Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 9, No. 1, p. 150-158, 2016
http://www.innspub.net

INNSPUB

RESEARCH PAPER OPEN ACCESS

Evaluation of *in-vitro* antibacterial activity of leaf extracts of three species of family Oleaceae

Rizwana Rafique[*1], Zaheer-Ud-Din Khan[1], Sundas Altaf[1], Anjum Parveen[2]

[1]*Department of Botany, GC University, Lahore, Pakistan*

[2]*Center for Plant Conservation, University of Karachi, Pakistan*

Article published on July 16, 2016

Key words: *Invitro* antibacterial activity, *Jasminum sambac* (L.) Aiton, *Olea europaea* L., *Olea ferruginea* Royle, agar well diffusion, MIC.

Abstract

The present study was carried out to determine the antibacterial potential of the extracts of *Jasminum sambac* (L.) Aiton, *Olea europaea* L. and *O. ferruginea* Royle leaf against *Staphylococus aureus* and *Bacillus subtilis* (Gram positive bacteria) and. *Escherichia coli* and *Pseudomonas aeruginosa* (Gram negative bacteria) by agar well diffusion method. The extracts were obtained by maceration in solvents, such as n-hexane, chloroform, ethanol and distilled water. The zone of inhibition of the leaf extracts recorded as antibacterial potential was compared with that of the different standard antibiotics, like amikacin (30µg), erythromycin (10µg) etc. The n-hexane extracts of *Jasminum sambac* showed highest activity (26.37±2.54 mm) against *E. coli*. Aqueous and ethanol extracts of all the plants exhibited comparatively higher antibacterial potential against Gram negative bacteria than the Gram positive bacteria. The Minimum Inhibitory Concentration (MIC) was also determined for all the extracts. The results supported the ethnomedicinal use of *Jasminum sambac* (L.) Aiton, *Olea europaea* L. and *O. ferruginea* Royle to treat various bacterial infections in man.

***Corresponding Author:** Rizwana Rafique ✉ rizrafiq@ymail.com

Introduction

Ethnopharmacology is related to the discovery of indigenous drugs from plants and also to ascertain the biological activities of the secondary metabolites from the plants. Use of plants as natural sources in pharmacology has many purposes including isolation, production and use of bioactive compounds as drugs. About 6000 plants species have been identified as medicinal plants (Mahmood *et al*., 2003). In the past, plant secondary metabolites were mostly ascertained as sources of antinutritional factors. Now restrictions and bans on the consumption of animal antibiotic growth promoters enthused interest in bioactive metabolites of plants sources as alternative growth enhancers (Greathead, 2003). Secondary metabolites behave as an alternative source for plants to overcome stress and act as protective substances from toxicity (Vasconsuelo & Boland, 2007). The resistant development of microbe to antibiotics causes treatment failure (Chambers, 2001; Casal *et al*., 2005). Due to increase in the multi resistant strains of bacteria and low susceptibility to traditional antibiotics, substances from other sources containing antimicrobial agents from herbal medicines are being used (Samuelsson, 2004). Plant-based antimicrobials have huge therapeutic potential. They are effective for the infectious diseases treatment and may reduce side effects that are often associated with synthetic drugs. Presence of secondary metabolites in plants plays an important role as antimicrobial agents. As a result some medicinal plants containing substances active against viruses, bacteria and fungi have been identified (Rybalchenko *et al*., 2010). Antimicrobial potential of extracts of different table olives was observed and extracts were found as good antioxidants and their strong antimicrobial activities suggesting olives as useful candidates against bacteria responsible for human respiratory tract and gastrointestinal infections (Adnan *et al*., 2014). Leaf extract of Olea was reported to interact with phospholipid bilayers (Micol *et al*., 2005). *Tagetes minuta* and *Usnea complanta* have been checked against the treatment of bacterial infections.

Bacopa monnieri has chemical compounds that are used in the cure of diseases like nervous disorders and epilepsy. In the treatment of anxiety and inflammation, use of chemicals from *Hypercicum hookerianum* has been reported (Dekanski *et al*., 2009).

Oleaceae, a dicotyledonous family includes 29 genera and about 600 species of deciduous trees and shrubs including olive tree and its relatives (Grohmann, 1974). Amongst the species, the most popular member of the genus Olea is *Olea europaea* L., only species of this genus is used as food mostly as olive oil or table olives. Plants of this family are therapeutically used in traditional medicine (Paiva-Martins *et al*., 2003; Khan *et al*., 2015). The phenolic compound, such as, oleuropein have strong antioxidant activity *in vitro* and *in vivo*. Medicinally it is used for its antimicrobial, gastroprotective, antioxidant, hypotensive, hypoglycaemic, antiarrhythmic, anti-atherosclerotic, antiviral, anti-tumor and anti-inflammatory properties (Haloui *et al*., 2010).

Olea ferruginea Royle is commonly known as Kao and Indian Olive.The decoction of its fresh leaves strengthen gums and relieve toothache hoarseness and throat ache (Shabir *et al*., 2015). Olive has antioxidant, antihypertensive potential due to presence of many potentially bioactive compounds (Hansen *et al*., 1996). *Jasminum sambac* (Linn.) Ait., commonly called as Mogra and Arabic jasmine has several active compounds, including flavonoids and coumarins enhance vascular health, heart function and phenolics help in the detoxification of the body. It is also considered a biological cure for jaundice, ulcers, skin diseases, tumor and eye disorders (Zhang *et al*., 1995; Kunhachan *et al*., 2012; Sabharwal *et al*., 2013).

The aim of present investigation is to evaluate the antibacterial potential of n-hexane, chloroform, ethanol and water extracts of *Olea europaea*, *Olea ferruginea* and *Jasminum sambac* leaves against clinically bacterial isolates.

Material and method

Plant Extraction

Leaves of *Olea europaea* were collected from PMAS-Arid University, Rawalpindi while the leaves of *Olea ferruginea* and *Jasminum sambac* from Botanical Garden of GCU, Lahore. After their identification with the help of taxonomic literature, these were deposited in GCU, herbarium, Lahore as vouch specimens. The leaves were dried in shade, ground by pistle and morter and macerated in n-hexane, chloroform, ethanol and water for 7 days in each solvent. The crude extracts thus obtained were stored at 4 °C.

Bactrerial strains used in the present test including *Staphylococcus aureus*, *Bacillus subtilis* as well as *E. coli* and *Pseudomonas aeruginosa* were collected from the Postgraduate Medical Institute, Lahore. Standard microbiological techniques were employed to sub-culturing of the microbes in nutrient broth.

Antimicrobial assay

The agar-well diffusion method was adopted after Pelczar *et al.* (1993) to evaluate the antimicrobial activity. The inoculum was carried out under aseptic condition spread on the medium homogenously. Afterward, the well was prepared in the center of the Petri-plate containing medium, and 1mL of extract was poured in the well. The inoculated plates were then incubated at 37±2°C for 24 hours. After which the zone of inhibition was measured in mm with the help of vernier caliper (Table 2, 3, 4 and 5). The same procedure was repeated by using standard antibiotics (Table 1) such as amikacin 30 µg, ampicillin 10 µg, erythromycin 15 µg, gentamicin 15 µg, streptomycin 10 µg and tetracycline 10 µg and also by taking the blank solvents as negative / positive control.

Minimum Inhibitory Concentration (MIC)

The agar dilution method was applied to determine Minimum inhibitory concentration after Jorgensen and Turnidge, 2007. MIC was checked with reference to negative (solvents) and positive (antibiotic dilutions) control. Different concentrations (10, 5, 2.5, 1.25 and 0.625mg/ml) of plant extract were prepared by serial dilution method. 18 ml of medium and 2ml of different concentrations of concentrated extracts of *Olea europaea* L., *O. ferruginea* Royle and *Jasminum sambac* (L.) Aiton were added in individual reaction plate (autoclaved). After solidification of media, the plates were inoculated by bacterial inoculum and plates were incubated at 37±2°C overnight. After incubation, plates were analyzed for the presence and absence of bacterial growth. The least concentration that had completely inhibited bacterial growth was considered as Minimum Inhibitory Concentration. All the tests were conducted in triplicates and data was analyzed statically, after Steel *et al.* (1997).

Result and discussion

The present study revealed that the tested medicinal plants; *Olea europaea*, *Olea ferruginea* and *Jasminum sambac* leaves extracts possessed good antibacterial potential against all the bacterial strains, used in the study. The ethanol extract of *Olea ferruginea* leaf had significant yield (7.25%) in comparison to other extracts, while the lowest yield was obtained by the n- hexane of the leaves of *jasminum sambac*, i.e. 2.19 % (Table. 1). The standard discs were used to check the susceptibility of the bacteria in present study (Table. 2). The zone of inhibition observed against the tested bacteria had revealed that *E. coli* and *S. aureus* exhibited intermediate response while resistance was observed by *B. subtilis* and *P. aeruginosa*.

Table 1. Extraction yield of the leaves of *Olea europaea, Olea ferruginea* and *Jasminum sambac.*

Plants	% extraction yield			
	n-hexane	Chloroform	Ethanol	Aqueous
Olea europeaea	2.99	3.65	6.01	5.67
Olea ferruginea	3.43	3.13	7.25	5.20
Jasminum sambac	2.19	4.12	4.34	3.92

The results indicated ethanol and aqueous extracts in all tested plants showed significant activities against both the types of bacterial strains. This might be due to the extraction of entire phenolic compounds in ethanol and water as compared to other solvents (Fazal *et al*., 2011).

Second and third effective solvents were n- hexane and chloroform, respectively. Nostro *et al*., 2000 also proved ethanol and water as efficient solvents for extraction procedures. Highest activity of leaf extracts of olive might be due to higher concentrations of oleuropein in the leaf.

Table 2. Zone of inhibition (mm) by the test organisms against standard antibiotic discs.

Antibiotics	Conc. (µg)	Zone of Inhibition (mm)			
		B. subtilis	*E. coli*	*P. aeruginosa*	*S. aureus*
Amikacin	30	18±1.3	13±0.3	14±0.8	18±0.5
Ampicillin	10	13±1.5	18±2.5	11±0.5	21±3.9
Erythromycin	15	11±0.6	12±0.7	-	18±1.0
Gentamicin	10	14±2.5	14±2.2	12±0.5	12±3.5
Streptomycin	10	-	14±1.5	14±0.9	17±0.8
Tetracycline	10	12±2.4	16±0.7	13±1.4	14±0.4
Final response		Resistant	Intermediate	Resistant	Intermediate

All the results are mean of three parallel replicates, ± indicates the Standard error.

Table 3. Inhibitory Zone (mm) by leaf of *Olea europaea* L. against bacterial test strains.

Solvents	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*
n-Hexane	12.53±1.86b	5.77±0.68c	23.17±1.26a	20.90±1.65a
Chloroform	6.43±1.69c	20.87±0.81a	17.83±3.32b	14.23±2.36b
Ethanol	6.53±0.92c	6.03±1.05c	17.17±2.02b	15.73±1.62b
Aqueous	19.77±2.25a	17.43±1.69b	17.57±1.40b	19.57±1.91a
LSD	3.29	2.12	4.07	3.59

All the results are mean of three parallel replicates, ± indicates the Standard error.

Results revealed that all plant extracts showed potential against bacterial strains (*B. subtilis, P. aeruginosa, E. coli* and *S. aureus*). Ethanol extracts of *O. ferruginea* and *J. sambac* and also exhibited good potential against *E. coli,* both extracts showed 20.47±2.24 mm and 20.23±2.66 zone of inhibition respectively (Table. 4, 5, Fig 4). n- hexane extracts of *Olea europaea* exhibited significant activity (23.17±1.26mm) followed by ethanol extracts of *J. sambac* with 20.00±2.65 mm of inhibition zone against *P. aeruginosa* (Table. 5, Fig 5) and our result is supported by Gopalakrishnan *et al.* (2012). n-Hexane extract of leaves of *J. sambac* showed maximum activity (26.37±2.54 mm) against *E. coli*.

Table 4. Inhibitory Zone (mm) by leaf of *Olea ferruginea* Royle against bacterial test strains.

Solvents	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*
n-Hexane	12.23±1.97b	5.23±1.57b	19.26±1.42a	16.47±3.23ab
Chloroform	5.56±1.25c	6.17±0.76b	9.90±2.85b	7.50±2.29c
Ethanol	8.23±1.36c	6.20±1.59b	15.13±0.81a	20.47±2.24a
Aqueous	18.50±2.78a	20.27±2.00a	17.50±3.77a	14.93±0.90b
LSD	3.56	2.91	4.71	4.37

Table 5. Inhibitory Zone (mm) exhibited by leaf of *Jasminum sambac* (L.) Aitonagainst bacterial test strains.

Solvents	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E.coli*
n-Hexane	8.57±0.98b	10.17±1.26b	18.27±1.42ab	26.37±2.54a
Chloroform	7.63±1.18b	6.27±1.55c	15.17±2.02b	5.63±1.52c
Ethanol	9.13±2.42b	11.20±1.59b	20.00±2.65a	20.23±2.66b
Aqueous	24.43±1.69a	15.00±2.00a	16.20±2.31ab	18.13±1.21b
LSD	3.13	3.05	4.04	3.91

The difference in the activity might be due to the varying degree of presence of different secondary metabolites in the different solvents (Majorie, 1999). Aquous extracts of *J. sambac*, *O. europaea* and *O. ferruginea* exhibited significant activity against *B. subtilis* that was 24.43±1.69 mm, 19.77±2.25 mm and 18.50±2.78 mm respectively (Fig 1) and this was in contrary to the Korukluoglu *et al.* (2010) who reported no antibacterial activity by aqueous extracts in their studies.

Table 6. MIC exhibited by *O. europaea, O. ferruginea*and *J. sambac* against bacteria.

Bacterial strains	Plant extracts (mg/ml)	*Olea europaea*				*Olea ferruginea*				*Jasminum sambac*			
		Hex	Chl	Eth	Aq.	Hex	Chl	Eth	Aq.	Hex	Chl	Eth	Aq.
B. subtilis	10	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	+	+	-	-	+	+	-	+	+	+	-
	2.5	+	+	+	-	+	+	+	-	+	+	+	-
	1.25	+	+	+	-	+	+	+	-	+	+	+	-
	0.625	+	+	+	+	+	+	+	+	+	+	+	+
S. aureus	10	-	-	-	-	-	-	-	-	-	-	-	-
	5	+	-	-	-	+	+	+	-	+	+	-	-
	2.5	+	-	+	-	+	+	+	-	+	+	+	-
	1.25	+	-	+	-	+	+	+	-	+	+	+	+
	0.625	+	+	+	+	+	+	+	+	+	+	+	+
P. aeruginosa	10	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-	-
	2.5	-	+	+	-	+	-	-	+	+	-	-	+
	1.25	-	+	+	-	+	-	+	+	+	+	+	+
	0.625	-	+	+	+	+	+	+	+	+	+	+	+
E. coli	10	-	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	+	-	-	-	-	-	-
	2.5	-	-	-	-	-	+	-	-	-	-	-	-
	1.25	-	-	-	-	+	+	-	+	-	+	-	+
	0.625	-	+	+	+	+	+	+	+	+	+	+	+

*Key: - = Absence of bacterial growth, + = Presence of bacterial growth.

*Macerates: Hex = *n*-hexane, Chl = Chloroform, Eth = Ethanol, Aq = Aqueous.

Maximum antibacterial activity against *S. aereus* was reported by aqueous extract of *O. ferruginea* (20.27±2.00) as well as by chloroform extracts of *O, europaea* (20.87±0.81 mm). From the results, more susceptibility of Gram negative bacteria was observed than Gram positive bacteria towards plant macerates due to the presence of lipopolysaccharide membrane and this correlates to the results of Hussain *et al.* (2014).

Leaf ethanol extracts of*O. europaea, O. ferruginea*and *J. sambac* against *S. aureus, B. subtilis, P. aeruginosa* and *E. coli* were tested for Minimum Inhibitory Concentration and found to be very effective against the growth of tested bacterial strains. *P. aeruginosa* and *E.coli* were found to be more susceptible towards the *Olea europaea* and *J. sambac*. n- hexane leaf extracts of *Olea europaea* and *Jasminum sambac* exhibited significant potential against *P. aeruginosa* and *E. coli* followed by ethanol extracts of *Olea europaea*, *Olea ferruginea* and *J.sambac* against *P. aeruginosa* and *E. coli* (Table. 6). Aqueous extract of *Olea europaea* presented significant potency against *B. subtilis* and *S. aureus*. Most resistance was showed by *B. subtilis* against n-hexane leaf extract of *Olea ferruginea*.

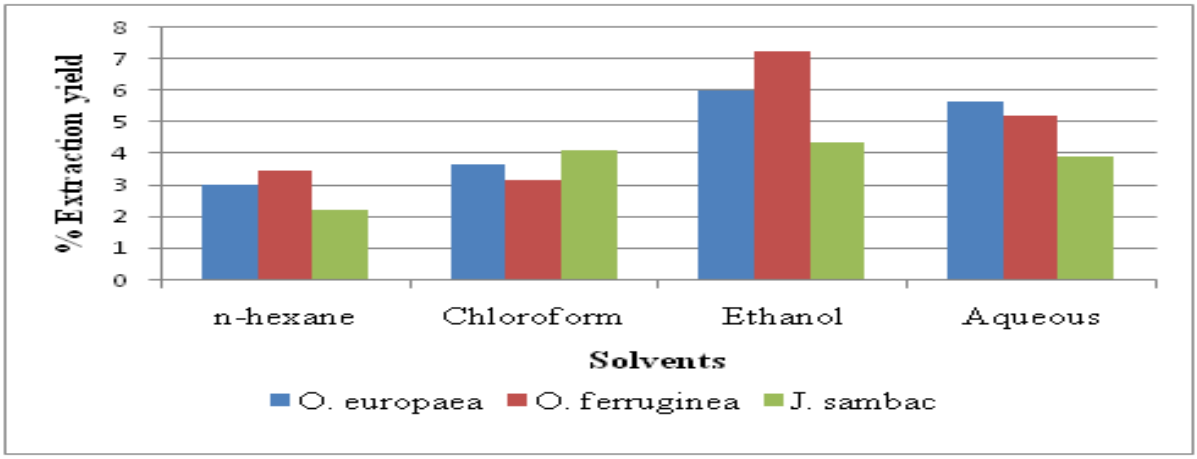

Fig. 1. Graphical image of the % extraction yield of the leaves of *O. ferruginea*, *O. europaea* and *J. sambac*.

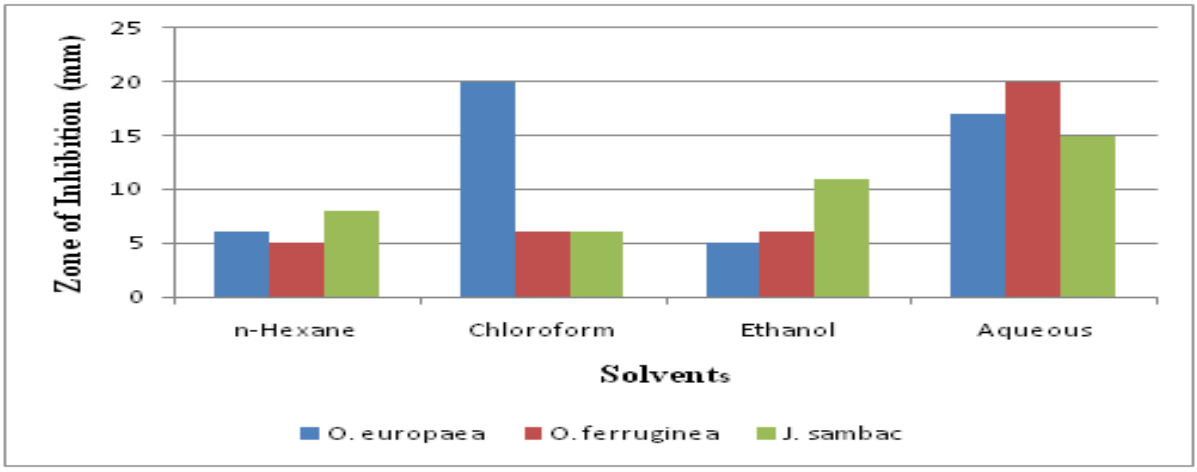

Fig. 2. Graphical image of the Zone of Inhibition by the leaf extracts of *O. ferruginea*, *O. europaea* and *J. sambac* against *S. aureus*.

Leaf extracts of *Olea ferruginea, Olea europaea* and *Jasminum sambac* showed more potential against Gram negative bacteria as compared to Gram positive bacteria and this is in consistence with the previous studies of Khan *et al*. (2009). Our present studies had proved the potential of *O. europeae, O. ferruginea* and *jasminum sambac* against resistant strains of *E. coli* and *P. aeruginosa* and this is in agreement with the studies of Auwal *et al*., 2013. Reason for the use of ethanol extracts against bacterial activity could be due to high extraction of the chemical constituents such as alkaloids, saponins and tannins in ethanol as compared to other solvents (Akinyemi *et al*., 2006).

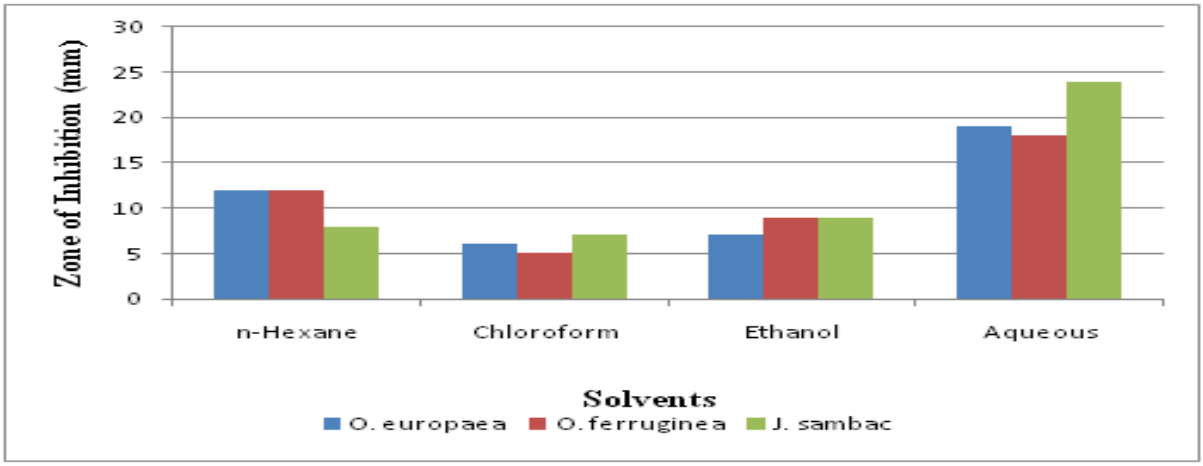

Fig. 3. Graphical image of the Zone of Inhibition by the leaf extracts of *O. ferruginea*, *O. europaea* and *J. sambac* against *B. subtilis*.

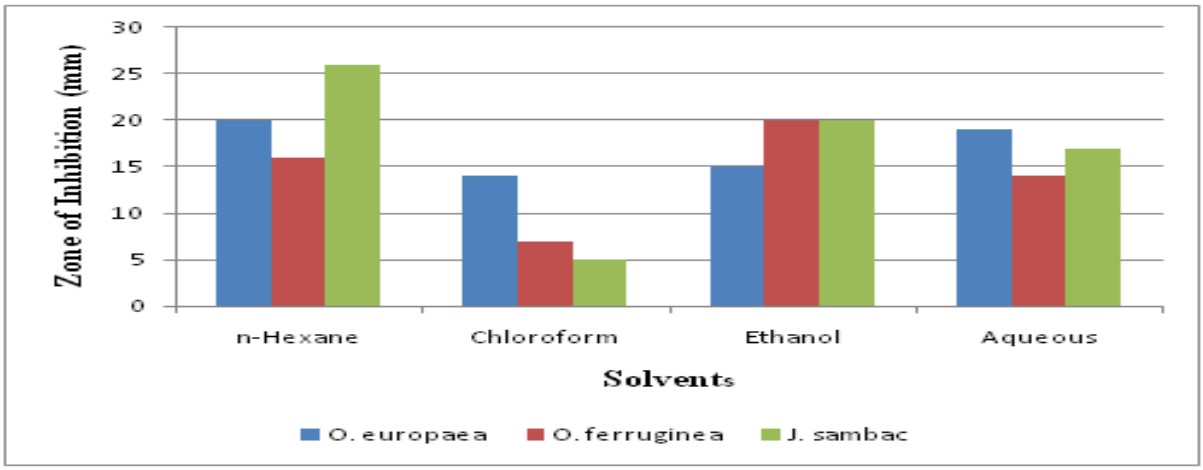

Fig. 4. Graphical image of the Zone of Inhibition by the leaf extracts of *O. ferruginea*, *O. europaea* and *J. sambac* against *E. coli*.

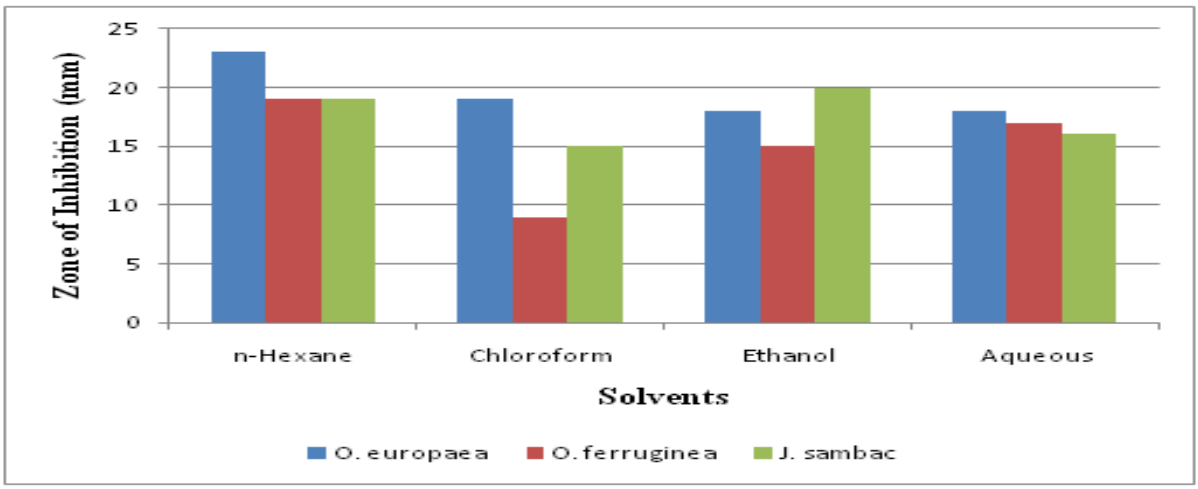

Fig. 5. Graphical image of the Zone of Inhibition by the leaf extracts of *O. ferruginea*, *O. europaea* and *J. sambac* against *P. aeruginosa*.

Conclusion

The present study concluded that leaf extracts of *Jasminum sambac* (L.) Aiton, *Olea europaea* L. and *O. ferruginea* Royle belonging to family Oleaceae have shown significant activity against the pathogenic bacterial strains. Results obtained suggest that these plants can be used in preparation of effective natural medicines.

References

Adnan M, Bibi R, Mussarat S, Tariq A, Shinwari ZK. 2014. Ethnomedicinal and phytochemical review of Pakistani medicinal plants used as antibacterial agents against *Escherichia coli*. AnnalsofClinicalMicrobiologyand Antimicrobials **13,** 40.

Akinyemi KO, Oluwa OK, Omimigbehin EO. 2006. Antimicrobial activity of crude extracts of three medicinal plants used in south- west Nigerian folk medicine on some food borne bacterial pathogens. African Journal of Traditional, Complementary and Alternative Medicine **3,** 13-22.

Auwal MS, Mairiga IA, Shuaibu A, Ibrahim A, Gulani IA, Wampana B, Lateef GI, Lawan FA, Sanda KA, Thaluvwa AB, Njobdi AN, Yagana KZ. 2013. Preliminary phytochemical and *in vitro* antibacterial evaluation of crude pericarp extract of *Hyphaene thebaica*(Doumpalm). Journal of Medicinal Plant and Herbal Therapy Research (JMPHTR) **1,** 1-7.

Casal M, Vaquero M, Rinder H, Tortoli E, Grosset J, Gutierrez J, Jarlier V. 2005. A case study for multi-drug resistant tuberculosis risk factor in four European countries.MicrobialDrug Resistance **11,** 62-67.

Chambers HF. 2001. The changing epidemiology of *Staphylococcus aureus*. Emerging Infectious Diseases **7,** 178-182.

Dekanski D, Janicijevic-Hudomal S, Tadic V, Markovic G, Arsic I,Mestrovic D. 2009. Photochemical analysis and gastroprotective activity of an olive leaf extract.Journal ofSerbian Chemical Society **74,** 367-377.

Fazal H, Ahmad N, Ulah I, Inayat H, Khan L, Abbasi BH. 2011. Antibacterial potential in *Parthenium hysterophorus*, *Stevia rebaudiana* and *Ginkgo biloba*. Pakistan Journal of Botany **43,** 1307-1313.

Gopalakrishnan S, Rajameena R, Vadivel E. 2012. Antimicrobial activity of the leaves of *Myxopyrum serratulum* A.W.Hill. International Journal of Pharmaceutical Sciences and Drug Research **4,** 31-34.

Greathead W. 2003. Extraction and antimicrobial effect of Thai herbs and spices extracts.J. Agricultural and Food Chemistry **43,** 361-371.

Grohmann F. 1974. Oleacceae. In: Flora of Pakistan. No.59.

Haloui E, Marzouk Z, Marzouk B, Bouftira I, Bouraoui A, Fenina N. 2010. Pharmacological activities and chemical composition of the *Olea europaea* leaf essential oils from Tunisia.Journal of Food, Agriculture & Environment **2,** 204-208.

Hansen K.1996. Isolation of an Angiotensin Converting Enzyme (ACE) inhibitor from *Olea europaea* and *Olea valancia*. Phytomedicine **2,** 319-325.

Hussain A, Qarshi IA, liaqat R, Akhtar S, Aziz I, Ullah I, Shinwari K. 2014. Antimicrobial potential of leaf and fruit extracts and oils of wild and cultivated edible Olive. Pakistan Journal of Botany **46,** 1463-1468.

Jorgensen JH, Turnidge JD. 2007. Susceptibility test methods: Dilution and disk diffusion methods. *In*: Murray, (Eds.), Manual of Clinical Microbiology. (9th Ed), ASM Press, Washington D.C., 1152-1172 p.

Khan MY, Panchal S, Vyas N, Butani A, Kumar V. 2015. *Olea europaea*: A phyto-pharmacological review. Pharmacognosy Review 1, 1.

Khan R, Islam B, Akram M, Shakil S, Ahmad A, ManazirAS, Siddiqui M, khan AU. 2009. Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules **14,** 586-597.

Korukluoglu M, Sahan Y, Yigit A, Gucer S. 2010. Antibacterial activity and chemical constitiutions of *Olea europaea* L. leaf extracts. Journal of Food processing and Preservations **34,** 383-396.

KunhachanP,BanchonglikitulC, Kajsongkram T, Khayung A, Leelamanit W. 2012. Chemical composition, toxicity and vasodilation effect of the flowers extract of *Jasminumsambac* (L.) Ait. Evidence- Based Complementary and Alternative Medicine **4,** 471312.

Mahmood A, Ahmad M, Jabeen A, Zafar M, Nadeem S. 2003. Pharmacognostic studies of some indigenous medicinal plants of Pakistan. Journal of Ethnobotanical LeafletsSIUC, USA.

Majori MC. 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews **12,** 564-582.

Micol V, Chaturla N, Fons LP, Mas V, Perez L, Estepa A. 2005. The olive leaf extract exhibits antiviral-activity against viral*haemorrhagic septicaemia rhabdovirus* (VHSV). Antiviral Research **66,** 129-136.

Nastro A, Germano MP, Angelo VD, Marino A, Cannatelli MA. 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology **30,** 379-384.

Paiva-Martins F, Gorden MH,Gameiro P. 2003. Activity and location of olive oil phenolic antioxidants in liposomes. Chemistry and Physics of Lipids **124,** 23-36.

Pelczar MJ, Chan ECS, Krieg NR. 1993. Microbiology, Int. Ed.McGraw. 578.

Rybalchenko NP, Prykhodko VA, Nagorna SS, Volynets NN, OstapchukAN, Klochko VV, Rybalchenko TV, Avdeeva LV. 2010. *In vitro* antifungal activity of phenylheptatriyne from *Bidens cernua* L. against yeasts. Fitoterapia **81,** 336-338.

Sabharwal S, Vats M, Sardana S, Aggarwal S. 2013. Pharmacological, Physico and Phytochemical evaluation of the leaves of *Jasminum sambac* Linn. InternationalJournalofPharmacy and Pharmaceutical Science ISSN: 0975-1491.

Samuelsson G. 2004. Drugs of Natural Origin: a textbookofPharmacognosy,5thSwedish. Pharmaceutical Press, Stockholm.

Shabir H, Khan MS, Rehman HU, Masood Z, Yousaf T, Majid A, Iqbal R.2015. Ethnomedicinal uses of xeric flora in tehsil Banda Daud Shah collected from district Karak KPK Pakistan. World Journal of Zoology **10,** 59-69.

Steel RGD, Torrie JH, Discky DA. 1997. Principles and Procedures of Statistics: A Biometrical Approach. (3rd Ed.), McGraw Hill Co., New York.

Vasconsuelo A, Boland R. 2007. Molecular aspects of the early stages of elicitation of secondary metabolites in plants. Plant Science **172,** 861-875.

Zhang ZYJ, Liu LYQ, Pu PX, Yang YC. 2008. Iridoidal glycosides from Jasminum sambac. Phytochemistry **38,** 899-903.