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RESEARCH ARTICLE

OLIVE (*OLEA EUROPAEA*) LEAF AND ARUGULA (*ERUCA SATIVA*) SEED EXTRACTS HAVE ANTIBACTERIAL PROPERTIES

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ABSTRACT

Background:Olive leaf and arugula seed crude extracts were tested for their in vitro antibacterial activities. Utilizing the disc diffusion technique, antibacterial activity against three microorganisms was assessed. Both the minimum bactericidal concentration (MBC) and the minimum inhibition concentration (MIC) have been established. The standard medication for the investigation of antibacterial activity was gallic acid.**Materials and Methods**: The antibacterial activity was selected against two Gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*; The extract's antibacterial activity was determined using a diffusion test on Mueller-Hinton agar that had previously been injected with 1 mL of an 18-hour-old bacterial solution (106 CFU/mL).**Results:** With an average zone of inhibition of 3 to 8 mm, the methanol extract of olive leaves and arugula seeds demonstrated strong antibacterial activity. Staphylococcus aureus was the bacterium with the highest level of sensitivity. In comparison to olive leaf extract, the antibacterial activity of arugula seed extract was higher. For *Staphylococcus aureus* and Bacillus cereus, the minimum inhibition concentration (MIC) values of the olive leaves and arugula seeds extract were 80 and 40 g/ml, respectively, while the minimum bactericidal concentration (MBC) values for *S. aureus* and *B. cereus*, respectively, were 60 and 600 g/ml. **Conclusion:** These findings imply that arugula seeds and olive leaves have intriguing antimicrobial properties.

KEYWORDS

Extracts, gallic acid, Antibacterial, microorganisms, Phytochemical Olive, Arugula.

1. INTRODUCTION

Olive is one of the most important fruit tree. It is native to the Mediterranean region such as Syria, Palestine, Algeria, Morocco, Turkey, Spain, Italy, Greece, France. It accounts for 98% of the world crop and cover about 8 million hectare area (Bensehaila et al., 2022). Many contemporary pharmaceuticals have been separated from natural sources, which has been a source of therapeutic agents for thousands of years. A large portion of this isolation was based on the applications of these agents in conventional medicine (SK et al., 2009). Scientists exploring for novel sources of effective medications to treat infectious illnesses have long been very interested in the study of biologically active chemicals from natural sources. Despite enormous advancements in human medicine, infectious illnesses brought on by bacteria, fungi, viruses, and parasites continue to pose a serious danger to public health. Due to the relative scarcity of medications and the advent of widespread drug resistance, their impact is more severe in underdeveloped nations (Chokshi et al., 2019). Both humans and other animals may get deadly diseases from bacteria. For instance, it was discovered that Staphylococcus aureus (S. aureus) causes food poisoning and superficial skin lesions (Karimi et al., 2019). Because there are few effective antimicrobial treatments against Pseudomonas aeruginosa (P. aeruginosa), it is a nosocomial organism that causes a considerable amount of hospital-acquired infections and healthcare facilities (Sadeq et al., 2021). Therefore, the herbal and medicinal plants have expected greater significance in recent days, because of the marvelous potential that they offer in expressing new medications against *Olea europaea* L (Eom et al., 2012).

Wild olea ferruginea Royle may be found in the Himalayas from Kashmir to Nepal at elevations of up to 2400 m. Throughout human history, the olive tree has been a significant source of food and medicine. They have a lot of potentially bioactive substances that might be antioxidant (Tavakoli et al., 2018). Antihypertensive, anti-inflammatory, anti-bacterial, hypoglycemic and hypocholesterolemic properties (O'Keeffe et al., 2017; Adem et al., 2020; Khemakhem et al., 2017; Acar-Tek and Ağagündüz, 2020). The survival and development of certain gram positive and gram negative bacterial strains of the American type culture collection (ATTC) were examined in the present investigation to determine the in vitro effects of olive leaf extracts and oils. *Eruca sativa* L. which is commonly known as Rocket is used in this study. It is a member of the Brassica plant family (Cruciferae) and is widely used as a vegetable and spice. It originated in the Mediterranean region and is now found all over the world (Marmiroli et al., 2022). The plant also has a wide spread medicinal use. Traditionally, it is used as astringent, diuretic, digestive, emollient, tonic, depurative, laxative, rubefacient and stimulant is well documented (Jaafar and Jaafar, 2019). Plants create a large number of antibacterial organic chemicals. Alliin/allicins, isothiocyanates, and plant pigments are present in diverse plant components such as stems, roots, leaves, bark, flowers, fruits, and seeds. The current study sought to evaluate the antibacterial activity of several medicinal plant extracts of Olea europaea leaves and E. sativa seeds in vitro.

2. MATERIAL AND METHODS

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The antibacterial activity was selected against two Gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*; These bacteria were obtained from the Microbiology Laboratory of Universal Technology Malaysia.

2.1 Sample Collection and Preparation

The olive leaves and arugula seeds were purchased at the Kajang market in Malaysia. Prior to extraction, samples were cleaned, dried in a drying oven at 50 0C for 48 hours, and ground to granulometry less than 250 µm. Buckner funnel and Whatmann's No. 1 filter paper were used to filter the extracts. Before using the disc diffusion experiment, the extracts were stored at 40 degrees Celsius to retain their antibacterial properties.

2.2 Determination of Antimicrobial Activity

Mueller-Hinton Agar was used to culture all of the bacteria tested. The extract's antibacterial activity was determined using a diffusion test on Mueller-Hinton agar that had previously been injected with 1 mL of an 18-hour-old bacterial solution (106 CFU/mL) (Wilson et al., 2016). Sterilized paper discs (6 mm) were impregnated with 20 µL of different concentrations of extract, (500, 1000, 1500, 2000 µg/mL) prepared in pure methanol, and placed onto nutrient agar. The plates were incubated at 40°C for 2 hours to allow the active chemicals in the medium to diffuse (Majeed et al., 2011). Negative controls were made with the same solvent that was used to dissolve the plant extract. Gentamicin discs (10 µg, Oxoid, UK) were used as control and positive controls. Incubation of plates was performed at 37 ⁰C for 24 h. Inhibition zones in mm (without disc paper diameter) around discs were measured. The diameter of inhibition zones created by the extract against test microorganisms was used to calculate antibacterial activity. The experiment was performed three times, and the mean diameter of the inhibitory zones was determined. Minimum inhibition concentration (MIC) was determined as described by (Giweli et al., 2012). Different concentrations (10–2000 µg/mL) of extract or standard (Gallic acid) were tested. 1 mL of each solution was mixed with 9 mL of Mueller-Hinton medium and poured into sterilized Petri plates. The plates were spot infected with 10µL of suspension containing 106 CFU/mL of each bacteria immediately after solidification. The inoculation plates were incubated for 24 hours at 37 ⁰C. The MIC values were determined by determining the lowest concentration of extract or standard at which no growth was seen. To calculate the minimum bactericidal concentration (MBC), nutrient broth tubes were infected with a sample obtained from a plate that did not display any growth. The mixture was incubated for 24 hours at 37 ⁰C. The minimum bactericidal concentration was determined to be the lowest concentration of the extract or standard with no observable growth following incubation.

2.3 Phytochemical Screening of Olive Leaves and Arugula Seeds

Ethanol, chloroform, hydrochloric, and aqueous extracts of olive leaves and arugula seeds were produced for phytochemical screening. In line with the standards, the extracts were submitted to phytochemical testing for secondary metabolites, tannins, steroid, saponins, alkaloids, terpenes , unsaturated sterol, and flavonoid in olive leaves and arugula seeds (Bakdi et al., 2021)

2.4 Statistical Analysis

The experiment was repeated three times. The results of this study were expressed as (mean ±SD. IBM SPSS version 21.0 ; SPSS Inc., Chicago, IL, USA was used to perform the data analysis using one-way ANOVA. Duncan triplicates range test revealed significant differences (P<0.05) across treatments (George et al., 2004).

3. Results and Discussion

3.1 Phytochemical Screening.

The presence of flavonoids, steroids, Sterols, terpenes and saponins was discovered during a phytochemical examination of Olea europaea leaves (Table 1). Phytochemical screening is often used to identify and describe the components present in a specific plant sample. Generally, secondary metabolites that have accumulated to some amount in specific organs of the plant are identified during phytochemical screening of any plant. When tested on animals, these metabolites, which are primarily employed by the plant to protect itself from herbivores, may have pharmacological effect. The presence of saponins, stérols, steroid, terpenes, and flavonoids was found in phytochemical screening of Olea europea leaves using different extracts. All of these chemicals have previously been found in olive leaf (Yaseen et al., 2019; Al-Gorany et al., 2020).

3.2 Antibacterial Activity

The widths of inhibition zones imposed by the extract and the standard on tested microorganisms are shown in Table 2. Although olive and arugula extracts were effective against two Gram-positive pathogens (Staphylococcus aureus and Bacillus cereus), they were ineffective against the Gram-negative strain (Bacillus cereus). Higher inhibition was seen against Staphylococcus aureus, one of the most frequent Gram-positive bacteria responsible for food poisoning. Olive leaf and arugula seed extracts have lesser activity than Gallic acid. Gallic acid inhibited Bacillus cereus at a similar zone (8.76 mm) to olive leaves and arugula seeds extracts (4.86 and 5.33mm, respectively). The sensitivity of S. aureus to olive leaves and arugula seeds is comparable with previously published data on eucalypt species, although results are difficult to compare because experiments in the literature were performed under various settings (Nikšić et al., 2012). Extracts of Eruca sativa were discovered to have a great antibacterial action against S. aureus and B. cereus. Olive leaves may be beneficial in circumstances where extended antibiotic usage promotes the development of opportunistic infections. The analysis is particularly efficient against Klebsiella and Pseudomonas, two bacterial taxa that constitute a significant resistance concern (Larsson, 2022; Lionakis and Hohl, 2020). Extracts of guava leaves (Psidium guajava) and cloves (Syzgium aromatica) inhibited S. aureus growth, with inhibition zones extending from 10 to 20 mm and 21 to 30 mm, respectively. The antibacterial activity of olive, arugula extract, and standards was quantified against selected microorganisms as in figure 1; the MIC and MBC of the tested substances are shown in Table 3. The MIC values of olive, arugula extract, and Gallic acid were lower for B. cereus, but higher for S. aureus. Indeed, extracts of olive leaves and bark have been shown to be efficient against B. subtilis. (Bombard et al., 2018). According to the findings, olive and arugula extracts were efficient against the two Gram-positive bacteria (S. aureus, B. subtilis). This is consistent with prior research that found Gram-negative bacteria to be more resistant to antimicrobials than Gram-positive germs because of their outer lipopolysaccharide membrane.

Table 1: Phytochemical Screening of olive and arugula extract.

Phytochemicals	Olive leaves	Arugula seeds
Alkaloids	-	+
Saponins	+	+
Tanin	-	-
Flavonoids	+	+
Sterol and steroid	+	+
sterols and terpens	+	+

(+) positive = present , (-) Negative = absent

Table 2: Antibacterial activity of olive leaves, arugula seeds and standard.

Microorganisms	Inhibition zone (mm)		
	Olive leaves extract	Arugula seeds extract	Gallic acid
S. aureus	6.32 ± 0.52	8.53 ± 0.24	8.87 ± 0.51
B. cereus	4.84 ±0.27	5.23 ± 0.13	5.77 ± 0.16

Differences were considered to be significant at $p < 0.05$.

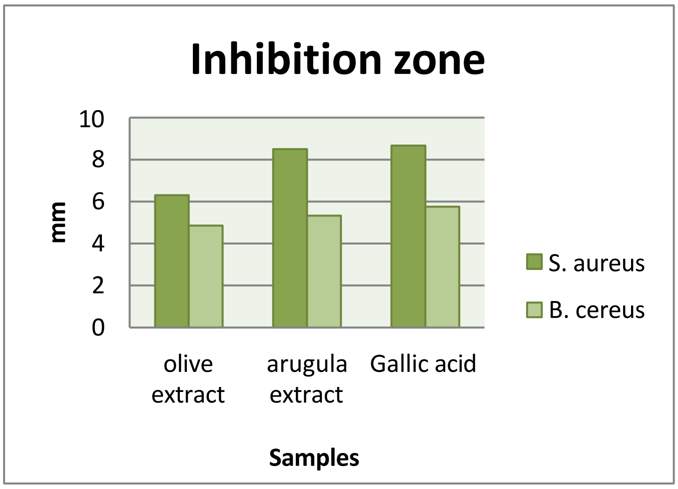

Figure 1: Antibacterial activity of olive arugula and standard. S. aureus: Staphylococcus aureus, B. cereus: Bacillus cereus.

Table 3: MIC and MBC for olive leaves, arugula seeds and standard.

Microorganisms	olive extract	arugula extract	Gallic acid	olive extract	arugula extract	Gallic acid
	MIC (µg/mL)			MBC (µg/mL)		
S. aureus	90	70	30	50	70	40
B. cereus	40	30	500	600	400	300

Differences were considered to be significant at $p < 0.05$.

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

4. Conclusion

In conclusion, the results of this study show that the use of arugula seeds and olive leaves as nutraceuticals may reduce the incidence of microbial infections, particularly in the respiratory and digestive tracts. This is primarily because these plants' phenolic chemicals have a protective effect. As a result, this extract and its constituent parts are suitable for therapeutic usage and alternative medicine.

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