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ANTIBACTERIAL ACTIVITY OF AROMATIC HERBS AQUEOUS EXTRACTS: OREGANO *(ORIGANUM VULGARE* L.), TURMERIC (*CURCUMA LONGA* L.), CLOVES (*SYZYGIUM AROMATICUM* L.), AND CORIANDER (*CORIANDRUM SATIVUM* L.) ON *ESCHERICHIA COLI* ATCC 25922

Anna Batjuka*, Inta Umbraško, Aleksandrs Petjukevičs, Nataļja Škute

Batjuka A., Umbraško I., Petjukevičs A., Škute N. 2024. Antibacterial activity of aromatic herbs aqueous extracts: oregano (*Origanum vulgare* L.), turmeric (*Curcuma longa* L.), cloves (*Syzygium aromaticum* L.) and coriander (*Coriandrum sativum* L.) on *Escherichia coli* ATCC 25922. *Acta Biol. Univ. Daugavp., 2024(2): 309-320.*

Abstract
Aromatic herbs have been used for both culinary and medicinal purposes for centuries. The antimicrobial properties of medicinal plants have been intensively investigated, and many have been used as therapeutic alternatives because of their antimicrobial qualities. The quest for suitable and affordable options in the face of increasing antimicrobial drug resistance is to explore using plant extract to treat infections. The antibacterial properties of cloves (*Syzygium aromaticum* L.), coriander (*Coriander sativum* L.), turmeric (*Curcuma longa* L.), and oregano (*Origanum vulgare* L.) against the bacteria *Escherichia coli* bacteria were assessed using the disc diffusion method, and gentamicin was used as a control. The results showed that aqueous extracts of aromatic herbs demonstrated different antibacterial activities against the test culture, which varies depending on the plant species. The zones of inhibition exhibited by aqueous extracts against the test organism ranged from 6.90 to 9.59 mm. The results of the current study revealed that cloves have the lowest pH (4.16), the highest oxidation-reduction potentials (Eh) (172.50 mV), and the largest inhibition zone (9.59) indicating the best properties to inhibit the growth of *E. coli* bacteria. This study reveals that the aqueous extracts of tested aromatic herbs have different antibacterial potentials against the selected organism and may be of great use to pharmaceutical industries in developing medicine to cure ailments. However, human clinical trials and quality control studies are needed to establish effective and safe doses.

Keywords: Redox potential, *E. coli*, aromatic herbs, antimicrobial effect, pH.

*Corresponding author: *Anna Batjuka. Department of Ecology, Institute of Life Sciences and Technologies, Daugavpils University, Parades Str. 1A, LV-5401, Daugavpils, Latvia. E-mail: anna.batjuka@du.lv https://orcid.org/0009-0009-0597-7906*

Inta Umbraško. Department of Ecology, Institute of Life Sciences and Technologies, Daugavpils University, Parades Str. 1A, LV-5401, Daugavpils, Latvia. https://orcid.org/0009-0009-5792-9033

Aleksandrs Petjukevičs. Department of Ecology, Institute of Life Sciences and Technologies, Daugavpils University, Parades Str. 1A, LV-5401, Daugavpils, Latvia. https://orcid.org/0000-0001-6917-2677

Nataļja Škute. Department of Ecology, Institute of Life Sciences and Technologies, Daugavpils University, Parades Str. 1A, LV-5401, Daugavpils, Latvia. ORCID: https://orcid.org/0000-0002-3584-0347

INTRODUCTION

Aromatic herbs have been used by people as an integral part of the culinary culture around the world for flavoring, coloring, and preserving food as well as for medicinal purposes. Aromatic herbs enhance the flavor, aroma, color of food and beverages, and also decide on their role in the prevention of acute and chronic diseases. Aromatic herbs help people maintain health and good mood. It has been reported that about 80% of the world's population depends on medicinal plants and their phytoconstituents (bioactive compounds) for their primary health care (Iweala et al. 2023). The preference for the use of medicinal herbs over orthodox medicines may be due to the efficacies of their bioactive agents as well as other contexts such as accessibility, affordability, availability, and their acclaimed less toxic effects.

Aromatic herbs such as cloves, coriander, rosemary, sage, turmeric, oregano, and cinnamon are excellent sources of antioxidants due to their high levels of phenolic compounds. On the other hand, recent findings evidence that aromatic herbs possess antioxidant, anti-inflammatory, antitumorigenic, anticarcinogenic, and glucose- and cholesterol-lowering activities as well as properties that affect cognition and mood (Alsulaim et al. 2024). Research over the past few decades has demonstrated the wide range of health benefits they possess due to their bioactive components, including sulphur-containing compounds, alkaloids, phenolic diterpenes, condensed tannins, and vitamins, especially flavonoids, and polyphenols. Aromatic herbs have been widely studied in different countries due to their beneficial effects on human health.

The scientific community has been paying plentiful attention to the biological activities of these plants, namely due to the interest in compounds, their mechanism of action, and bioavailability which are largely consumed as a dietary component and supplement all around the world. The long historical use of aromatic herbs for medicinal purposes is fully acknowledged, and there are a growing number of reports describing the benefits to society.

Curcuma longa L.*,* commonly known as turmeric is one of the most consuming rhizomatous herbaceous perennial aromatic herbs belonging to the Zingiberaceae family and is extensively cultivated in tropical areas of Southeast Asia and India. The roots of turmeric have been widely used as a spice in cooking for a long time, especially in Asian countries. The typical yellow color of turmeric is due to curcuminoids, a class of phenolic compounds in turmeric. The therapeutic properties of *Curcuma longa* L. are attributed to existing polyphenolic curcuminoid compounds, especially diferuloymethane or curcumin which are commonly present in the rhizomes of *Curcuma longa* L. (Paultre et al. 2020, Razavi et al. 2021). This pleiotropic compound is a yellow-colored and crystallisable powder with high hydrophobicity that shows poor solubility in the aqueous phase. Turmeric extract is used in traditional medicine as an efficient drug for psoriasis therapy, inflammation, gum pains, snake bites, and scorpion stings (Cheraghipour et al. 2018).

It is also used for various diseases including dyslipidemia, stomach and skin disorders, diabetes, arthritis, and hepatic diseases (Ahmad et al. 2020). It promotes blood circulation, removes stagnation, alleviates depression, and serves as a natural flavoring agent that strongly affects food's color, taste, and nature (Masella & Cirulli 2022).

Moreover, curcumin has low inherent toxicity and various properties with great impact and applications on a wide range of pharmacological developments, including anti-osteoarthritis, anti-inflammatory, antioxidant, hepatoprotective, neuroprotective, antidiabetic, antiviral, antidiarrheal, antimicrobial, anti-atherosclerotic, anti-arthritic, antidepressant (Cheraghipour et al. 2018, Sharifi-Rad et al. 2020). The use of turmeric in traditional medicine is supported by the presence of more than 300 bioactive components such as polyphenols, sesquiterpenes, diterpenes, triterpenoids, sterols, and alkaloids (Iweala et al. 2023). Coriander (*Coriandrum sativum* L.) belonging to the Apiceae family, is a widely used herbal plant due to its medicinal and biological properties. It is used as a carminative in the treatment of dyspepsia, flatulence, and diarrhea, for the relief of respiratory and urinary problems, and as an antiemetic remedy (Scandar et al. 2023). For centuries it has been used in culinary as spice or seasoning as well as ingredient in perfumes and cosmetics in many regions of the world. This versatility of using the coriander plant is due to the heterogeneous chemical composition that it possesses.

Syzygium aromaticum L., commonly called clove, is a common culinary spice for pastries, condiments, and sauces with its essential oil also used in medicine, particularly in the preparation of gums and teeth (Pandey et al. 2024). Pharmacologically, cloves are considered a major source of phenolic acids such as hydroxybenzoic acids, flavonoids, hydroxyphenyl propens, hydroxycinnamic acids, and eugenol which is the main bioactive molecule, as well as gallic acid derivatives such as hydrolysable tannins, which are found in large quantities in fresh form (Batiha et al. 2020).

Oregano (*Origanum vulgare* L.) is a herb known in the Mediterranean diet that has several bioactive properties including antioxidant, antimicrobial, anti-inflammatory, and analgesic properties and the most common compounds present in its essential oil include diterpenes, carvacrols, and thymols (Veenstra & Johnson 2019). In recent times, there has been an increased impetus to reconnoiter the medicinal properties of oregano.

Despite its global significance, medicinal herbs and their bioactive constituents have received enormous research attention from various disciplines leading to their exploitation as natural therapeutic agents in developing new drugs and pharmacological products in the treatment of various diseases.

The antimicrobial properties of plant extracts exert their inhibitory effects on the microorganism through multiple mechanisms, including inhibition of protein synthesis, cell wall disruption, nucleic acid inhibition and possible influence on processes in epigenetics regulation in cells. These properties act on ribosomal subunits to disrupt protein folding, thereby interfering with microbial activity. They can also disrupt the integrity of the cell wall, leading to increased membrane permeability, leakage of intracellular components, DNA damage, and possible influence on processes in epigenetics regulation in cells, like methylation level changes and others. (Petjukevičs et al. 2017, Savicka et al. 2018, Škute et al. 2020, Lobo et al. 2021, Umbrasko et al. 2022).

Therefore, the aim of this study was a better understanding of the effectiveness of the antibacterial activity of different spices extracts: coriander (*Coriander sativum* L.), cloves (*Syzygium aromaticum* L.), turmeric (*Curcuma longa* L.), and oregano (*Origanum vulgare* L.) on the growth of *Escherichia coli*.

MATERIAL AND METHODS

Plant material and preparation of aqueous extracts

Four varieties of dried spices were used to obtain samples for the study: cloves (*Syzygium aromaticum* L.), coriander (*Coriander sativum* L.), turmeric (*Curcuma longa* L.), and oregano (*Origanum vulgare* L.). Two grams of material from each spice sample were taken and grounded to a fine powder using CryoMill (Retch, Finland), after that, the powder of herbs was mixed with 10 mL of sterile distilled water at room temperature, and then centrifuged at 3000 rpm for 15 min. Each sample was transferred into sterile laboratory flasks and the resulting mixture was sterilized (HSP Laboklav Steriltechnik AG, Germany) for 5 minutes at 100°C. The extracts were appropriately labeled and stored in a refrigerator for further analysis. In the present study, the antibiotic gentamicin was used to compare the bacterial zones.

Antimicrobial Agent use as a control

Gentamicin is a transparent, colorless, or slightly greenish-yellow liquid that is characterized by a broad spectrum of antimicrobial bactericidal action (Heydariyan et al. 2023). It is effective against many gram-negative and gram-positive microorganisms and proved to be highly effective against aerobic gram-negative bacteria: *E. coli*. There are contraindications to the use of gentamicin and its side effects. Furthermore, microorganism resistance to gentamicin develops slowly and does not act on anaerobes, fungi, viruses, or protozoa.

The aminoglycoside gentamicin as antibiotic was used as a control for susceptibility to *Escherichia coli*, for which the zone of diameter interpretive Stds (mm) were interpreted as resistant ≤12, intermediate 13-14, susceptible ≥15 (CLSI guidelines – Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated of Fastidious Bacteria).

Control strains used to monitor accuracy of Disk Diffusion testing

A control strain of the *Escherichia coli* bacteria (Enterobacteriaceae): ATCC 25922 was used for the experimental study. The organism groups covered in that manuscript are Enterobacteriaceae a large family of Gram-negative bacteria. ATCC appeared as a registered trademark of the American Type Culture Collection. Strains sored in the ATCC International Strain Collection are used as standard reference strains worldwide. We take a culture *Escherichia coli* (Enterobacteriaceae) bacteria (ATCC 25922) were inoculated into 9 ml of BRAIN HEART INFUSION BROTH (BioMaxima, Poland). The tubes were placed in a thermostat for 24 hours at a temperature of +25°C. Then 1 ml of the inoculum was seeded on a dense agar medium Plate Count LabAGAR (BioMaxima, Poland) by the surface method and cultivated at 25°C for 48 hours (refrigerated incubator FTC 90E). After 48 hours of cultivation, the culture gave abundant growth (Umbrasko et al. 2024). The nutrient agar powder Mueller-Hinton Agar (ThermoFisher Scientific, US) was used. Mueller–Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing, specifically disk diffusion tests, and contains:

- 2.0 g beef extract
- 17.5 g casein hydrolysate
- 1.5 g starch
- 17.0 g agar
- 1 L of distilled water.
- pH adjusted to 7.4 at 25°C.

Mueller-Hinton agar (MHA) is primarily used for antimicrobial susceptibility testing (AST) and has become the standard medium for the Bauer-Kirby method. To minimize or eliminate variability in this testing, (Batiha et al., 2020). developed a standardized procedure in which Mueller Hinton Agar was chosen as the test medium.

Preparation of susceptibility Antibiotic Assay Discs and antibacterial susceptibility testing of plant extracts

Using a paper punch, 5 mm diameter disks were cut using Whatman® (UK) filter paper (No. 1). The disks were sterilized by autoclaving at 121 °C for 15 minutes (HSP Laboklav Steriltechnik AG, Germany) and then stored in a dry sterile place until use. Impregnation was achieved by placing four sterile paper disks in the prepared spice extract and then placed on Mueller–Hinton agar (Thermo Fisher Scientific, US) with bacteria colonies using sterile tweezers. Petri dishes were incubated aerobically at 37 °C for 24 h and then the antibacterial activity was assessed by measuring the zone of inhibition (mm). The boundary of the zone should be considered to be the area where there is no obvious visible growth that can be detected with the unaided eye. The antibacterial activity was determined using the disk diffusion method with some modifications. The average result was calculated with three replicates for each treatment using Acquisition & Analysis software Ver. 8.20 (Vision Works, US).

pH determination

The pH values of the plant aqueous extracts were measured with a pH meter equipped with inLab® Expert Pro electrode (Mettler Toledo, Germany). 2 grams of plant material powder was mixed with 10 mL of autoclaved, distilled water, mixed well with a magnetic stirrer for 10 minutes at room temperature. After that, the mixture was filtered. The pH reading was taken by placing the electrode of the pH meter into aqueous solutions. A pH value below 7.0 indicated the presence of organic acids in the solutions.

Redox potential (mV) measurements

Redox potential or Oxidation-reduction potentials (Eh) measurements were carried out on the four chosen media, as detailed above using Mettler Toledo pH meter equipped with inLab® Expert Pro electrode (Germany). The electrode was used according to the producer's instructions. Before use, the electrode was rinsed with bi-distilled water. When making measurements, the electrode was carefully placed in a vial containing the chosen media; a sufficient liquid sample must cover the sensing element. The electrode was carefully stirred and then placed in a fixed position, slightly above the bottom of the container.

Data processing and analysis

Each experiment in this study was done in triplicates, and each data was reported in mean values and standard deviations (mean ± SD). The measurement error was in the range of +/- 0.04-0.11 mm. Microsoft Excel Ver. 14.0.7214.5 (US) was used for the data processing.

RESULTS AND DISCUSSION

Escherichia coli (*E. coli*) are gram-negative, non-spore-forming, motile rods with peritrichous flagellation, and facultative anaerobes, capable of obtaining energy through fermentation and respiration. This bacterium of the genus *Escherichia* is widely distributed in many places in the environment, including the gastrointestinal tract of humans and warm-blooded organisms, where it is part of the intestinal microbiota. *E. coli* has a lipopolysaccharide layer attached to the outer membrane with a hydrophobic bond, so it might be more resistant to antimicrobials. *E. coli* has been established as a highly effective model system in molecular and cellular biology. It should be noted that gentamicin is a broad-spectrum antibiotic and is highly effective against various types of gram-negative microorganisms: *Escherichia coli*, *Proteus* spp. (both indole-positive and indole-negative), *Pseudomonas aeruginosa*, *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp., *Salmonella* spp., *Shigella* spp., and *Staphylococcus* spp. (including penicillin- and methicillin-resistant strains). However, Gentamicin also has several significant contraindications. It was chosen for the study in an attempt to find natural antibiotics with effective antimicrobial properties. This antibiotic has many side effects, including hypersensitivity to gentamicin and other aminoglycoside antibiotics, which can cause auditory neuritis, severe renal dysfunction, uremia, and affect pregnancy and lactation (breastfeeding). Gentamicin is contraindicated during pregnancy. Therefore, there is a need to find alternative antimicrobial drugs with less toxicity.

The ability of each bacterium to fight antibacterial activity varies depending on the thickness and composition of the cell wall (Godoy-Gallardo et al. 2021). However, the effectiveness of plant extracts sometimes changes after the separation and purification process so it can be said that the efficacy of plant extracts may vary depending on the solvent type and method of extraction (Al-Manhel & Niamah 2015). The researchers' attention is focused on identifying factors that could influence the occurrence of inhibitory zones in aqueous extracts, such as the diffusion capacity of the antimicrobial materials into the media and their interaction with the tested microbes, the number of tested microbes, the growth rate of the tested microbes, and the level of microbial sensitivity to antimicrobial materials.

Our study's results show that aqueous extracts of cloves (*Syzygium aromaticum* L.), turmeric (*Curcuma longa* L.), oregano (*Origanum vulgare* L), coriander (*Coriander sativum* L.), and the antibiotic gentamicin had different antibacterial activity against *E. coli*, with zones of inhibition ranging from 6.90 mm to 23.81 mm. The observation of antibacterial activity in aqueous extracts on opportunistic bacteria using the agar diffusion method showed that the growth inhibition zones were 9.59 mm for cloves (*Syzygium aromaticum* L.), 7.56 mm for turmeric (*Curcuma longa* L.), 7.09 mm for oregano (*Origanum vulgare* L.), 6.90 mm for coriander (*Coriander sativum* L.) and 23.81 mm for antibiotic gentamicin. The results of the research are represented in Figure 1.

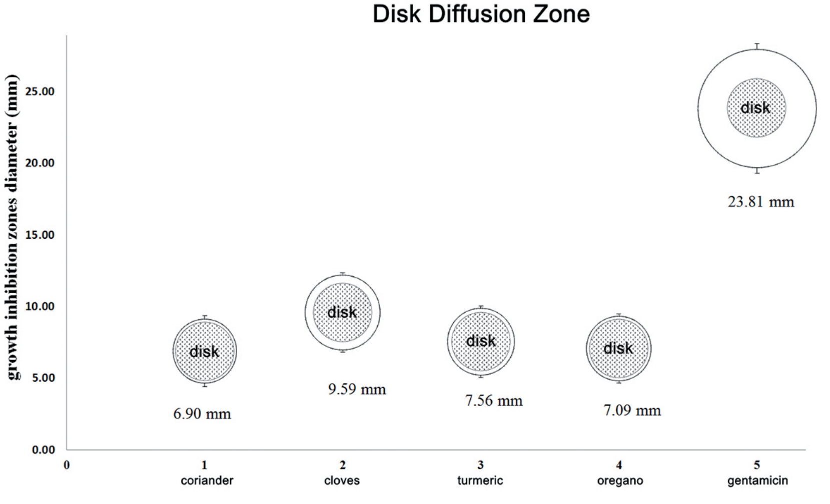

Figure 1. Zones of suppression of bacteria *Escherichia coli* antibacterial activity by various aromatic herbs aqueous extracts: 1 – coriander (Coriander sativum L.) 2 - cloves (*Syzygium aromaticum*) 3 - turmeric (*Curcuma longa* L.) 4 - oregano (*Origanum vulgare*) 5 - standard drug (gentamicin) by the disk diffusion assay. Data are expressed as mean ± SE (diameter inhibition mm).

The antibacterial activity of aqueous extracts of these tested plants may be attributed to the presence of anionic components such as thiocyanates, nitrates, chlorides, and sulfates as well as many other bioactive compounds naturally present in plants that can act alone or in synergy, as demonstrated in different research fields (Darout et al. 2000, Razavi et al. 2021). (Gengatharan & Rahim 2023) found that cloves (Syzygium aromaticum L.) contain flavones as well as phenolic constituents, which have an important role in discouraging the growth of bacteria that influence on the inhibition of the enzymes responsible for the metabolic basis and interfere with interactions with proteins, leading to the metamorphosis of proteins and then the inability of bacteria to continue their activities. In turn, cloves contain also other bioactive compounds such as flavonoids, eugenols, and other phenolic compound types as well as hydroxybenzoic acids and hydroxycinnamic acids, which have instrumental roles in increasing the antimicrobial effectiveness and these results are consistent with a study by (Zhang et al. 2021) which shows that the high acid works to change the nature of living material, in particular proteins in the cell membrane through the process and deformed proteins that lose their function leading to a crash in the cell membrane of bacteria. In addition to this, (Odo et al. 2023, Dai et al. 2022) reported that turmeric was effective against E. coli, possibly due to the presence of curcuminoid, a phenolic compound. Turmeric root extract (Curcuma longa L.), contains as a main active ingredient is curcuminoids, and is widely used as an anti-inflammatory agent. Furthermore, (Ahmad et al. 2020) reported that tumerone and curlone components of turmeric possessed better antibacterial activity against a wide range of microbes including B. subtilis, S. aureus, B. cereus, B. coagulans, E. coli and P. aeruginosa that is reported to be effective against carcinogenesis. The antimicrobial activity of turmeric is also reported to be due to the presence of active components including essential oils, curcumins, curcuminoids, turmeric oils, and valeric acids (Razavi et al. 2021, Azhari et al. 2018). This is probably the reason some people use these spices for the treatment of bacterial infections and also spices have garnered increasing significance in recent times as promising reservoirs of natural food preservatives.

Oxidation-reduction potentials (Eh) and acid-base reactions (pH) are essential for the maintenance of the energy functioning of cells and strongly affect plant metabolism and catabolism of microorganisms (Husson 2013). It is known that each type of microorganism is adapted to certain oxidation-reduction (Eh) conditions and is characterized by the ability to develop in a wider or narrower Eh range. For example, anaerobic bacteria can only grow in a narrow range of very low Eh values (Husson 2013). However, aerobic microorganisms, such as Azotobacter sp. or Actinomyces sp., require a higher Eh but can grow in a much wider range (Rabotnova & Schwartz 1962). Moreover, fungi grow more than bacteria under moderately reducing conditions (Eh> 250 mV), whereas bacteria are more abundant than fungi and grow under highly reducing conditions (Eh< 0 mV) (Seo & DeLaune 2010). pH is considered also as the main variable but Eh and pH respectively jointly affect the development of microorganisms. One of the proven indicators (pH) suggests that the effectiveness of the solution's action on the microorganism is dependent on this factor. Increased acidity possibly enhances bacterial inhibition. The antibiotic gentamicin is also acidic (pH). The obtained results show that the oxidation-reduction potential (Eh) was 172.50 mV for the tested plant aqueous extract of cloves (Syzygium aromaticum L.), 24.00 mV for turmeric (Curcuma longa L.), 74.68 mV for oregano (Origanum vulgare L.), 56.50 mV for coriander (Coriander sativum L.) (Fig. 2). It is well known that plant extracts with good antibacterial activity have high levels of total polyphenols and titratable acidity, as well as low pH values (Oulahal & Degraeve 2022). Bacterial growth is directly related to changes in Eh (Kimbrough et al. 2006) and E. coli growth bacteria are inhibited by decreasing pH. It is well known that E. coli is adapted to certain Eh and pH conditions and can grow only in certain ranges.

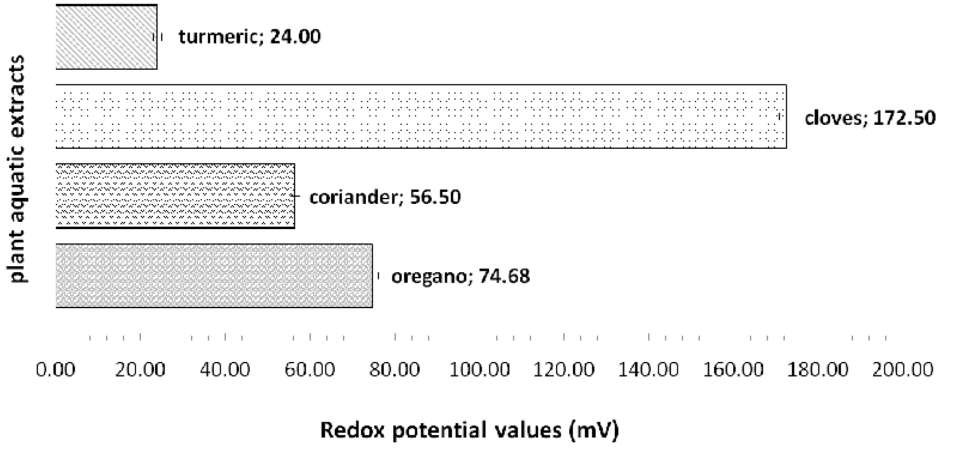

Figure 2. The pH values of plant aquatic extracts: 1 – coriander (*Coriander sativum* L.) 2 - cloves (*Syzygium aromaticum* L.) 3 - turmeric (*Curcuma longa* L.) 4 - oregano (*Origanum vulgare*). Data are expressed as mean ± SE.

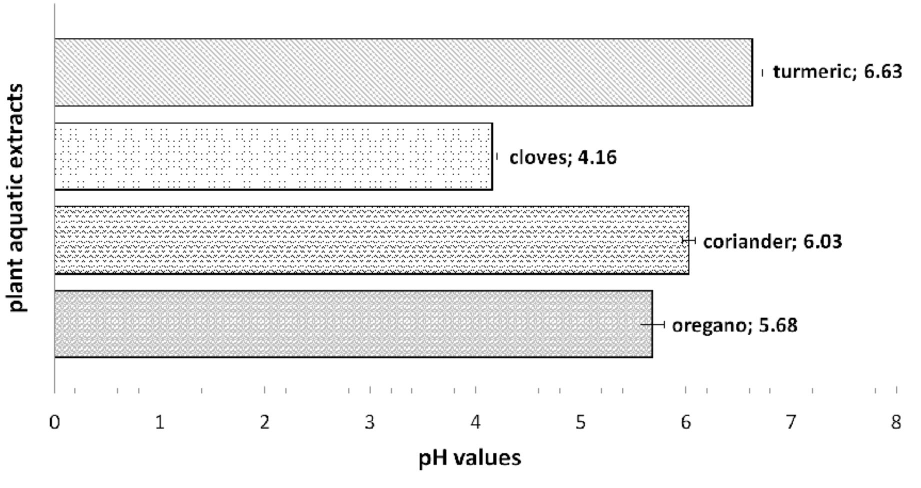

Figure 3. The redox potential values of plant aquatic extracts: 1 coriander (*Coriander sativum* L.) 2 - cloves (*Syzygium aromaticum*) 3 - turmeric (*Curcuma longa* L.) 4 - oregano (*Origanum vulgare*). Data are expressed as mean ± SE.

Our results indicate that the acid-base reactions (pH) were 4.16 for cloves (*Syzygium aromaticum* L.), 6.63 for turmeric (*Curcuma longa* L.), and 5.68 for oregano (*Origanum vulgare* L.), 6.03 for coriander (*Coriander sativum* L.) (Fig. 3). Obtained results indicated that cloves have the lowest pH 4.16, the highest oxidation-reduction potentials (Eh) 172.50 mV and the largest inhibition zone 9.59 mm indicating the best properties to inhibit the growth of *E. coli* bacteria. These results are similar to the results of many who have studied the plant's leaf extracts in inhibiting the growth of many organisms (Mostafa et al. 2018, Ceruso et al. 2020). At the same time, the results of our previous investigations revealed that the aqueous extracts of traditional medicinal plants: common thyme (*Thymus vulgaris* L.), pot marigold (*Calendula officinalis* L.), and common wormwood (*Artemisia absinthium* L.) have noticeable antifungal effect against yeast *Saccharomyces cerevisiae* (Umbrasko et al. 2024).

(Khatri et al. 2023) suggested that the mechanism of antibacterial action of spices involves hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, membrane disruption and disruption of the electron transport system, and cell wall destruction. If the development of microorganisms leads to undesirable consequences, then it must be restrained. In the case of infectious diseases, it is necessary to suppress microbial activity, antimicrobial drugs are used. Among antimicrobial agents, an important place is occupied by antibiotics - special low-molecular products of cellular activity, possessing high physiological activity. Each antibiotic suppresses or destroys a certain range of organisms sensitive to it. However, the increased use of antibiotics is accompanied by the emergence of an increasing number of pathogenic microorganisms resistant to these compounds. Currently, there is great interest in herbal medicine (from the ancient Greek φυτόν "plant" + θεραπεία «treatment»), which is a method of treating and preventing diseases in humans and animals based on the use of medicinal plants. Many plants synthesize substances that are beneficial for maintaining the health of people and animals. These include, in particular, aromatic substances. Many people believe that the «naturalness» of herbal preparations, their «natural» origin, as well as the long history of their use in folk medicine, can serve as a guarantee of their safety and effectiveness. Further studies could be conducted to examine other bacteria, including gram-positive bacteria, with appropriate conclusions drawn in subsequent studies especially gram-positive bacteria which are quite diverse.

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