International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Preliminary Phytochemical and Antibacterial Studies of *Olea europaea* and *Polygonum maritimum* Libyan Plants

Salem Edrah[1], Ashok Kumar[2]

[1]Department of Chemistry, Faculty of Sciences, Al-Khoms,

[2]Al-Mergheb University, Al-Khoms, LIBYA

Abstract: ***Polygonum maritimum and Olea europaea are the useful plants used in folk medicines for the treatment of various diseases. The present study was conducted to examine preliminary phytochemicals screening of aqueous and ethanlolic leaves extracts and antibacterial activity of ethanolic extracts of both plants of Libyan origin. The qualitative phytochemical studies of aqueous and ethanolic extracts of the plants leaves were carried out using standard testing procedures for metabolites viz. tannins, saponins, phlobatanins, flavonoids, terpenoids, cardiac glycosides and alkaloids. The aqueous and ethanolic extracts of Olea europaea showed the presence of tannins, saponins, phlobatanins, flavonoids, terpenoids and cardiac glycosides metabolites, however, the aqueous and ethanolic extracts of Polygonum maritimum exhibited the presence of tannins, saponins flavonoids, terpenoids and cardiac glycosides metabolites . The antibacterial activity of ethanolic leaves extract of both plants was tested using Gram positive bacterial strains (Staphylococcus epidermidis ,Staphylococcus saprophyticus) and Gram negative bacterial strains (Proteus vulgaris, Eschericia coli, Citrobactor freundii). In general, the extracts of both plants exhibited considerable activity on the bacterial species. Both plants extracts selectively inhibited the growth of both gram positive and gram negative bacteria with zones of inhibition ranging from 8 mm to 10 mm at concentrations of 50 mg/ml. Present findings suggest that Polygonum and Olive leaves extracts exhibit antibacterial effect against both gram positive and gram negative bacteria and these plants seems to be considered for detailed investigation in an attempt to find out the chemical entities combating against pathogenic microorganisms.***

Keywords: Libyan plants, *Polygonum maritimum, Olea europaea,* Phytochemicals screening, Antibacterial activity.

1. Introduction

Starting from ancient times plants have given us several remedial agents for the treatment of deseases and have an important place in the therapeutic armory of mankind . At least 25% of the prescribed drugs used for the treatment of diseases in the USA and Canada contain bioactive ingredients derived from plant natural products. Therefore such plants should be investigated to understand better about their medicinal properties, safety and efficacy[1-3]. .Mcrobial infections constitute a major cause of morbidity and mortality in humans and medicinal plants are well known as important source of obtaining antimicrobial drugs[4-7].

The olive tree, *Olea europaea* (family Oleaceae) is an evergreen tree or shrub native to the Mediterranean, Asia and Africa. Olive tree parts have shown useful medicinal properties and possess antibacterial activity [8] has the capacity to lower blood pressure in animals [9] , increase blood flow in the coronary arteries , relieve arrhythmia and prevent intestinal muscle spasms [10] and its oil has a number of common phenolic compounds that might possess positive health effect to the human body, as well as a good source of Vitamin E [11].

Polygonum maritimum L. (family polygonoceae*)* is a perennial herb or small shrub 20-50 cm in height and found in coastal sites in Europe, America, South Africa, and the Mediterranean region [12]. It has been reported as good source of phytochemicals known for biological actions The genus *Polygonum* is well known for producing a variety of plant secondary metabolites such as phenylpropanoids, acetophenones, chalcones, coumarins, flavonoids, lignans, naphtoquinones, anthraquinones, sesquiterpenoids, triterpenoids, stilbenoids and tannins described in many publications [13-22]. According to our knowledge, there are very few publications available about the characterisation of the antimicrobial activity of crude extract of *P. matitimum* [20-21].

So far no specific study has been carried out for the preliminary phytochemical screening and antibacterial activity of *Olea europaea* and *Polygonum maritimum* grown in Libya. Therefore, in this present investigation, the preliminary phytochemical testings and antibacterial activity of leaves extracts of both these plants against Gram positive and Gram negative bacteria were investigated and described.

2. Materials and Methods

2.1. Collection of Plants materials:

Olea europaea and *Polygonum maritimum* plants were collected from different parts of the Al-khoms city (Libya) in April-May months of 2013. Identification of both these plants was confirmed by Plant taxonomist of the Department of Biology of Al- mergheb University, Al-Khoms, Libya. Leaves of each plant were collected, washed under running tap water and dried under shade and ground into fine powder in the electronic grinder, the powder was stored in plastic bags at room temperature under low humidity condition.

2.2 Preparation of Extract

The crude powdered plants leaves were extracted in water and ethanol separately at a 40% (w/v) concentration (200g leaves powder in 500 ml water) by using Soxhlet method for 8 h and filtered. The extracts were stored in refrigerator at 4°C until further use

2.3 Phytochemicals screening

Chemical tests were performed for the aqueous and ethanolic leaves extracts of both the plants using standard procedures to identify the presence of various phytochemicals as described by Sofowora, 1993[23] and Raman, 2006 [24].

2.3.1 Tannins
A small quantity of each extracts was heated on water bath and filtered. A few drops of ferric chloride was added to the filtrate. A dark green solution indicated the presence of tannins.

2.3.2 Saponins
Small amount of filtered plant extract was shaken and heated to boil. Frothing (appearance of creamy mass of small bubbles) shows the presence of saponins.

2.3.3 Phlobatanins
Small amount of filtered extract was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins.

2.3.4. Flavonoids
Filtered extract **of** each plant was mixed with diluted NaOH and HCl was added. A yellow solution that turns colorless indicates the presence of flavonoids.

2.3.5. Terpenoids
Small amount of each extract was filtered and mixed with 2 ml of chloroform ($CHCl_3$) and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

2.3.6. Cardiac glycosides
Small amount of each extract was filtered and shaken with 1ml of glacial acetic acid. A drop of ferric chloride and a drop of concentrated sulfuric acid was added. Green blue color to upper layer and reddish brown color at the junction of two layers indicates the presence of cardiac glycosides.

2.3.7. Alkaloids
Small amount of the extracts was filtered and warmed with 2% H_2SO_4 for two minutes. It was filtered and few drops of Dragondorff reagent were added. Orange red precipitate indicated the presence of alkaloids.

2.4. Antibacterial screening

2.4.1 Test Organisms
Two Gram positive strains (*Staphylococcus epidermidis, Staphylococcus saprophyticus*) and three Gram negative bacterial strains (*Proteus vulgaris, Eschericia coli, Citrobactor freundii)* from standard cultures were used as test strains. Standard bacterial strains were procured from Microbiology Medical Laboratory, Central Hospital, Al-Khoms, Libya.

2.4.2 Determination of Antibacterial activity by disc diffusion method:
Ethanolic leaves extracts of both plants were investigated for antibacterial activity against two Gram positive bacterial strains and three Gram negative bacterial strains by the paper disc diffusion method [25]. From the 50 mg/ml stock solution of ethanolic extracts of both plants, 40 µl aliquots were tranferred onto blank sterile paper discs (6 mm diameter). Dried discs were placed onto nutrient agar medium (UK) previously inoculated with a bacterial suspension and incubated at 37ºC for 24 h. Ethanol solvent was used as control to determine the sensitivity of the tested strains. After incubation, plates were examined for the presence of zones of growth inhibition, and the diameters of these zones were measured in mm. Tests were performed in duplicate under sterile conditions.

3. Results

3.1. Phytochemicals screening:

Table 1 shows the results of phytochemicals screening of aqueous and ethanolic leaves extracts of *Olea europaea* and *Polygonum maritimum* plants. *Olea europaea* leaves extracts revealed the presence of tannins, saponins, phlobatanins, flavonoids, terpenoids and cardiac glycosides , however, *Polygonum maritimum* leaves extracts revealed the presence of tannins, saponins flavonoids , terpenoids and cardiac glycosides.

Table 1: Phytochemical screening of aqueous and ethanolic leaves extracts of *O. europea* and *P. maritimum*:

Chemical - Component	*Olea europea*		*Polygonum maritimum*	
	aq. extr	eth. extr.	aq. extr.	eth. extr.
Tannins	+	+	+	+
Saponins	+	+	+	+
Phlobatanins	+	+	–	–
Flavonoids	+	+	+	+
Terpenoids	+	+	+	+
Cardiac glycosides	+	+	+	+
Alkaloids	–	–	–	–

Key: + = present ; - =bsent

3.2. Antibacterial activity

Table 2 shows the results of antibacterial activity of both of plants leaves ethanolic extracts.The ethanolic extract of *Olea europaea* exhibited antibacterial activity against a Gram positive bacterial strains (*S. Epidermidis) among the two tested Gram positive bacterial strains* and a Gram negative bacterial strain (*Citrobactor freundii*) among the three tested Gram negative bacterial strains, while the ethanolic extract of *Polygonum maritimum* revealed antibacterial activity against both tested Gram positive bacterial strains (*S. epidermidis*

S.saprophyticus) and a Gram negative strain *Proteus vulgaris* among the three tested Gram negative bacterial strains. *Olea europaea* and *Polygonum maritimum* plants *exhibited maximum antibacterial activity against* Gram negative bacteria *Citrobacter freundii* (zone of inhibition:9 mm) and *Proteus vulgaris* (zone of inhibition: 10 mm) , respectively.

Table 2: Antibacterial activity of leaves of ethanolic extracts (50 mg/ml) of *Olea europea* and *Polygonum maritimum* :

Bacterial strains	*O. europea*	*P. maritimum*
	Zone of inhibition (mm)	
S. epidermidis	8	9
S. saprophyticus	-	8
Proteus vulgaris	-	10
Eschericia coli	-	-
Citrobactor freundii	9	-

Note: Discs injected with 40 µl of ethanol solvent served as controls against each bacterial strain and showed nil zone of inhibition.

4. Discussion

The results of the screened phytochemicals (tannins, saponins, phlobatanins, flavonoids, terpenoids, cardiac glycosides and alkaloids) of aqueous and ethanolic extracts of both the plants showed the common presence of. tannins, saponins ,flavonoids, terpenoids and cardiac glycosides, however, phlobatanins was present in ethanolic extract of *Olea europaea* but not in *Polygonum maritimum* . Further, ethanol extraction is widely used to obtain crude extracts of phytochemicals from plant materials in the herbal medicine industry for therapeutic applications and most of the previous studies have indicated that ethanolic extracts of the plants leaves contains more biological activity and showed richer in phytochemicals concentrations than the aqueous and other organic solvents extracts of other parts of the plants, therefore, ethanolic leaves extract of the plants was chosen for the preliminary investigation of both the plants for antibacterial activity. Previous some reports have indicated that the aqueous and alcoholic extract of *Olea europaea* and *Polygonum maritimum* are found to have remarkable biological and therapeutic effects including antibacterial action[10],[15],[19],[21-22]**.** The results on both of the studied plants of Libyan origin lacking in previous reports prompted us for the present study. The results of anti bacterial activities of both of the plants leaves ethanolic extracts have shown good antibacterial effects against standard strains of Gram positive and Gram negative bacterial strains. Zone of inhibition produced by the extracts (50 mg/ml) in paper disc diffusion method against the susceptible bacteria ranged from 8 to 10 mm diameter. It was interesting to note that *Proteus vulgaris* (Gram negative bacterial strain) indicated maximum sensitivity to the investigated plants leaves extracts of *Polygonum maritimum*. Results have shown that the anti-bacterial activities of *Olea europaea* and *Polygonum maritimum are not comply well with the previous reports[21][22]*. This may be due to the effect of the regional variation of environment and soil composition in different places. The extracts contain many phytochemicals substances including flavonoids. Antibacterial activity shown by both of these plants are assumed to be due to the presence of flavonoids content due to its ability to form complex with extracellular and soluble proteins and to complex with bacterial cell walls. Lipophilic flavonoids may also disrupts bacterial membranes. However, search of exact phytochemical as antibacterial agent from both of these plants extracts warrants further detailed study involving the isolation , purification and antibacterial screening of different constituents of both of the plants to find out the bio-active ingredient(s) .

5. Conclusion

The present study revealed antibacterial activity of ethanolic leaves extracts of *Olea europaea* and *Polygonum maritimum* of Libyan origin. Aqueous and ethanolic leaves extracts of both these plants showed the presence of tannins, saponins, flavonoids, terpenoids and cardiac glycosides phytochemicals. The phytochemical constituents present in the plant may be responsible to exert such medicinal properties. Future study that involves the isolation and purification of the bioactive compound that may help to investigate the bio-active ingredient(s) of both the plant's leaves extracts.

6. Acknowledgement

The authors are grateful to the authorities of Faculty of Sciences, Al-khums, Al-mergheb University, Libya for their technical support, and providing the necessary laboratory facilities.

References

[1] N.R. Farnsworth, "The role of medicinal plants in drug development", In: Krogsgaard-Larsen, editor. Natural products and drug development. London: Balliere, Tindall and Cox, pp. 8-98, 1984.
[2] G. Addis, D. Abebe , K. Urga, "A survey of traditional medicinal plants in shirka district, ARSI zone", Ethio Pharm J.,19(2),pp. 30-35,2001.
[3] G G.F. Nascimento ,J. Lacatelli , P.C. Freitas G.L. Silva, "Antibacterial aitctivy of plant extracts and Phytochemicals on antibiotic-resistant bacteria", Braz J Microbiol, 3, pp.886-891,2000.
[4] A. Sofowora , "Medicinal plant and traditional medicine in Africa II". John Wiley Chiechester; pp.178, 1986.
[5] A. Duncan, C. Okunji , "New Antimicrobials of plant origin", IN: J.Janick (ed), perspectives on new crops and new uses. Alexandria, VA, ASHS PRESS, 6, pp.457- 462,1999
[6] K. Asres, S. Taddese , T. Gebremariam ," In vitro antimicrobial activities of some selected topically applied medicinal plants of Ethiopia. Ethio Pharm J., 21, pp. 34-4, 2003.
[7] R.Sharma, C.Sharma,., B. Kapoor,, 'Antibacterial resistance:current problems and possible solutions", *Indian. J. Med. Sci*., *59*(3), pp.120-129, 2005.
[8] N.B. Nora, K. Hamid, M. Snouci, M.Boumedien, M. Abdellah, **"**Antibacterial Activity and Phytochemical Screening of Olea *Europaea* Leaves from Algeria', The

Open Conference Proccedings Journal, 3, (Suppl 1-M11),pp. 66-69,2012.
[9] Samuelsson, G. "The blood pressure lowering factor in leaves of *Olea europaea*", *Farmacevtis Revy 15*,pp. 229–239,1951.
[10] A.Zarzuelo, Duarte, J, Jimenez, J., Gonzales, M, Utrill, "Vasodilator effect of olive leaf", *Planta Med. 57*, pp.417-419, 1991.
[11] O.B. Garcia, J. Castillo, J. Lorente, A. Ortuno, J.A. Del-Rio, "Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves". *Food Chem.*, *68*,pp. 457-462, 2000.
[12] G.Kazantzoglou, P. Magiatis , E. Kalpoutzakis , A.L. Skaltsounis , 'Polygonophenone, the first MEM - substituted natural product, from *Polygonum maritimum*", J Nat Prod. 72, pp.187–189, 2009.
[13] D.G. Wang, W.Y. Liu, G.T.Chen, "A simple method for the isolation and purification of resveratrol from *Polygonum cuspidatum*", J Pharmaceut Anal. , *10, pp.1016, 2012*.
[14] Z.F., Peng, D. Strack , A. Baumert , R. Subramaniam , N.K. Goh , T.F. Chia et al. "Antioxidant flavonoids from leaves of *Polygonum hydropiper* L.", Phytochem. 62, pp.219–228, 2003.
[15] D.Q.Han, J. Zhao, J. Xu, H.S. Peng , X.J. Chen , S.P., Li , "Quality evaluation of *Polygonum multiflorum* in China based on HPLC analysis of hydrophilic bioactive compounds and chemometrics", J Pharmaceut Biomed Anal. , 72, pp.223–230, 2013.
[16] K.J. Wang, Y.J. Zhang, C.R. Yang, 'Antioxidant phenolic compounds from rhizomes of *Polygonum paleaceum* J Ethnopharmacol., 96,pp.483–487,2005.
[17] D. Fan, X. Zhou C., Zha, H. Chen, Y. Zhao, X. Gong , "Anti-inflammatory, antiviral and quantitative study of quercetin-3-O-β-D-glucuronide in *Polygonu perfoliatum* L. Fitoterapia", 82,pp.805– . 810,2011.
[18] S.N. Lopez , M.G. Sierra, S.J. Gattuso , R.L. Furlan S.A. Zacchino , "An unusual homoisoflavanone and a structurally-related dihydrochalcone from *Polygonum ferrugineum* (Polygonaceae)", Phytochem,,54, 201–205,2006.
[19] S. Hussein, U. EL-Magly, M. Tantawy , S. Kawashty, N. Saleh, 'Phenolics of selected species of *Persicaria* and *Polygonum* (Polygonaceae) in Egypt", Arab J Chem. 2012 doi : 10,1016/ j. arabjc.2012.06.002.
[20] B.K. Datt, S.K. Datta, S.D. Sarker , "Quercetin 3- O-(6″-galloyl)-b-D-galactoside from *Polygonum viscosum (*Polygonaceae)", Biochem Syst Ecol.,28pp.,805–807,2000.
[21] I.A. El-Haci, F. Atik-Bekkara, W. Mazari, M.A. Hassani, Didi, "Screening of biological activities of Polygonum maritimum L. From Algerian coast", *Asian* Pac J Trop Biomed., 3(8), PP. 611–616, 2013.
[22] I.A. El-Haci, F.A. Bekkara, W. Mazari, F.I. Hassani, M.A. Didi, "Screening of biological activities of Polygonum maritinum L. From Algerian coast", Asian Pacific J of topical biomedicine,4(1),pp. 1-84, 2014.
[23] A. Sofowora., "In Phytochemical screening of Medicinal plants and traditional medicine in Africa", Spectrum Books Ltd Nigeria,; 2nd ed : pp. 150 – 156,1993.
[24] N.Raaman, "Phytochemical techniques, New Delhi", pp19-22, 2006.
[25] A.W.Bauer, W.M.M Kirby, J.C.Sherris, M. Turck, "Antibiotic susceptibility testing by a standardized single disc method", Am. J. Pathol. 49**:**pp. 493-496, 1966.

Author Profile

Dr Ashok Kumar: 1977, 1982: received the Masters and Ph D degrees in Chemistry from Kanpur University and Banaras Hindu University, respectively. **1983-1997 :** worked as PDF, RA, in Universities in India & abroad (Germany, Sweden), and as R &D Executive in industries in India .**1998 onwards :** worked / working as Faculty member and Principal in academic Institutions situated in abroad (Libya) & Kanpur, India .**Teaching Specializations :** Organic Chemistry, Biochemistry. **Research Specializations:** Synthetic Organic Chemistry, Neurochemistry, Neuropharmacology, Phytochemistry etc.

Dr. Salem Edrah: 2001, 2008: received the Masters and Ph. D degrees in Chemistry from Pardubice University. **2013-2014:** worked as PDF, RA, in Universities in Pardubice (Czech Republic). **1998 onwards:** worked / working as Faculty member and Principal in academic Institutions situated in El-Mergeb University, Al-khums (Libya). **Teaching Specializations:** Organic Chemistry, Biochemistry. **Research Specializations:** Synthetic Organic Chemistry, Phytochemistry etc.