Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(13):118-124
(http://www.derpharmachemica.com/archive.html)

Antibacterial Activity of Aqueous and Methanolic Extracts of Olive (*Olea europaea* L.) Leaves Collected from Different Regions in Morocco

Lahdibi Sahraoui H[1,2*], El Berny H[1], El Karkour M[3], Quasmaoui H[2], Charof R[2], Mennane Z[2]

[1]*Department of Biology, Laboratory of Biotechnology, Environment and Quality (LABEQ), University Ibn Tofail, Kenitra, Morocco*
[2]*Department of Medical Bacteriology, National Institute of Hygiene, Rabat, Morocco*
[3]*Department of Chemistry, Laboratory of Naturel Substances and Thermolyse-eclaire, University Mohamed V, Rabat, Morocco*

ABSTRACT

The aim of this work was to investigate the antibacterial activity of Aqueous and Methanolic Extracts of Olive (Olea europaea L.) Leaves Collected from Different Regions in Morocco against 31 bacteria strains: five represent ATCC strains and 26 strains are from nosocomial infections (Escherichia coli, Klebsiella pneumonia, Enterobacter cloacae, Proteus mirabilis, Morganella morganii, Pseudomonas aeruginosa, Acinetobacter baumanii and Staphylococcus aureus from different origins and phenotypes. These strains were collected from the National Institute of Hygiene (NIH) Rabat-Morocco). The Powder of leaves O. europaea L., was extracted by Soxhlet extraction. The antibacterial activity was determined by agar diffusion method. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) have been determined. The extracts were found to be effective against all the bacterial strains, but they were effective against Gram-positive more than Gram-negative bacteria. The maximum inhibitory zone was noted against multiresistant Staphylococcus aureus (20 mm), imipenem resistant A. baumanii (18 mm), they were also active against Extended-Spectrum Beta-Lactamase-(ESBL): E. coli (1 and 3) and Pseudomonas aeruginosa (1) (16 mm and 14 mm respectively). The MIC observed from the aqueous and methanolic leaf extract is 12.5-50 µg/ml and 1.56-12.5 µg/ml respectively. For All the tested strains, bactericidal activity was observed neither for the aqueous extract nor for the methanolic extract. The results indicate that olive can be used for the treatment of various infections and for the development of new antibacterial agents.

Keywords: Antibacterial activity, Aqueous extracts, Methanolic extracts, *Olea europaea* L., Nosocomial infections

INTRODUCTION

Nosocomial infections are considered as acute, and they are characterized by high morbidity and mortality rates [1]. According to the World Health Organization (WHO, 2008) every 14 h, over 1500 people die from an infectious disease of which more than half of them are children under 5 y old. It has been observed that 19% of deaths in developed countries and 43% of deaths in developing countries are due to infectious diseases; this is depended to the eruption of drug resistant microorganisms and the emergence of undisclosed disease causing microbes [2]. The solution to this problem is therefore pivotal and requires the search for new and more sustainable antibiotics [3].

In this context, resistance in Gram-negative bacteria offer a major challenge for the antibacterial therapy and significantly narrows the treatment options of human infections. Extended Spectrum β-lactamase (ESBL) producing bacteria are span worldwide [4]. The treatment of diseases began long time ago with the use of plants. They were the only medicinal system before modern or orthodox medicine could be developed [5]. Medicinal plants have been used for thousands of years in view of their active components have efficacy in the treatment of diseases because he constitute a potential reservoir of effective antibacterial molecules [6].

Clinical microbiologists have two reasons to be interested in the topic of antibacterial herb extracts. First, it is very likely that these phytochemicals will find their road into the arsenal of antibacterial drugs approved by physicians; several are already being tested in humans. Worldwide spending on discovery new anti-infective agents is awaited for increase 60% from the spending levels in 1993 [7]. New sources, especially plant sources, are also being studied. Second, the public is becoming increasingly conscious of problems with the over prescription and misuse of traditional antibiotics. In addition, many people are concerned by having more independence over their medical care [8]. The first formal report of medicinal use was made in 1854, when olive leaf extract was described to be effective in treating fever and malaria [9].

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 presents 98% of the world crop and covers about 8 million hectare area [10]. A phytochemical investigation announced that oleuropein was extracted from the leaves of *O. europaea* [11]. This compound is known for possessing a broad range of pharmacologic and health promoting properties including antiarrhythmic, spasmolytic, immunostimulant, cardioprotective, hypotensive, antihyperglycemic, antimicrobial and anti-inflammatory effects [12,13].

Various reports have shown that olive leaf extract has the capacity to lower blood pressure in animals and increase blood flow in the coronary arteries [14], relieve arrhythmia and prevent intestinal muscle spasms [15]. In addition, leaves may be used in infusions, allowing a considerable intake/uptake of bioactive compounds. The reports describing antibacterial properties of phenolic compounds in olive products refer to compounds obtained from olive fruit, especially hydroxytyrosol and oleuropein [16].

MATERIALS AND METHODS

Plants collection

Olive leaves were collected in September-December 2014 from for Moroccan Provinces (Meknes, Fquih Ben Salah, Taza and Ouezzane). The leaves were cleaned from extraneous matter and properly washed then dried at room temperature for 15 days. The dried leave material were ground to fine powder using an electric grinder and stored in an air-tight container in a dark place until extraction procedure to prevent oxidation.

Preparation of the olive leaves extract

Aqueous extract

The Powder of leaves *O. europaea* L. 100 g was extracted by Soxhlet extraction for 24 h in about 700 ml of cold sterile distilled water. After this step, the decoction was filtered and then freeze-dried (aqueous extract) [17].

Organic extracts

Soxhlet extraction of 100 g of leaves for 24 h in about 700 ml of solvent used obtained methanolic extract (ME). The resultant was subjected to drying in a rotary evaporator, after which the leaf extract was used for other analyses [18].

Microorganisms used

The test organisms used included 31 bacteria strains: five represent American Type Culture Collection (ATCC) strains and 26 strains are from nosocomial infections from different origins (Tables 1 and 2). These strains were collected from the National Institute of Hygiene (NIH) Rabat-Morocco).

Table 1: American Type Culture Collection (ATCC)

Escherichia coli ATCC 25922
Staphylococcus aureus ATCC 25923
Pseudomonas aeruginosa ATCC 27853
Proteus vulgaris ATCC 13315
Citrobacter freundii ATCC 8090

Table 2: bacterial strains from different origins

Microbial group	Organisms used	Strain origin	Phenotype
Enterobacteriae (Gram-negative)	*Escherichia coli* (1)	CBUE	ESBL
	Escherichia coli (2)	Central Catheter	ESBL
	Escherichia coli (3)	Pus	ESBL
	Klebsiella pneumonia (1)	CBUE	ESBL
	Klebsiella pneumonia (2)	Central Catheter	ESBL
	Klebsiella pneumonia (3)	Pus	ESBL
	Enterobacter cloacae (1)	CBUE	ESBL
	Enterobacter cloacae (2)	Central Catheter	ESBL
	Enterobacter cloacae (3)	Pus	ESBL
	Proteus mirabilis (1)	CBUE	ESBL
	Proteus mirabilis (2)	Pus	ESBL
	Morganella morganii	Pus	ESBL
	Escherichia coli (4)	CBUE	HLP
	Escherichia coli (5)	CBUE	HLC
	Escherichia coli (6)	CBUE	LLC
	Escherichia coli (7)	CBUE	LLP
	Escherichia coli (8)	Ascites Liquid	HLP
	Escherichia coli (9)	Pus	LLP
Not *Enterobacteriae* (Gram-negative)	*Pseudomonas aeruginosa* (1)	CBUE	ESBL
	Pseudomonas aeruginosa (2)	CBUE	Multiresistant
	Acinetobacter baumannii (1)	CBUE	IPM-R
	Acinetobacter baumannii (2)	Central Catheter	IPM-R
	Acinetobacter baumannii (3)	Pus	IPM-R
	Acinetobacter baumannii (4)	Traumatology	IPM-R
Gram positive	*Staphylococcus aureus* (1)	Central Catheter	Meti-R
	Staphylococcus aureus (2)	Pus	Meti-R

CBUE: Cytobacterioligical Urine Exam, HLP: High Level Penicillinase, HLC: High Level Cephalosporinase, LLC: Low Level Cephalosporinase, LLP: Low Level Penicillinase, IPM-R: Imipenem Resistant, Meti-R: Methicillin-Resistant

Culture media and antibacterial assay

Bromo Cresol Purple (BCP), lactose agar, mannitol salt agar and *Pseudomonas* cetrimide agar were used for *Enterobacteriae*, *S. aureus* and *P. aeruginosa* growth, respectively. Microbial cultures, freshly grown at 37°C for 24 h were appropriately diluted in sterile normal saline solution (NaCl 0.9%) to obtain the cell suspension previously adjusted using the standard 0.5 McFarland and turbidity at 10^5 CFU/ml. The antibacterial activity was performed using the diffusion method on agar media Muller-Hinton Agar (MHA) as recommended by Nongpanga et al. [19]. Thus, the organisms were spread on MHA: Sabouraud Dextrose Agar (SDA) plates by cotton swab. Wells of 6 mm diameter were punched into the agar medium and filled with 50 µl of plants extracts. The plates were incubated for 24 h at 37°C. Antibacterial activity was evaluated by measuring the inhibitory zone against the test organism.

Minimum Inhibitory Concentration (MIC)

The determination of MIC of the plants extracts against bacterial strains was performed according to the micro titration technique described by Eloff [20].

Minimum Bactericide Concentration (MBC)

In order to evaluate MBC, 100 µl of each case, in which bacterial growth was not observed, was spread plated in MH agar. Plates were incubated at the appropriate temperature for 24 h. The MBC was defined as the lowest concentration in which the growth of bacteria was completely inhibited [21,22].

RESULTS AND DISCUSSION

The results of antibacterial activity of the aqueous and methanolic extracts of *O. europaea* leaves are presented in Table 3. The aqueous extracts of olive leaves from different regions. The plant extract had inhibitory effect on the entire test organisms: Gram-positive more than Gram-negative bacteria. Meknes aqueous extract was the most active; it was active against all bacteria tested by maximum inhibitory zone against Multiresistant *S. aureus* (MRSA) (20 mm) while the minimum inhibitory zone was against *E. coli* (8) (11 mm). It was also active against ESBL bacteria: *E. coli* (1 and 3) and *P. aeruginosa* (1) (16 mm and 14 mm respectively). It was effective against imipenem resistant *A. baumanii* (18 mm) which are pathogenic nosocomial germs.

Aqueous extract

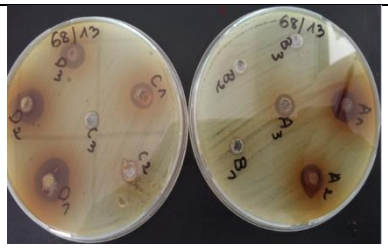

Photo 1: ***Acinetobacter baumanii***

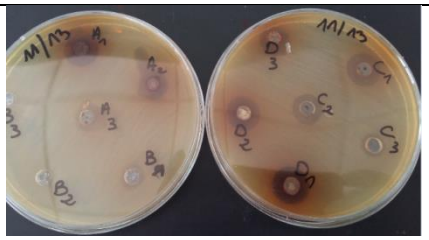

Photo 2: ***Proteus mirabilis***

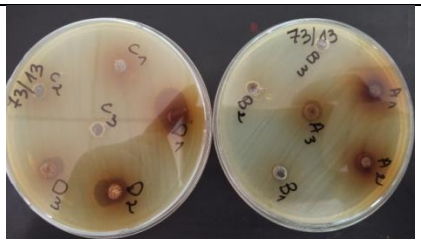

Photo 3: ***Enterobacter cloacae***

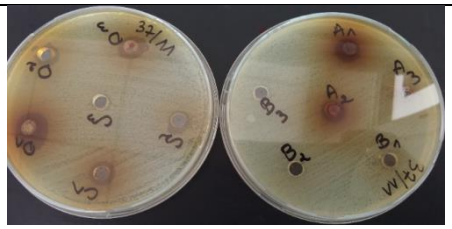

Photo 4: ***Pseudomonas aeruginosa***

Methanolic extract

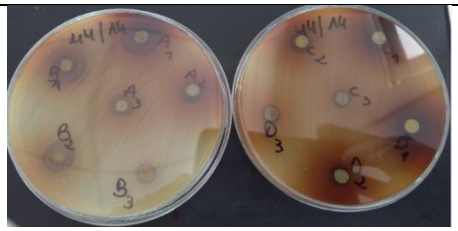

Photo 5: ***Acinetobacter baumanii***

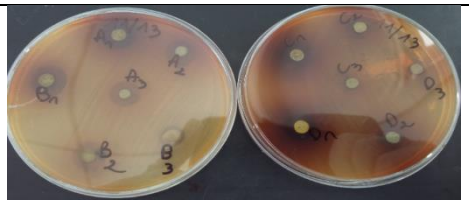

Photo 6: ***Proteus mirabilis***

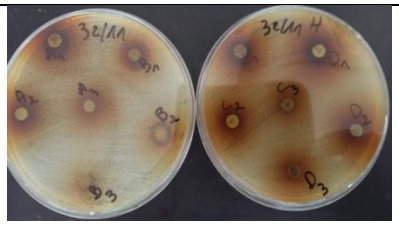

Photo 7: ***Staphylococcus aureus***

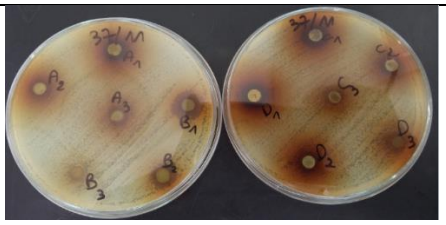

Photo 8: ***Pseudomonas aeruginosa***

Figure 1: Agar-well diffusion assay: growth inhibitory zone of some strains bacteria

The methanolic extracts has also an activity against the different microorganisms tested, it exhibited the highest inhibitory potential on imipenem resistant *A. baumanii* (4) and MRSA (2) with inhibitory zone (16 mm and 15 mm respectively). It was effective against ESBL *P. mirabilis* (2) and ESBL *P. aeruginosa* (1) by 12.25 mm and 11.5 mm respectively (Figure 1). The Dimethyl Sulphoxide (DMSO) (10%)) that served as the control did not show any activity against the test microorganisms (negative control).

MIC of the aqueous and methanolic leaf extracts of the bacterial isolates is presented in Figures 2 and 3. The MIC observed from the aqueous and methanolic leaf extract is 12.5-50 µg/ml and 1.56-12.5 µg/ml respectively witch therefore shows that the plant extracts were effective on the test bacteria. The methanolic extract has showed the higher MIC against tested bacterial strains, it was for ESBL *Enterobacteriae* presented by *E. coli* (3), *E. cloacae* (2), *P. mirabilis* (1) and imipenem resistant *A. baumanii* (3) (MIC was 1.56 µg/ml).

Aqueous and methanolic extracts have a good activity against ATCC and the inhibition diameter of these extracts was between 9-19 mm. For All the tested strains, bactericidal activity was not observed neither for the aqueous extract nor for the methanolic extract. Then we can say that our extracts are bacteriostatic.

Table 3: Screening antibacterial activity of olive (*Olea europaea* L.) leaves extracts collected from 4 different regions of Morocco

Bacterial strains tested	Inhibition zone diameters (mm)							
	Meknes extract		Fquih Ben Salah extract		Taza extract		Ouezzane extract	
	AE	ME	AE	ME	AE	ME	AE	ME
Escherichia coli ATCC 25922	9	9	0	14	9	10	13	12
Staphylococcus aureus ATCC 25923	12	14	0	13	15	14	17	14
Pseudomonas aeruginosa ATCC 27853	12	13	9	13	12	10	19	12
Proteus vulgaris ATCC 13315	11	10	0	12	11	10	14	10
Citrobacter freundii ATCC 8090	15	0	11	13	11	9	11	10
Escherichia coli (1)	12	10	0	13	12	10	16	10
Escherichia coli (2)	10	11	0	14	0	9	12	10
Escherichia coli (3)	11	10	0	13	0	11	16	11
Klebsiella pneumonia (1)	10	9	0	12	0	9	14	10
Klebsiella pneumonia (2)	8	9	0	11	0	10	13	10
Klebsiella pneumonia (3)	10	10	0	11	9	10	15	11
Enterobacter cloacae (1)	11	10	0	11	10	10	14	10
Enterobacter cloacae (2)	10	10	0	13	0	0	13	10
Enterobacter cloacae (3)	10	10	0	13	0	10	16	10
Proteus mirabilis (1)	12	9	8	13	8	10	13	10
Proteus mirabilis(2)	14	12	9	13	14	12	15	12
Morganella morganii	11	10	0	11	9	10	14	11
Escherichia coli (4)	10	12	0	14	0	10	14	10
Escherichia coli (5)	10	10	0	13	9	10	14	10
Escherichia coli (6)	9	10	0	14	0	10	11	11
Escherichia coli (7)	12	10	0	12	10	10	14	10
Escherichia coli (8)	11	11	0	14	0	10	11	11
Escherichia coli (9)	9	11	0	14	12	9	11	10
Pseudomonas aeruginosa (1)	11	10	0	14	10	12	14	10
Pseudomonas aeruginosa (2)	10	11	0	12	9	10	13	10
Acinetobacter baumanii (1)	13	11	0	12	9	13	15	13
Acinetobacter baumanii (2)	13	11	0	13	11	12	18	12
Acinetobacter baumanii (3)	11	10	0	10	0	10	13	10
Acinetobacter baumanii (4)	13	16	0	16	12	11	16	11
Staphylococcus aureus (1)	19	12	0	14	17	10	20	10
Staphylococcus aureus (2)	17	13	0	15	13	12	16	11

AE: Aqueous Extracts; ME: Methanolic Extracts; 0: No activity

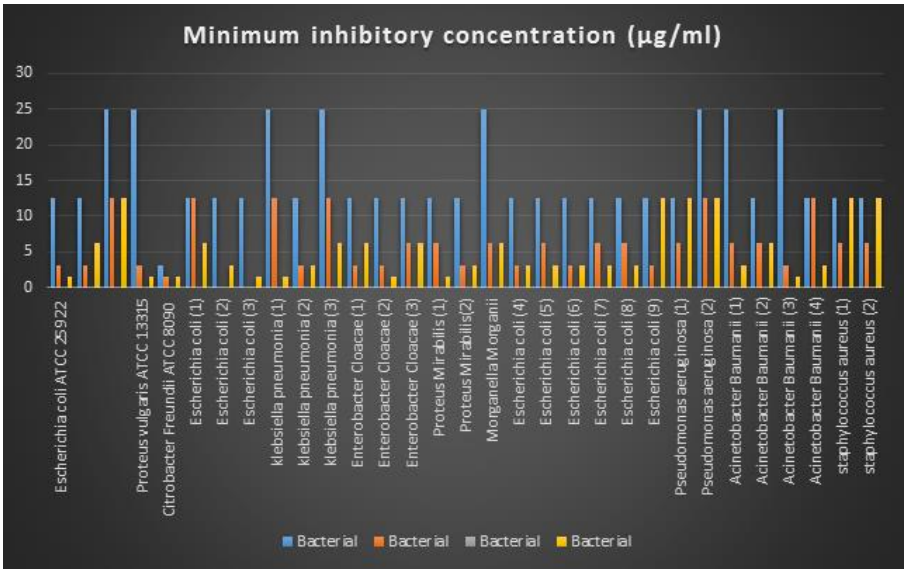

Figure 2: Minimum inhibitory concentration of olive (*Olea europaea* L.) leaves extracts collected from Meknes and Fquih Ben Salah

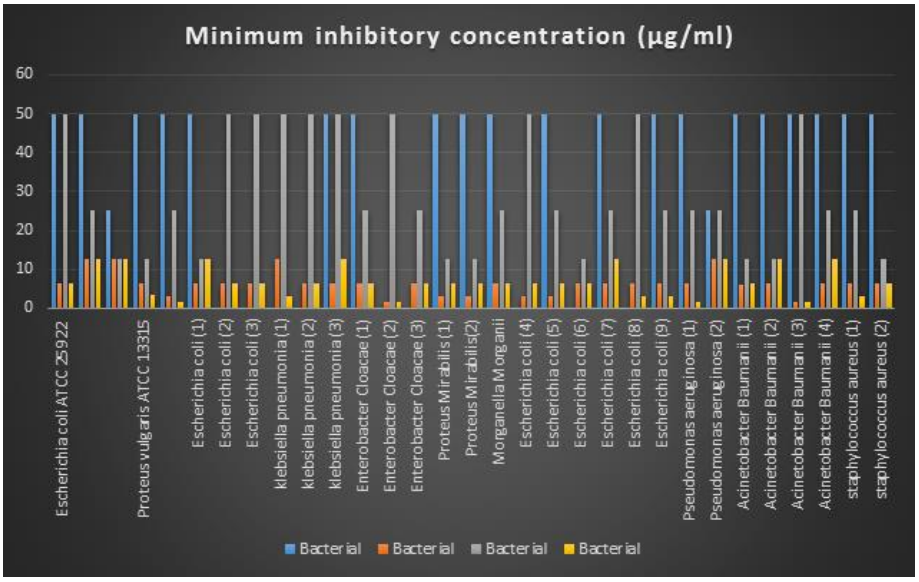

Figure 3: Minimum inhibitory concentration of olive (*Olea europaea* L.) leaves extracts collected from Taza and Ouezzane

The results obtained in this study determined antibacterial efficacy of the aqueous and methanolic leaf extracts of *O. europaea* on test isolates. A major challenge of using water for extraction is that non-polar bioactive compounds cannot be extracted. The type of solvent used in the extraction procedure discovered the success of isolating compounds from plant material [23].

Many studies confirm positive role of olive leaf in inhibitory pathogenic bacteria. Morteza Azizollahi reported that olive leaf aqueous extract showed good Antibacterial abilities and highest inhibition of 11.5 mm against *Salmonella typhimurium* PTCC 1639 [24].. In one study from West Anatolia, Turkey showed that aqueous extract of olive leaves studied in this work showed has antibacterial activity against some of the test microorganisms with the exception of *Bacillus cereus* CCM99, *Enterobacter aerogenes* ATCC 13048 and *Enterobacter cloacae* ATCC 13047 that showed no such activity [25]. In another study, Korukluoglu et al. reported that water extract of olive leaves did not show any inhibition effects on all the tested bacteria. They collected samples from Trilye region in Bursa, Turkey [26]. Korukluoglu et al. determined in another study that the aqueous extract had no antibacterial activity against any of the test bacteria in this study, as expected [27]. He explained these differences by the olive cultivars, the content of the antibacterial agents present in the extracts, or the sample preparation methods used [28].

Peter Masoko and David M Makgapeetja showed that of the nine solvents used, methanol was the best extractant, extracting greater quantity of plant material than the other solvents used [29]. In one study realized by E.O. Dada, the methanolic leaf extracts of *O. europaea* inhibited all the bacteria examined while the aqueous leaf extracts of *O. europaea* were non-inhibitory to *E. coli*, *Salmonella enterica* and *Enterococcus faecalis* at a concentration of 15 mg/ml. This data means that methanol has extracted the active ingredients more than the water from the plant [30]. Results indicated that own extracts are effective against *P. mirabilis* (2) ESBL and *P. aeruginosa* (1) ESBL by 12.25 mm and 11.5 mm respectively. This was confirmed by a study that showed that the highest antibacterial activity against ESBL producing *E. coli* and *K. pneumoniae* was mainly manifested by *Rheum rhaponticum* and *O. europaea* [31].

Furthermore, the minimum inhibitory concentrations values observed from the methanolic leaf extracts of the leaf are in the range of 1.56-12.5 and 12.5-50 mg/ml for the aqueous extract. The results in our study have similarity between the other studies [32,33]. It can be deduced from the result recorded in (Figures 2 and 3; Table 3), that *A. baumanii* (2) have the widest inhibitory zone and lowest MIC, while *K. pneumonia* (3) and *P. aeruginosa* (2) has the lowest inhibitory zone and the highest MIC. This result correlates with the report that microorganisms vary in their degree of susceptibility against antibacterial agents and that antibacterial agents with low activity against an organism have high MIC while an antibacterial with high activity have a low MIC [34].

Bactericidal effects (MBC) of the aqueous and methanolic extracts were not detected; this result is similar to a result presented in study realized by Ziad et al. [31]. A large number of authors have recorded that oleuropein is among the most powerful phenolic compounds of olive leaves for its antibacterial properties [35-38]. It would therefore be probable that the results obtained in this work stem from the presence of oleuropein in the various extracts of olive leaves studied.

In our study, it was found that *S. aureus* was the most sensitive microorganisms, presenting maximum inhibitory zone (20 mm). *S. aureus* is frequently connected to cases of bacteraemia, septicaemia, endocarditis, osteomyelitis, furuncle, etc. It is also frequently implicated in both nosocomial and community acquired infections. The successful inhibition of this bacteria and its contemporary etiology of gastroenteritis (*E. coli*) is a good development, mostly when the registrations of its resistance to various conventional antibiotics is considered [39-41]. *S. aureus* is one of the most common pathogens causing food poisoning [42].

This is consistent with previous studies reporting that the strength and spectrum of antibacterial activity varied depending on the extract type and the Gram of the bacteria. However, Gram-positive bacteria are overall the most sensitive to the effects of these polyphenolic extracts. This higher generally resistance in Gram-negative bacteria is imputed to the presence of an outer lipopolysaccharide membrane impermeable to lipophilic compounds. The absence of this barrier in Gram-positive bacteria lets direct contact of the hydrophobic constituents of the extracts with the phospholipid bilayer of the bacterial cell membrane, resulting in increased ion permeability and leakage of vital intracellular constituents or alteration of Bacterial enzymatic systems [43,44]. Therefore, this aqueous and methanolic extracts have hydrophilic properties and its can steal out the bacterial cells Gram negative.

A study by Galal Al Askari et al. At the National Institute of Hygiene, They were tested the antibacterial activity of leaves of *Vitis vinifera* collected from different regions in morocco (Fez, Meknes, El Jadida, Skhirat and Marrakech) against several bacteria such as *E. coli*, *K. pneumonia*, *P. aeruginosa*, *A. baumanii* and *S. aureus.* They approved that the aqueous and ethanolic extracts of *V. vinifera* leaves from different region has a good activity against Gram-positive more than Gram-negative bacteria [45]. N. Benayad et al. carried out another study on the same range of bacteria with different extracts of essential oil and some extracts of *Cistus ladaniferus* from Oulmes in Morocco; she noticed that methanolic and aqueous extracts had strong antibacterial activity against these strains [46].

Finally, we can go out with a recommendation that olive leaves may be useful in cases where prolonged use of antibiotics hearten development of opportunistic infections [47], being mostly effective against *Klebsiella* and *Pseudomonas*, two bacterial genera that pose a major resistance problem [48].

CONCLUSION

This study emphasizes antibacterial properties of aqueous and methanolic extracts of *O. europaea* leaves against human pathogenic bacteria. The maximum inhibitory zone was noted against multiresistant *S. aureus*, imipenem resistant *A. baumanii*, they were also active against ESBL bacteria: *E. coli* (1 and 3) and *P. aeruginosa* (1). The lowest MIC observed from methanolic leaf extract than the aqueous leaf extract.

The data obtained in this study reveal that the use of olive leaves seed as nutraceuticals may lower the risk of bacterial infections, notably in the intestinal and respiratory tract, mainly due to the protective action provided by its phenolic compounds. Generally, phenolic compounds were shown to be more effective against Gram-positive than Gram-negative bacteria. The observed antibacterial effects of this medicinal plants on the organisms used, though *in vitro* seem interesting and promising and may be effective as a potential source of novel antibacterial drugs. Further research is required in order to obtain information regarding the practical effectiveness of *O. europaea* L. extracts to prevent the growth of a broad spectrum of bacteria under specific conditionals of application.

REFERENCES

[1] K.B. Kirkland, J.P. Briggs, S.L. Trivette, W.E. Wilkinson, D.J. Sexton, *Infect. Control Hosp. Epidemiol*., **1999**, 20, 725-730.
[2] World Health Organisation (WHO), Traditional Medicine, **2008**.
[3] J.L. Rios, M.C. Recio, *J. Ethnopharmacol*., **2005**, 100, 80, 84.
[4] R. Sharma, C. Sharma, B. Kapoor, *Indian J. Med. Sci*., **2005**, 59(3), 120-129.
[5] L. Dyubeni, L.V. Buwa, *South Africa J. Med. Plants Res*., **2012**, 6(14), 2721-2726.
[6] S. Hemaiswarya, A.K. Kruthiventi, M. Doble, *Phytomedicine*., **2008**, 45, 639-652.
[7] J. Alper, *ASM News*., **1998**, 64, 440-441.
[8] A.M. Clark, *Pharm. Res*., **1996**, 13.
[9] S.L. Huang, L. Zhang, P.L. Huang, Y.T. Chang, P.L. Huang, *Biochem. Biophys. Res. Commun*., **2003**, 307, 1029-1037.
[10] A.P. Pereira, I.C.F.R. Ferreira, F. Marcelino, P. Valentao, P.B. Andrade, R. Seabra, L. Estevinho, P.A. Pereira, *Molecules*., **2007**, 12, 1153-1162.
[11] R. Briante, F. La Cara, F. Febbraio, M. Patumi, R. Nucci, *J. Biotechnol*., **2002**, 93, 109-119.
[12] H.F. Al-Azzawie, M.S.S. Alhamdani, *Life Sciences*., **2006**, 78, 1371-1377.
[13] A.N. Sudjana. C.D. Orazio, V. Ryan, N. Rasool, J. Ng, N. Islam, T.V. Riley, K.A. et Hammer, *I. J. Antimicrob. Agents*., **2009**, 33, 461-463.
[14] A. Zarzuelo, J. Duarte, J. Jimenez, M. Gonzales, *Planta Med.*, **1991**, 57, 417-419.
[15] O.B. Garcia, J. Castillo, J. Lorente, A. Ortuno, J.A. Del Rio, *Food Chem.*, **2000**, 68, 457-462.
[16] M.G. Soni, G.A. Burdock, M.S. Christian, C.M. Bitler, R. Crea, *Food Chem. Toxicol*., **2006**, 44, 903-915.
[17] J.B. Harbone, Halsted press, John Wiley and Sonc, Inc: New York, **1976**, 1-26.
[18] A.M. Dub, A.M. Dugani, *Libyan J. Med*., **2013**, 8, 20947.
[19] K. Nongpanga, W. Aporn, M. Duangtip, T. Sukon, *Kmitl Sci. Tech. J*., **2008**, 8, 1.

[20] J.N. Eloff, *Plant Medical*., **1998**, 64, 711-713.
[21] M.A. Janssen, J.J.C. Scheffer, A.B. Svendsen, *Pharmaceutische Weekblad*., **1987**, 9, 193-197.
[22] Clinical and Laboratory Standards Institute-M07-A8, **2008**.
[23] P. Masoko, M.P. Mokgotho, V.G. Mbazima, L.J. Mampuru, *Afri. J. Biotechnol*., **2008**, 20, 3743-3748.
[24] M.A. Aliabadi, R.K. Darsanaki, M.L. Rokhi, M. Nourbakhsh, G. Raeisi, *Ann. Biol. Res*., **2012**, 3(8), 4189-4191.
[25] D. Keskın, N. Ceyhan, A. Uğur, A. Durgan Dbeys, *J. Food Agric. Environ*., **2012**, 10(2), 99-102.
[26] M. Korukluoglu, Y. Sahan, A. Yıgıt, E. Tumay-Ozer, S. Gucer, *Fourth Aegean Analytical Chemisrty Days*., **2004**, 569-571.
[27] A.A. Martínez-Delgado, S. Khandual, S.J. Villanueva-Rodríguez, *J. Food Process. Preserv*., **2010**, 34, 383-396.
[28] A. Ranalli, S. Contento, L. Lucera, M. Di Febo, D. Marchegiani, V. Di Fonzo, *J. Agric. Food Chem*., **2006**, 54, 434-440.
[29] P. Masoko, D.M. Makgapeetja, *BMC Compl. Altern. Med*., **2015**, 15, 409.
[30] E.O. Dada, *J. Biol*., **2013**, 1, 118-124.
[31] D. Ziad, A. Elias, A.M. Roula, *IJPSR*., **2011**, 2(7), 1669-1678.
[32] M. Gökmen, R. Kara, L. Akkaya, E. Torlak, A. Önen, *Ame. J. Microbiol*., **2014**, 5(2), 37-40.
[33] N. Bouderba Nora, K. Hamid, M. Snouci, M. Boumedien, M. Abdellah, *The Open Conference Proccedings Journal*., **2012**, 3, 66-69.
[34] A. Banso, P.O. Ayodele, *Nigerian J. Appl. Arts Sci*., **2005**, 1, 35-42.
[35] N.H. Aziz, S.E. Farag, L.A. Mousa, M.A. et Abo-Zaid, *Microbios*., **1998**, 93, 43-54.
[36] G. Bisignano, A. Tomaino, R. Lo Cascio, G. Crisafi, N. Uccella, A. Saija, *J. Pharm. Pharmacol*., **1999**, 51, 971-974.
[37] E. Medina, M. Brenes, C. Romero, A. García et De Castro, *J. Agric. Food Chem*., **2007**, 55, 9817-9823.
[38] A.G. Ponce, R. Fritz, S.I. del Valle C et Roura, *LW u.-Technol*., **2003**, 679-684.
[39] A. Voss, *Chemother. J*., **1996**, 5, 5-6.
[40] B. Wiedemann, *Drugs*., **1996**, 52, 95-100.
[41] G.A.J. Ayliffe, *Clin. Infect. Dis.*, **1997**, 24, 74-79.
[42] A. Mastoraki, I. Kriaras, E. Douka, S. Mastoraki, G. Stravopodis, S. Geroulanos, *Cardiovasc. Thorac. Surg*., **2008**, 7, 452e6.
[43] R. Khan, B. Islam, M. Akram, S. Shakil, A. Ahmad, A.S. Manazir, M. Siddiqui, A.U. Khan, *Molecules*., **2009**, 14, 586-597.
[44] M. Wendakoon CN et Sakaguchi, *J. Food Prot*., **1995**, 58, 280-283.
[45] G. Al Askari, A. Kahouadji, K. Khedid, M. Mousaddak, L. Ouaffak, R. Charof, Z. Mennane, *American-Eurasian J. Agric. Environ. Sci*., **2012**, 12(1), 85-90.
[46] N. Benayad, Z. Mennane, R. Charof, A. Hakiki, M. Mosaddak, *J. Mater. Environ. Sci*., **2013**, 4(6), 1066-1071.
[47] F.M. Verduyn-Lunel, J.F. Meis, A. Voss, *Diagn. Microbiol. Infect. Dis*., **1999**, 34(3), 213-220.
[48] H.C. Neu, *Science*., **1992**, 257, 1064-1073.