulant: restore balance to a dysfunctional immune system either by stimulating a weakened immune system or suppressing an overactive one. They can act through modulation of cytokines, enhancing the activity and

proliferation of immune cells including T-cells, natural killer cells, and macrophages.

13-Vermifuge:the action help to expel parasitic worms from the gastrointestinal tract by causing paralysis or irritation of the parasites, thereby promoting their removal from the body via bowel movements.

14- ulnerary. healing wounds ,promote tissue repair, and reduce bleeding.

Anti-inflammatory

Anti-inflammatory herbs reduce inflammation in the body by modulating molecular and cellular inflammatory response pathways. Anti-inflammatories inhibit pro-inflammatory enzymes, such as cyclooxygenase (COX) and lipoxygenase (LOX), reducing the production of inflammatory mediators like prostaglandins and leukotrienes. Anti-inflammatory Activity.

The aromatic constituents of EO are used as inhibitor of the production and synthesis of tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), leukotriene B₄, and thromboxane B₂ in human blood monocytes, suggesting that eucalyptol is a strong inhibitor of cytokines that might be suitable for long term treatment of airway inflammation in bronchial asthma and other steroid-sensitive disorders [32].

Antimicrobial: Antiseptic and Antibacterial Effects

Eucalyptus EO has broad spectrum antimicrobial effects against many bacteria, including Mycobacterium tuberculosis and methicillin-resistant Staphylococcus aureus (MRSA), fungi (including Candida) and viruses (16). Topical application of the EO in a carrier oil, for the treatment of respiratory tract infections [23]: the herb is, in fact, very helpful for colds, flu, sore throats and chest infections including bronchitis and pneumonia [15]. Several studies showed a moderate antimicrobial activity of EO both on Gram-negative (Salmonella enteritidis, Escherichia coli and Pseudomonas aeruginosa) and Gram-positive bacteria (Staphylococcus aureus, Enterococcus faecium, Listeria monocytogenes 4b and Listeria monocytogenes EGD-e) and a bacteriostatic activity against Pseudomonas aeruginosa) [24].

Safety

Cytotoxic and Toxic Features . the concentration of sample that inhibited 50% of cell growth (IC₅₀). [38]. Eucalyptus oil should be avoided internally and externally during pregnancy and when breast-feeding eucalyptus leaf medicine should only be used by adults and children over 12 years old (26).

2.2 Active compounds or Chemical Composition

is a rich source of phytochemical compounds such as phenolic compounds and alkaloids, glycosides, flavonoids essential oils, terpenes, tannins and propanoids, extracted in the leaf, stem and root of the plant [11]. several volatile constituents as 1,8-cineole (eucalyptol) aromadendrene, α -gurjunene, globulol, β -pinene, pipertone, terpinen, caproic acid, citral, eudesmol, fenchone, p-menthane, myrecene, myrtenol, α -terpineol, verbinone, asparagine, cysteine, glycine, glutamic acid, ornithine ,threonine [16], also formic acid, dextrin and sucrose were extracted from flowers [17]. Despite the fact that more

than 18 compounds were identified in EO, eucalyptol represents the 79.85% of the total chemical composition .

Pathogenic Bacteria

1-Staphylococcus

Staphylococcus bacteria, commonly known as staph, are among the most widespread bacteria found in humans. They naturally inhabit the skin and mucous membranes, particularly in areas such as the nose, mouth, and armpits. These bacteria belong to the family Staphylococcaceae and are spherical in shape, Gram-positive, and typically appear under the microscope as clusters resembling bunches of grapes (33)

Morphological and Physiological Characteristics

1. Spherical in shape, arranged in clusters resembling bunches of grapes
2. Gram-positive
3. Non-motile and non-spore-forming
4. Facultative aerobes (can grow in the presence or absence of oxygen)
5. Produce the enzyme catalase (3)

Locations of Staphylococcus

Staphylococcus aureus is a very common bacterium found widely in nature Human Skin, (armpits, nose, and groin region). Mucous Membranes (nasal vestibule) its main permanent habitats. It can also be found in the throat and upper respiratory tract. present on contaminated surfaces, medical instruments, clothing, and food, In Animals, domestic or food-producing animals, (10) Also as Normal Flora of Nose (Nasal Vestibule), Pharynx and Upper Respiratory Tract ,Gastrointestinal Tract, and Skin, such as the Armpits, groin, and around the mouth.

Klebsiella

Klebsiella is a Gram-negative bacterium belonging to the Enterobacteriaceae family. It is an opportunistic microorganism that can normally inhabit the intestines but may cause serious diseases when it spreads to other parts of the body or in cases of weakened immunity. Its main characteristics are as follows:

- 1 . Morphological Characteristics Rod-shaped. - Non-motile. - Surrounded by a thick capsule that gives it a shiny appearance under the microscope when stained with India ink.
2. Physiological Characteristics, Facultative anaerobe, meaning it can grow with or without oxygen. - Ferments sugars such as lactose, producing acids and gases. - Non-spore-forming
3. Cultural Characteristics: - Grows well on media such as MacConkey agar ,forming pink colonies due to its ability to ferment lactose. - On (Blood agar) it produces large, shiny, and mucoid colonies because of its capsule. (16). The most prominent of these factors include: Capsule: protects the bacteria from phagocytosis and the effects of the immune system. It also helps the bacteria adhere to tissues and form biofilms. Lipopolysaccharide (LPS): It forms part of the bacterial outer membrane and contributes to resistance against the complement system, while also triggering a strong inflammatory response in the body. Biofilm Formation: It enables the bacteria to adhere to medical surfaces such as catheters and ventilators, making it more difficult to eliminate

them with antibiotics. Siderophores: These are molecules secreted by the bacteria to capture iron from the surrounding environment, which is an essential element for their growth inside the human body. β -lactamase Enzymes: Including ESBLs and Carbapenemases, which provide the bacteria with broad resistance to antibiotics, increasing the severity of infections and making them more difficult to treat. Fimbriae and Adhesins: They help the bacteria adhere to host cells, particularly in the urinary tract and respiratory system (35).

The most notable effects include:

Pneumonia, Urinary Tract Infections, Septicemia .Wound and Soft Tissue Infections Meningitis and Liver Abscess. (44)

2.3 Antibiotic Resistance ,Klebsiella bacteria are among the most antibiotic-resistant pathogens, especially in hospital settings. Their danger lies in their high ability to acquire new resistance genes through plasmids and mobile genetic elements. The main mechanisms of their resistance include(11).

Enterobacter

rod-shaped of the family Enterobacteriaceae . *Enterobacter* are gram-negative bacteria that are classified as facultative anaerobes, which means that they are able to thrive in both aerobic and anaerobic environments. Many species possess flagella and thus are motile. Features such as motility, as well as certain biochemical properties, including the ability to synthesize an enzyme known as ornithine decarboxylase, are used to distinguish *Enterobacter* from the very similar and closely related *Klebsiella* bacteria. *Enterobacter* is named for the organisms' predominant natural habitat, the intestines of animals.

Virulence characteristics

For *Enterobacter* species, flagella are used for adhesion, biofilm formation, and protein export as well as motility. [7]. The lipopolysaccharide capsule of *Enterobacter* spp. helps members of this genus avoid phagocytosis and initiate the host inflammatory response.

Pathogenic strains of Enterobacter spp. It have been found in the sputum, blood, wounds, and stool of humans. *Enterobacter* spp. are associated with common nosocomial infections including respiratory, endocarditis, bacteremia, urinary tract infections, osteomyelitis, among others.[8] *Enterobacter*-associated bacteremia presents as fever but can progress to systemic inflammatory response syndrome and shock.[8] For pneumonia caused by *Enterobacter* spp., symptoms include coughing and shortness of breath [8].

2.4 Antioxidant Properties of Eucalyptus EO

The infection process frequently induces inflammation which determines the release of free radicals from the phagocytes. Antioxidants are molecules able to scavenge reactive oxygen species or free radicals, protecting cells from damage and death. At a physiological level, these free radicals play important roles in energy production, synthesis of some biomolecules, phagocytosis, and cell growth in living systems

[27]. An imbalance between free radical generation and unfavorable antioxidant defenses leads to oxidative stress, resulting in DNA or tissue damage [28, 29]. It is possible to distinguish among two categories of antioxidants, the natural and the synthetic. Recently, due to the adverse effects demonstrated by synthetic antioxidants, the interest in finding naturally antioxidant molecules in foods has increased considerably [30]. A study by Akolade and colleagues was conducted with an aim to determine the antioxidant effects of EO from *E. globulus* grown in Nigeria [18]. The antioxidant activity was evaluated by the ability of EO to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in methanol (DPPH assay) and the results showed that, although its activity resulted lower when compared with ascorbic acid, the EO depending on concentration, exerts radical scavenging activity. The low antioxidant capacity of the EO may be attributed to the absence of phenolics compounds (such as thymol and cavacrol) in Nigerian *E. globulus* leaf [18]. The major derivative compound from *E. globulus* are called Globulisin A and Eucaglobulin which demonstrated a suppressive effect on DPPH free radical development were examined. These molecules, in fact, scavenged DPPH free radical in a concentration dependent manner, and revealed an inhibitory activity stronger than ascorbic acid [31]. According to all these assessments, and considering the various experimental methods used, eucalyptus plant is an important source of antioxidants.

3. MATERIAL AND METHODS

3.1: Materials

3.1.1: Apparatuses and equipment: The apparatuses and equipment that are used in the study were shown in table (3-1)

Table (1): Apparatuses and equipment that used in this study.

Culture media	Company /origin	Purpose
Congo Red Agar	Himedia / India	growth ,activation and maintenance of bacteria
Muller –Hinton agar	Lab/ United kingdom	Susceptibility of bacteria and yeast to certain antibiotic

3.1.3: Culture media

The culture media used in this study are listed in table (3-3).

Table (2): The culture media used in this study.

Equipment & Instrument	Manufacturing company	Origin
Autoclave	Labtech	Korea
Burner	Amal	Turkey
Cotton swab	MDIC	India
Distillator	Buchi	Switzerland
Filter unite	Wertheim	Germany
HOT plate with magnetic	Gallenkamp	England
Incubator	Memmert	Germany
PH – Meter	LKB	Sweden
Sensitive balance	Sauter	Switzerland
Shaker water bath	Memmert	Germany
Microscope	Olympus	Japan
Syringe	ISO9001	Germany

3.2: Method

3.2.1: Plants collection and preparation

The plant *Eucalyptus* was collected from the gardens our Faculty. during September 2025 and January . Plant parts were separated as leave, and bark then washed two times with tap water and once with distilled water . After that, put under shade until dry. Each dried part was grinding by electrical grinder . The powdered parts were kept in plastic tubes in refrigerator at 4°C until the time of use (Harborne, 1984).

3.2.2: Preparation of plant extracts

The plants were extracted with four types of solvents, which were cold and hot (distilled water) , ethanol and methanol (alcohol). In both cases the extracted parts of plants were *Eucalyptus* leaves, and bark (Harborne, 1984).

3.2.2.1: hot aqueous extracts

Take 20 grams of finely powdered material and 200 ml of distilled water were refluxed in flask 500ml and the suspension was put on magnetic stirrer plate for 48 hours at room temperature to prepare cold extract , while hot water extract put in boiling water and put in magnetic stirrer plate for 48 hour at room temperature , then filtered by multi- layer of muslin cloth then by filter paper type Whattmann, No. 1 and dried in oven (40-30)°C. After that the extract was kept in refrigerator until it was used. [12]

1- Storage, The pure oil is poured into a dark bottle (to protect it from light) and stored in a cool, dark place to preserve its quality and properties.

3.3 Extraction of Eucalyptus

oils using the Clevenger Apparatus. Preparation of the Sample and Solvent A measured quantity of representative dry eucalyptus leaves is weighed. The sample is ground to facilitate the extraction process. The ground sample is placed inside a tube of thick filter paper (thimble). solvents, loaded with the extracted oils, return to the boiling flask. Mueller–Hinton agar Weigh 7.5 g of Mueller's agar medium Prepare 200 ml of water, pour it into a flask, add the agar medium, and close the flask with cotton wool. Place the flask on the stove and bring to a boil, then cool and pour into dishes. Then prepare the MacFarland Put 5ml of distilled water in the tube and inoculate it with bacteria (E. coli) Place the tubes in the incubator for 2 hours. Then inoculate the dish with (E. coli) bacteria. Make holes in the plates. We put it in the incubator for 24 hours. then observe the growth inhibition in the plate and measure it with a ruler.

Antibiotics Testing

Pour the culture medium into Petri dishes and let them cool. Inoculating of the culture medium with bacteria .Use two from the dishes to inoculate staphylococcus and entero bacteria using the streaking method. put Antibiotics discs. Place the following antibiotic discs in the two dishes. Oxacillin: 5 mcg (OX), TOB: 30mcg (TOB), Chloramphenicol: 10 mcg (C), Cefoxitin: 30 mcg (FOX), Vancomycin: 30 mcg (VA), Experiment/5

Congo Red Agar

prepare Congo Red Agar medium for culture staphy and enterococcus bacteria, Weight the above ingredients according to the quantities indicated. and Sterilize the medium in an autoclave at 121°C for 15 minutes. After sterilization, allow it to cool to approximately 50°C. Add Congo Red stain and allow to solidify at room temperature. Divide the dish into two parts using the loops 14- Inoculating each part with a type of bacteria Part one staphylococcus bacteria and Part two enterococcus bacteria .Place the dish in the incubation for 24 hours

Blood Agar

is a medium used to detect the type of hemolysis performed by bacteria, such as α (alpha), β (beta), γ (gamma) hemolysis. preparation of Blood Agar Medium After sterilization, allow the medium to cool to approximately 45-50°C (it should not be hot enough to prevent blood coagulation). Adding the blood Add 50 ml of sterile or fresh blood (5%) per liter of medium. Mix gently to avoid bubbles.

Table (3). Interaction between Eucalyptus with concentration mg/ml of extract from bark, leaves and VO in inhibition zone by mm

Plant part extract	Conce.mg/ml	Enterobacter mm	Staphylococcus mm	Klebsiella mm
Leaves V.O.	V.O	15	15	25
Leaves	200	15	28	19
Leaves	300	26	33	25
Bark	200	20	20	15
Bark	300	24	29	23

pathogenic bacteria

Clinical bacteria culture was obtained from microbiology Lap. Bacteria were grow in nutrient agar and Muller-Hinton Agar (MHA).

McFarland Standard

McFarland standard turbidity 0.5 was prepared by adding 0.5 ml of a 1.175 percent (wt/vol) BaCl₂. 2H₂O solution to 99.5 ml of 1% (vol/vol) H₂SO₄ solution. Sweating the turbidity criterion into similar test tubes to those used to prepare the inoculum suspension was performed. The McFarland standard tubes were coated with Para film to avoid evaporation and kept in the dark at a temperature of 24°C for an additional six months (Wayne, 2011).

3.4 Antioxidant Activity

Antioxidant activities: Various concentrations of extracts (100, 75, 50 and 25 mg/ml) stayed mixed by way of 5.0 ml of methanolic solution comprising DPPH radicals (0.004% w/v) to determine the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity. The mixture was shaken vigorously and left to stand in a microtiter plate for 30 minutes in the dark until stable absorbance values were obtained. The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. Radical-scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the following equation: % RSA = (AOPPH – A_s)/AOPPH x 100.

The solution absorbance of sample extract is added at a certain level, AOPPH means the solution absorbance of the DPPH [17][18].

4.RESULTS AND DISCUSSION

After added plant extracts to the medium of Enterobacter, Klebsiella and Staphylococcus the results showed an inhibitory activity for each extract, and volatile oil, The results showed a clear inhibitory activity of the plant extracts against the bacteria used in the study, the effect of the concentrations and type of plant extracts . The in inhibiting the two types of bacteria separately. The effect of Eucalyptus Leaves V.O. ranged 15mm (Enterobacter and Staphylococcus) While the around inhibition zone Klebsiella was 25mm.. The effect of Eucalyptus bark extract Conc.200-300 mg/ml., it more effect on Staphylococcus and it had an inhibitory effect on Klebsiella by 25 mm. the leaves extractit had an inhibitory effect on bacteria (Conc. 200mg/ml) by 15mm at Enterobacter and 19mm at Klebsiella as G-ve bacteria but in G+ve but the Conc. 300mg/ml showed more effect as in the figure below G-ve 26mm and 26mm, G+ve the most inhibition zoon 33mm. . Plants bark extracted interact Conc. 300mg/ml Staphylococcus (G+ve) as 29mm more effect than Enterobacter 24mm and Klebsiella 23mm (G-ve) , but low results inhibition zoon at 200mg/ml as in the followed Enterobacter, Staphylococcus and Klebsiella , 20mm and 15mm.

4.2 Antiseptic and Antibacterial Effects

Eucalyptus EO has broad spectrum antimicrobial effects against many bacteria, including *Mycobacterium tuberculosis* and methicillin-resistant *Staphylococcus aureus* (MRSA), fungi (including *Candida*) and viruses (16). Topical application of the EO in a carrier oil, Eucalyptus oil is antimicrobial, immunomodulant, anti-inflammatory, (16). Eucalyptus oil has antibacterial and antiviral activity against a broad range of organisms and strains (1,10,13). This antimicrobial activity of eucalyptus oil has been demonstrated against *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae*, which are some of the most common pathogens causing respiratory tract infections, such as influenza, otitis media (ear infection), sinusitis, bronchitis and pneumonia (3,18). The treatment of many of these conditions with antibiotics is threatened by the development of worldwide antibiotic resistance (18). However, the complex mixture of constituents in EOs is advantageous because pathogens have difficulty in developing resistance mechanisms to the multiple constituents (19). The immune stimulating properties of eucalyptus make it a powerful ingredient to add to an immune-supporting formula, Eucalyptus stimulates the circulation, which enhances blood flow to the skin and promotes sweating (11). This helps to lower a fever, clear toxins from the blood and speed up the resolution of a fever. Eucalyptus thins phlegm and mucus making it less viscous and easier for the body to expel, as well as inhibiting the secretion of more mucus via an anti-inflammatory cascade (3). The purpose of the holes is to define areas of growth: The holes help define specific areas for bacterial growth, making it easier to monitor their growth and determine their response to various factors. Testing effects: They can be used to test the effect of antimicrobials or chemicals on bacteria. Then, on the same medium, we inoculate (Entero) and (Staph) bacteria. using the streaking method, make five holes, place the antibiotic in the holes, and put it in the incubator for 48 hours. After that, we observe the inhibition and measure it with a ruler. Before entering the incubator antimicrobial activity of EO from both on Gram-negative (*Salmonella enteritidis*, *Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecium*, *Listeria monocytogenes* and a bacteriostatic activity. Antimicrobial herbs prevent the growth and activity of microorganisms responsible for causing disease including bacteria, fungi and viruses. Antimicrobials act through multiple mechanisms such as cell wall disruption, nucleic acid inhibition, protein synthesis inhibition or suppression of biofilm formation dependent on the microorganism. Many of these herbs also contain phytoconstituents including phenolic compounds, terpenoids, and alkaloids that contribute to their antimicrobial effects. These herbs help to support immune function and protect against infections affecting the skin, respiratory, gastrointestinal or urinary systems [24].

4.3 Hemolysis Production

Hemolysin production was detected phenotypically using blood agar supplied with 5% fresh human blood. More virulent bacteria are more expected to exhibit the alpha (partial)

otherwise beta (complete) type of hemolysis than other which frequently exhibit gamma (absent) hemolysis that found in three isolates of the present study. The ability of *Enterobacter*, *Klebsiella* and *staphylococcus* to produce Hemolysin as a result was found that positive for beta and alpha type of hemolysis, *Staphylococcus* (beta) and *Enterobacter* (alpha). Non Hemolysis (Gamma Hemolysis) at *Klebsiella*.

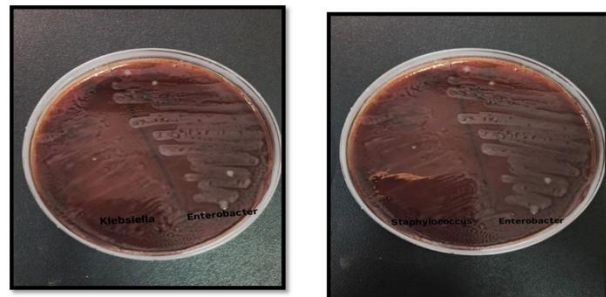


Figure 1. Hemolysin production

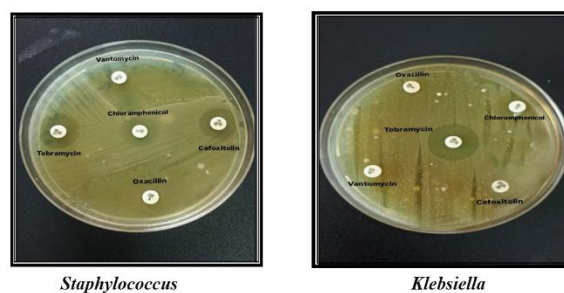


Figure 2. *Staphylococcus* and *Klebsiella*

4.4 Detection of inhibitory growth for Antibiotics

The results indicate that all antibiotics were not showed same effectiveness to inhibiting growth of pathogenic bacteria, Oxacillin was non inhibition zone around the tested bacteria, indicating resistant bacteria. But the others were effectiveness. such as Chloramphenicol: the inhibition zone as *Enterobacter* (16) and *staphylococcus* (18) and *Klebsiella* (11mm). Cefoxitin: low inhibition zone of 13mm at *Klebsiella*, 14mm at *Enterobacter* and 15mm at *staphylococcus*. Vancomycin: *Klebsiella* non inhibition zone (Resistant bacteria), *Enterobacter* and *staphylococcus* equal to 15mm as inhibition zone. The inhibition zone (Resistant bacteria or susceptible) on all pathogenic bacteria depending on factors as the concentration of the antibiotic. Minimum inhibitory concentrations (MIC), also the defense tools of bacteria act as an important role such as the enzymatic system, Slime layer (weak or strong), G+ve or G-ve (thickness of peptidoglycan layer) and other virulence factors.

Table(4).of Klebsiella Enterobacter Staphylococcus

	Klebsiella	Enterobacter	Staphylococcus	
Oxacillin		Non	None	None
Chloramphenicol		11	16	18
Cefoxitin		13	14	15
Vantomycin		Non	15	15

4.5 Phenotypic Detection of Biofilm among

The ability of Enterobacter, Klebsiella and staphylococcus. to produce biofilm by Congo red agar method to show their ability to produce slime layer and biofilm formation. the result were appear that change the color of colony to black on Congo-red agar Enterobacter and Klebsiella as Positive for biofilm while Staphylococcus negative for biofilm production (non-biofilm) The medium remained red/pink, (Figure 4-1).

The slime layer works on the bacterial cell packaging, forming thin, living membranes known as biofilm its act as a layer that inhibits the antibiotic influence within the bacteria cell and thus confers resistance (Al-Khafaji, 2018). Biofilm is an aggregate of sessile microorganisms, which prevents the antibiotics from coming into contact with bacteria, leading to repeated infections (Liu et al., 2022).

Antioxidant Activity

The antioxidant activity of the extraction of plant species parts by methanol against free radicals in DPPH was examined at different concentrations of the methanolic extract, as well as in a control group using DPPH only. The results in Table 3 showed strong antioxidant activity at high concentrations of 100 mg/ml, 0.75 mg/ml, 0.50 mg/ml and 0.25 mg/ml, and showed significant differences among all concentrations compared with the control group for different types. The same table also investigated significant differences among other plant types, with the highest antioxidant activity found in EO. The major derivative compound from *E. globulus* are called Globulisin A and Eucaglobulin which demonstrated a suppressive effect on DPPH free radical development were examined. These molecules, in fact, scavenged DPPH free radical in a concentration dependent manner, and revealed an inhibitory activity stronger than ascorbic acid [31]. According to all these assessments, and considering the various experimental methods used, eucalyptus plant is an important source of antioxidants.

5. Conclusion

1-hot water extraction is suitable to extract active ingredients by and was a very effective inhibitor, against *E. coli* also this extract was little cost, simple and easy for usages.

2- Most pathogen bacteria exhibited multi drug resistance (MDR), that is resistant to drugs

3- may be have many virulence factors such as production of haemolysin. In addition, to biofilm formation

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL. Not applicable.

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