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Research Article

Antibacterial Activity of Olive *(Olea europaea)*Leaves and Arugula *(Eruca sativa)* Seeds Extract

*Salma Nassrullah Malik
Radiotherapy Department, Institute of Medical Technology, Baghdad, Iraq

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ABSTRACT
Crude extracts from olive leaves and arugula seeds were screened for its in vitro antibacterial activity. Antibacterial activity was determined by using disc diffusion method against three bacteria (, *Staphylococcus aurous* and *Bacillus cereus*). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) have been determined. Gallic acid was used as a standard drug for the study of antibacterial activity. Phytochemical screening revealed the presence of some active substances flavonoids, saponins and steroid, to express the desired activities. Results show that the methanol extract was active against all 5 bacterial strains. The methanol extract of olive leaves and arugula seeds showed good antibacterial activity with the average zone of inhibition 3-8mm. The most sensitive bacteria were bacteria: *Staphylococcus aureus*. The arugula seeds extract had higher antibacterial activity than olive leaves extract. The minimal inhibitory concentration (MIC) values of the olive leaves and arugula seeds extract on *Staphylococcus aureus* and *Bacillus cereus* were 80 and 40 µg/ml, respectively, and the minimal bactericidal concentration (MBC) values of the olive leaves and arugula seeds extract on *S. aureus* and *B. cereus* were 60 and 600 µg/ml, respectively. These results suggest that leaves of olive and seeds of arugula have interesting antibacterial activities.

Key words: Antibacterial activity, *S. aurous* and *B.cereus*, Phytochemical Olive, Arugula

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; much of this isolation was based on the uses of these agents in traditional medicine[1]. The study of biologically active compounds from natural sources has always been of great interest to scientists looking for new sources of useful drugs for treating infectious diseases. Infectious diseases caused by bacteria, fungi, viruses, and parasites remain a major threat to public health, despite tremendous progress in human medicine. Their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance [2]. Bacteria cause serious infections in humans as well as other animals. For example, it was found that Staphylococcus aureus (S. aureus) causes superficial skin lesion and food poisoning [3]. Pseudomonas aeruginosa (P. aeruginosa) is a nosocomial pathogen accounting for a significant percentage of hospital-acquired infections and health care centers because there are a little effective antimicrobial agents against it [4]. Thus, the medicinal and herbal plants have assumed greater importance in recent days, due to the tremendous potential that they offer in formulating new drugs against. *Olea europaea* L. (Olive) is one of the most important fruit tree. It is native to the Mediterranean
region such as Palestine, Syria, Spain, Italy, Greece, France, Turkey, Algeria and Morocco. It accounts for 98% of the world crop and cover about 8 million hectare area [5]. *Olea ferruginea* Royle is found wild in the Himalayas from Kashmir to Nepal up to 2400 m altitude. The olive plant is an important source of nutrition and medicine throughout the history of civilization. They contain many potentially bioactive compounds that may have antioxidant [6], antihypertensive [7] , anti-inflammatory, anti-bacterial [8-10] , hypoglycemic [11,] and hypocholesterolemic properties. In the present study, the *in vitro* effect of olive leaf extracts and oils on the survival and growth of certain gram positive and gram negative bacterial strains of American type culture collection (ATTC) was investigated. *Eruca sativa* L. which is commonly known as Rocket is used in this study. It belongs to the Brassica plant family (Cruciferae),and is immensely used as vegetable and spice ,it originated in Mediterranean region and now is found around the word [12]. 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 [13]. Plants produce a multitude of organic compounds that have antimicrobial activity. The compounds are found in various plant parts such as stems, roots, leaves, bark, flowers or fruits and seeds and include alliin/allicins, isothiocyanates, and plant pigments. The aim of the present study was to assess the in vitro antibacterial activity of different medicinal plants extracts of *Olea europaea* leaves and *E. sativa* seeds.

MATERIALS AND METHODS

Author for Correspondence

Table 1. Phytochemical Screening of olive and arugula extract

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

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	6.31 ± 0.50	8.50 ± 0.21	8.67 ± 0.50
B. cereus	4.86 ±0.24	5.33 ± 0.11	5.76 ± 0.15

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

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

Sample Collection and Preparation of Olive Leaves and Arugula Seeds Extract

The olive leaves and arugula seeds were obtained from the market in Kajang, Malaysia. Samples were cleaned, dried in the drying oven at 50 ^{0}C during 48 h, and ground to granulometry lower than 250 _m prior to extraction. The extracts were filtered using Buckner funnel and Whatmann's No. 1 filter paper. Extracts were kept at 4^{0}C to preserve the antibacterial property before they were used for disc diffusion assay.

Determination of Antimicrobial Activity

All the bacteria tested were grown on Mueller Hinton Agar. The antibacterial activity of the extract was measured by a diffusion test using Mueller–Hinton agar previously inoculated with 1 mL of 18 h old of bacterial suspension (10^6 CFU/mL) [14]. 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 4 ^{0}C for 2 h to allow diffusion of the active compounds in the medium [15] . Negative controls were prepared using the same solvent employed 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 ^{0}C for 24 h. Inhibition zones in mm (without disc paper diameter) around discs were measured. The antibacterial activity was expressed as the diameter of

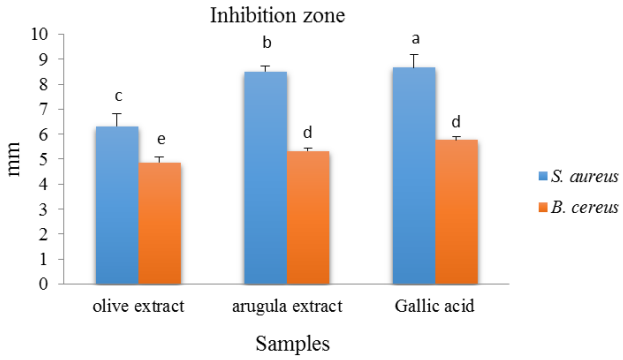

Fig 1. Antibacterial activity of olive arugula and standard.

inhibition zones produced by the extract against test microorganisms. The experiment was repeated in triplicate and the mean of diameter of the inhibition zones was calculated. Minimum inhibition concentration (MIC) was determined as described by [16]. 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 Muller Hinton medium and poured into sterilized Petri plates. Immediately after solidification, the plates were spot inoculated with 10 µL of suspension containing 10^6 CFU/mL of each bacterium. The inoculated plates were incubated at 37 ^{0}C for 24 h. The MIC values were determined as the lowest extract or standard concentration at which no growth was observed. To determine the minimum bactericidal concentration (MBC) values, nutrient broth tubes were inoculated with a sample taken at the spot of the plates which did not show any growth. The mixture was incubated at 37 ^{0}C for 24 h. The lowest concentration of the extract or standard with no visible growth after incubation was taken as the minimum bactericidal concentration.

Phytochemical Screening of Olive Leaves and Arugula Seeds

Ethanol; chloroform; hydrochloric and aqueous extracts were prepared for phytochemical screening of olive leaves and arugula seeds. The extracts were subjected to phytochemical tests for olive leaves and arugula seeds secondary metabolites, tannins, saponins, steroid, alkaloids, flavonoid, unsaturated sterol and terpen in accordance with [17].

Statistical Analysis

The experiment was carried out in triplicate. Statistical analysis of the data was performed by one-way ANOVA using (SPSS 19 software). Significant differences ($p<0.05$) between the treatments were analyzed by Duncan triplicates range test[18]

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

Microorganisms	Olive extract	Arugula extract	Gallic acid	Olive extract	Aarugula extract	Gallic acid
	MIC (µg/mL)			MBC (µg/mL)		
S. aureus	80	60	20	60	80	40
B. cereus	40	20	400	600	400	200

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

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical analysis conducted on *Olea europaea* leaves revealed the presence of flavonoids, steroids and saponins (Table 1). Phytochemical screening is usually carried out to screen for and to characterized the constituents available in a given plant sample. Generally, in the phytochemical screening of any plant one normally identifies secondary metabolites that have accumulated to some extent at specific organ of the plant. These metabolites that are mainly used by the plant for protection against herbivores may have pharmacological activity when tested on animals. Result of phytochemical screening of *Olea europea leaves* us of the various extracts showed the presence of saponins, stérols, steroid, terpen and flavonoids. All these compounds were previously reported to occur in olive leaf [19-20].

Antibacterial Activity

Table 2 presents diameters of inhibition zones exerted by the extract and the standard towards tested microorganisms. Olive and arugula extract were effective against the two Gram-positive strains (*Staphylococcus aureus* and *Bacillus cereus*) but low activity was observed against the Gram-negative strain (*Bacillus cereus*). Higher inhibition was detected against *Staphylococcus aureus*, which is one of the most common of the Gram-positive bacteria causing food poisoning. The activity of olive leaves and arugula seeds extract are lower than that of Gallic acid. In the case of *Bacillus cereus*, Gallic acid gave comparable inhibition zone (8.76 mm) compared to that of olive leaves and arugula seeds extract 4.86 and 5.33mm respectively. The sensitivity of *S. aureus* to olive leaves and arugula seeds is consistent with published data about eucalypt species, but the results are difficult to compare because literature assays were carried out at different conditions[21] It found a remarkable antibacterial effect of extracts of *Eruca sativa against S. aureus and B. cereus*. Olive leaves may be useful in cases where prolonged use of antibiotics encourage development of opportunistic infections [22] being especially effective against *Klebsiella and Pseudomonas*, two bacterial genera which pose a major resistance problem [23]. The extracts of leaves of guava (*Psidium guajava*) and cloves (*Syzgium aromatica*) showed inhibitory effects on growth of *S. aureus*, with inhibition zones ranging from 10 to 20 mm and from 21 to 30 mm, respectively [24]. Quantitative evaluation of the antibacterial activity of the olive, arugula extract and of the standards was carried out against selected microorganisms; the MIC and MBC of the tested samples are presented in Table 3. MIC values of olive, arugula extract and Gallic acid were lower for *B. cereus* while olive, arugula extract and Gallic acid was a more potent inhibitor of *S. aureus*. Indeed, extracts of leaves and bark of olive leaves have been reported to be effective against *B. subtilis* [25]. This study shows that Olive and arugula extracts were effective against the two Gram-positive strains (*S. aureus, B. subtilis*). This is consistent with previous studies reporting that Gram-negative bacteria are more resistant to antimicrobials than Gram-positive microorganisms due to their outer lipopolysaccharide membrane [25].

CONCLUSION

In conclusion, the data obtained in this study demonstrate that the use of olive leaves and arugula seeds as nutraceuticals may lower the risk of microbial infections, particularly in the intestinal and respiratory tract, mainly due to the protective action provided by its phenolic compounds. Hence, this extract and their components can be recommended for therapeutic purposes and be used as an alternative medicine.

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