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Original Article

Antioxidant Properties and Antimicrobial Activity of *Pimpinella anisum* against Pathogenic Bacteria in a Laboratory Setting

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Abstract:

Anise, scientifically known as Pimpinella anisum, is a fragrant herbaceous flowering plant that is utilised for its aromatic and flavourful properties as well as its many culinary and cosmetic applications. Many traditional Arab dishes, especially those from Iraq, call for anise seeds or anise essential oils (EOs). This study set out to accomplish just that—extract the essential oils from aniseeds, identify their chemical composition, and investigate their antioxidant and antibacterial properties. Gas chromatographic-mass spectrometry (GCMS) has uncovered twenty previously unknown chemicals. The secondary metabolites detected are: dodeca-5,7-diyne-1,12-diol, Eucalyptol, 2-9,12-Octadecadienyloxy, Estragole, trans-Caryophyllene, trans-Caryophyllene, alpha-Linolenic acid, 1,3-Benzodioxole-5-carboxylic, L-Fenchone, Gibberellic acid, palmitic acid, Gadoleic acid, and Cyclopropanedodecanoic acid. Aniseed was found to posses powerful antioxidant activity for the scavenging of Nitric oxide radical scavenging and Hypochlorous acid scavenging in a concentration dependant way, the highest antioxidant activity was registered in a concentration of 10000 ppm. The scavenging of the nitric oxide radical and hypochlorous acid scavenging of Pimpinella anisum in methanol, ethanol and standards of antioxidant activity. The various types of extracts found included crude, Ethanol fraction and standard Curcumin extracts at Nitric oxide radical scavenging 40.58±0.47, 57.13±0.60 and Curcumin (standard) 94.73±0.98 respectively. The percentage of Sequestration of hypochlorous acid were recorded 180.26±3.92, 191.59±4.01 and Ascorbic acid (standard) 219.26 ± 4.74 respectively. Antimicrobial Activity of (Methanol, Ethyl acetate and Ethanolic extract) of Pimpinella anisum against Bacillus cereus observed was: 29.34±0.45, 22.40±0.31 and 31.15± 0.48 respectively, while recorded 23.42±0.32, 18.47±0.25 and 28.13±0.42 respectively in Enterococcus faecalis. In the same times antimicrobial activity of Pimpinella anisum against Streptococcus pyogenes and Staphylococcus aureus recorded 26.38±0.39, 20.28±0.28 and 23.45±0.29 and 19.34±0.27, 26.34±0.40 and 29.00±0.41 respectively (Figure 3 and 4) comparison with two standard antibiotics RF-Rifampicin 33.07±0.52 and AP-Ampicillin 35.08±0.57.

Keyword: Antioxidant, Antimicrobial, *Pimpinella anisum*, Pathogenic Bacteria.

Introduction:

On a global scale, plants have the ability to produce a vast variety of chemicals, including novel antimicrobials. It was formerly believed that plant extracts containing aromatic volatile oils (EOs) had antibacterial qualities. Essential oils are a kind of secondary metabolite that plants produce in various parts of their bodies. They have many important ecological roles, such as protecting plants from herbivores and microbes, but they also have other uses, such as as an antiseptic, antibacterial, antiviral, antioxidant, antiparasitic, antifungal, and insecticide. However, essential oils are typically characterised by two or three main components, which are present in relatively high concentrations (20–70%) relative to other trace components, and which determine their biological capabilities. For ages, people have turned to plant essential oils (EOs) as a natural remedy against a wide variety of infectious diseases. The antibacterial properties of certain essential oils are due to their ability to rupture cell walls and membranes, resulting in the release of cell contents and the inhibition of proton motive force. One of the earliest medicinal herbs in Iraq, anise (Pimpinella anisum) is a member of the Apiaceae family and a herbal flowering plant that grows in many regions around the world. It produces aromatic chemicals. In reality, there is evidence supporting the efficient elimination of microbes without promoting the evolution of their resistance, with minimal damage to mammalian cells and relatively easy accessibility. Since they break down quickly in both water and soil, they are considered to be eco-friendly compounds. Our study aimed to identify aniseed hydro-distilled EOs, assess their antibacterial and antibiofilm potentials against Pseudomonas aeruginosa, an MDR burns wound isolate, and investigate their antioxidative activity. Research into the pharmacological effects of medicinal plants' active components has revealed that they possess antimicrobial, antifungal, and antiviral properties. This proves that they are reliable and efficient techniques of curing illnesses. People without access to treatment facilities sometimes cannot afford them because they are often less expensive than synthetic medications. In order to determine the antioxidant value and phytochemical composition of aniseed methanolic extract, this in vitro study used comprehensive GC-MS and HPLC methods to characterise its bioactive components and phenolics, and to quantify the extract's efficacy against multidrug resistant bacteria. The presence of phenolic hydroxyl groups gives phenolic compounds a high radical scavenging capacity. Actually, phenolic compounds can serve as a redox agent, donor of hydrogen and electrons, quencher of singlet oxygen, and chelator of metal ions, among their many biological functions, all thanks to their redox activities. Due to their high concentrations of total phenolic components, aniseed essential oils are known to have antioxidant qualities. The polyphenol content of fruits and vegetables is supposedly a major contributor to their antioxidant capacity. In the current research, monoterpenes (phenolic compounds) were the main identified components of aniseed EOs, these have been regarded as natural high antioxidants agents and as additives in food supplements to preclude the oxidative stress, which is the defining etiological factor of degenerative diseases in human. But the effect of small EOs' constituents should be also taken into consideration, and synergic interactions between these components may be responsible for antioxidant activity (9). The antioxidative abilities of some of the belonging to umbelliferae belonged plants have been reported in a research conducted in Iran. The plants in the study, P. animus had the highest reducing activity on DPPH (IC50= 109.80), the correlation between the studied fractions' antioxidant potency and phenolic contents was also positive. (6). The ethanolic extract of the aniseed EOs in a concentration dependent fashion displayed reducing scavenging activity against the nitric oxide, superoxide and DPPH. The alcoholic and water extract of aniseeds Eos demonstrated a significant antioxidative action in linoleic acid system and liposome, water extract displays more actions against free radicals than the corresponding water fractions. The achievement in inhibiting cellular link can be described as the cellular connection, namely the first step in the development of the biofilm. Our research had the objects to determine antioxidant properties and antimicrobial activity of Pimpinella anisum against pathogenic bacteria.

Materials and Methods:

Aromatic essential oils are extracted from anise seeds (Pimpinella anisum). The seeds were sourced from the neighbourhood market, and the Clevenger equipment was used for hydro-distillation extraction. Carefully crushed and combined were 100g of anise seeds. Clevenger apparatus, originally designed for distillation, was subsequently applied to them. After three hours of distillation, the liquid was poured into a separate funnel and allowed to settle for twenty-four hours. At this point, two layers of varying densities emerged; the top layer, which stood for the EOs layer, was removed and set aside.

Gas chromatographic–mass spectral (GCMS) analysis of aniseed Eos:

Using GC-MS (Shimadzu GC-MS-QP2010 Plus), the components of aniseed EOs were identified and quantified. Helium was utilised as the carrier gas with a flow rate of 13 ml.min-1, and the analyser was responsible for determining the primary chemical components in aniseed oil. We programmed the mass spectrometer to scan between 12,000 and 50,000 m/z. The injector was set to 280 degrees Celsius, and samples were injected at a ratio of 5.0. While the flow rate of the column was 1.69 ml/min, the total flow rate was 13.1 ml/min. A temperature of 50°C and a hold period of 2–5 minutes were used in the column oven. The entire duration was thirty minutes.

Antioxidant potential of aniseed:

Nitric oxide radical scavenging:

The Griess-Illosvoy assay is used to determine the formation of nitrite ions when oxygen, nitrate oxide combines with a sodium nitroprusside (SNP) solution in water at a physiological pH. These were made with 3 mL of pH 7.4 phosphate buffered saline (PBS), test solutions with different concentrations ranging from 0 to 70 µg/mL, and 10 mM of SNP. One millilitre of the incubated solution was mixed with one millilitre of sulfanilamide (0.33% in 20% glacial acetic acid) after 150 minutes of incubation at 25°C. For 5 minutes, the mixture was allowed to stand at room temperature. After that, 1 millilitre of napthylethylenediamine dihydrochloride (NED) (0.1 percent w/v) was incorporated, and the mixture was after that left to incubate at 25 ˚C for 30 minutes. In order to determine how many pink chromophores are created during the conversion of nitrite ions to diazonium salt using sulphanilamide and NED, the blank sample was utilised for this measurement. Its spectrophotometric wavelength was 540 nanometres. Six times were conducted for each test. Curcumin served as a benchmark.

Hypochlorous acid scavenging:

Just prior to the experiment, hypochlorous acid (HOCl) was made by combining 0.6 M H2SO4 with 10% NaOCl to get a pH of 6.2. The concentration of HOCl was determined by calculating the absorbance at 235 nm with an extinction value of 100 M -1 cm -1. We followed the identical approach as outlined in the work of Aruoma and Halliwell for this test, with the understanding that there may be some small modifications. By analysing the variations in absorbance at 404 nm, we were able to determine the extent to which the compound inhibited catalase action. A 50 mM phosphate buffer with a pH of 6.8, 7.2 µ M catalase, 8.4 mM HOCl, and varying concentrations of plant extract ranging from 0µg/mL to 100 µg/mL in 1 ml of solution were used to make the reaction mixture. After diluting the aliquots of the combination and developing them for 20 minutes at 25°C with a blank, the absorbance of the coloured complex that was generated could be measured. There were a total of six independent testing. Ascorbic acid is going to be used as a reference because it is known to interact easily with HOCl and interfere with its synthesis.

Antibacterial activity of aniseed:

Bacteria, and growth conditions and In vitro antibacterial activity

Identification of isolates was confirmed by using Vitek 2 compact system. Antibacterial activity of aniseed was carried out using agar well assay along Mueller-Hinton agar (MHA, Himedia/India). Wells were made in 5 mm size with sterile cork borer; 80 µL of aniseed EOs were added in wells, plates were incubated at 37°C for 24 hr; antibacterial activity was defined through the measurement of the diameter of the zone of inhibition (IZD); sensitivity was referred to three categories.

Statistical Analysis

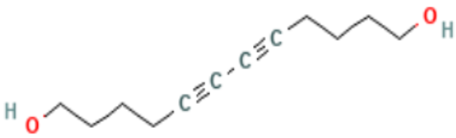
IBM (New York, NY, USA) software on statistics and Tukey's test to determine statistically significant differences (HSD) for an analysis of variance (ANOVA) on averages Mean values were used at 95% or 99% confidence interval. Statistical significance was determined by a p-value lower than 0.05.

Results and Discussion:

A large number of pharmaceuticals are derived from plants used in herbal medicine. hence, medicinal plants play an important role in the field of biotechnology. There are a lot of herbal components used in Indian cuisine, perfumes, pharmaceuticals, and food colouring. The vast majority of these herbal remedies were made with extracts from plants that included various phytochemical components. The therapeutic impact was determined by comparing the amount and identity of the chemical produced with the correlation. The gas chromatography–mass spectrometry (GC–MS) method was used to analyse the extracted extract, which helps determine the amount of active ingredients in herbal plants used in cosmetics, medications, food, and pharmaceutical products The secondary metabolites detected are: dodeca-5,7-diyne-1,12-diol, Eucalyptol, 2-9,12-Octadecadienyloxy, Estragole, trans-Caryophyllene, trans-Caryophyllene, alpha-Linolenic acid, 1,3-Benzodioxole-5-carboxylic, L-Fenchone , Gibberellic acid, palmitic acid, Gadoleic acid, and Cyclopropanedodecanoic acid.

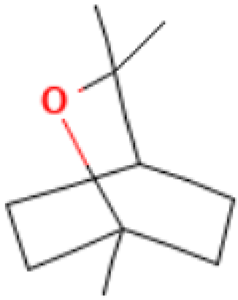
dodeca-5,7-diyne-1,12-diol

M.W.: 194.27 g/mol



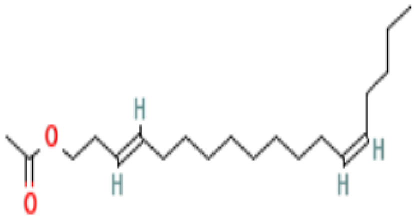
Eucalyptol

M.W.: 154.25 g/mol



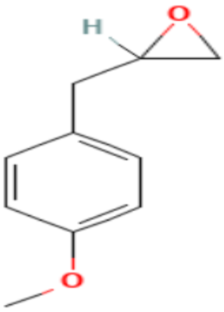
2-9,12-Octadecadienyloxy

M.W.: 308.5 g/mol



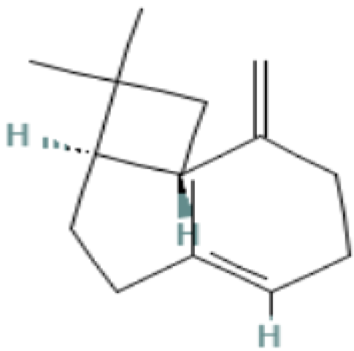
Estragole

M.W.: 164.20 g/mol



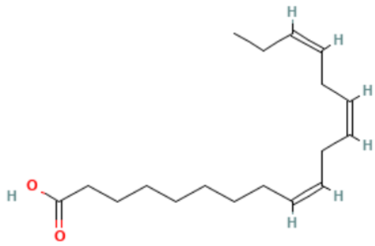
trans-Caryophyllene

M.W.: 204.35 g/mol



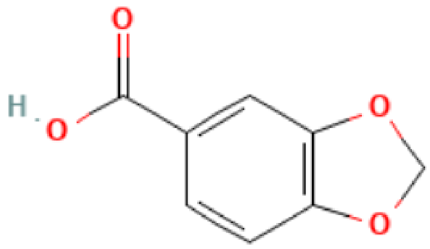
alpha-Linolenic acid

M.W.: 278.4 g/mol



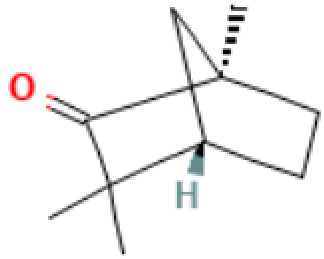
1,3-Benzodioxole-5-carboxylic acid

M.W.: 166.13 g/mol



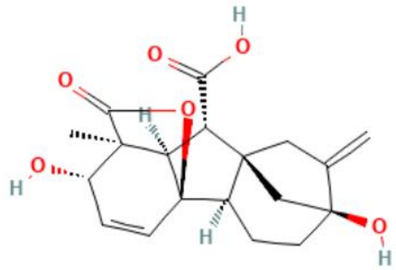
L-Fenchone

M.W.: 152.23 g/mol



Gibberellic acid

M.W.: 346.4 g/mol

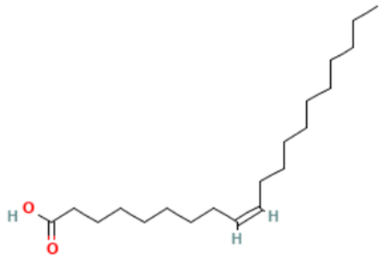


palmitic acid

M.W.: 256.42 g/mol

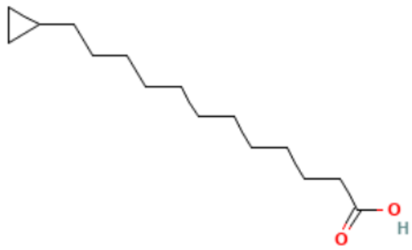
Gadoleic acid

M.W.: 310.5 g/mol



Cyclopropanedodecanoic acid

M.W.: 240.38 g/mol



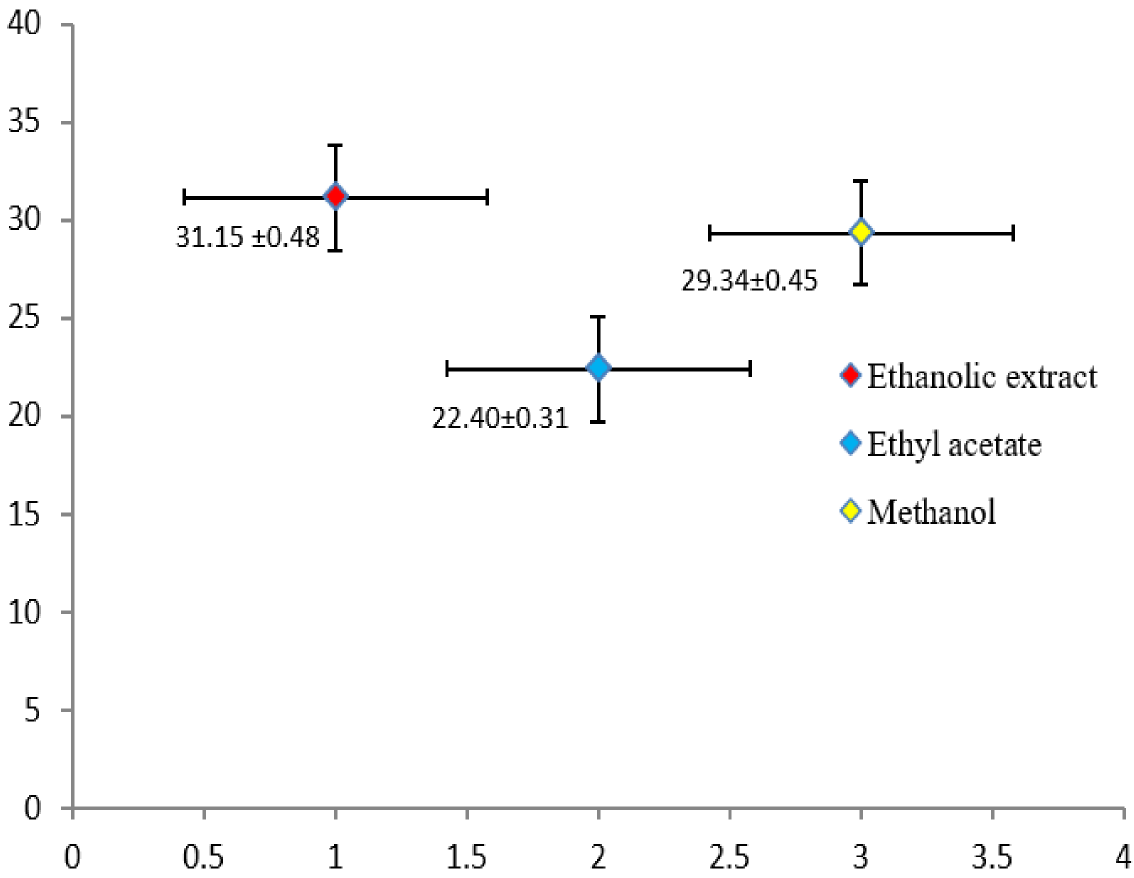


Figure 1. Antimicrobial Activity of (Methanol, Ethyl acetate and Ethanolic extract) of *Pimpinella anisum* against *Bacillus cereus*

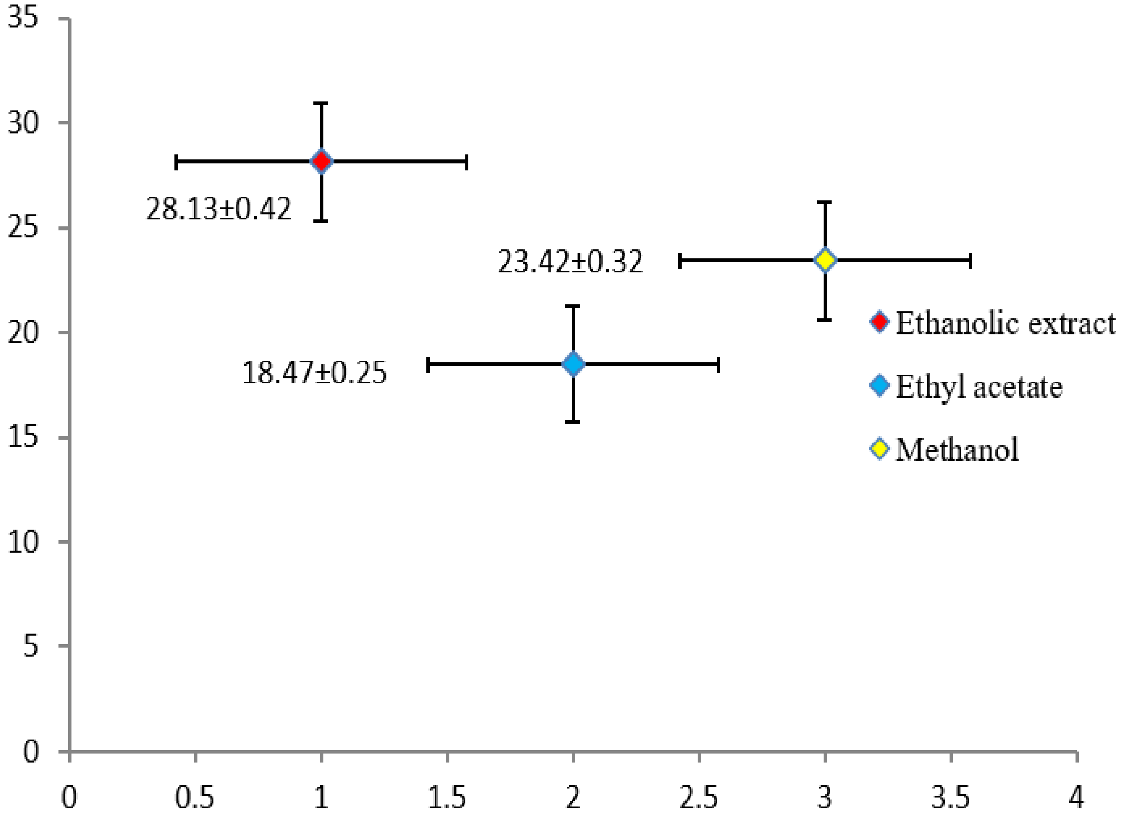


Figure 2. Antimicrobial Activity of (Methanol, Ethyl acetate and Ethanolic extract) of *Pimpinella anisum* against *Enterococcus faecalis*

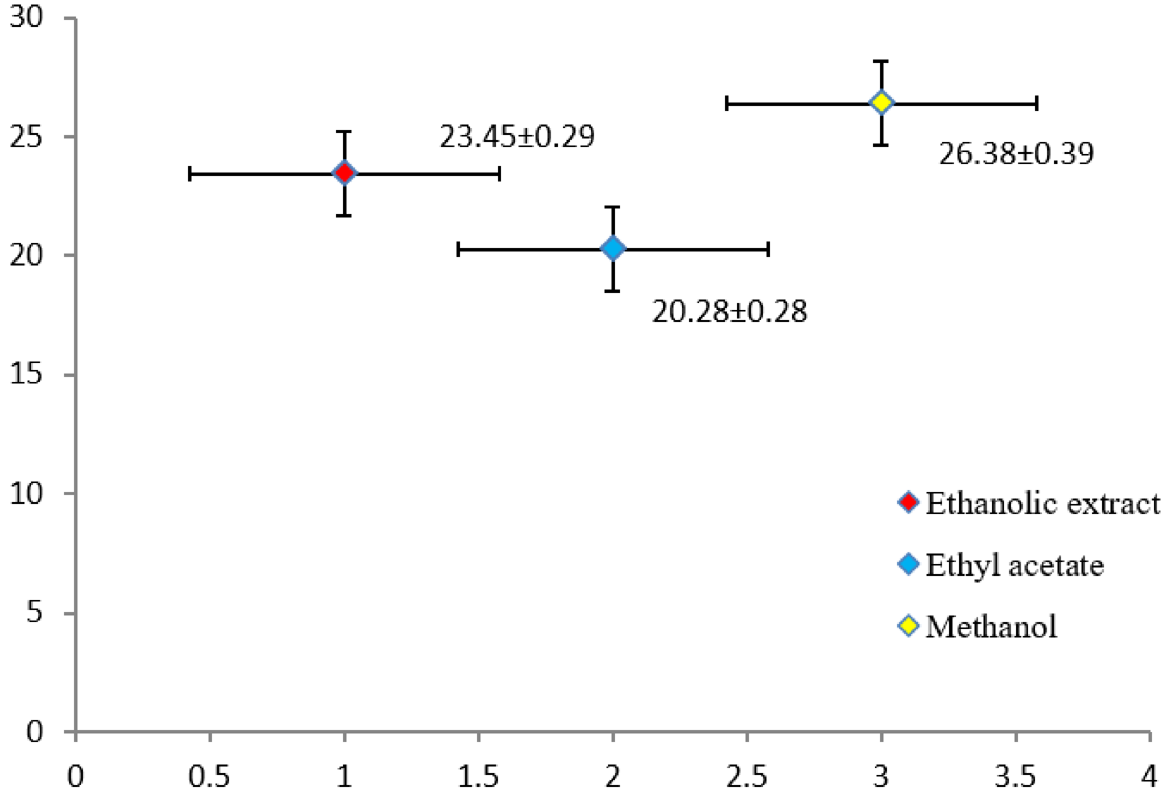


Figure 3. Antimicrobial Activity of (Methanol, Ethyl acetate and Ethanolic extract) of *Pimpinella anisum* against *Streptococcus pyogenes*

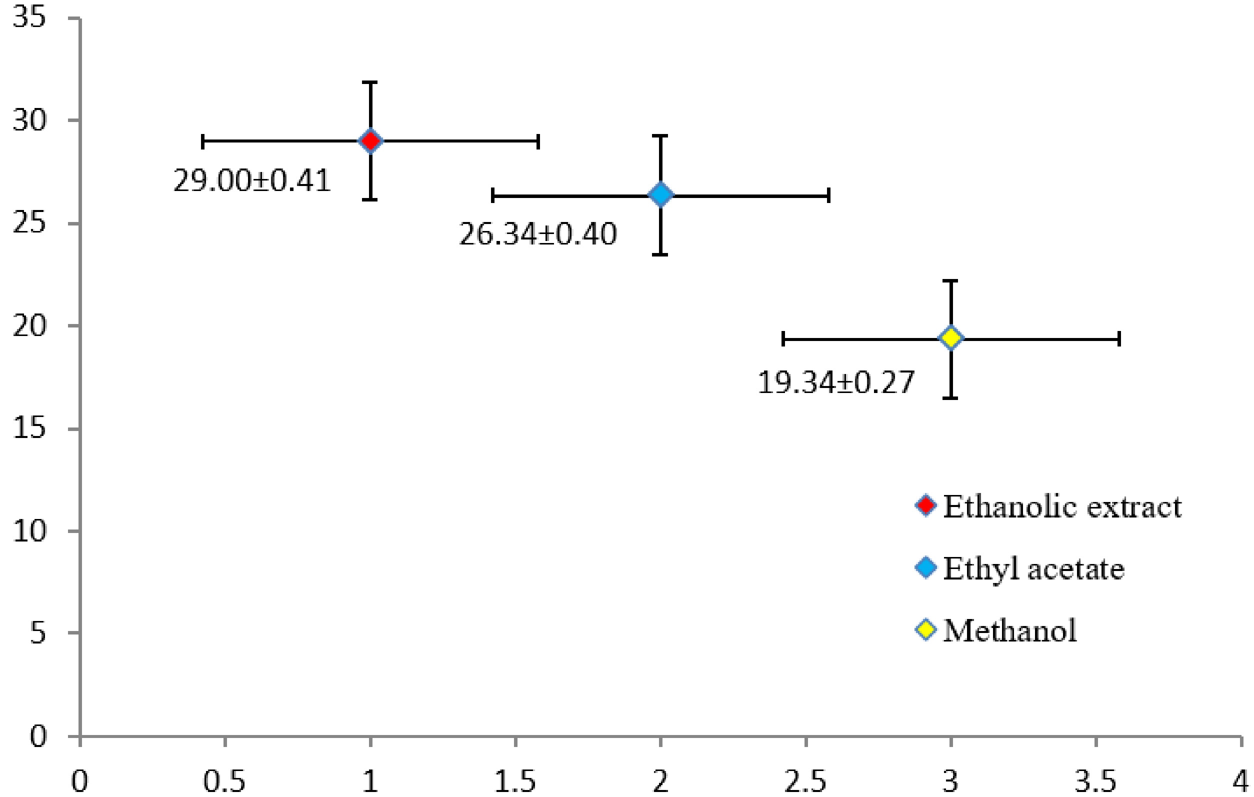


Figure 4. Antimicrobial Activity of (Methanol, Ethyl acetate and Ethanolic extract) of *Pimpinella anisum* against *Staphylococcus aureus*

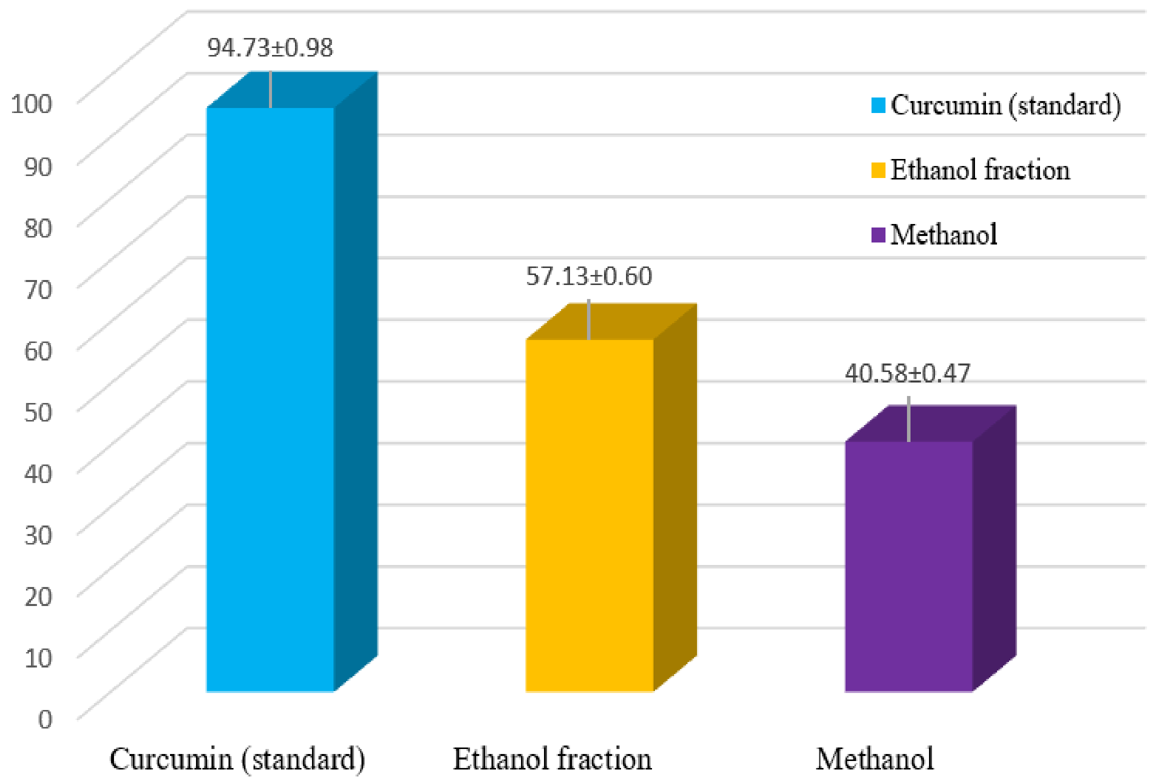


Figure 5. Antioxidant activity of Nitric oxide radical scavenging Methanol, Ethanol fraction and Curcumin (standard) of *Pimpinella anisum*

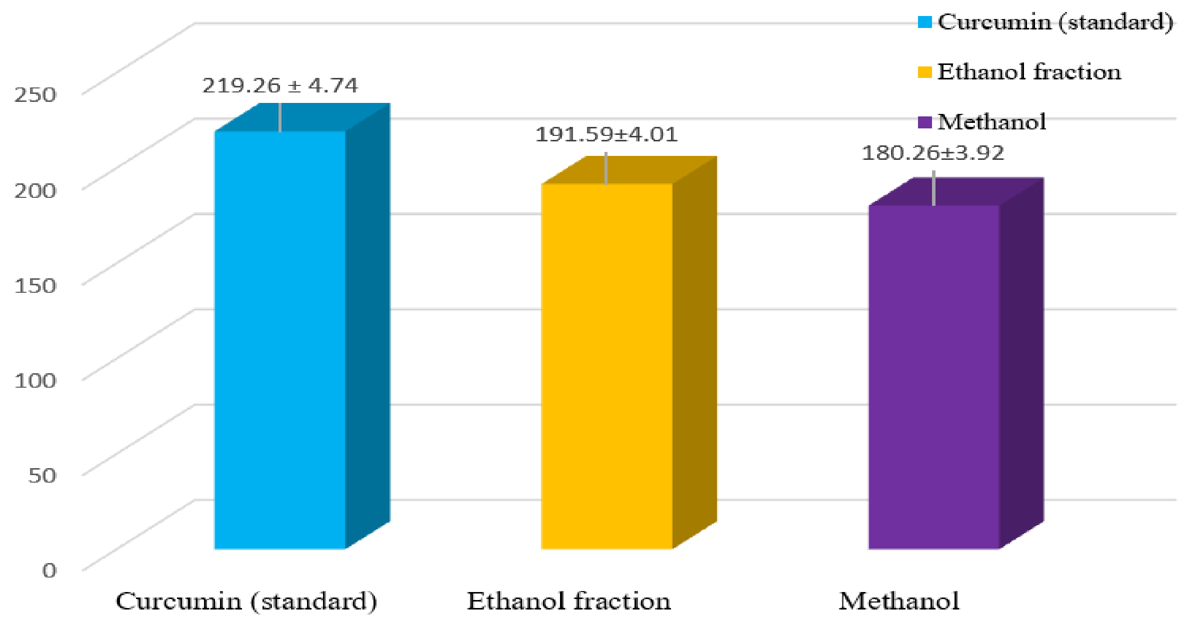


Figure 6. Antioxidant activity of Hypochlorous acid Methanol , Ethanol fraction and Ascorbic acid (standard) of *Pimpinella anisum*

Antimicrobial Activity of (Methanol, Ethyl acetate and Ethanolic extract) of *Pimpinella anisum* against Bacillus cereus recorded 29.34±0.45, 22.40±0.31 and 31.15± 0.48 respectively (Figure 1), while recorded 23.42±0.32, 18.47±0.25 and 28.13±0.42 respectively (Figure 2) in Enterococcus faecalis. In the same times antimicrobial activity of Pimpinella anisum against Streptococcus pyogenes and Staphylococcus aureus recorded 26.38±0.39, 20.28±0.28 and 23.45±0.29 and 19.34±0.27, 26.34±0.40 and 29.00±0.41 respectively (Figure 3 and 4) comparison with two standard antibiotics RF-Rifampicin 33.07±0.52 and AP-Ampicillin 35.08±0.57. Antibacterial activity against carbapenem-resistant enterobacter hormaechei (CREH) as effected by vanillic acid was investigated by measuring changes in the intracellular ATP level, the intracellular pH, and the membrane potential. Among the different species of Artemisia, it was revealed that they produce antibacterial metabolites. Further, in ethanolic extract, high amount of chlorogenic acid was detected in a tall species of the genus Asteraceae (A. gmelinii). Current studies reveal that chlorogenic acid binds to outer membrane, penetrates it, depletes the intracellular potential, and discharges macromolecules from cytoplasm, thus, killing a cell. People in the poor nations particularly in the developing world have been exposed to cultures, wherein people used herbs as medicine. The growing importance of new technologies and scientific investigation procedures could be seen as continues research in actions and medical values in phyto-active compounds of plants. In various pharmaceutical forms and packing as per the pharmacopoeia single herbal extracts in form of plant galenicals are marketed.

The antioxidant activity of different standards. The Pimpinella anisum in methanol, ethanol and standards, hypochlorous acid scavenging, nitric oxide radical scavenging. The different types of extracts present were; crude, Ethanol fraction, Standard Curcumin extracts at Nitric oxide radical scavenging 40.58±0.47, 57.13±0.60 and Curcumin (standard) 94.73±0.98 respectively. Percentage of Sequestration of hypochlorous acid were taken as 180.26±3.92, 191.59±4.01 and Ascorbic acid (standard) 219.26 ± 4.74 respectively. The results are shown in (Figure 5 and 6) showing greater %inhibition of crude and other fractions as compared to standard mannitol ($P<0.05$) against Hypochlorous acid activities. Process of contribution of nitric oxide in various inflammatory events is well established. This radical is toxic straight to tissues and the occurrence of a radical of this kind causes vascular collapse that is observed in septic shock. On the contrary, conditions that involve inflammations like ulcerative colitis, juvenile diabetes, multiple sclerosis, arthritis and carcinomas are characterized by the chronic nitric oxide radical.

Infections in burn wounds can be caused by a variety of bacteria. One of the most prevalent bacteria in nosocomial infections is Pseudomonas aeruginosa, a Gram-negative opportunistic pathogen. Bacteria are among the microbes that cause infections in burn wounds. The most important factor in burning wound infections is Pseudomonas aeruginosa, which is within the group of community and hospital acquired pathogens for burn units. Pseudomonas aeruginosa has developed remarkable resistance to many antibiotics, which has greatly restricted the options available for treating pseudomonal infections. The development of multidrug resistance (MDR) by P. aeruginosa has been remarkably expedited in recent years. It sparked a surge in the desire for novel treatments as it emerged as the most common multidrug-resistant bacterium. Pseudomonas aeruginosa is a multidrug-resistant (MDR) bacterium with a huge genome that can alter its outer membrane permeability, produce antibiotic-degrading enzymes, produce alginate, and transfer resistance genes, among other things. Persistent and frequently fatal infections are caused by P. aeruginosa because one of its defence mechanisms makes the bacterium harder to cure than its planktonic counterparts. Bacteria develop resistance to therapy through indirect modes of action, one of which is the creation of biofilms. High levels of antibiotic resistance in biofilms, non-responsive bacteria within the biofilms, and biofilm-specific resistance genes could all be explained by the physical barrier formed by encapsulating exopolymer substances. There needs to be a new way to get rid of bacterial biofilms because of all the characteristics shared by bacteria that become resistant to antibiotics. Regardless of these issues. In addition to being the subject of substantial research, these plants are also commercially lucrative because to their many uses in cosmetics, pharmacology, and agriculture (due to the large variety of antibacterial and antioxidant chemicals they contain). Before antibiotics and other modern medicines were available, people all over the world employed traditional remedies to cure infectious diseases and microbial pathogenicity. Aniseed, scientifically known as Pimpinella anisum, has several medicinal uses, including alleviating pain associated with osteoarthritis, gastritis, skin irritation, and toothaches. Not only does it fight the flu and HIV, but it also has chemopreventive, antibacterial, antiseptic, and insecticidal effects. The aforementioned extract contains a number of commonly found compounds, such as aniseed catechin, isochiapin, trans-anethole, estragole, trans-ferulic acid, and quercitin rhamnoside. Some think that secondary metabolites in plants play a significant role in their defence system against infections since they can stop microbes from growing and infecting plants. Analgesic, anti-inflammatory, antibacterial, and anticancer effects are exhibited by some active components of medicinal plants, such as alkaloids, terpenoids, and flavonoids. Natural plant substances are less hazardous than manmade pharmaceuticals, making them a safer alternative for treating illness.

Another possible treatment for MRSA is a combination of rosmarinic acid and vancomycin. Flavonoids and their glycosides provide multiple health benefits, as shown by numerous epidemiological studies. In spite of being produced in plants in reaction to microbial assaults, flavonoids have broad antibacterial efficacy in both laboratory and living organism settings. On the cellular level, there is more than one target. Our research revealed the presence of several flavonoids, including catechin, apegenin-7-O-glucoside, rutin, quercitin rhamnoside, kaempferol-O-rutinoside, trans-ferulic acid, eucalyptol, gibberellic acid, and isochiap. The eugenol rutinoside is found in a variety of plants and is a volatile phenolic chemical that belongs to the phenylpropene class. It also has antimicrobial activities. Eugenol has an antibacterial action against bacteria, according to previous study. This includes antibiotic-resistant bacteria like Acinetobacter baumannii and Staphylococcus ssp. Glycosylated eugenol has demonstrated antibacterial characteristics, such as an increased capacity to fight Staphylococcus spp. and E. coli, among others. Aniseed methanol extracts outperformed vitamin C in DPPH radical scavenging activities (IC50 of 17.92 g/mL). Findings revealed that fennel extracts had strong anti-radical characteristics. There is a lack of information regarding these aniseed chemicals. At dangerous levels, free radicals produced by the metabolic process known as oxidative stress can cause damage to the body. With its abundance of phenolic and flavonoid compounds, aniseed extract showed promise as an antioxidant. According to Danilenko et al., aniseed polar components have potential in the biopharmaceutical industry as antioxidants and antibiotics. The natural metabolites of specific lactobacilli exhibit antibacterial, antifungal, antioxidant, and anti-inflammatory properties; they can be utilised as a metabiotic in medicine as a replacement for plant extracts.

Conclusions:

Since the selected bacterial isolates were significantly suppressed by the anis extract, it suggests that this natural substance may become an attractive anti-virulence commodity for the treatment of infections caused by drug-resistant bacteria. There are phenolic chemicals in the extract, which are responsible for its antibacterial effect. In addition, the antibacterial activity may be due to this active ingredient, since its presence at higher concentrations suggests as much. Aniseed extracts have the ability to act as antioxidants, which suggests they may have health benefits. In addition, the compounds found in aniseeds have a synergistic effect with existing antibiotic treatments, suggesting that they could be a superior option for treating bacterial infections. It is more accurate to say that aniseed essential oils are rich in compounds with powerful antioxidant and antimicrobial properties. This might be because of the synergistic effects between various components or the high concentration of the main ingredients, which together produce an outstanding biocidal effect against harmful bacteria.

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