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Evaluation of antibacterial effects of some traditional plants against pathogen microorganisms

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Abstract. Antimicrobial resistance has been an increasing problem in public health around the world. Natural plants can be utilised as an important source for antimicrobial agents to be used in place of synthetic drugs due to their costs and side effects. Thus, it is essential to find out new, effective, safer natural antimicrobial products. In the present study, in vitro antibacterial activities of different traditional plants against pathogen microorganisms were tested. The extracts of all plants were prepared in four different solvents (ethanolic, methanolic, hexane and aqueous) and then used for disc–diffusion method. Each assay was repeated in triplicate. The analysis was carried out using two–way analysis of variance (ANOVA). The highest antimicrobial activities were obtained by methanolic extracts of *Sorbus domestica* against *E. coli*, while Methanolic Extracts of *Epilobium angustifolium* were effective against *Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa*. In addition, Hexanic Extract of Tragacanth gum and *S. domestica* had high antibacterial activity against *K. pneumoniae and P. aeruginosa,* respectively.
Keyword*: Antibacterial activity, Tragacanth gum, methanolic/ Hexanic extracts, traditional plants.*

Around the world and particularly in Europe, antimicrobial resistance has been monitored since 1998 by the European Antimicrobial Resistance Surveillance System (EARSS) and thus the emergence of resistance particularly to multiple antibiotics among such pathogens as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Mycobacterium tuberculosis* caused a huge concern, seeking alternative tools to combat these public health challenges [BRONZWAER *et al.*, 2002; LOWY, 2003; PRASAD, 2014; SANTAJIT and INDRAWATTANA, 2016].

The ESKAPE pathogens encompassing both Gram–positive and Gram–negative species (*Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* species) are the leading cause of nosocomial infections throughout the world in both high– and low–income countries [RICE, 2008; RICE, 2010; BUSH and JACOBY, 2010].

Among these pathogens, *S. aureus* is part of the normal skin flora, especially of the nose and causes perineum of humans and animals.

K. pneumoniae is mainly found to be related to infections in healthcare settings, as well as *E. coli* and *P. aeruginosa* which is a part of the normal gut flora, causing high infection rates in hospital, especially in immuno compromised hosts.

According to World Health Organization (WHO) reports; 80 % of the population living in developing countries generally prefer traditional medicines with herbal origin for their basic health needs.

Approximately 20 000 plant species are used for treatment purposes [CERİT, 2008].

At present, at least 25 % of the active substances of pharmacologically produced drugs are obtained from plants [SEKAR and KANDAVEL, 2010; FAYDALIOĞLU and SÜRÜCÜOĞLU, 2013].

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Table 1.

Properties of the studied plants

Latin name	Synonyms	Family	Common name	Benefits	Distribution	References
Rhamnus cathartica L.	*Cervispina cathartica* (L.) Moench.	Rhamnaceae	The buckthorn, common buckthorn, purging buckthorn	Chronic skin diseases, dropsy, gout, and liver disorders, purgative, treat skin irritation, laxative	Most of Europe, Scandinavia, Western Asia, Iran, Turkey, Northwest Africa Algeria, Morocco and North America	[KURYLO *et al.*, 2007; QADERI *et al.*, 2009; KURYLO and ENDRESS, 2012]
Fumaria officinalis L.	*Fumaria officinarum* Neck.	Papaveraceae	Fumewort, fumitory, drug fumitory	Antirheumatic, antispasmodic, aperient, cholagogue, diaphoretic, diuretic, laxative, tonic	Most of Europe, Britain, Mediterranean and Iran.	[CLAPHAM *et al.*, 1987; KUMARASAMY *et al.*, 2007]
Erica arborea L.	*Ericoides arboretum* L., *Calluna* sp.	Ericaceae	Heather leaf, briar root, root heather, tree heath, tree heath	Anti–inflammatory, antinociceptive activity, diuretic, urinary antiseptic and laxative	Southern and West Europe, The Mediterranean, South Africa, Turkey	[NAYEBI *et al.*, 2008; AKKOL *et al.*, 2008; GUVENÇ and KENDIR, 2012; OK *et al.*, 2014]
Epilobium angustifolium L.	*Chamaenerion angustifolium* L.	Onagraceae	Willow herb, French–willow, great willow herb, rosebay willow herb	Anti–inflammatory, antiandrogenic, antiproliferative, antimicrobial, antinociceptive, and antioxidant effects treatment of disorders of the prostate, kidneys, and urinary tract, immunomodulatory activity	Widespread in Canada, North America, Himalayas, Western Europe, Siberia, China, Japan	[MYERSCOUGH, 1980; OSTROVSKA *et al.*, 2017; KADAM *et al.*, 2018]
Cassia angustifolia	*Cassia senna, Folliculj sennae*	Leguminosae	Senna	Anthelmintic, digestive, and to treat piles, skin diseases, and abdominal troubles	America, tropical Africa, Madagascar, Australia, southeaster Asia and Europe	[RANDELL and BARLOW, 1998; MARAZZI *et al.*, 2006; SRIVASTAVA *et al.*, 2006]
Cormus domestica L. Spach	*Sorbus domestica* L., *Pyrus sorbus*	Rosaceae	Sorb, service tree	Anti–diarrheal diuretic, anti–diabetic, antimicrobial, cytotoxic and antioxidant activities, vasoprotective, broncho / vaso–relaxant, treatment of anaemia, dyspepsia and various digestive disorders	Central and Southern Europe in particular in the Balkan peninsula, Italy and Southern France, and rarely in parts of North Africa and Western Asia	[ZLOBIN *et al*, 2012; UDDIN *et al.*, 2013; ENESCU *et al*, 2017; MRKONJIĆ *et al.*, 2017]
Anastatica hierochuntica L.	*Myagrum hierochunticum* L. Crantz	Brassicaceae	Rose of Jericho, Kaff Maryam	Antimicrobial, antioxidant activities, anti–inflammatory activity, anti–melanogenic, treat asthuma and respiratory disease, dysentery, salmonella typhi, typhod fever, diabetes	The arid regions of Saudi Arabia, Egypt, Jordan, Iraq, the UAE, Iran, Kuwait, and North Africa	[NAKASHIMA *et al*, 2010; MOHAMMD *et al.*, 2015; ZIN *et a.*, 2017]
Zea mays		Poaceae	Corn, Maize	Cystitis, gout, kidney stones nephritis and prostatitis, anti-diabetes	Central Europe, surrounding Mediterranean regions, Mexico, South America, Canada, Russia	[GUO *et al.*, 2009; ROMERO, 2016]
Citrus sinensis		Rutaceae	Orange	Intake of vitamin C is associated with a reduced risk of colon, skin, lung, breast, stomach cancer. Prevents us from asthma, osteoarthritis, and rheumatoid arthritis	Colombia, United States, South Africa, Australia, the Mediterranean area, and subtropical areas of South America and the Caribbean, Brazil, China, India, Mexico, Spain, Egypt, Italy, Turkey	[MORTON, 1999; PEREA, 2017]
Astragalus gummifer	*Astragalus gummifer* Labill., *Astracantha gummifera*	Leguminosae	Tragacanth gum, Goat's thorn, Green dragon, Gum dragon, Gum Tragacanth, Gummi Tragacanthae, Hog gum, Milkvetch, Syrian Tragacanth	Inhibit the growth of cancer cells, antitumor activity, analgesic effects, laxative, diarrhea	Near East and the Middle East, Southern Europe, central parts of Africa, Iran, Turkey, Iraq, Syria, Lebanon, Afghanistan and parts of Russia	[MORTON, 1977; SUN, 2007; KHAN, 2010; YAZDANSHENAS *et al*, 2014; BAGHERI, 2015]

The main reasons for use of herbal medicine raw materials are;

- some of the synthetic drug raw materials used in treatment show dangerous side effects;
- the inability of some natural drug raw materials to be made synthetically;
- toxic effects;
- antibiotic resistance developed by microorganisms that cause hospital infections;
- herbal medicines have several different effects, easy and inexpensive use [SEKAR and KANDAVEL, 2010; BAYTOP, 1984].

In recent years, there has been worldwide interest in functional food that

can provide not only basic nutrition requirements but also physiological benefits.

Thus, plants have been regarded as useful, self–generating machines, which produce many useful bioactive products.

The crude plant extracts and their compounds are used for the treatment of infectious diseases caused by bacteria, viruses and fungi.

Ten different plants used in the present study were common buckthorn (*Rhamnus cathartica* L.) berries, corn (*Zea mays*) tassel, fumewort (*Fumaria officinalis* L.), heather leaf (*Erica arborea* L.), orange (*Citrus sinensis*) pell, rose of Jericho (*Anastatica hierochuntica*), senna (*Cassia angustifolia*), sorb (*Sorbus domestica* L.), Tragacanth gum (*Astragalus gummifer* Labill.), willow herb (*Epilobium angustifolium* L.)] were obtained from local market in Diyarbakır, Turkey.

Table 1 shows about some properties of the studied plants, such as synonyms, Family, common name, benefits and distribution.

Material and methods

Plants and preparation of extracts

In this study, 10 different plants [common buckthorn (*R. cathartica*) berries, corn (*Z. mays*) tassel, fumewort (*F. officinalis*), heather leaf (*E. arborea*), orange (*C. sinensis*) pell, rose of Jericho (*A. hierochuntica*), senna (*C. angustifolia*), sorb (*S. domestica*), Tragacanth gum (*A. gummifer*), willow herb (*E. angustifolium*)] were obtained from local market in Diyarbakır, Turkey.

The dried plant material of each plant was grinded with a blender and separately dissolved in sterile distilled water, ethanol (99 %, Sigma), methanol (99.7 %, Sigma) and *n*–hexane (95 %, Merck) at room temperature for 24 h.

After incubation all samples were centrifuged (10.000 rpm) for 10 min and the supernatants were transferred in clean tubes and stored at room temperature.

The filtrates were evaporated and dried at 40 °C under reduced pressure using rotatory vacuum evaporator.

Bacterial strains

The antibacterial potency of each plant extract was evaluated using four bacterial strains [*E. coli* ATCC 25922, *S. aureus* ATCC 25923, *K. pneumoniae* and *P. aeruginosa*] used as test microorganisms were provided from the culture collections of the Medical Microbiology Laboratory, Research Hospital of Dicle University in Diyarbakir, Turkey.

The isolates were separately sub–cultured in liquid Mueller–Hinton Broth (MHB, Sigma) and incubated at 37 °C for overnight (18–24 h) in a shaker.

The culture was harvested using 5 mL of sterile 0.9 % salt solution, its absorbance was adjusted at to a 0.5 McFarland turbidity standard (5×10^5 CFU/mL) using spectrophotometer (Biochrom).

Antibacterial activity

The disk diffusion test for determining the antibacterial activity was carried out according to the method by Murray and collab., [MURRAY *et al.*, 1995].

Mueller–Hinton agar (MHA, Sigma) was sterilized and dispensed into sterile plates, then all plates were dried at 37 °C for 2 h under the sterile conditions.

The cultures inoculated on MHA were then allowed the surface of the medium to dry for 3–5 minutes. 20 µL of re–dissolved plant extracts were applied to sterile 6 mm diameter paper discs (Oxoid) and discs were carefully placed onto the surface of the MHA plates. The plates were kept in the fridge at 5 °C for 2 h to permit plant extracts diffusion then incubated at 37 °C for 24 h.

An antibiotic disc of Gentamycin (10 µg/ disc) was also used as positive control. Absolute solvents (distilled water, ethanol, methanol and *n*–hexane) were used as negative controls.

After which in all experiments, the diameter of the zones of inhibition around disc was measured in millimetres. All antibacterial tests were performed under sterile conditions in triplicate.

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Determination of combined effect of plants

The single and combined antimicrobial effects of the selected plants were also investigated.

The binary combinations of plant samples extracted in the same type of solvent were used.

All disk diffusion tests were carried out with the same instructions described above for each strain.

Statistical analysis

Two–factor analysis of variance (two–factors ANOVA) was used for different bacteria to distinguish the antibacterial effect of plant extracts in different solvents with SPSS software. Two factors were examined for plants with 10 levels and solvents with 4 levels.

Because ANOVA is based on the assumptions that the observations are random samples from normal distributions and the populations have the same variance and the observations are independent of each other, the data was examined to determine whether these assumptions are satisfied before carrying out the test.

One–Sample Kolmogorov–Smirnov test is used to determine the observations are random samples from normal distributions. If p values are smaller than 0.05 for each bacteria, it means that observations are random samples from normal distributions. Chi–Square test is used to determine the observations are independent of each other. If p values are smaller than 0,05 for each bacteria, it means that observations are independent of each other.

Levene's test significance values determine the homogeneity of variances of populations. While for *E. coli* it is assumed that the error variance of the dependent variable is equal across groups, for others it has been assumed that the error variance of the dependent variable is not equal across groups. Hence, we used the information above while performing the two–factor ANOVA.

Results and discussion

In this study, antibacterial assay was performed using plant extracts in different solvents [aqueous (QE), ethanolic (EE), methanolic (ME) and hexanic (HE)] against some of the pathogenic microorganisms such as *E. coli*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa*. All samples from various extracts showed an inhibition zone against the pathogenic microorganisms determined by statistical analysis.

Table 2.

Antibacterial effect of aqueous extracts (QE) on bacteria

Plant extract	Inhibition zone			
	E. coli	***S. aureus***	***K. pneumoniae***	***P. aeruginosa***
R. cathartica (common buckthorn berries)	22±1.41	13±0.01	11.5± 6.36	20.05±0.71
F. officinalis (fumewort)	ND	ND	ND	ND
E. arborea (heather leaf)	8±0.01	11±0.7	13±2.83	ND
E. angustifolium (willow herb)	11.5±0.71	5±7.07	13± 2.83	4±0.01
C.angustifolia (senna)	5.5± 0.71	ND	7± 0.01	8.5± 0.71
S. domestica (sorb)	5± 0.7	4± 0.01	ND	10± 0.01
A. hierochuntica (rose of Jericho)	ND	ND	8.5± 0.71	5± 0.01
Z. mays (corn) tassel	ND	5± 1.41	5.5± 0.71	ND
C. sinensis (orange) pell	ND	ND	ND	10± 0.01
A. gummifer (Tragacanth gum)	ND	ND	10±0.01	ND

Antibacterial activities were expressed as inhibition diameter zones in millimetres (mm±SD). n=3.
ND = Not Determined

Plants are traditionally used as tea or spices in the kitchens and are often brewed in water or directly added onto foods. For this reason, in this study, QE of plants were also used to determine antibacterial effect.

The highest antimicrobial activity of aqueous extract of *R. cathartica* was 22±1.41 and 20.5±0.71 mm against *E. coli* and *P. aeruginosa*, respectively (Table 2), while 13±0.01 mm inhibition zone was obtained against *S. aureus*.

Aqueous extract of *E. arborea* and *E. angustifolium* showed 13±2.83 mm inhibition zone against *K. pneumoniae.* However, aqueous extract of *F. officinalis* had no effect on these test microorganisms.

On the other hand, QE of *C. angustifolia*, *A. hierochuntica* and *S. domestica* had moderate effects on the microorganisms tested, whereas Tragacanth gum had a moderate effect only on *K. pneumoniae.*

Zaouia and collab., previously found that aqueous extract of *Rhamnus* sp. showed a high antibacterial effect on *E. coli*, *P. aeruginosa* and *K. pneumonia,* but showing a moderate effect on *S. aureus* [ZAOUIA *et al.*2010].

The Aqueous extract of *A. hierochuntica* was also found to have an antibacterial effect on *E. coli*, *P. aeruginosa* [TAYEL and EL–TRAS, 2009; AL–SAEED and JABER, 2013], *S. aureus* [TAYEL and EL–TRAS, 2009], *Salmonella typhi*, *S. paratyphi, P. mirabilis* and *K. pneumoniae* [AL SOBEAI et al., 2016].

In the present study, EE of *R. cathartica,* showed antibacterial activities against all test microorganisms.

F. officinalis showed a strong antibacterial activity against *E. coli*, *K. pneumoniae* and *P. aeruginosa* and a slight antibacterial activity against *S. aureus*.

The EE from *E. arborea,* corn tassel*, E. angustifolium, C. angustifolia, S. domestica* and *A. hierochuntica* showed a strong antibacterial activity, whereas orange pell and Tragacanth gum had a slight effect on all test microorganisms.

As shown in Table 3, the highest antibacterial activity (20.5±0.71 mm) was obtained by EE of *R. cathartica* against *E. coli*, *K. pneumoniae* and *P. aeruginosa*, while the highest antibacterial activity (19±1.41 mm) against *S. aureus* was by EE of *S. domestica.*

Table 3.

Antibacterial effect of ethanol extracts on bacteria

Plant extract	**Inhibition zone** [diameter zones in mm (mm±SD)]			
	E. coli	***S. aureus***	***K. pneumoniae***	***P. aeruginosa***
R. cathartica (common buckthorn berries)	17.5±1.41	10±0.71	20.5± 0,71	19.5±1.41
F. officinalis (fumewort)	13±2.83	6±1.41	12±1.41	13.5±0.71
E. arborea (heather leaf)	13±1.41	15±2.83	13.5± 2.12	12.5±3.54
E. angustifolium (willow herb)	15±2.83	17±4.24	14± 1.41	18.5±4.95
C. angustifolia (senna)	10.5±4.95	14.5±2.83	19± 0.01	17.5±3.54
S. domestica (sorb)	14.5±2.12	19±1.41	17± 2.83	19±0.7
A. hierochuntica (rose of Jericho)	8.5±3.54	11.5±3.54	16.5± 3.54	8.5±2.12
Z. mays (corn) tassel	11.5±0.71	13±2.83	9.5±0.71	17.5±7.78
C. sinensis (orange) pell	9±1.41	9±1.41	8±0.01	10.5±0.71
A. gummifer (Tragacanth gum)	10±0.01	11.5±3.54	10.5±0.71	10±4.24

Antibacterial activities were expressed as inhibition diameter zones in millimetre's (mm±SD). n=3.
ND = Not Determined

The antibacterial properties of EEs of several plants including *Rhamnus frangula* against six pathogenic bacteria (*Bacillus cereus* ATCC 10876, *Enterococcus faecalis* ATCC 14506, *Salmonella choleraesuis* ATCC 10708, *S. aureus* ATCC 11632, *Proteus mirabilis* ATCC 12453 and *E. coli* ATCC 10536) have been studied. It was shown that a high antibacterial potential was exibited by EE of *R. frangula* [LJUBIŠA *et al.*, 2009].

Moreover, Battinelli *et al.* [BATTINELLI *et al.*, 2001] stated that ethanol extract of *E. angustifolium* was effective on Gram (+) and Gram (–) bacteria including *E. coli*, *P. aeruginosa*, *S. aureus* and *K. pneumoniae*.

Antibacterial effects of EE of *A. hierochuntica* on *E. coli*, *P. aeruginosa* and *S. aureus* were also detected [TAYEL and EL–TRAS, 2009; Al–SAEED and JABER, 2013].

ME of all plant samples studied showed strong antibacterial activity (Table 4). It is clear that ME *S. domestica* caused the highest antibacterial activity (22.5±3.54 mm) against *E. coli,* while ME of *E. angustifolium* and orange pell had an antibacterial activity against *S. aureus.*

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On the other hand, *E. angustifolium, E. arborea* and *R. cathartica* had very strong antibacterial activity against *K. pneumoniae.*

Table 4.

Plant extract	Inhibition zone [diameter zones in mm (mm±SD)]			
	E. coli	*S. aureus*	*K. pneumoniae*	*P. aeruginosa*
R. cathartica (common buckthorn berries)	22±1.41	10±0.01	20.5± 0.71	12.5±2.12
F. officinalis (fumewort)	18.5±2.12	10±4.24	10.5±0.71	19±2.83
E. arborea (heather leaf)	13±1.41	12±4.24	22± 4.24	17±0.01
E. angustifolium (willow herb)	14.5±6.36	21.5±3.54	23.5± 4.95	25.5±2.12
C. angustifolia (senna)	11.5±7.78	19.5±0.71	15± 2.83	18±0.01
S. domestica (sorb)	22.5±3.54	10.5±0.71	14± 1.41	19±4.95
A. hierochuntica (rose of Jericho)	8±1.41	8±2.83	12.5± 3.54	9±2.83
Z. mays (corn) tassel	12±2.83	13.5±0.71	12±1.41	12.5±3.54
C. sinensis (orange) pell	14.5±3.54	20±0.01	7.5±0.71	17.5±3.54
A. gummifer (Tragacanth gum)	9.5±0.71	12±1.41	11±2.83	7.5±0.71

Antibacterial activities were expressed as inhibition diameter zones in millimetre's (mm±SD). n=3.
ND = Not Determined

Our results are also in agreement with the previous reports: significant antibacterial activity of ME of *Rhamnus wightii* [RAJA *et al.*, 2018] and *Rhamnus* sp. [ZAOUIA *et al.*, 2010] against *K. pneumoniae, S.aureus* and *E. coli*; *Rhamnus prinoides* [MOLLA, 2015] against *P. aeruginosa, S. aureus* and *E. coli; R. cathatrica* [MOSS *et al.*, 1999] and *R. californica* [CARRANZA *et al.*, 2015] against *S. aureus.*

Furthermore, ME of *E. angustifolium* had very strong antibacterial activity on *P. aeruginosa* [BATTINELLI *et al.*, 2001; MOLLA, 2015], *S. aureus* [BORCHARDT *et al.*, 2008], *K. pneumoniae* [BATTINELLI *et al.*, 2001] and *E. coli* [ZENG *et al.*, 2016], while ME of *A. hierochuntica* had effects only on *Bacillus subtilis* and *K. pneumoniae* [MOHAMMD *et al.*, 2015].

Table 5.

Plant extract	Inhibition zone [diameter zones in mm (mm±SD)]			
	E. coli	*S. aureus*	*K. pneumoniae*	*P. aeruginosa*
R. cathartica (common buckthorn berries)	9±1.41	ND	16± 5.66	19±1.41
F. officinalis (fumewort)	10±0.01	10±0.01	8.5±0.71	16±1.41
E. arborea (heather leaf)	ND	7±1.41	16± 1.41	9±0.01
E. angustifolium (willow herb)	12±0.71	18±5.66	13± 2.83	19±1.41
C. angustifolia (senna)	9±0.71	4±4.95	11± 2.83	4±0.01
S. domestica (sorb)	13±0.71	ND	14.5± 0.71	22.5±3.54
A. hierochuntica (rose of Jericho)	8±0.01	6±3.54	ND	13.5±0.71
Z. mays (corn) tassel	ND	ND	19±1.41	13±2.83
C. sinensis (orange) pell	7.5±4.24	5±1.41	12.5±3.54	15±0.7
A. gummifer (Tragacanth gum)	9.5±0.71	9±0.01	24±4.24	7±0.01

Antibacterial activities were expressed as inhibition diameter zones in millimetres (mm±SD). n=3.
ND = Not Determined

To evaluate statistical significance of antimicrobial assays performed using plant extracts in different solvents (aqueous, ethanolic, methanolic and hexanic) against some of the pathogenic microorganisms such as *E. coli*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa*, we have analysed inhibition zones. The analysis was carried out using two–way analysis of variance (ANOVA).

The normality assumption required for the variance analysis to be applied was tested against the values of skewness and kurtosis.

The ANOVA analysis in Table 6 gives F statistics and p values for plants, solvents and their interaction.

All signicance values are smaller than 0.05. So, both plants and solvents are needed, as well as their interaction, to explain inhibition zone of bacteria.

For statistical analysis to determine antibacterial effect, plants, solvents and their interactions with bacteria were evaluated.

Table 6.

Analysis of variance [two–way ANOVA] for different bacteria

Bacteria	R^2	Adjusted R^2		Between plants	Between solvents	Interactions
E. coli	0.856	0.716	F value	7.231	38.642	2.125
			Sig.	0.000	0.000	0.015
S. aureus	0.924	0.849	F value	9.439	81.811	5.686
			Sig.	0.000	0.000	0.000
K. pneumoniae	0.923	0.,848	F value	12.880	53.412	7.564
			Sig.	0.000	0.000	0.000
P. aeruginosa	0,918	0,837	F value	12.476	59.762	5.678
			Sig.	0.000	0.000	0.000

These suggest that mean inhibition zones for *E. coli* and *S. aureus* are significantly lower for HE and QE than ME and EE (p=0.000) and for *K. pneumoniae* and *P. aeruginosa* mean inhibition zones are significantly lower for QE than ME, EE and HE (p=0.000).

Comparing different solvent extracts of plant samples, it was revealed that QE of *R. cathartica* showed antibacterial activity against all test microorganisms, while the EE of *R. cathartica* showed antibacterial activity against *K. pneumoniae* and *P. aeruginosa*.

It can clearly be seen that ME were more effective on all test microorganisms, whereas the very strong antibacterial activity of hexane extracts was determined against *P. aeruginosa* by *S. domestica* and *K. pneumoniae* by Tragacanth gum.

Al–Saeed and collab., studied the chemical content and antibacterial activity of extracts from *A. hierochuntica* leaves by n–hexan, EE and QE preparations and showed that plant extracts were more active against Gram–negative bacteria (*E. coli*, *P. aeruginosa* and *Proteus vulgaris*) than Gram–positive bacteria (*S. pyogens* and *S. aureus*) and that alcoholic extract has antibacterial activity stronger than hexan and QE [AL–SAEED *et al.*, 2013].

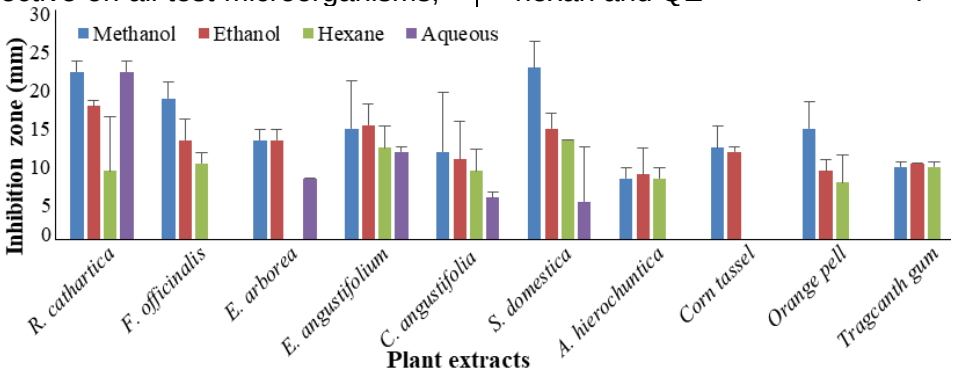

Figure 1. Effect of different extract of plants on *E. coli*

In statistics, the post hoc tests display the differences; for example, as shown in Figure 1 for *E. coli*, ME of *R. cathartica*, corn tassel, orange pell, *F. officinalis, S. domestica and* EE of Tragacanth gum were found to have higher values in terms of antibacterial effects compared to plant extracts in other solvents.

However, ME or EE of *E. arborea and E. angustifolium* and M, E or HE of *C. angustifolia and A hierochuntica* appeared to give the higher values.

Although QE of *F. officinalis, A hierochuntica,* corn tassel, orange pell and Tragacanth gum have no effect against *E. coli*, the QE of *E. arborea, E. angustifolium, C. angustifolia* and *S. domestica* have rather less effect against *E. coli*. HE of *R. cathartica*, *F. officinalis,* corn tassel, *E. angustifolium, C. angustifolia, S. domestica*, orange pell

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and Tragacanth gum have a slight effect on *E. coli*.

Mean inhibition zones determined for different extracts in different solvents were found to be higher with *R. cathartica*, *E. angustifolium* and *S. domestica* compared to other plant extracts against *E. coli*.

Figure 2 indicates that QE of *R. cathartica*, ME and HE of *F. officinalis*, ME of *E. angustifolium*, *C. angustifolia*, corn tassel and orange pell, and Tragacanth gum, EE of *E. arborea, S. domestica*, *A. hierochuntica* cause antimicrobial activity against *S. aureus*.

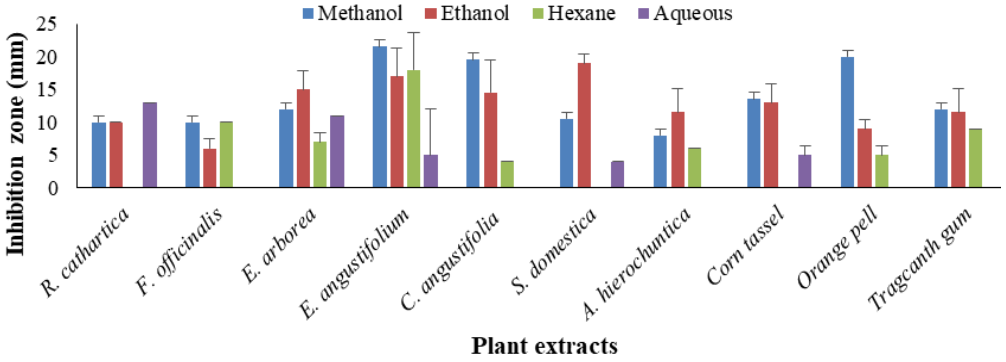

Figure2. Effect of different extract of plants on *S. aureus*

The results on mean inhibition zones showed that the antibacterial effects of the ME and EE of *R. cathartica*, ME of Tragacanth gum and corn tassel and ME and HE of *F. officinalis* were similar against *S. aureus*.

EE of *E. arborea, S. domestica* and Tragacanth gum, ME of *E. angustifolium, C. angustifolia,* corn tassel and orange pell seemed to give higher values against *S. aureus* than other plant extracts.

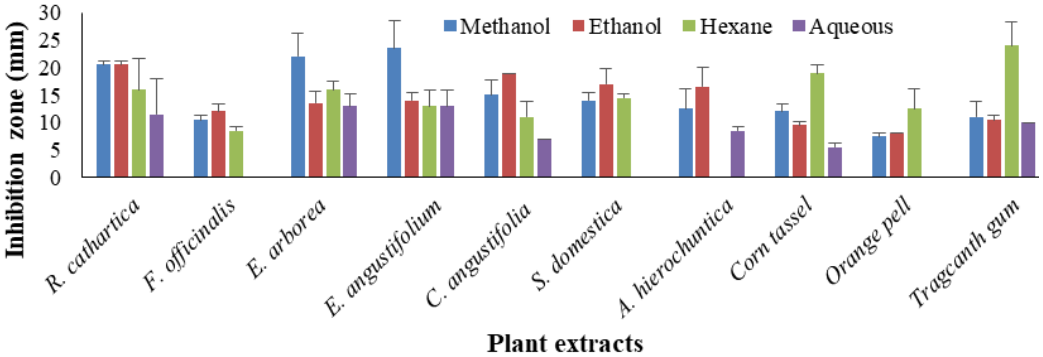

Figure 3. Effect of different extract of plants on *K. pneumonia*

In Figure 3, it can clearly be seen that ME and EE of *R. cathartica*, ME of *E. arborea* and *E. angustifolium*, EE of *F. officinalis*, *C. angustifolia, S. domestica* and *A. hierochuntica,* HE of corn tassel, orange pell and Tragacanth gum had higher antibacterial effect on *K. pneumoniae*.

ME and EE of *R. cathartica* has similar the mean inhibition zone.

Figure 4 shows that QE of *R. cathartica*, ME of *F. officinalis*, *E. arborea, E. angustifolium C. angustifolia* and orange pell, EE of corn tassel and Tragacanth gum, HE of *S. domestica* and *A. hierochuntica* have antibacterial activity against *P. aeruginosa*.

Comparing mean of inhibition zones, EE and HE of *R. cathartica* and EE and ME of *C. angustifolia* had similar effect against *P. aeruginosa*.

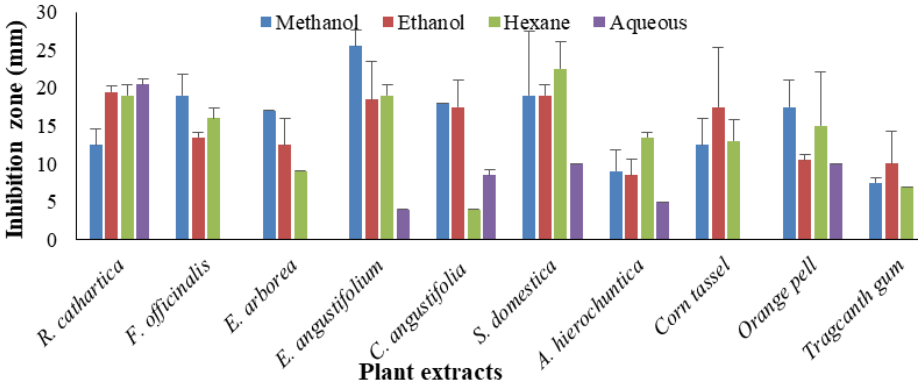

Figure 4. Effect of different extract of plants on *P. aeruginosa*

As can be seen in Table 7 (Combined effects of plant extracts on pathogen microorganisms), the single and combined antibacterial effects of the selected plants were investigated.

Table 7.

The combined effects of plant extracts on pathogen microorganisms

Pathogen microorganism	Plants A	Plants B	Single effect (m*) A	Single effect (m*) B	Combined effect (m*) A+B	Single effect (e*) A	Single effect (e*) B	Combined effect (e*) A+B
E. coli	*R. cathartica*	*F. officinalis*	22±1.41	18.5±2.12	10±2.12	13.5±0.71	13±2.83	ND
	R. cathartica	*S. domestica*	22±1.41	22.5±3.54	10±2.83	13.5±0.71	14.5±2.12	**20±1.41***
	F. officinalis	*S. domestica*	18.5±2.12	22.5±3.54	10±2.12	13±2.83	15.5±2.12	ND
S. aureus	*E. arborea*	*E. angustifolium*	12±4.24	21.5±3.54	ND	15±2.83	17±4.24	15±4.24
	E. arborea	*C. angustifolia*	12±4.24	19.5±2.12	ND	15±2.83	14.5±2.83	14±1.21
	E. angustifolium	*C. angustifolia*	21.5±3.54	19.5±2.12	18±2.21	17±4.24	14.5±2.83	**21.5±3.54***
	R. cathartica	*C. angustifolia*	20.5±0.7	15±2.83	ND	20.5±0.7	19±0.01	20.5±0.7
	R. cathartica	*S. domestica*	20.5±0.7	14±1.41	ND	20.5±0.7	17±2.83	20.5±0.7
	R. cathartica	*A. hierochuntica*	20.5±0.7	12.5±3.54	ND	20.5±0.7	16.5±3.54	20.5±0.7
	R. cathartica	*E. angustifolium*	20.5±0.7	18±4.95	13±0.7	20.5±0.7	14±1.41	20.5±0.7
	R. cathartica	*E. arborea*	20.5±0.7	22±4.24	14±0.3	20.5±0.7	13.5±2.12	20.5±0.7
K. pneumoniae	*E. arborea*	*E. angustifolium*	17±4.24	18±4.95	**24±0.7***	14±1.41	13.5±2.12	**17±4.24***
	C. angustifolia	*S. domestica*	15±2.83	14±1.41	ND	19±0.01	17±2.83	15±2.83
	C. angustifolia	*A. hierochuntica*	15±2.83	12.5±3.54	ND	19±0.01	16.5±3.54	15±2.83
	S. domestica	*A. hierochuntica*	14±1.41	12.5±3.54	ND	17±2.83	16.5±3.54	14±1.41
	R. cathartica	*E. angustifolium*	12.5±2.12	25.5±2.12	ND	19.5±0.7	18.5±4.95	13±0.7
	R. cathartica	*C. angustifolia*	12.5±2.12	18±0.01	ND	19.5±0.7	17.5±3.54	9±0.21
	R. cathartica	*S. domestica*	12.5±2.12	19±4.95	ND	19.5±0.7	19±1.41	14±0.21
	E. arborea	*E. angustifolium*	12.5±3.54	22±0.21	12.5±3.54	18.5±4.95	ND	12.5±3.54
P. aeruginosa	*E. arborea*	*F. officinalis*	19±2.83	15±1.41	12.5±3.54	13.5±0.71	ND	**19±2.83***
	E. arborea	*S. domestica*	19±4.95	18±2.1	12.5±3.54	19±1.41	ND	19±4.95
	E. angustifolium	*C. angustifolia*	18±0.01	ND	18.5±4.95	17.5±3.54	13±0.7	**18±0.01***
	E. angustifolium	*S. domestica*	19±4.95	ND	18.5±4.95	19±1.41	23±0.21	19±4.95

Antibacterial activities were expressed as inhibition diameter zones in millimeters (mm±SD). n=3. ND = Not Determined (no zone of inhibition, 0 mm). (e*): ethanolic extract, (m*): methanolic extract

The binary combinations of plant samples extracted in the same type of solvent were used.

The results clearly showed that binary combinations of EE of *R. cathartica* + *S. domestica* increased antibacterial effects on *E. coli* (20±1.41 mm) compared to single effects of *R. cathartica* (13.5±0.71) and *S. domestica* (14.5±2.12), indicating a synergistic effect.

However, the effect of binary combinations of ME of *R. cathartica* + *S. domestica* (10± 2.83) was found to be less than that of the single ones, indicating an antagonistic effect.

Combination of EE of *R. cathartica* + *S. domestica* (20.5±0.7) seemed not to cause any changes on *K. Pneumoniae*, compared to the single preparations, whereas ME of these plants caused a

decrease in antibacterial activity for *K. Pneumoniae* and *P. aeruginosa*.

Moreover, there was an increase in antibacterial activity by combination of ME and EE of *E. arborea* + *E. angustifolium* (24.5±0.7 and 17±4.24) against *K. Pneumoniae,* but a decrease was detected against *S. aureus* and *P. aeruginosa*. In addition, an increase in antibacterial activity was detected in combinations of EE of *E. arborea* + *F. officinalis* (19±2.83) and *E. angustifolium*+ *C. angustifolia* (18±0.01) against *P. aeruginosa*.

Conclusions

The results indicated that there was strong evidence for which the mean inhibition zones varied with different plant extracts and solvents used.

The presence of interaction between plant extracts and solvents means that inhibition zones change depending on different plant extracts and on different solvents.

When comparing the results in the present study, all plant extracts used for antibacterial effects on pathogen strains were effective, all EE and ME used were more effective, least effective with QE, HE were more effective against *K. pneumoniae* and *P. aeruginosa,* and maximum effect with plant extracts was observed against *K. pneumoniae,* whereas *S. aureus* was minimally effected.

Moreover, the single and binary combined antibacterial effects of the selected plants showed that some of the binary combinations of plant extracts were more effective than single ones and that some binary combinations caused less effect compared to single extract applications.

The present work has revealed that the studied plants are a potential source of antimicrobial agents and demonstrates the importance of such plants in medicine and in assisting local primary health care, as well as providing basic nutrition requirements.

Conflict of Interest: The authors declare that they have no conflict of interest.

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