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Original Articles

Anti-Microbial Activity and Chemical Constituents of Ethanolic Leaves Extract of *Ocimum Basilicum* L., Lamiaceae Using GC-MS Technique

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Abstract:

Background: The *Ocimum basilicum* plant, which belongs to the Lamiaceae family, has been used for a very long time in traditional medicine to treat a variety of conditions, including cancer, convulsions, epilepsy, gout, nausea, sore throat, toothaches, bronchitis, rhinitis, mental weariness, cold, spasms, and as a first aid therapy for snakebites and wasp stings. For medical purposes, the leaves, seeds, and essential oils of the plant are utilized to treat a variety of ailments, including but not limited to: cough, pyrexia, anxiety, gripe, worms, infectious disorders, acne, diarrhea, headaches, constipation, warts, and kidney dysfunction.

Objective: In the current study, the bioactive components of the extract of *Ocimum basilicum* leaves are investigated, as well as the anti-microbial properties of these fractions in *vitro*.

Materials and Methods: In order to conduct the GC–mass spectrometry (MS) study of the ethanolic Leaves Extract of *Ocimum basilicum* L., a Thermo Scientific Trace Ultra GC that was interfaced with a Thermo Fisher Scientific SpA was utilized. Antimicrobial activity of the *Ocimum basilicum* is being investigated by screening. Phytochemicals and the solvents that were employed for each extract served as the controls for this experiment.

Results: The major constituents were menthone lactone, Diterpenoid SP-II, Pulegone, Sesquiterpene lactone, menthyl acetate, alpha-Cadinol, Linalool, alpha-Bergamotene, beta-Bisabolol, 2,3-dihydroxypropyl elaidate, isopropyl palpitate, 2-methoxy-4-(1-propyl)phenol, α-cubene, vanillin, 1-methyl-3-(1-methyl)benzene, 1,4-diethylbenzene, hexadecanoic acid methyl ester. According to the type of extract (methanol, Ethyl acetate fraction, and Ethanol fraction) recorded 22.41±0.31, 21.39±0.30, and 23.05± 0.32 respectively in *Streptococcus faecalis*. While recorded 16.58±0.25, 17.32±0.26, and 22.00±0.30 for *Bacillus subtilis* . At the same time record 13.09±0.19, 17.00±0.26, and 19.07±0.28 *Streptococcus pyogenes*. While recorded 15.00±0.25, 21.96±0.30, and 24.65±0.32 *Pseudomonas aeruginosa* in comparison with Rifambin 21.00±0.30 and Bacteracin 23.41±0.31. The metabolites of *Ocimum basilicum* exhibited significant activity against *Staph. aureus* (20.39±0.31).

Keywords: Anti-Microbial activity, Chemical constituents, *Ocimum basilicum* L, GC-MS.

Introduction:

The sweet basil, also known as *Ocimum basilicum* L., is a member of the Lamiaceae family, which is comprised of over 200 species and can be found in a wide range of botanic variants and forms [1]. As a traditional medicinal plant, sweet basil has been utilized for the treatment of a variety of ailments, including but not limited to headaches, coughing, diarrhea, constipation, warts, worms, and failures of the kidneys [2]. In addition to being a well-liked culinary herb, *Ocimum basilicum* L. is a source of essential oils that are collected through steam distillation from the flowering tops and the leaves of the plant. These oils are utilized to impart taste to dishes, as well as in dentistry and oral merchandise, and in perfumes [3-5]. In basil, the aromatic quality of each variety is defined by the genotype of the plant and is influenced by the primary chemical components of the essential oils, which are predominantly composed of monoterpenes and phenylpropanoids [6]. In addition to its antibacterial, antifungal, and insect-repelling properties, the essential oil also possesses anticonvulsant, hypnotic, and antioxidant properties. As a result of the anticancer potential of medicinal herbs, there has been a growing acknowledgment of their usage in conjunction with conventional medicines for the treatment of cancer [7]. It has been suggested that the phytochemical composition of medical herbs, which includes flavonoids, phenolic acids, and essential oils, is responsible for the medicinal herbs' beneficial effects in the treatment of tumors. Researchers have discovered that these bioactive chemicals are powerful immunomodulators, which means that they have the ability to reduce the adverse effects that are caused by cancer treatment [8]. Particularly noteworthy are the blooming plants belonging to the Lamiaceae family, which are able to be discovered in virtually every region of the planet. There are numerous members of the family that are utilized as sources of essential oils, as well as components of medical preparations and as spices. Essential oils, triterpene, alkaloids, flavonoids, saponins, coumarin, steroids, gycoside, and tannins are only some of the components that can be found in the *Ocimum basilicum* L. plant. Monoterpene hydrocarbons, oxygenated monoterpene, sesquiterpene hydrocarbons, and oxygenated monoterpene are all components that are found in organic essential oils. There have been applications for essential oils produced from *Ocimum* plants, including aromatherapy, fragrance, and the preservation of food. These oils have also been used to restrict the growth of bacteria. Profiling the oil metabolites with the use of the gas chromatography-mass spectrometry method is a method that may be utilized for the purpose of studying volatile ingredient components [9]. There is also a connection between the reaction and mechanism of a medicine and the process of determining secondary metabolites that have particular features. As a result, the purpose of this research was to investigate the chemical constituents of essential oils derived from *Ocimum basilicum* L. and to assess the antibacterial properties of these oils. The current study investigates the anti-microbial properties of *Ocimum basilicum* L. extract fractions in vitro, as well as the bioactive components of the extract.

Materials and Methods:

Preparation of Ethanolic Leaves Extract of (*Ocimum basilicum* L., Lamiaceae)

The hilla city in Iraq was the location where the *Ocimum basilicum* was gathered. After being macerated in methanol at a concentration of 90%, the powdered form of *Ocimum basilicum* was filtered. A crude extract was obtained by first filtering the extract and then concentrating it using a Boeco Rotary Evaporator RVO 400 SD, which was manufactured by Boeco in Germany. In a separating funnel, the crude extract was dissolved in the least amount of deionized distilled water, and then it was combined with hexane. Following a vigorous shaking of the mixture, it was laid aside for a day to allow it to be settled. A mixture of dichloromethane (DCM) was used to re-mix the residual after the hexane fractions of the settling extract were separated as distinct components. The exact same method was carried out in order to gather the DCM fractions [10]. The EA fraction was obtained by treating the residual from the DCM fraction with ethyl acetate (EA), giving rise to the EA fraction. After all is said and done, the separation will result in the ethyl acetate fraction, while the remaining component will be an aqueous/water fraction.

Gas Chromatography-Mass Spectrometry

The GC–mass spectrometry (MS) study of the ethanolic Leaves Extract of *Ocimum basilicum* L. was performed using a Thermo Scientific Trace Ultra GC that was interfaced with a Thermo Fisher Scientific SpA. Strada Rivoltana, which is located in Rodano-Milan, Italy. a TG-5 column with a 30 m × 0.25 mm internal diameter and a 0.25 μm film thickness was installed. Using helium as a carrier gas at a rate of 1.0 mL/min, the temperature of the oven was raised at a rate of 3 degrees Celsius per minute between 60 and 220 degrees Celsius. The injector was set at a temperature of 230°C, with an injection size of 0.1 μL. The injection was made in n-hexane, and the split ratio was 1:50. At a power of 70 eV, mass spectroscopy was performed with a range of 40-450 amu. The numbers. The identification of constituents was accomplished by the utilization of retention index (RI), [11] which was obtained by referring to the homologous series of n-alkanes C8-C25, under the same experimental conditions. Additionally, the MS library search (both NIST and WILEY) and the comparison with MS literature data were utilized. A calculation was made based on the GC peak area (FID response) to determine the relative amounts of the different components, but the correction factor was not given any consideration.

Screening for the antimicrobial potential of the chemical constituents of Ethanolic leaves extract of *Ocimum basilicum* L

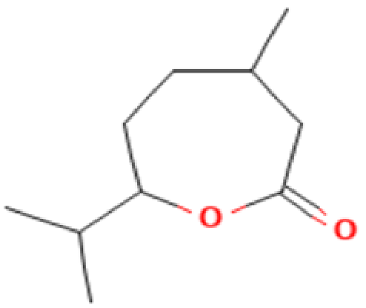
At a temperature of 37 degrees Celsius, the cultures of bacteria were cultured in Brain Heart Infusion liquid medium. Following the completion of a growth period of six hours, each microorganism was injected onto the surface of Mueller-Hinton agar plates at a concentration of 106 cells per milliliter. After that, filter paper discs with a diameter of six millimeters were placed on the surface of each inoculation plate. These discs were saturated with either extract or phytochemicals, having a volume of fifty microliters. In order to assess the effectiveness of the process, each extract was concurrently introduced into a hole that was created in new plates, with a volume of fifty microliters. One day was spent incubating the plates at 37 degrees Celsius. It was feasible to observe the inhibitory zone after this length of time had passed [12]. In general, it was determined that cultivated bacteria with halos that were around 7 millimeters in diameter or larger were vulnerable to either the extract that was examined or the phytochemical. In the preliminary investigations, the solvents that were employed for each extract and the phytochemicals served as the controls, and they did not exhibit any instances of inhibition.

Statistical analysis

The GraphPad Prism 5 Statistical Package (GraphPad Software, USA) was utilized in order to carry out the various statistical analyses. One-way analysis of variance (ANOVA) was used to assess the data, and then the Bonferroni test was performed on the results. For triplicate determinations, the results of the in vitro IC50 were represented as the mean plus or minus the standard error of the mean.

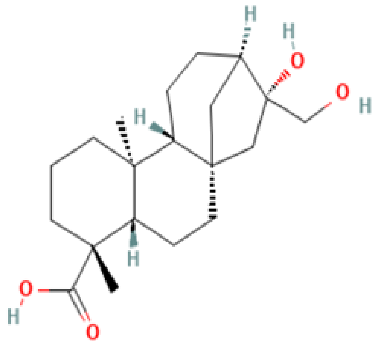
Results and Discussion:

The major constituents were menthone lactone, Diterpenoid SP-II, Pulegone, Sesquiterpene lactone, menthyl acetate, alpha-Cadinol, Linalool, alpha-Bergamotene, beta-Bisabolol, 2,3-dihydroxypropyl elaidate, isopropyl palpitate, 2-methoxy-4-(1-propyl)phenol, α-cubene, vanillin, 1-methyl-3-(1-methyl)benzene, 1,4-diethylbenzene, hexadecanoic acid methyl ester. Studies of significant importance have been carried out with the purpose of determining the phytochemical composition of *Ocimum basilicum* by utilizing essential oils that have been extracted from *Ocimum basilicum*. Essential oils of *Ocimum basilicum* are mixes of aromatic, volatile organic, and natural chemicals that are created by the plant as secondary metabolites. These compounds have a protective effect on the plant and may be extracted from a variety of components of the plant, including the flowers, roots, bark, leaves, and seeds. The presence of these complex mixes of volatile secondary metabolites can be seen in the form of saturated and unsaturated hydrocarbons, ethers, ketones, alkaloids, phenolics, flavonoids, tannins, saponins, reducing sugars, cardiac glycosides, steroids, and glycosides. The majority of investigations, on the other hand, have demonstrated that the primary components are estragole, linalool, eugenol, methyl chavicol, methyl eugenol, 1,8-cineole, eucalyptol, and bergamotene, with the quantities of these chemicals changing [13]. On the basis of their genetic traits, developmental stage, climatic conditions, drying conditions, and storage conditions, as well as the characteristics of their soils, the chemical compounds that make up *Ocimum basilicum* essential oil go through a process of quantitative and qualitative transformation.



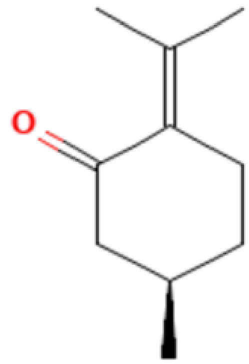
Menthone lactone

Molecular Weight: 170.25 g/mol



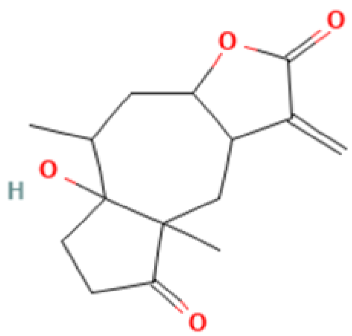
Diterpenoid SP-II

Molecular Weight: 336.5 g/mol



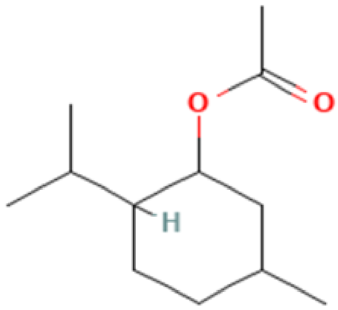
Pulegone

Molecular Weight: 152.23 g/mol



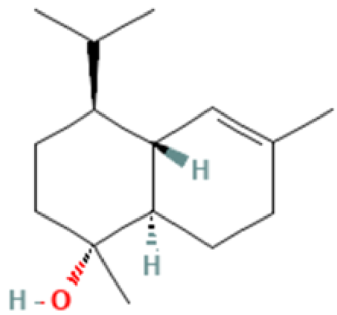
Sesquiterpene lactone

Molecular Weight: 264.32 g/mol



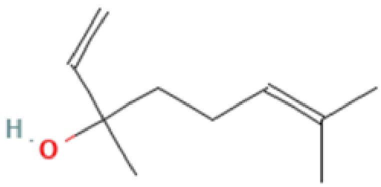
menthyl acetate

Molecular Weight: 198.30 g/mol



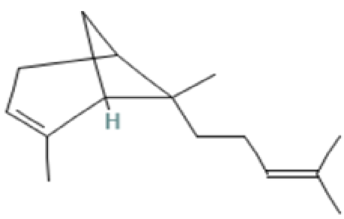
alpha-Cadinol

Molecular Weight: 222.37 g/mol



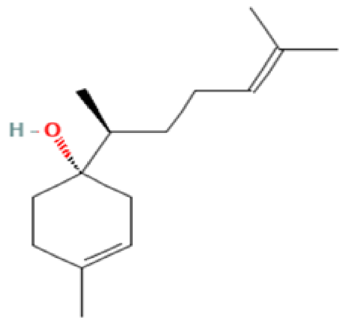
Linalool

Molecular Weight: 154.25 g/mol



alpha-Bergamotene

Molecular Weight: 204.35 g/mol



beta-Bisabolol

Molecular Weight: 222.37 g/mol

In *vitro* antimicrobial activity of *Ocimum basilicum* leaves extracts on four microorganism

According to the type of extract (methanol, Ethyl acetate fraction, and Ethanol fraction) recorded 22.41±0.31, 21.39±0.30, and 23.05± 0.32 respectively in *Streptococcus faecalis*. While recorded 16.58±0.25, 17.32±0.26, and 22.00±0.30 for *Bacillus subtilis*. At the same time record 13.09±0.19, 17.00±0.26, and 19.07±0.28 *Streptococcus pyogenes*. While recorded 15.00±0.25, 21.96±0.30, and 24.65±0.32 *Pseudomonas aeruginosa* in comparison with Rifambin 21.00±0.30 and Bacteracin 23.41±0.31. The metabolites of *Ocimum basilicum* exhibited significant activity against *Staph. aureus* (20.39±0.31). In addition, the majority of essential oils that were tested for their antibacterial capabilities indicated a higher level of effectiveness against Gram-positive bacteria in contrast to Gram-negative bacteria. The reason for this is that Gram-negative bacteria are able to protect themselves by preventing the entrance of hydrophobic chemicals like essential oil. This is made possible by the presence of an outer membrane that is constituted of lipopolysaccharides. Therefore, it is possible that the essential oil is unable to effectively assault the phospholipid layers of bacterial cells, which subsequently compromises the permeability and integrity of the bacterial cells [14, 15]. Due to the essential oil's primary components, specifically the phenolic component known as estragole and the monoterpenoid compound known as linalool, *Ocimum basilicum* essential oil demonstrated a significant level of antibacterial activity against Gram-positive bacteria. By causing a disruption in the permeability and integrity of bacterial membranes, causing intracellular ATP and potassium ion leaks, and ultimately resulting in cell death, the presence of these components in essential oil may be able to boost antibacterial action. Additionally, among the types of bacteria, *Staphylococcus aureus* is extremely susceptible to alcoholic extracts at concentrations of 8 mg/mL. Inhibition of vancomycin-resistant enterococci was achieved with minimal inhibitory doses of 14, 16, and 20 mg/mL of ethanol, methanol, and aqueous extracts, respectively. Furthermore, it was observed that the floral oil extract had the most potent antibacterial activity against *Staphylococcus aureus*. It exhibited the greatest inhibition zone, measuring 15.47 mm, the lowest minimum inhibitory concentration (MIC) of 0.09 µg/mL, and a minimum bactericidal concentration (MBC) of 0.19 µg/mL. Because it contains a high concentration of phenolic chemicals, such as eugenol, methyl eugenol, benzoic acid, 4-hydroxybenzoic acid, salicylic acid, and phenol, *Ocimum basilicum* extract has the capacity to prevent the growth of bacteria. Phenolic compounds have a wide range of effects on microorganisms, including the modification of the permeability of microbial cell membranes as a result of the accumulation of hydrophobic groups in the phospholipid bilayer, the disruption of membrane integrity, the induction of leakage in intracellular components, and ultimately the death of the cell [16, 17]. In addition, phenolic compounds have the ability to bind to enzymes and block their functions, including those functions that are associated with the creation of proteins, DNA, and RNA.

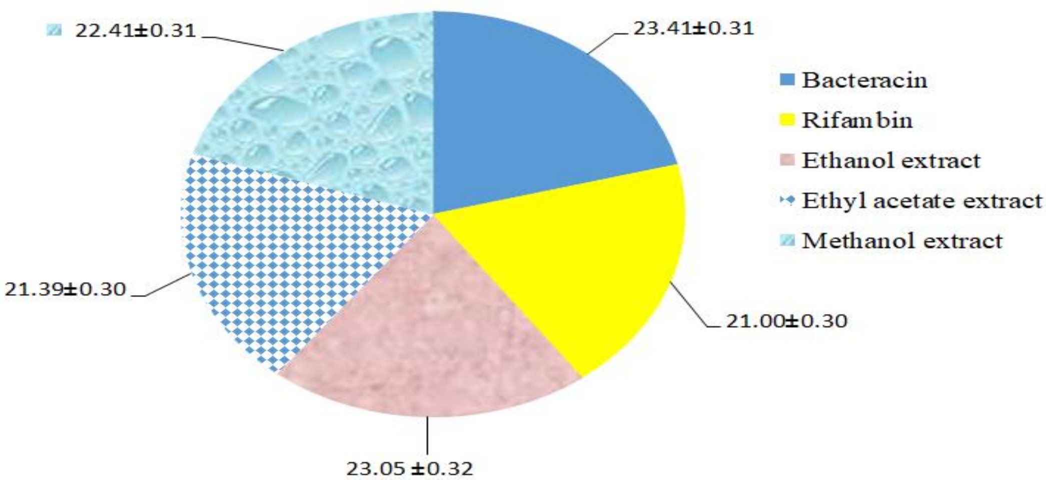


Figure 1. Zone of inhibition (mm) of various bioactive compounds derived from *Ocimum basilicum* and conventional antibiotics against *Streptococcus faecalis*

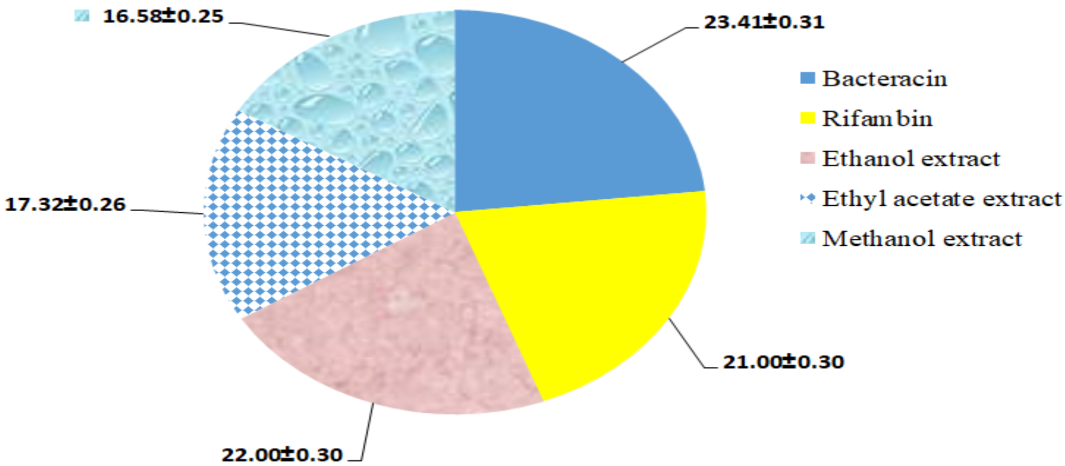


Figure 2. Zone of inhibition (mm) of various bioactive compounds derived from *Ocimum basilicum* and conventional antibiotics against *Bacillus subtilis*

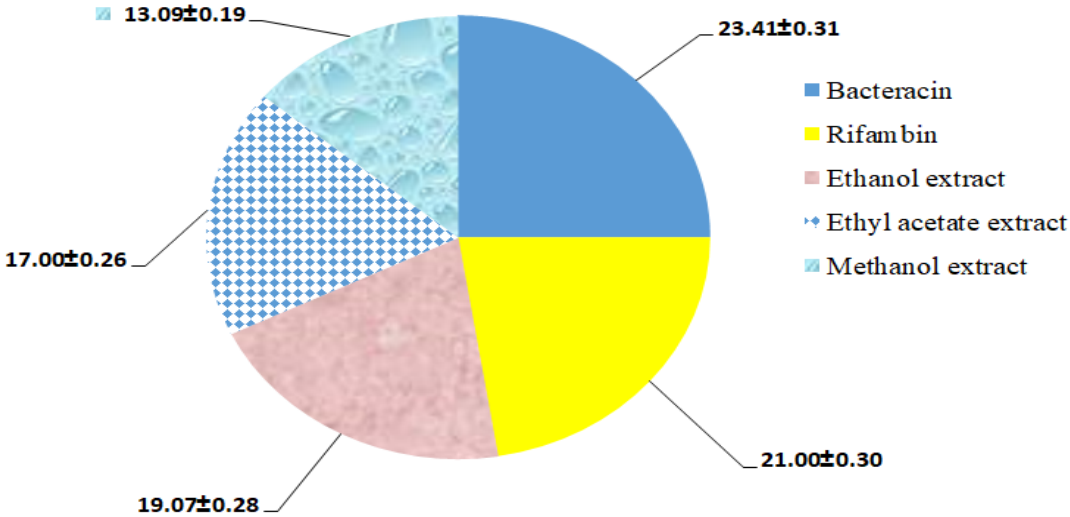


Figure 3. Zone of inhibition (mm) of various bioactive compounds derived from *Ocimum basilicum* and conventional antibiotics against *Streptococcus pyogenes*

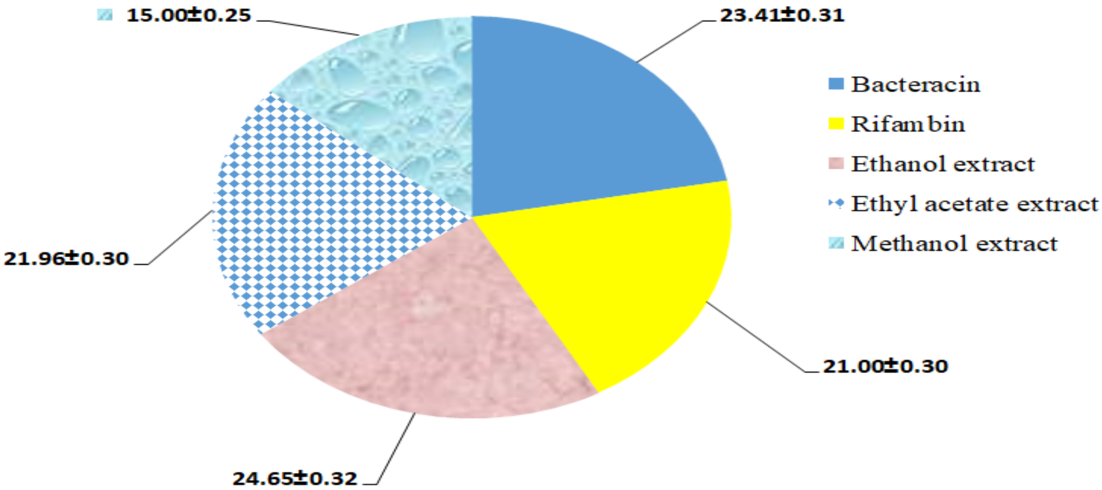


Figure 4. Zone of inhibition (mm) of various bioactive compounds derived from *Ocimum basilicum* and conventional antibiotics against *Pseudomonas aeruginosa*

Conclusions

Ocimum basilicum has a wide range of potential applications in medicine, and this potential is always developing. In vivo and in vitro studies have shown that *Ocimum basilicum* possesses the majority of the pharmacological activities that have been reported. Several clinical trials have demonstrated that it is useful in treating a wide range of medical conditions. The ethnopharmacological profile of *Ocimum basilicum*, on the other hand, requires additional research that are comparable in order to be determined and confirmed. For this reason, there is a need for additional research to be conducted using clinical illness models in order to evaluate and validate the efficacy of plants in treating a variety of ailments. It is essential to keep in mind that, in contrast to the effects of other medications used to treat the disease, the curative properties of herbs and extracts of those herbs do not result in any adverse effects. However, it is essential to keep in mind that, despite the fact that the active components originate from natural sources, excessive dosages have the potential to result in adverse effects.

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