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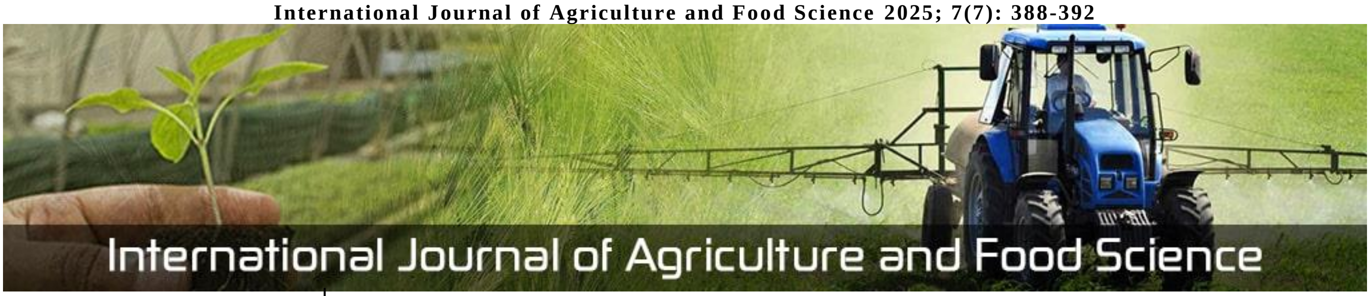

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Study of the effectiveness of nano-Arugula oil in inhibiting pathogenic bacteria and its antioxidant activity

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Abstract
The study aimed to evaluate the bioactivity of nano-Arugula Oil (Arugula sativa) in terms of antibacterial and antioxidant activity using laboratory-validated techniques. The results showed that nano-Arugula Oilpossessed significant inhibitory activity against the tested bacterial isolates, recording the highest activity against *Escherichia coli* with an inhibition diameter of 16 mm, followed by *Staphylococcus aureus* (14 mm), while *Salmonella typhi* showed the least sensitivity with an inhibition diameter of 12 mm. This variation in response is attributed to biochemical differences in cell walls and resistance mechanisms, as well as the ability of the active nanocomposites in the oil to penetrate cell membranes. Regarding the antioxidant activity, the results showed a gradual decrease in absorbance (Abs) and an increase in the inhibition rate with increasing oil concentration from 25 to 100 µg/mL, where the maximum inhibition rate reached 88.54% at the highest concentration. These results reflect the presence of strong antioxidant activity attributed to the oil content of phenolic compounds and flavonoids, which work to inhibit free radicals by donating electrons or hydrogen atoms.

Keywords: Nano Arugula oil, inhibitory activity, antioxidant activity

Introduction
The Importance of Medicinal Plants
Medicinal flowers are a few of the most critical organic assets used because ancient times to deal with sicknesses and promote preferred health. They include effective herbal compounds consisting of phenols, alkaloids, flavonoids, and volatile oils, which are characterized with the aid of their antimicrobial, antioxidant, and anti-inflammatory homes Medicinal flora remain utilized in people and contemporary medicinal drug as a number one supply for the discovery of recent drugs, in particular with the developing resistance of microbes to conventional antibiotics (Sasidharan *et al*., 2011) [1].Watercress (*Arugula sativa*) is an important medicinal plant belonging to the Brassicaceae circle of relatives it is rich in energetic compounds together with glucosinolates, flavonoids, and carotenoids, further to its excessive content material of vitamins and minerals. Multiple studies have tested that watercress extracts possess awesome organic interest, along with antioxidant, antibacterial, and anticancer results, making it a promising candidate for use in meals and pharmaceutical packages (Akinmoladun *et al*., 2010; D'Antuono *et al*., 2008) [2, 3].

Arugula oil and its medicinal uses
Arugula oil is extracted from the seeds of the watercress plant. It is a vegetable oil rich in biologically energetic compounds, which encompass unsaturated fatty acids, isothiocyanates, nutrients A, C, and E, as well as natural antioxidants along with flavonoids and carotenoids. The significance of this oil lies in its precise chemical composition, which offers it precious medicinal and nutritional houses. Recent research mean that watercress oil possesses antimicrobial hobby, demonstrating clean effectiveness closer to several sorts of pathogenic bacteria which incorporates *Staphylococcus aureus* and *Escherichia coli*. The oil moreover possesses antioxidant houses that help lessen oxidative strain and therefore shield cells from unfastened radical harm, highlighting its preventive role in persistent diseases consisting of coronary heart disorder and cancer (Youssef *et al*., 2021; Mohammed *et al*., 2022) [4, 5].In

addition, Arugula Oilhas shown anti-inflammatory and analgesic results in animal fashions, which supports its use in opportunity medication to deal with inflammatory situations. It is likewise used topically in people medicinal drug to improve hair and pores and skin fitness because of its nutrition and antioxidant content material (El-Sayed *et al*., 2019) [6].

Nano technology and its Application in Improving the Efficiency of Vegetable Oils

Nano technology is defined because the technological know-how, engineering, and design of substances on the nanometer scale (1-100 nm). Materials in this range exhibit unique bodily, chemical, and organic residences which can be basically specific from their properties in the traditional bulk nation. Nanotechnology is widely used in the fields of medicine, pharmacy, agriculture, and the meals industry, due to its capability to improve the bioactivity of energetic substances and gain particular focused on (Wang *et al*., 2021) [7]. In the sector of vegetable oils, nanotechnology has contributed to improving oxidative stability, antioxidant activity, and antibacterial homes through reworking herbal oils into nanoemulsions or nanocapsules. These systems shield active compounds from degradation and increase their water solubility and bioabsorption inside the body, which complements their biological efficiency when used topically or orally (Salvia-Trujillo *et al*., 2020; Ali *et al*., 2023) [8, 9]. Numerous research have proven that vegetable oils of their nanoform showcase a higher potential to face up to unfastened radicals and pathogens, compared to their conventional shape, which complements their use in therapeutic, preventive, and pharmaceutical fields (Sharma *et al*., 2021) [10].

Pathogenic bacteria and their health impact

Pathogenic bacteria are a number of the maximum prominent reasons of infectious illnesses in human beings and animals. They pose a developing global health threat, especially with the growing resistance of many lines to standard antibiotics. These bacteria encompass an extensive range of species, along with *Escherichia coli, Staphylococcus aureus*, *Salmonella spp.*, *Pseudomonas aeruginosa*, *and Klebsiella pneumoniae*, which could cause serious infections of the gastrointestinal, respiratory, urinary, and skin structures, as well as sepsis and death in excessive instances (Ventola, 2020) [11]. The indiscriminate use of antibiotics in human, veterinary, and agricultural medicinal drug has brought about the emergence of distinctly resistant strains, referred to as antimicrobial resistance (AMR). The World Health Organization (WHO) has categorized bacterial resistance as a public fitness emergency that threatens the success of cutting-edge treatments and will increase contamination-related mortality prices. (WHO, 2022) [18] Recent studies suggest that the search for powerful natural options, such as plant oils and nanocomposites, might also represent a promising strategy to address these challenges. Some critical and plant oils have verified inhibitory interest in opposition to the growth of drug-resistant pathogenic microorganism, without causing extensive toxicity (Liu *et al*., 2023) [19].

The importance of antioxidants in reducing oxidative stress

Oxidative stress is a physiological state on account of an imbalance between the manufacturing of loose radicals (ROS) and the body's capacity to neutralize them with antioxidants. When free radicals gather uncontrolled within cells, they purpose damage to proteins, lipids, and DNA, main to the improvement of many continual sicknesses inclusive of cancer, coronary heart ailment, diabetes, and neurological disorders (Liguori *et al*., 2020) [20]. Antioxidants play a key position in mobile protection in opposition to oxidative damage by means of inhibiting unfastened radical activity and preventing harmful oxidative reactions. They include natural antioxidants inside the frame (which include glutathione and superoxide dismutase) in addition to exogenous antioxidants acquired from meals which includes vitamins C and E, flavonoids, and polyphenols extracted from plants (Pham-Huy *et al*., 2022) [21]. Recent research have shown that reliance on plant antioxidants is a promising course in preventive and healing remedy, specially given that those compounds show off effective organic interest thru a couple of mechanisms, without inflicting substantial mobile toxicity. Therefore, the usage of antioxidants within the prevention of diseases related to oxidative stress has grown to be a growing recognition of hobby in modern-day clinical and dietary research (Saini *et al*., 2023) [22].

The importance of this study is with the ability to publish nano-rocate oil as a multifunctional natural compound in nano technology, which can contribute to the development of safe and effective options of traditional antibiotics and synthetic anti-Ox kissants, supporting public health trends and pharmaceutical applications.

Materials and Methods

Preparation of nano-Arugula Oil loaded with silver nano particles (AgNPs)

In the southern Iraqi city of Amarah, we purchased Arugula t (*Arugula sativa*) seeds from local markets. After careful cleaning to eliminate any dust and contaminants, the oil was extracted from the seeds using a hydraulic press by means of a cold-press method. To obtain pure oil free from impurities, it was filtered using a fine mesh cloth after extraction. The filtered oil was stored in opaque bottles in a refrigerator prior to being used in the subsequent studies. Silver nitrate (0.169 g) was dissolved in distilled water to make 100 ml of 0.01 M silver nitrate solution ($AgNO_3$). The solution was kept in a colored glass bottle so light would not affect its stability (Ali and Alkahtani, 2021) [14]. Tween 80 was employed as a surfactant in the preparation process to ensure a uniform dispersion of the material and to minimize agglomerations in the pelleted liquid. For vegetable oil-based nano systems, the low-energy emulsification system was used for preparing the Arugula Oilnanoemulsion as per recent publications (Rani *et al*., 2023; Singh *et al*., 2022) [12, 13]. Two milliliters of the surfactant, Tween 80, was mixed with one milliliter of Arugula t oil, which had been cold-pressed. Gradual addition of seven milliliters of distilled water to the mixture while stirring with a magnetic stirrer was performed. Eventually, to guarantee a uniform Nano emulsion, the blend was subjected to ultrasonic agitation for 10 min. Silver nitrate solution was then gradually added to load silver into the Nano emulsion while continuously mixing. The mixture was then subjected to a 60ºC water bath for 30 min. A greyish-brown hue of the mixture, which is a characteristic of formation of silver nanoparticles (AgNP), was meanwhile an indicator of AgNP formation (Zhang *et al*., 2022). The UV-Visible spectrum displayed a characteristic absorption around 420 nm, and this absorption is associated with the surface plasmon resonance of the silver nanoparticles, which confirmed their characterization (Ravindran *et al*., 2023) [16]. The final product was kept in the dark in glass vials at low temperatures (4°C) until it was to be used, to preserve its stability while minimizing oxidation or photoreaction. Sustainable nanotechnology principles and biosafety were followed in utilizing a low concentration of silver nitrate to prevent any possible biotoxicity (Kumar *et al*., 2024) [17].

Biological efficacy

Pathogenic bacterial isolates had been acquired from the Maysan Health Department/Central Public Health Laboratory. The isolates blanketed *Salmonella typhi, Escherichia coli,* and *Staphylococcus aureus*. Mueller Hinton Agar changed into used, that is the usual medium for inhibitory efficacy exams because of its high capability to assist the increase of most bacterial species and its ease of use in various biological assays. The efficacy become evaluated the usage of the Well Diffusion Method, where small holes (about 6 mm in diameter) were made within the Mueller Hinton medium inoculated with pathogenic bacteria. These holes had been packed with nano-Arugula t oil, and the plates were then incubated at 37°C for 24 hours (CLSI, 2023) [23].

Antioxidant activity

The antioxidant activity of nano-Arugula oil was evaluated using the DPPH (2,2-Dipanile-1-Percharylhydrisile) method, which is a common and widely used method to assess the ability to carry free radical manual scavenging. A 0.004% DPPH solution was designed in ethanol and used as a reagent to determine antioxidant activity. Four separate concentrations of nano Arugula Oilwere designed to evaluate antioxidant activity using DPPH reagent. The finished concentrations included 25%, 50%, 75%and 100%. These concentrations were prepared by taking back 25 µg, 50 µg, 75 µg, and 100 µg nano Arugula t oil. Each volume was placed in 1 mL ethanol to prepare four concentrations gradually. Each concentration was later mixed in 1: 1 ratio with the DPPH solution (at a concentration of 0.004%) in independent test tubes. The tubes were incubated in the dark for 30 minutes at room temperature to avoid the effect of light on the response. Again, the absorption at a wavelength of 517 nm was measured using the UV-Viz spectrophotometer to determine the antioxidant activity (brand-viliums *et al*., 1995) [24].

Results and Discussion

Table and Figure (1) show that nano-Arugula Oilis quite potent against the tested bacterial isolates. Gram-negative bacteria such as Escherichia coli showed maximum efficacy with a diameter of inhibition zone of 16 mm, followed by Gram Bacteria: Staphylococcus aureus, which had 14 mm of inhibition zone more than the least inhibited Salmonella typhi with an inhibition zone of 12 mm. Physiochemical and biochemical facts common to each of the bacterial species above, including the attributes of active components in nano Arugula t oil, can be used to analyze the results. In this study, E. coli was found to be the most sensitive to nano-oil, while normally they have an outer membrane that is rich in lipopolysaccharides (LPS), which makes them resistant to many bioactive substances. This finding can cause the impression that different Gram-negative isolates would have different membrane permeabilities. Because the cell wall of E. Coli is thinner than that of Salmonella typhi, active nano-composites like sulphur compounds and isothiocyanates found in the oil may penetrate more easily and inhibit growth effectively (Nikaido, 2003; Bassolé & Juliani, 2012) [25, 26]. As noted before, Staphylococcus aureus being a Gram-positive bacteria, has no outer membrane, hence, allowing the active compounds to penetrate cell wall and affect internal components of the cell. This explains why it showed a good response rate to the oil. But there are some underlying reasons as to why Salmonella spp. give a smaller inhibition diameter. The reasons include the outer membrane's more complex structure and some efficient resistance mechanisms such as efflux pumps, which allow these isolates to counteract with the biological effects of nano-Arugula Oil (Poole, 2005) [30]. The concentration of the active compounds in the oil may well not reach a level sufficient to cross the barriers posed by this isolate's cells. Nano-Arugula t oil, then, has a very high inhibitory effect on both types of bacteria- Gram positive and Gram negative premises- actually even more obviously against E. coli. This complies with that shown by Bassolé & Juliani (2012) [26] concerning the possibility of some vegetable oils being used against Gram-negative bacteria, provided they possess substances capable of penetrating their outer membrane, rich in lipopolysaccharides. These new studies, including that of Salem *et al*. (2021) argue, however, in some respect against earlier research that found Gram-positive bacteria tend to be more sensitive to vegetable oils compared with Gram-negative bacteria (Al-Jaafari *et al*., 2022; Nazzaro *et al*., 2013) [29, 27]. Such difference could perhaps be explained with the type of oil and the concentration of its active ingredients.

Table 1: Effect of nano Arugula Oil on the growth of some bacterial isolates by measuring the diameters of inhibition zones (mm).

Bacterial species	Gram type	Zone of inhibition (mm)
Escherichia coli	Gram negative	16
Staphylococcus aureus	Gram positive	14
Salmonella typhi	Gram negative	12

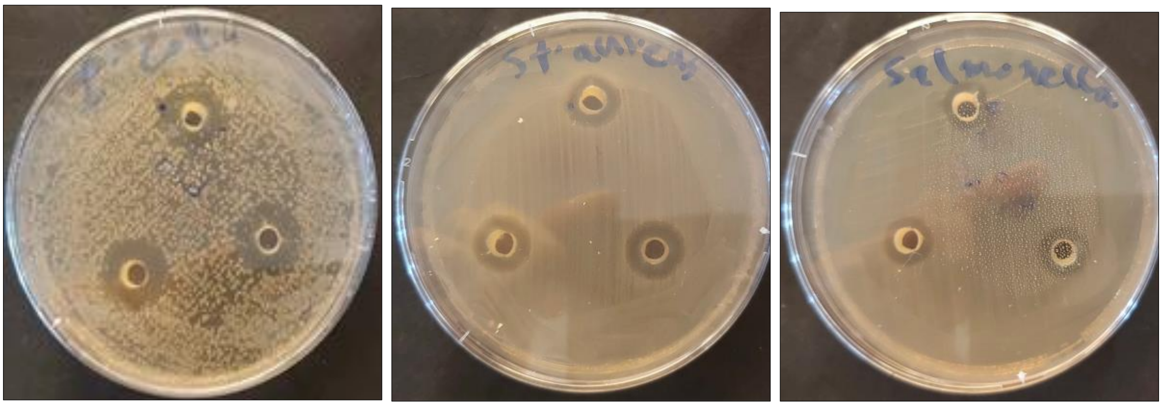

Fig 1: The effect of nano Arugula Oil on pathogenic bacteria *Escherichia coli, Staphylococcus aureus,* and *Salmonella typhi*.

Antioxidant activity

The results in Table (2) indicate an assessment of the antioxidant activity of nano-Arugula Oilby measuring the absorbance (Abs) at a wavelength of 517 nm using a DPPH detector, and calculating the inhibition percentage (%). It was found that as the concentration of Arugula Oilincreased from 25 to 100 µg/mL, the absorbance gradually decreased (from 0.194 to 0.068), while the inhibition percentage increased from 67.28% to 88.54%. This demonstrates an inverse relationship between absorbance and inhibition percentage, an expected result in DPPH tests, as antioxidants reduce DPPH absorption at 517 nm due to their interaction with free radicals (Brand-Williams *et al*., 1995) [24]. Arugula Oilcontains phenolic compounds and flavonoids known for their ability to donate electrons or hydrogen atoms to free radicals, reducing their harmful activity and leading to a decrease in absorbability and an increase in the inhibition rate (Gulcin, 2020) [31]. High inhibition rates (88.54%) in concentrations of 100 µg/ml indicate good effectiveness as a natural anti -Ox Kiss, which highlights the potential applications of oil in food or pharmaceutical industries as a natural preservative. These results were compatible with Kamal and Sharma (2021), indicating that plant oils such as Arugula Oiland thyme oil showed an anti -Ox kissing activity of more than 85% in high concentrations using DPPH tests. This was similar to the study by Abdelwahab *et al*. (2022) [33], which shows that Arugula Oilin Ka Racted by Cold Pressing has a barrier activity of up to 80% in concentrations of 100 µg/ml. However, this study does not agree with a study conducted by Al-Nanafi (2019) [34], which found that Arugula Oilmay be less effective than other oils, such as olive oil or clove oil in terms of barrier, in some experiments not exceeding 70%. This may be due to the difference in extraction methods, soil type, or phenolic material.

Table 2: The absorbance and inhibition rate of nano Arugula Oil concentrations.

Arugula Oil concentration (µg/mL)	Absorption (Abs)	Inhibition ratio (%)
25	0.194	67.28
50	0.150	74.70
75	0.139	76.56
100	0.068	88.54

Conclusions

The study results indicate that nano-Arugula Oilhas a remarkable antimicrobial activity, recording the highest efficacy against Gram-negative bacteria Escherichia coli, reflecting the nanocomposites' ability to penetrate the outer membrane of this bacterium, contrary to what would be expected from its traditional resistance. The oil also demonstrated good efficacy against Gram-positive bacteria Staphylococcus aureus, enhancing its potential as a natural, broad-spectrum antimicrobial. In contrast, Salmonella typhi demonstrated a lower response, likely due to its complex membrane and effective resistance mechanisms such as centrifugal pumps. Regarding antioxidant activity, the DPPH test results showed that nano-Arugula Oilhas strong efficacy, with the inhibition rate increasing significantly with increasing concentration, reaching 88.54% at the highest tested concentration (100 µg/mL). This confirms that it contains active phenolic compounds that contribute to neutralizing free radicals. These results demonstrate that converting oil into a nano-form enhances its biological effectiveness and supports its potential use as an effective natural substance in the food and pharmaceutical industries, whether as an antimicrobial or a safe and effective antioxidant.

Recommendations

Study the effect of the oil on other types of bacteria or fungi, study its toxicity and side effects, and its potential use in the pharmaceutical or food industries.

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