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Research Article

Antimicrobial Activity of Oleuropine and Thyme Extracts Against Selected Pathogenic Microorganisms and their Potential Uses as Natural Preservatives

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ABSTRACT

Introduction: Currently, there is an increasing desire for food, cosmetic, and pharmaceutical products with fewer synthetic chemicals, and longer shelf life. Thus, there is an urgent need to develop these products with self-preservation and /or protection from microbial growth using natural substances. The purpose of this study was to confirm the antimicrobial activity of Oleuropein and thyme oil (pharmacopeial grade). In addition, we combined oleuropein and thyme oil at different concentrations to determine their antimicrobial synergistic effects and the potential to use them as natural preservatives in food, cosmetic, and pharmaceutical preparations.

Methods: Oleuropine and thyme oil were evaluated for their activity against human pathogenic microorganisms such as Candida albicans (ATCC 10231) Aspergillus niger (ATCC 16404), Escherichia coli (ATCC 8739), Pseudomonas aeruginosa (ATCC 9027), Staphylococcus aureus (ATCC 6538). The antimicrobial activity of the extracts was performed by antimicrobial preservative tests used for topical formulations according to the United States Pharmacopeia (USP).

Results: The efficiency of Oleuropine against the microbes tested showed an optimum concentration of 0.6%. Whereas thyme oil at a concentration of 0.1% v/v. showed that it was effective against the three bacteria only The antimicrobial activity of oleuropine (0.4 w/v %) and thyme oil (0.1 v/v %) showed synergistic activity against all pathogenic microorganisms tested.

Conclusion: The combination can be considered very promising for use as antimicrobial natural preservatives in food, cosmetic, and pharmaceutical products. In addition, it can be of great significance in the treatment of infectious diseases such as superficial skin infections caused by pathogenic microorganisms.

Keywords: Oleuropine, thyme oil, antimicrobial, Extract, Natural Preservatives

INTRODUCTION

Synthetic preservatives and antioxidants provide high antimicrobial and antioxidant activities. However, they are usually associated with adverse reactions and implicated in potential harmful effects due to chronic consumption(Raymond C Rowe; Paul J Sheskey; Marian E Quinn, 2009). Currently, there is an increasing desire for high quality products, with fewer synthetic chemicals, and longer shelf life . Thus, there is an urgent

need to develop food, cosmetic, and pharmaceutical products with self-preservation and /or protection from microbial growth using natural substances. The microbial growth and the subsequent deterioration in these products are usually prevented by using chemical preservatives. But most of these chemical preservatives are toxic and have many side effects (Chen et al., 2018; Elder DP, 2012).

Antimicrobial activity of olive leaf extract (OLE) and thyme oil

Several reports have shown that different plants including olive leaf extract (OLE) and thyme have the ability to act as antidiabetic, anti-hypertensive, anti-obesity, anticancer, hepatoprotective, gastroprotective and cardioprotective agents (Afsheen et al., 2018; Arulselvan et al., 2016; Desai et al., 2008; Erdohan & Turhan, n.d.; Kanetkar et al., 2007; Kasote et al., 2015; Tabassum & Ahmad, 2011). They are also have potential activety against microbes by inhibiting the growth of several groups of bacteria (Hickl et al., 2018), fungi(Lewu et al., 2008) and viruses (Ganjhu et al., 2015). The beneficial effects of the olive components (Olea europaea L.) had been confirmed and proven in many studies (Atai et al., 2007; El & Karakaya, 2009; Vogel et al., 2015) . The olive tree contains a high percentage of biophenols such as oleuropein, verascascide, ligstrosides, tyrosol or hydroxyl tyrosol (Dilek Keskın , Nur Ceyhan , Aysel Uğur, 2012; Larussa et al., 2019).

A significant antimicrobial activity of olive leaves extract has been recently reported in a review article by Atai et al (Atai et al., 2016). Most of the reviewd studies demonstrated that the method used to obtain olive leaf extract using different solvents and concentration had presented significant effectiveness against microorganisms. Figures (1, 2).

The purpose of this study was to evaluate the antimicrobial activity of Oleuropein and thyme oil (pharmacopeial grade). In addition, we combined oleuropein and thyme oil at different concentrations to determine their antimicrobial synergistic effects on five microorganisms (3 bacteria and 2 fungi) as per the pharmacopeia in order to use the combination as natural preservative in topical pharmaceutical preparation , cosmetics and food preperations to replace the chemical preservatives.

MATERIALS AND METHODS

All materials used were of analytical grade. These materials and reagents were donated by Beit-Jala Pharmaceutical Co, Ltd Bethlehem - Palestine. Thyme oil was purchased from the manufacturer (Batch No. SO1162769/3, TREATT), and analyzed by Beit- Jala Pharmaceutical Co, Ltd and was found to meet the requirements of the European Pharmacopoeia(Community Herbal Monograph on Thymus Vulgaris L., Thymus Zygis Loefl. Ex L., Aetheroleum Final Discussion in Working Party on Community Monographs and Community List (MLWP), 2010). Oleuropine was extracted and tested by Beit- Jala Pharmaceutical Co, Ltd according the monograph of the European Medicines Agency(Medicines Agency, n.d.). Oleuropine 98.3% assay and thyme oil 99.4% assay samples were also donated by Beit- Jala Pharmaceutical Co, Ltd Bethlehem - Palestine in December 2018.

Human pathogenic microorganisms

Human pathogenic microorganisms used in this research are the following: Candida albicans (ATCC 10231), Aspergillus niger (ATCC 16404), Escherichia coli (ATCC 8739), Pseudomonas aeruginosa (ATCC 9027) and Staphylococcus aureus (ATCC 6538). All were donated by Beit-Jala Pharmaceutical Co, Ltd and were maintained in nutrient agar and stored at 4° C until use.

Antimicrobial effectiveness testing

This procedure was designed to evaluate the antimicrobial effectiveness of antimicrobial preservatives used in semisolid formulations (General Chapters: <51> ANTIMICROBIAL EFFECTIVENESS TESTING, n.d.).

Media

For the cultivation of the test organisms, agar medium that optimally support their growth and the growth of the stock cultures were used. Soybean Casein Digest Agar and Broth and Sabouraud's Dextrose Agar and Broth were used. In addition, a suitable inactivator (neutralizer) for the specific antimicrobial properties in the product was added to the broth (General Chapters: <51> ANTIMICROBIAL EFFECTIVENESS TESTING, n.d.). The Culture conditions for the inoculum preparations are described in Table (1).

Growth promotion of the media

The selected media were used to cultivate and grow the appropriate microorganisms. Solid agar media was used for growth promotion testing. The pour plate method was used to determine the number of colony forming units (CFU) which must be ≥ 70% of the microorganism inoculum's calculated value (General Chapters: <51> ANTIMICROBIAL EFFECTIVENESS TESTING, n.d.).

Preparation of inoculums

Preparatory to the test

Inoculums were prepared by taking few colonies from the surface of an agar plate incubated for 18 to 24 hour. The Culture conditions for the inoculum preparations were described in Table (1) USP (General Chapters: <51> ANTIMICROBIAL EFFECTIVENESS TESTING, n.d.).

Harvesting the bacterial and Candida albicans cultures

Sufficient sterile saline TS was added to obtain a microbial count of about $1x10^8$ CFU per mL. The number of cells were counted by measuring the turbidity using a spectrophotometer at □□650 nm to obtain an optical density (O.D.) of:

A. 0.3-0.45 for S. aureus (~1-3 $X10^8$ CFU/ ml).

B. 0.2-0.3 for P. aeruginosa and E. coli (~1-3 $X10^8$ CFU/ ml).

C. < 1.0 for C. albicans (~ 1-3 x 10^8 CFU / ml)

Several dilutions were made (10^{-3} – 10^{-6}) and cultivated by the pour plate method. Bacteria was inoculated for 24 hours at 35°C± 2, and C. albicans at 23° C ± 2 for 2-3 days and the CFUs were counted (count plates having between 30-100 CFU).

Harvesting the Aspergillus Niger cultures

The surface growth was washed using sterile saline TS containing 0.05% of polysorbate 80. A sufficient sterile saline TS was added to obtain a microbial count of about $1x10^8$ CFU per mL. Several dilutions were made (10^{-3} – 10^{-8}) and seeded by pour plate method. Cultures were incubated between 2-4 days at 23 ± 2°C and the CFUs were counted (count plates having between 10-100 CFU). To determine the number of CFU per mL in each suspension, the condition of media and microbial recovery incubation times were used as listed in Table (1) to confirm the initial CFU per mL estimate.

Results and Discussion

Antimicrobial effectiveness testing

Oleuropein

Three concentrations of Oleuropein were tested, 0.2, 0.4 and 0.6% w/v. The results abtained using the concentration 0.2% w/v of Oleuropein, are shown in Table (2). There was more than 1 log reduction (10 folds) in the counts (CFU) of the three bacterial strains tested from the initial count at 14 days (for Staphylococcus aureus there is complete inhibition after 7, and 14 days of incubation at all dilution used). The same for Pseudomonas aeruginosa there were more than 10 fold (1 Log) reduction in counts after 7 and 14 days using all dilutions. The same results (as for Pseudomonas aeruginosa) were obtained for Escherichia coli. Furthermore, there was complete inhibition of these three bacterial strains after 28 days (i.e. no increase from the 14 days count to 28 days). The results obtained imply that the concentration of 0.2% w/v Oleuropein can be used as antimicrobial agent against the three strains of bacteria tested. However, the Oleuropein to be used as a preservative at this concentration should also be effective against yeast and mold in addition to these three bacterial strains tested. As shown from the results for Oleuropein at 0.2% w/v, there was an increase in the count of yeast (Candida albicans) and mold (Aspergillus niger) at 14 days, which indicates that Oleuropein at this concentration cannot be used as preservative since it is not effective against yeast and mold but can be used as antimicrobial agent against the three types of bacteria tested.

Therefore, it became necessary to increase the concentration from 0.2% to 0.4% w/v. At this concentration, Oleuropein was found to be effective against the three bacterial strains same as 0.2% concentration, but more than 10 fold reduction in the counts were obtained at 14 days and no increase in the counts after 28 days as shown in Table (3). Regarding the activity against yeast and mold, the results showed that it wasn't effective against yeast and mold, as shown in Table (3). Increasing the concentration of Oleuropein to 0.6% has resulted in complete inhibition of all three bacterial strains after 7, 14 and 28 days. In addition, there was no increase in the yeast and mold counts from initial inoculum at 14 and 28 days as shown in Table (4). It was apparent that the results obtained with Oleuropein at the new higher concenteration of 0.6% w/v has the desirable antibacterial and antifungal effects as well as adequate preservation effects.

Thyme oil

The same tests conducted with Oleuropine has been performed using thyme oil at concentration of 0.1% v/v, taking into consideration to use the lowest concentration of the oil in order to minimize the strong stinging effects due to its odor and it is also the highest concentration that can be dissolved in water. At this concentration, the results showed that it was effective against the three bacteria only, as shown in Table (5).

Synergistic effect between Oleuropine and thyme oil

The use of a combination of thyme oil and oleuropein at concentrations of 0.2% and 0.4% w/v respectively showed inhibitory activity against the three bacterial strains as well as the yeast and mold tested as shown in Table (6). This implies that thyme oil/ Oleuropein combination

can be used as natural preservative system in pharmaceutical preparations. Combination of the two oils produced adequate synergistic effect against the pathogens included in this study at the determined concentrations. As for the results, bold black did not specify the conditions, which increase from the 14 days count at 28 days for the bacteria and increase from the initial calculated count at 14, and 28 for the yeast and molds. Therefore, these results cannot be taken at these concentrations and cannot be used as a preservative, until concentration is increased or combination are used. Data analysis of the antimicrobial activity of oleuropine (0.4 w/v %) and thyme oil (0.1 v/v %) against the three types of bacteria , the yeast and the molds showed ,

DISCUSSION

Oleuropein has antimicrobial activity against gram positive, gram negative bacteria and mycoplasma. It is also able to inhibit the production of enterotoxin B by S. aureus and the germination of B. cereus spores(Omar, 2010). Consequently, the results obtained in our study revealed that Oleuropein at concentration 0.6% has resulted in complete inhibition of all three bacterial strains and there was no increase in the yeast and mold counts from initial inoculum after 7, 14 and 28 days. These resuls is in agreement with previous studies conducted to determine the antibacterial properties of Oleuropine in different concentrations(Omar, 2010; Pereira et al., 2007; Starliper et al., 2015). The mechanism of action of oleuropein against microorganisms occurs by damaging the bacterial cell membrane and disrupting the peptidoglycan layers in the cell wall (Omar, 2010). In addition to its antimicrobial activity, it was found that Oleuropein ameliorated the nephrotoxicity induced by amikacin in rats (Abdel-Gayoum et al., 2015; M Abd El-Rahman & El-Rahman HSM, 2016).

Thyme oil at the concentration of 0.1% v/v, has antimicrobial activity against standard and clinical isolates of S. aureus, E. coli, Enterococci and P. aeruginosa, but with lower efficacy against P. aeruginosa clinical isolates. The results of the present study are similar to previous studies(Sienkiewicz et al., 2012; Starliper et al., 2015) conducted on thyme oil. It was found that clininical isolates of Enterococcus and S. aureus were susceptible to thyme oil with MIC values of 0.25 and 0.50 ul/ml respectively(Sienkiewicz et al., 2012). On the other hand it was found that , the thyme oil, at higher concentration than the concentation used in our sstudy, showed antimicrobial activity against standard and clinical strains of S. aureus, En. faecalis, En. faecium, En. durans, Es. coli, and P. aeruginosa(Sienkiewicz et al., 2012; Sim et al., 2019)

As the results of the current study indicated the combination of thyme oil and Oleuropein showed inhibitory activity against the three bacterial strains as well as the yeast and mold tested. This indicates that the thyme oil and Oleuropein combination can be used as a natural preservative system in wide range of pharmaceutical, food and cosmetics preperations. A combination of the thyme oil and Oleuropein produced an adequate synergistic effect against the pathogens included in this study at the determined concentration. Literature reviews showed a wealth of data about the use of oleuropein and thyme oil individually. However, little information was found about the antimicrobial effects of the combination of both of these two natural substances. It has been reported that the administration of both extracts into rats gave a synergistic effect in the antioxidant status and enhanced bioavailability of phenolic compounds in the presence of thyme phenolics(Rubió et al., 2014). Therefore, the results of the combination of oleuropein and thyme oil obtained in this study can contribute additional significant information. Our results showed the synergistic effects of the combination of Oleuropein and thyme oil against tested microorganisms with lower concentrations as compared to individual use.

Limitation of study

No clinical isolates were included in this study.

CONCLUSIONS

The efficiency of Oleuropein against microbes was verified, with the determined optimal concentration of 0.6%. This concentration showed a desirable antibacterial and antifungal effects as well as adequate preservation effects. Efficiency of thyme oil against microbes was also conducted, taking into consideration to use the lowest concentration of 0.1% of the oil (the maximum concentration that can be dissolved in water), which was effective against the three bacteria only. The antimicrobial activity of Oleuropine (0.4 w/v %) and thyme oil (0.1 v/v %) showed synergistic activity against all pathogenic microorganisms tested. This combination can be considered very promising to be used as a natural antimicrobial preservative in pharmaceutical,food and cosmetics products as well as antibacterial and antifungal supplements in the development of herbal formulations. In addition, it can be of great significance in treatment of infectious diseases such as superficial skin infections caused by pathogenic microorganisms.

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Authorship contribution statement:
Yousef Sahoury: design the study, carried out the experiments, wrote the introduction and the results, Hani Naseef: design the study, carried out the experiments, wrote the discussion, supervision, Mohammad Farraj: review- editing, Moammal Qurt: wrote the methods, review – editing, Lina El-Khairy: review- editing

Conflict of Interest
The authors declare no conflicts of interest in publication of this study.

Data Availability
The data used to aid the outputs of this research are available from Yousef Sahoury (sahoury1987@yahoo.com) upon request.

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Table (2): Antimicrobial activity of Oleuropein (0.2% w/v) in distilled water against three types of bacteria, one yeast and one mold

Microorganism 10^6 CFU/ml	ATCC NO.	Day	Test dilution					Control dilution (without preservative)				
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10-4	10-5	10-6
S. aureus	6538	0	>>10$^{3..}$	>10^3	>10^3	1201	406				>10^3	
		7	0	0	0	0	0				>10^3	1600
		14	0	0	0	0	0				>10^3	>10^3
		28	0	0	0	0	0				770	60
P. aeroginosa	9027	0	>>10^3	>10^3	>10^3	317	120			180	260	
		7	>10^3	512	230	51	0				>10^3	1200
		14	103	25	10	0	0				>10^3	400
		28	0	0	0	0	0			>10^3	375	
E.coli	8739	0	>>10^3	>10^3	>10^3	>10^3	450			449	32	
		7	>10^3	501	220	85	10				>10^3	>10^3
		14	100	29	0	0	0				>10^3	590
		28	0	0	0	0	0				>10^3	100
C. albicans	10231	0	>10^3	>10^3	>10^3	>10^3	420		920	115	25	
		7	>10^3	>10^3	>10^3	1533	450			>10^3	1450	
		14	>10^3	>10^3	>10^3	>10^3	**215**			>10^3	>10^3	
		28	>10^3	>10^3	>10^3	490	**240**				1260	
A.niger	16404	0	>>10^3	>10^3	400	45	20	>10^3	480	69	5	
		7	>10^3	>10^3	120	30	5		90	6	1	
		14	>10^3	>10^3	306	10	4		25	4	1	
		28	>10^3	370	30	5	0		65	10	2	

Table (1): Culture conditions for inoculum preparation

Organism	Suitable Medium	Incubation Temperature	Inoculum Incubation Time	Microbial Recovery Incubation Time

Escherichia coli ATCC No. 8739	SCD, SCDA	32.5 + 2.5°C	18 – 24 hours	3 – 5 days
Pseudomonas aeruginosa ATCC No. 9027	SCD, SCDA	32.5 + 2.5°C	18 – 24 hours	3 – 5 days
Staphylococcus aureus ATCC No. 6538	SCD, SCDA	32.5 + 2.5°C	18 – 24 hours	3 – 5 days
Candida albicans ATCC No. 10231	SAB, SABDA	22.5 + 2.5°C	44 – 52 hours	3 – 5 days
Aspergillus niger ATCC No. 16404	SAB, SABDA	22.5 + 2.5°C	6 – 10 days	3 – 7 days

Table (3): Antimicrobial activity of Oleuropein (0.4% w/v) in distilled water against three types of bacteria, one yeast and one mold

Microorganism 10^6 CFU/ml	ATCC NO.	Day	Test dilution					Control dilution (without preservative)				
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10-6
S. aureus	6538	0	>>$10^{3..}$	>10^3	>10^3	>10^3	380			525	40	
		7	0	0	0	0	0				>10^3	>10^3
		14	0	0	0	0	0				>10^3	>10^3
		28	0	0	0	0	0				750	60
P. aeruginosa	9027	0	>>10^3	>10^3	>10^3	400	120			165	10	
		7	>10^3	310	35	10	0				>10^3	>10^3
		14	0	0	0	0	0				>10^3	430
		28	0	0	0	0	0			>10^3	360	
E.coli	8739	0	>>10^3	>10^3	>10^3	>10^3	200			449	32	
		7	>10^3	500	281	120	5				>10^3	>10^3
		14	0	0	0	0	0				>10^3	580
		28	0	0	0	0	0				892	100
C. albican	10231	0	>>10^3	>10^3	620	105	10		890	100	20	
		7	>>10^3	>10^3	>10^3	>10^3	300				>10^3	
		14	>10^3	>10^3	>10^3	600	**60**			>10^3	>10^3	
		28	>10^3	>10^3	>10^3	311	**120**				1205	
A.niger	16404	0	>>10^3	>10^3	400	75	5	>10^3	475	65	5	
		7	>10^3	300	100	25	4		85	8	1	
		14	>10^3	>10^3	200	11	2		25	3	1	
		28	477	220	10	1	0		60	5	1	

Table (4): Antimicrobial activity of Oleuropein (0.6% w/v) in distilled water against three types of bacteria, one yeast and one mold

Microorganism 10^6 CFU/ml	ATCC NO.	Day	Test dilution					Control dilution (without preservative)				
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10-6
S. aureus	6538	0	$>10^{3..}$	$>10^3$	$>10^3$	419	80			565	40	
		7	0	0	0	0	0				$>10^3$	1670
		14	0	0	0	0	0				$>10^3$	825
		28	0	0	0	0	0				770	60
P. aeruginosa	9027	0	$>10^3$	$>10^3$	300	35	13			185	20	
		7	$>10^3$	70	15	5	0				$>10^3$	1200
		14	0	0	0	0	0				$>10^3$	450
		28	0	0	0	0	0			$>10^3$	365	
E.coli	8739	0	$>10^3$	$>10^3$	$>10^3$	565	45			435	30	
		7	$>10^3$	400	85	15	9				$>10^3$	$>10^3$
		14	0	0	0	0	0				$>10^3$	550
		28	0	0	0	0	0				$>10^3$	85
C. albican	10231	0	$>>10^3$	$>10^3$	633	100	18		928	115	28	
		7	$>10^3$	$>10^3$	$>10^3$	$>10^3$	308			$>10^3$	1380	
		14	511	417	312	155	9			$>10^3$	$>10^3$	
		28	200	280	80	5	0				1200	
A.niger	16404	0	$>10^3$	$>10^3$	265	30	5	$>10^3$	472	60	5	
		7	330	55	5	0	0		98	8	1	
		14	160	31	3	0	0		38	5	1	
		28	240	21	5	0	0		62	15	2	

Table (5): Antimicrobial activity of thyme oil (0.1 %) in distilled water against three types bacteria , one yeast and one mold

Microorganism 10*6	ATCC NO.	Day	Test dilution	Control dilution (without preservative)

CFU/ml			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10-6
S. aureus	6538	0	$>10^3$	$>10^3$	$>10^3$	300	79			577	45	
		7	$>10^3$	411	0	0	0				$>10^3$	1748
		14	0	0	0	0	0				$>10^3$	838
		28	0	0	0	0	0				770	68
P. aeruginosa	9027	0	$>10^3$	$>10^3$	267	21	2			180	16	
		7	318	43	3	0	0				$>10^3$	1270
		14	0	0	0	0	0				$>10^3$	440
		28	0	0	0	0	0			$>10^3$	375	
E.coli	8739	0	$>10^3$	$>10^3$	$>10^3$	435	32			449	32	
		7	$>10^3$	218	79	11	3				$>10^3$	$>10^3$
		14	0	0	0	0	0				$>10^3$	597
		28	0	0	0	0	0				912	100
C. albican	10231	0	$>10^3$	$>10^3$	910	401	290		928	115	28	
		7	$>10^3$	$>10^3$	$>10^3$	445	246			$>10^3$	1450	
		14	$>10^3$	$>10^3$	431	212	**109**			$>10^3$	$>10^3$	
		28	$>10^3$	$>10^3$	**450**	**320**	**150**				1269	
A.niger	16404	0	$>10^3$	$>10^3$	$>10^3$	$>10^3$	495	$>10^3$	484	69	5	
		7	$>10^3$	$>10^3$	$>10^3$	732	435		90	6	1	
		14	850	255	513	**420**	**390**		28	4	1	
		28	820	240	500	**450**	**400**		66	10	2	

Table (6): Antimicrobial activity of Oleuropine (0.4 w/v %) and thyme oil (0.1v/v %) in distilled water against three types of bacteria, one yeast and one molds.

Microorganism 10*6 CFU/ml	ATCC NO.	Day	Test dilution					Control dilution (without preservative)				
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	**10-4**	**10-5**	10-6
S. aureus	6538	0	$>10^{3..}$	$>10^3$	$>10^3$	311	61			577	45	
		7	0	0	0	0	0				$>10^3$	1748
		14	0	0	0	0	0				$>10^3$	838
		28	0	0	0	0	0				770	68

P. aeruginosa	9027	0	$>10^3$	$>10^3$	215	31	5		180	16	
		7	419	23	9	0	0			$>10^3$	1270
		14	0	0	0	0	0			$>10^3$	440
		28	0	0	0	0	0		$>10^3$	375	
E.coli	8739	0	$>10^3$	$>10^3$	$>10^3$	255	52		449	32	
		7	$>10^3$	202	99	21	8			$>10^3$	$>10^3$
		14	0	0	0	0	0			$>10^3$	597
		28	0	0	0	0	0			912	100
C. albican	10231	0	$>10^3$	$>10^3$	$>10^3$	58	15	928	115	28	
		7	$>10^3$	$>10^3$	560	245	223		$>10^3$	1450	
		14	501	400	280	109	8		$>10^3$	$>10^3$	
		28	210	250	31	0	0			1269	
A.niger	16404	0	$>10^3$	$>10^3$	185	21	0	$>10^3$	484	69	5
		7	$>10^3$	24	2	0	0		90	6	1
		14	192	16	18	0	0		28	4	1
		28	113	9	0	0	0		66	10	2

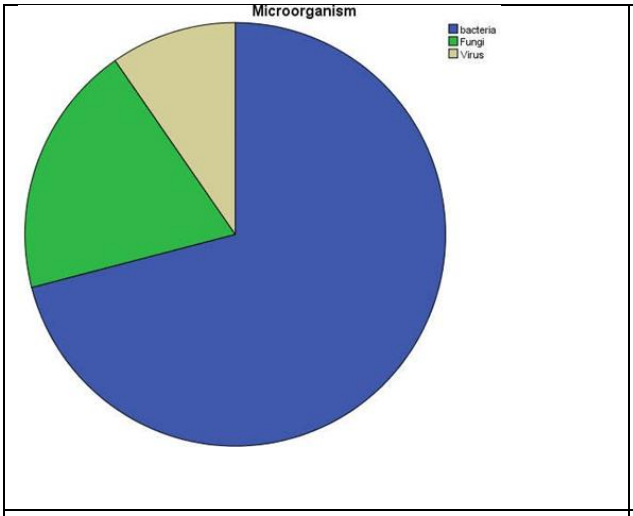

Figure (1): Types of microorganisms used in antimicrobial assessment of OLE

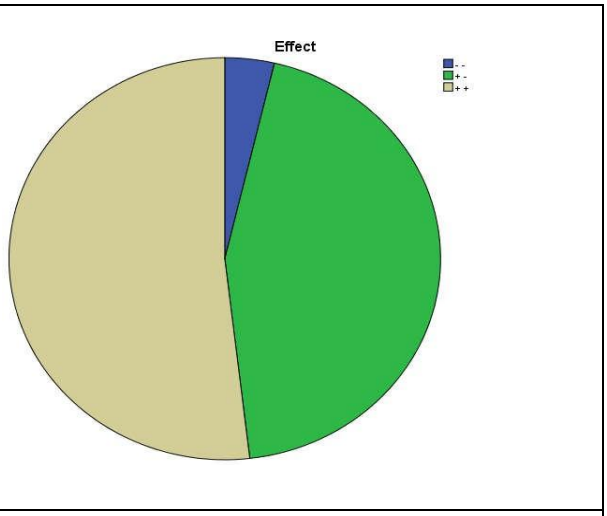

Figure (2): Type of antimicrobial effect OLE. ++: studies which were effective against all tested m.o. +-: studies which were effective against some strains of tested m.o.--: no effect