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Comparative study of plant extracts as broad-spectrum antibacterial agents

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Abstract : *Plants with an established history of medicinal uses were tested against various microorganisms in this study. We compared the antibacterial activity of methanol, ethanol, and water extracts of three plant species, including Origanum syriacum, Thymus vulgaris, and Carica papaya, which commonly grow in open habitats or mountainous areas with well-drained soil. The methanol and ethanol extracts of the three plants had high antibacterial activity against Escherichia coli, Campylobacter jejuni, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, and Shigella sonnei. Water extracts of O. syriacum and T. vulgaris showed an inhibitory effect against the tested bacteria, while the water extract of C. papaya was less effective. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were reflected in the inhibitory activity with MICs of 2 µL after treating with O. syriacum and T. vulgaris extracts, and MBCs of 4 and 8 µL with O. syriacum and T. vulgaris extracts. An assay of secondary compounds revealed the presence of alkaloids, flavonoids, glycoside, cardiac glycoside, terpenoids, anthraquinones, and tannins. We conclude that O. syriacum, T. vulgaris, and C. papaya have bioactive compounds that combat a broad spectrum of bacteria.*
Keywords: *antibacterial agents, Origanum syriacum, Thymus vulgaris, Carica papaya.*

I. INTRODUCTION

Throughout human history, certain plants have been used for medicinal purposes, with the earliest applications dating to more than 60,000 years ago [1]. Consequently, thousands of medicinal plants have been discovered and used to combat disease. The most popular aromatic medicinal plants commonly grow in open habitats or mountainous areas with well-drained soil. These species include *Thymus* spp. and *Origanum* spp., whose essential oils have been investigated with regard to their antimicrobial activity and found to contain bioactive components. In the in vitro study to evaluate the antibacterial activity of *Thymus vulgaris*, Moradi *et al*. [2] reported that a methanol extract of *T. vulgaris* was effective against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Several studies have tested the activity of essential oils derived from *Thymus* spp., including *T. vulgaris* T., *T. vulgaris*, *T. migricus*, *T. fallax*, and *T. pubescens* var. *pubescens*, and *Origanum* spp., including *O. vulgare* ssp. *viride and O. vulgare* ssp. *hirtum*, against the microorganisms *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *P. mirabilis*, *Pseudomonas aeruginosa*, *Campylobacter jejuni*, *Enterobacter aerogenes*, *Salmonella typhi* Ty2, *E. coli* O157:H7, *Acinetobacter calcoacetica*, *Aeromonas hydrophila*, *Citrobacter freundii*, *Clostridium sporogenes*, *Candida albicans*, *Aspergillus flavus*, *A. niger*, *Penicillum expansum*, *P. lanosum*, and *Alternaria alternata*. The extracted essential oils had a significant effect against the tested microorganisms, with activity being dependent on the type of microorganisms; for example, gram-negative bacteria were more sensitive than gram-positive bacteria [3–8].

The tropical plant *Carica papaya* has also been investigated for the antimicrobial activity associated with several of its parts. The aqueous and alcohol extracts of leaves, roots, fruits, and seeds were determined to have bactericidal activity against *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, *P. mirabilis*, *Streptococcus pyogenenes*, *S. pneumoniae*, *B. cereus*, *Salmonella typhi*, *Shigella flexneri*, *Enterobacter aerogenes*, and *Providencia stuartii* [9–12].

Plants synthesize a wide variety of chemical compounds that perform important biological functions and defend against microorganisms and other environmental threats. Medicinal plants contain aromatic compounds that have been shown to have antimicrobial, antioxidant, anticancer, and anti-inflammatory properties. Analyses of the bioactive compounds in *Thymus* spp., *Origanum* spp., and *C. papaya* have revealed primary and secondary metabolic compounds, including α-terpinene, γ-terpinene, sabinene, thymol, carvacrol terpenoids, organic acids, alkaloids, caffeic acid, thymol phenolic alcohol, tannins, polyphenols, flavones, β-citronellol, citronellol acetate, *trans*-geraniol, fatty acids, crude protein, crude fiber, papaya oil, carpaine, caricin, glucotropacolin, the enzyme myrosin, and *o*-cymene [3, 13–17].

The aim of this study was to investigate *O. syriacum*, *T. vulgaris*, and *C. papaya* extracts as potential broad-spectrum antibacterial agents.

II. MATERIALS AND METHODS

Study Bacteria

Eleven gram-negative bacteria were tested in this study, including *Escherichia coli* ATCC 8239, *Campylobacter jejuni* ATCC 33291, *Haemophilus influenza* ATCC 49247, *Klebsiella pneumoniae* ATCC 13883, *Neisseria gonorrhoeae* ATCC 31426, *Proteus mirabilis* ATCC 35659, *P. vulgaris* ATCC 33420, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028, *Shigella sonnei* ATCC 9290, and *Vibrio parahaemolyticus* ATCC 17802. The seven gram-positive bacteria included in the study were *Enterococcus faecalis* ATCC 29212, *Staphylococcus saprophyticus* ATCC 15305, *S. aureus* MRSA ATCC 33591, *S. epidermidis* ATCC 12228, *Stenotrophomonas maltophilia* ATCC 51331, *Streptococcus agalactiae* (group B) ATCC 12386, and *S. pyogenes* ATCC 19615. Assay plates were prepared by inoculating 100 µL of each sample (1×10^5 colony-forming units [cfu]) by using Mueller-Hinton agar (OXOID CM 337).

Study Plants and Extract Preparation

Frish *Carica papaya* seeds and *O. syriacum* and *T. vulgaris* leaves were collected from local markets, washed with distilled water several times, then spread on plates, and dried at 40°C. After drying, the plant materials were ground and solubilized in methanol, ethanol, and sterilized water at 50 g of material per 100 mL of solvents. The yelled mixtures were kept on a 120 rpm shaker at 30°C for 24 h and then filtered using Whatman No. 1 filter paper. The filtered solvents were dried under reduced pressure at 40°C, and the resulting deposits were used as crude extracts [18].

Antimicrobial Assays

The antimicrobial activity of each crude plant extract was determined against the gram-positive and gram-negative bacteria in vitro. The activity of each extract was measured by disc diffusion and broth dilution methods per the Clinical and Laboratory Standards Institute (CLSI) protocol (CLSI, 2007) [19, 20]. For the disc diffusion method, each extract was dissolved in dimethylsulfoxide (DMSO) at 50 µg mL^{-1} and filtered through a 0.22 µm pore filter (Millipore, Billerica, MA). 5 mm diameter of paper discs was placed with 100 µl of each solution, then they set on a pre-inoculated agar surface. Plates were incubated at 37°C for 24 h and the inhibition bacterial growth of each disc was measured. All tests were performed in triplicate [21–23].

Minimum Inhibitory Concentration

The extracts that inhibited bacterial growth were tested to determine the minimum inhibitory concentration (MIC) by using a broth micro dilution method [24]. The bacteria were cultured overnight on Mueller-Hinton agar and then suspended in 1 mL of Mueller-Hinton broth (OXOID CM 405) to give a final concentration of 5×10^5 cfu mL^{-1}. Each extract was serially diluted with Mueller- Hinton broth in a 96-well microplate and the plates were inoculated with the bacteria and incubated at 37°C for 16–20 h. After incubation, plates were evaluated for the visible presence or absence of microbial growth. The MIC was defined as the lowest concentration of an extract for which there was no visible growth compared to the control [22–25].

Minimum Bactericidal Concentration

Minimum bactericidal concentration (MBC) was determined for *O. syriacum*, *T. vulgaris*, and *C. papaya* by inoculating 0.1 mL of broth from negative growth wells in the MIC assay onto sterile nutrient agar by using streak plates. The plates were incubated at 37°C for 24 h. The concentration that showed no growth of the tested organisms was considered to be the MBC; the negative control was a plate with medium only [26–28].

Screening of Phytochemical Compounds

The bioactive components present in *O. syriacum*, *T. vulgaris*, and *C. papaya* were identified using previously described techniques [28–32] and included alkaloids, flavonoids, tannins, resins, saponins, glycoside, cardiac glycoside, terpenoids, anthraquinones, and carbohydrates.

Statistical Analysis

The results were analyzed by paired-samples *t*-test using the IBM SPSS 20 statistical software to compare the mean values of each treatment. The results are expressed as means ± SE. Probability levels of less than 0.01 were considered highly significant.

III. RESULTS

In this study, we investigated the antibacterial activity of methanol, ethanol, and water extracts of *O. syriacum*, *T. vulgaris*, and *C. papaya*. The results in **Table 1** show the inhibitory effects of the plant extracts against the tested bacteria. The highest antibacterial activity was associated with the *O. syriacum* methanol extract, with growth inhibition percentages of 62.04%, 65.19%, 59.63%, 56.48%, 59.63%, and 59.63% for *E.*

coli, *C. jejuni*, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*, and *S. sonnei*, respectively. The treatment with the *T. vulgaris* methanol extract showed bacterial growth inhibition of 63.15%, 60.56%, 57.78%, 57.96%, 58.33%, 55.74%, and 59.63%, for *E. coli*, *C. jejuni*, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*, *S. aureus*, and *S. pyogenes*, respectively. The *C. papaya* methanol extract was less effective against the tested bacteria, the bacteria that were the most sensitive to the extract were *E. coli*, *C. jejuni*, *P. aeruginosa*, and *K. pneumoniae*, with growth inhibition of 58.89%, 58.15%, 55.00% and 57.59%, respectively. Ethanol extracts inhibited bacterial growth similarly to the methanol extracts. Growth inhibition was 55.74%, 59.63%, and 55.74% for *E. coli*, *C. jejuni*, and *K. pneumoniae*, respectively, treated with *O. syriacum*; 60.93%, 58.14%, and 55.74% for *E.coli*, *C. jejuni*, and *P. vulgaris*, respectively, treated with *T. vulgaris*; and 55.37% and 52.78% for *E. coli* and *C. jejuni* treated with *C. papaya*. Water extracts of *O. syriacum* had a high inhibitory effect *E. coli* and *C. jejuni* (65.85% and 56.67%, respectively), while *T. vulgaris* and *C. papaya* water extracts had lower inhibitory activity. Growth inhibition was 46.11%, 44.63%, and 46.48% for *E. coli*, *C. jejuni*, and *S. aureus*, respectively, treated with *T. vulgaris*. The *C. papaya* water extract had a minimal inhibition effect on gram-negative bacteria, with percentages of 30.74%, 28.15%, 28.15%, and 28.89%, for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. epidermidis*, respectively, while the inhibition effect on gram-positive bacteria was higher, with percentages of 31.11%, 35.74%, 35.00%, 32.41%, and 31.67% for *S. saprophyticus*, *S. aureus*, *S. maltophilia*, *S. agalactiae*, and *S. pyogenes*. The results were highly significant ($P \leq .01$).

MICs and MBCs are shown in Tables 2 and 3. **Table 2** presents data indicating that *E. coli*, *C. jejuni*, *K. pneumoniae*, and *P. aeruginosa* were more sensitive to the methanol extract, with an MIC of 2 µL of the *O. syriacum* and *T. vulgaris* extracts. The distilled water extracts were less effective than the alcohol extracts, with MICs of 8, 16, and 32 µL of *O. syriacum*, *T. vulgaris* and *C. papaya* for the treated *E. coli* and *C. jejuni*. Further, the MBCs of *O. syriacum* and *T. vulgaris* methanol and ethanol extracts on *E.coli* and *C. jejuni* in **Table 3** were 4 and 8 µL.

Table 4 summarizes the bioactive compounds found in the plant extracts that could potentially affect bacterial growth. The compounds included alkaloids, flavonoids, glycoside, cardiac glycoside, terpenoids, anthraquinones, and tannins, which were present in *O. syriacum*, *T. vulgaris*, and *C. papaya* water, methanol, and ethanol extracts. Saponins were absent from all extracts.

IV. DISCUSSION

Investigation is needed for antibacterial agents that offer broad-spectrum activity against microorganisms, while offering minimal side effects, selective toxicity, and long-term stability. Secondary products from plants can have bioactivity against microorganisms.

Plants are a renewable source of natural products that have been used to treat various diseases, and many plants have been studied as sources of novel drugs [33]. Plant derived medicines contain natural substances that can promote health and alleviate illness. Extracts of numerous plant species such as *Alepidea amatymbica*, *Achillea millefolium*, *Caryophyllus aromaticus*, *Melissa officinalis*, *Ocimun basilucum*, *Psidium guajava*, *Rosmarinus officinalis*, *Salvia officinalis*, *Syzygium jambolanum*, *Thymus vulgaris*, *Lambertia inermis*, *Syzygium aromaticum*, *Piper pulchrum*, *P. paniculata* L., *Rhamnus globosa*, *Tecoma stans*, and *Coleus forskohlii* have been shown to have antimicrobial activity against a broad array of pathogens, including *S. aureus*, *B. cereus*, *S. epidermidis*, *S. saprophyticus*, *S. pyogenes*, *S. agalactiae*, *P. aeruginosa*, *S. paratyphi*, *S. dysenteriae*, *E. coli*, *B. subtilis*, *Enterobacter aerogenes*, *E. aerogenes*, β-hemolytic *Streptococcus* sp., and *K. pneumoniae*, with most extracts having broad-spectrum antimicrobial activity [34–39]. The antibacterial activity of alcohol extracts of *O. syriacum*, *T. vulgaris*, and *C. papaya* against the gram-negative and gram-positive bacteria tested in the current study agreed with results from several previous studies [40–44]. The high activity of the alcohol extracts may be due to the secondary metabolites of the plants, as suggested by Nikolić *et al* [40], who linked the antimicrobial activity of *T. algeriensis*, *T. speryllum*, and *T. vulgaris* with bioactive compounds, including thymol, carvacrol, *p*-cymene, and terpinene. Studies on *O. syriacum* and *C. papaya* extracts showed the presence of phenols, β-carotene, anthocyanins, and flavonoids [45–47].

Using fresh and dried materials for extractions of *U. rigida*, Tuney *et al*. [48] found that dried samples had no activity against *S. aureus*. However, the extract prepared from fresh material showed high inhibitory activity for the same strain. *U. fasciata* showed a broad spectrum of antibacterial activity, inhibiting the growth of both Gram-positive and Gram-negative tested organisms [49].

The evaluation of *O. syriacum*, *T. vulgaris*, and *C. papaya* extracts indicates that they may be suitable sources of novel antibiotics. This study found that the crude extract affected by several solvents, including distilled water, ethanol and methanol, and further studies are recommended to evaluate the effect of these extracts on the bacterial cell wall, the permeability of the cytoplasmic membrane, and the integrity of the DNA genome. In addition to the development of new drugs, we suggest to study the environmental conditions that may affect their potency.

V. CONCLUSION

A comparison of the antibacterial activity of methanol, ethanol, and water extracts of *O. syriacum*, *T. vulgaris*, and *C. papaya* showed that the methanol and ethanol extracts were highly effective against *E. coli*, *C. jejuni*, *P. aeruginosa*, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa*, S. *aureus*, *S. pyogenes*, and *S. sonnei*. An assay of secondary compounds revealed the presence of alkaloids, flavonoids, glycoside, cardiac glycoside, terpenoids, anthraquinones, and tannins in the extracts. We conclude that *O. syriacum*, *T. vulgaris*, and *C. papaya* contain potential bioactive compounds against a broad spectrum of bacteria.

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Table 1. Inhibition of bacterial growth (mm) after treating with *O. syriacum, T. vulgaris and C. papaya* and incubated for 24 h. (Mean±SD)

	O. syriacum			*T. vulgaris*			*C. papaya*		
	M	E	W	M	E	W	M	E	W
E. coli ATCC 8239	33.5±**0.0 96****	30.1±**0.59 2****	30.7±**0.09 6****	34.1±**0.56 3****	32.9±**0.09 6****	24.9±**0.59 2****	31.8±**0.09 6****	29.9±**0.09 6****	16.6±**0.09 6****
C. jejuni ATCC 33291	35.2±**0.5 92****	32.2±**0.56 3****	30.6±**0.09 6****	32.7±**0.59 2****	31.4±**0.59 2****	24.1±**0.09 6****	31.4±**0.09 6****	28.5±**0.09 6****	14.4±**0.09 6****
H. influenza ATCC 49247	27.4±**0.5 63****	22.5±**0.11 0****	25.1±**0.09 6****	18.9±**0.11 0****	19.8±**0.11 0****	0.0	20.3±**0.11 5****	21.6±**0.11 5****	0.0
K. pneumoniae ATCC 13883	32.2±**0.5 92****	30.1±**0.59 2****	28.1±**0.09 6****	31.2±**0.09 6****	29.6±**0.59 2****	11.1±**0.09 6****	31.1±**0.09 6****	23.8±**0.09 6****	15.2±**0.09 6****
N. gonorrhoeae ATCC 31426	20.3±**0.5 63****	18.6±**0.09 6****	15.4±**0.59 2****	19.1±**0.11 5****	19.0±**0.11 0****	15.1±**0.09 6****	17.3±**0.09 6****	15.4±**0.09 6****	0.0
P. mirabilis ATCC 35659	29.2±**0.5 92****	29.3±**0.09 6****	27.8±**0.59 2****	26.8±**0.59 2****	24.9±**0.09 8****	16.7±**0.59 2****	29.1±**0.09 6****	27.9±**0.59 2****	13.9±**0.59 2****
P. vulgaris ATCC 33420	30.5±**0.5 92****	28.7±**0.09 6****	25.9±**0.11 0****	31.3±**0.11 0****	30.1±**0.09 6****	11.9±**0.11 0****	28.4±**0.09 6****	24.4±**0.09 8****	13.7±**0.11 0****
P. aeruginosa ATCC 27853	32.2±**0.1 10****	27.1±**0.09 8****	26.8±**0.09 6****	31.5±**0.59 2****	19.8±**0.09 6****	11.1±**0.09 8****	29.7±**0.09 6****	20.8±**0.09 6****	15.2±**0.09 8****
S. typhimurium ATCC 14028	31.2±**0.1 10****	29.5±**0.11 0****	28.2±**0.09 6****	29.2±**0.09 8****	13.2±**0.09 6****	11.9±**0.56 3****	25.3±**0.09 6****	18.3±**0.09 6****	13.4±**0.11 0****
S. sonnei ATCC 9290	32.3±**0.0 98****	29.3±**0.56 3****	29.7±**0.09 6****	22.2±**0.59 2****	21.0±**0.09 6****	11.5±**0.11 0****	25.9±**0.56 3****	18.1±**0.09 6****	14.8±**0.56 3****
V. Parahaemolyticus ATCC 17802	28.7±**0.1 10****	23.8±**0.11 0****	20.5±**0.56 3****	28.4±**0.56 3****	20.7±**0.11 0****	15.9±**0.56 3****	29.6±**0.11 0****	18.9±**0.09 8****	12.2±**0.56 3****
E. faecalis ATCC 29212	29.9±**0.5 63****	26.4±**0.56 3****	21.1±**0.59 2****	27.2±**0.56 3****	20.5±**0.09 6****	17.8±**0.56 3****	28.1±**0.11 0****	19.5±**0.09 6****	12.6±**0.09 6****
S. saprophyticus ATCC 15305	25.4±**0.5 92****	20.8±**0.11 0****	21.2±**0.56 3****	26.1±**0.11 0****	22.8±**0.56 3****	17.4±**0.09 6****	24.2±**0.56 3****	15.2±**0.11 0****	16.8±**0.56 3****
S. aureus MRSA ATCC 33591	26.3±**0.1 10****	23.7±**0.09 6****	22.9±**0.09 6****	30.1±**0.56 3****	23.7±**0.11 0****	25.1±**0.56 3****	24.5±**0.11 0****	17.8±**0.09 8****	19.3±**0.11 0****
S. epidermidis ATCC 12228	28.7±**0.5 92****	26.7±**0.09 8****	23.6±**0.56 3****	22.5±**0.11 0****	23.4±**0.09 8****	10.0±**0.09 6****	29.5±**0.09 8****	16.4±**0.11 0****	18.9±**0.09 6****
S. maltophilia ATCC 51331	25.1±**0.1 10****	22.8±**0.11 0****	22.1±**0.09 6****	27.0±**0.09 8****	21.9±**0.11 0****	10.7±**0.56 3****	28.2±**0.11 0****	15,8±**0.59 2****	15.6±**0.59 2****
S. agalactiae (group B) ATCC 12386	29.9±**0.1 10****	20.1±**0.09 6****	19.4±**0.56 3****	25.8±**0.09 6****	22.3±**0.56 3****	11.1±**0.09 6****	27.3±**0.56 3****	15.3±**0.11 0****	17.5±**0.11 0****
S. pyogenes ATCC 19615	29.4±**0.0 96****	19.8±**0.11 0****	19.7±**0.09 6****	32.2±**0.56 3****	24.7±**0.09 6****	12.8±**0.11 0****	25.1±**0.11 0****	15.9±**0.56 3****	17.1±**0.09 6****

M: methanol, E: ethanol, W: water, extracts

Table 2. MICs of bacterial growth (µl) after treating with *O. syriacum, T. vulgaris and C. papaya* and incubated for 24 h.

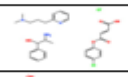
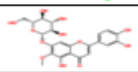
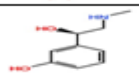
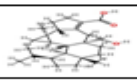
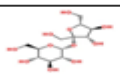
	O. syriacum			*T. vulgaris*			*C. papaya*		
	M	E	W	M	E	W	M	E	W
E. coli ATCC 8239	2	2	8	2	4	16	4	4	32
C. jejuni ATCC 33291	2	2	8	2	4	16	4	8	32
H. influenza ATCC 49247	16	32	32	8	16	-	8	16	32
K. pneumoniae ATCC 13883	2	4	16	2	32	32	2	8	32
N. gonorrhoeae ATCC 31426	16	32	32	4	8	8	16	32	-
P. mirabilis ATCC 35659	4	8	32	4	16	32	8	8	32
P. vulgaris ATCC 33420	4	4	16	4	16	16	8	16	32
P. aeruginosa ATCC 27853	2	4	8	4	16	8	8	8	16
S. typhimurium ATCC 14028	8	8	32	8	32	16	8	8	32
S. sonnei ATCC 9290	8	8	32	16	32	32	4	8	8
V. Parahaemolyticus ATCC 17802	16	8	32	16	32	32	4	8	32
E. faecalis ATCC 29212	8	16	32	8	16	32	4	16	32
S. saprophyticus ATCC 15305	8	8	16	4	32	32	8	16	32
S. aureus MRSA ATCC 33591	8	16	32	4	32	16	8	8	32
S. epidermidis ATCC	4	8	16	4	8	16	4	4	32

12228									
S. maltophilia ATCC 51331	4	8	16	8	8	16	16	16	32
S. agalactiae (group B) ATCC 12386	4	8	16	8	16	16	16	8	32
S. pyogenes ATCC 19615	8	8	32	8	16	16	8	8	32

Table 3. MBCs of bacterial growth (µl) after incubation for 24 h.

	O. syriacum			*T. vulgaris*			*C. papaya*		
	M	E	W	M	E	W	M	E	W
E. coli ATCC 8239	4	8	16	8	8	32	8	8	32
C. jejuni ATCC 33291	4	8	16	8	8	32	8	16	32
H. influenza ATCC 49247	32	32	32	32	32	-	32	32	32
K. pneumoniae ATCC 13883	4	8	32	4	32	32	8	32	32
N. gonorrhoeae ATCC 31426	32	32	32	8	16	32	32	32	-
P. mirabilis ATCC 35659	8	16	32	8	32	32	32	32	32
P. vulgaris ATCC 33420	8	16	32	8	32	32	32	32	32
P. aeruginosa ATCC 27853	8	8	32	8	32	32	32	32	32
S. typhimurium ATCC 14028	16	16	32	16	32	32	32	32	32
S. sonnei ATCC 9290	32	16	32	32	32	32	8	32	32
V. Parahaemolyticus ATCC 17802	32	16	32	32	32	32	16	32	32
E. faecalis ATCC 29212	32	32	32	16	2	32	8	32	32
S. saprophyticus ATCC 15305	16	32	16	16	32	32	16	32	32
S. aureus MRSA ATCC 33591	16	32	32	16	32	32	6	32	32
S. epidermidis ATCC 12228	16	32	32	16	32	32	16	16	32
S. maltophilia ATCC 51331	32	32	32	32	32	32	32	32	32
S. agalactiae (group B) ATCC 12386	32	32	32	32	32	32	32	32	32
S. pyogenes ATCC 19615	32	32	32	32	32	32	32	32	32

Table 4. The secondary compounds in *O. syriacum*, *T. vulgaris* and *C. papaya* methanol, ethanol and water extracts

Name	*Structure	*O. syriacum*			*T. vulgaris*			*C. papaya*		
		water	methanol	ethanol	water	methanol	ethanol	water	methanol	ethanol
alkaloids		+	+	+	+	+	+	+	+	+
flavonoids		+	+	+	+	+	+	+	+	+
tannins		+	+	+	+	+	+	+	+	+
resins		+	+	-	+	+	+	+	+	-
saponins		-	-	-	-	-	-	-	-	-
glycoside		+	+	+	+	+	+	+	+	+
cardiac glycoside		+	+	+	+	+	+	+	+	+
terpenoids		+	+	+	+	+	+	+	+	+
anthraquinones		+	+	+	+	+	+	+	+	+
carbohydrates		+	+	-	+	+	-	+	+	+

*The structures of the bioactive compounds were made by using the software http://isciencesearch.com/iss