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Antibacterial and Antifungal Activities of *Flueggea anatolica* (Euphorbiaceae)

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Flueggea anatolica Gemici (Euphorbiaceae) is a relict endemic shrub remnant from the tertiary period, has been collected from the north of Tarsus (Icel) in Turkey. The antibacterial and antifungal effects of the chloroform extracts of plant leaves were tested against the different bacteria *e.g.*, *Echerichia coli* ATCC-12228, *Salmonella thyphimirium* CCM-5445, *Proteus vulgaris* ATCC 29905, *Pseudomonas aeroginosa* ATCC-27853, *Bacillus subtilis* ATCC-6633, *Staphylococcus aureus* ATCC 6538-P and the yeast *Candida albicans* ATCC-10239 by micro dilution techniques *in vitro*. The growth of *E. coli* , *P. aeroginosa* and *P. vulgaris* the Gram-negative bacteria and *Candida albicans* yeast have been inhibited by the chloroform extract of the leaves of *Flueggea anatolica*. The extract did not prevent the growth of the other test organisms. This improved the existence of the antibacterial and antifungal activity of the plant. The results showed that leaf extract of *F. anatolica* had the strong antibacterial and antifungal effect with a minimal inhibitory activity of 0.0025 µg/mL against the *E. coli*, 0.005 µg/mL against *P. aeriginosa* bacteria and 0.0025 µg/mL against *C. albicans* yeast-like fungus used.

Key Words: Antimicrobial activities, Antifungal activities, *Flueggea anatolica* Gemici.

INTRODUCTION

Turkey has a rather rich flora due to its geological and morphological structure and climatic features. According to the latest records[1], the total number of vascular plant species in the flora of Turkey area comes to 9222, of which 8988 are native. These include accounts of an 11014 numbered taxa at species distributed among 1251 genera and 174 families. So, with the joining of subspecies and varietes, the number of taxon in the flora of Turkey equals approximately to the number of Europian species and is incomparably so high from the flora of the most of the countries of the North Africa and Asia.

The reason of the richness of the flora is historical. Anatolia is known to have a very dense and rich plant cover in Tertiary which seems to represent the tropicals of todays. During the glacier movement in Quarterner period, while similar flora largely disappeared in Europe, a lot of species were saved in the sheltered areas in Anatolia. Today, these species continue on living in their natural habitats with their close

relatives. The flora of Eastern Black Sea is the continuity of the flora of the Tertiary period.

Because of these interesting physical geography and historical reasons, Anatolia is rather rich about endemic taxons. Totally, there are 3432 endemic taxon and the ratio of this to the flora is 33.5 %. Within the endemics there are 15 taxon in genus level. *Flueggea* is primarily an Old World genus. The genus *Flueggea* has almost 15 species worldwide, with extending into warm temperate zones at tropics and subtropics regions.

The overall distribution of the genus is relicttual. *F. anatolica* is closely releated to *F. virosa* is a widespread paleotropically the nearest species to *F. anatolica*, being present around the Nile river in Egypt. *F. anatolica*, can be regarded as a Tertiary relict and Southern Anatolia harbours other such species, *e.g*. *Ajuga postii* and, further away in SW Anatolia, Liquidambar orientalis[2].

Flueggea anatolica[2,3] is a relict paleoendemic or conservative endemic shrub remnant from Tertiary and includes Euphorbiaceae[4] which consists of monoic or dioic herbs, brushes and trees which have laticifer. Leaves are alternate, rarely decussate and verticillate, simple or united, most of all stipulate. Flowers are solitary or in groups of spika or panicula. Sepals at male flowers are in 0-5 numbers, free or united; petals 0-6, sometimes united. They have one or a lot of stamens. Sepals at female flowers are 0-6, free, petals are 0-6 and gynekeum has one pistyll.

Flueggea anatolica is a dioic brush which can reach 5 m height of up. They have never thorns or hairs. Leaves are spirally arranged, petioled and oval or elliptic shaped, 2.5-6.5 cm × 1.5-3.5 cm long. Flowers are at the leafy branches with the groups of 13 flowers, female flowers are in the groups[1-3].

F. anatolica is known only from the type locality and from an area of approximately 7000 m^2. The number of individuals is approximately 500. Therefore, it should be regarded as belonging to World Conservation Union (IUCN) Critically Endangered (CR) threat category[5].

The medicinal use of *Flueggea* sp. comes from bergenin, a C-glucoside of 4-O-methyl gallic acid. Bergenin isolated from aereal parts of *Flueggea virosa* exhibited antiarrhythmic activity[6] and bergenin and norbergenin, two isocoumarins isolated from the leaves and roots of *Flueggea microcarpa Blume* gave significant protection again pylorus ligation and aspirin induced gastric ulcers in rats because of the increased prostaglandin production[7]. The roots of *Flueggea virosa* Roxb. Ex Willd, have been used for a treatment of rheumatism, pruritus, cephalic eczema, leucorrhoea injuries and is known as a traditional Chinese medicine[8]. Flueggenins A and B, C, C-linked dimeric indolizidine alkaloids isolated from the roots *F. virosa* and showed strong cytotoxicity, only A showed weak activity against the P-388 tumor cell line[9]. Bergenin was found also to inhibit the powdery mildew isolated from *Flueggea microcarpa Blume* and have antifungal effects against the plant pathogenic fungi, namely, *Alternaria alternata*, *A. brassicae*, *A. carthami*, *Fusarium udum*, *F. oxysporum f.sp. ciceri*, *Curvularia lunata* and *Erysiphe pisi*[10].

Because of the resistance acquiries and genetic transmitting abilities of bacteria to drugs which are utilized as therapeutic agents, antibacterial and antifungal drugs have gain great importance in drug industry. Although new synthetic chemical antibiotics have been produced last three decades, resistance to these drugs by microorganisms has increased[11]. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments by studies conducted in different countries[12-18]. The microbial traits of many plants come from the secondary metabolism of plants known as phenolic compounds which are parts of the essential oils[19] as well as tannins[20].

In this study, chloroform extracts prepared from the leaves of *Flueggea anatolica* plant were evaluated for the first time for antibacterial and antifungal activities. *F. anatolica* is facing a threat of extirpation as well as the other tertiary endemics, like *Liquidambar orientalis*.

EXPERIMENTAL

Plant specimens were taken from the north of Tarsus (Icel) valley of Kadincik in 1998 and autenticated by Yusuf Gemici of the Section of Botany, University of Ege (Gemici: 6330). Voucher specimens have been deposited in the Herbarium of the above-cited department (No; EGE 33639).

Preparation of plant extract: The fresh leaves (20 g) of plant were ground in a mortar and then extracted with chloroform (125 mL) (Riedel de Haen) for 24 h by using Soxhlet equipment. The extracts were filtered using Whatman filter paper (No. 1) and then concentrated *in vacuo* at 58-59 °C. The residues were stored in a refrigerator until subsequent use. For the bioassay, the extracts were suspended in chloroform at a concentration of 10 mg/mL.

Microorganisms: All organisms were obtained from the Aegean University Faculty of Science, Basic and Industrial Microbiology Department, Izmir, Turkey and included: *Staphylococcus aureus* ATCC 6538-P and *Bacillus subtilis* ATCC-6633 as gram-positive bacteria, *Echerichia coli* ATCC-12228, *Salmonella typhimurium* CCM-5445, *Pseudomonas aeroginosa* ATCC-27853, *Proteus vulgaris* ATCC 29905 as gram-negative bacteria and fungi *Candida albicans* ATCC-10239 as yeast-like fungus. Gentamycin and clotrimazole were used as standard antibacterial and antifungal agents, respectively.

Antimicrobial and antifungal activity: The antimicrobial activities of chloroform extract of the leaves were evaluated *in vitro* against an assortment of two gram-positive and four gram-negative bacteria and one fungus. The bacteria were grown in nutrient agar (Oxoid) at 37 °C and maintained on nutrient agar slants at 4 °C. *Candida albicans* was grown at 37 °C and maintained on Sabouraud-dextrose agar slants at 4 °C (Oxoid).

The minimum inhibitory concentrations (MICs) of chloroform extract of the leaves and reference antibiotics were determined by microdilution techniques in Mueller-Hinton broth (Oxoid) for bacteria and Sabouraud-dextrose Broth (Oxoid)

for fungus[21]. Breifly, the chloroform extract was first dissolved at a concentration of 20 mg/100 µL in dimethyl sulfoxide (% 10 v/v) contaning Tween 80 (% 5 v/v)[22]. Reference antibiotics were initially tested using a concentration of 0.40 mg/mL for gentamycin in distilled water and 0.50 mg/mL for clotrimazole in ethanol. Then two-fold dilutions of each compound were performed. Inocula for assays were prepared from activated cultures in broth media by dilution in growth medium to give a final viable cell count of 4.0-5.5 × 10^5 CFU/mL. Each drug solution (25 µL) and inoculum of microorganism (25 µL) were added into each well of a flat-bottom, 96-well microtiter plate prefilled with 200 µL of medium to give a total volume of 250 µL. Microtiter plates were incubated at 37 °C for 24 h for bacteria and 48-72 h for *C. albicans*. The solvents,dimethyl sulphoxide and ethanol, were used as the negative control for all experiments. After incubation, MIC value was detected by adding 50 µL of 0.5 % triphenyl tetrazolium chloride (TTC, Merck) aqueous solution[23,24]. MIC was defined as the lowest concentration of extract that inhibited visible growth as indicating by the TTC reduction. In the presence of bacterial growth by reduction reactions, TTC changes the color of microbial cells from colorless to red. This provide clearly defined and easily readable end points. All tests were repeated three times to confirm the results.

RESULTS AND DISCUSSION

The antimicrobial activity of *F. anatolica* Gemici was given in Table-1. As can clearly be seen from this Table-1, the extract provided from the leaves of *F. anatolica* were found to be effective against *Echerichia coli* ATCC-12228, *Pseudomonas aeroginosa* ATCC-27853, *Proteus vulgaris* ATCC 29905 as gram-negative bacteria and *Candida albicans* ATCC-10239 yeast-like fungus, showing MIC values 0.0025 µg/mL for *E. coli* and yeast-like fungus and 0.005 µg/mL for *P. vulgaris* and *P. aureginosa*. However, *F. anatolica* was not effective against *B. subtilis* ATCC-6633, *S. thyphimirium* CCM 5445 and S aureus ATCC-6538-P as gram-positive bacteria.

TABLE-1
ANTIMICROBIAL EFFECT OF *Flueggea anatolica* GEMICI

Microorganisms	Minumum inhibitory concentration (MIC)		
	Extract (µg/mL)	Gentamycin (µg/mL)	Clotrimazole (µg/mL)
Bacillus cereus CCM 99	-	1.25	n.t*
Escherichia coli ATCC 12228	0.0025	1.25	n.t
Salmonella thyphimirium CCM 5445	-	1.25	n.t
Staphylococus aureus ATCC 6538-P	-	1.25	n.t
Proteus vulgaris ATCC 29905	0.005	1.25	n.t
Pseudomonas aureginosa ATCC 27853	0.005	2.5	n.t
Candida albicans ATCC 10239	0.0025	n.t	0.78

*: not tested.

The microorganism *E. Coli* which is already known to be multi-resistant to drugs had its growth inhibited by the extract of *F. anatolica*. On the other hand, *P. aeroginosa*[25]

which is also resistant to different antibiotics, had its growth inhibited also by *F. anatolica* extract. Such results are interesting because the control of these bacteria was noticed to be very difficult by therapeutic means[26]. While the control of resistant bacteria is becoming a threat to human health, the studies regarding the mode of action for these compounds in the bacterial cell should be done. The synergistic effect of *F. anatolica* extract from the association of antibiotic against resistant bacteria will lead to new choices for the treatment of infectious diseases. This effect enables the use of respective antibiotic when it is no longer effective by itself during therapeutic treatment.

Many plant phenols are reported as fungi-toxic agents and the action of bergenin isolated from *Flueggea microcarpa*[27] can be declared to be similar to that of other phenols. Yeast and antifungal activity evaluate together and tannins can be toxic to filamentous fungi, yeast and bacteria[28]. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity[29].

REFERENCES

1. A. Guner, N. Ozhatay, T. Ekim and K.H.C. Baser, Flora of Turkey and the East Aegean Islands (Supplement 2). Vol. 11, p. 656 (2000).
2. Y. Gemici and E. Leblebici, *The Karaca Arboretum Magaz.*, **3**, 79 (1995).
3. Y. Gemici, *Edinburg J. Bot.*, **50**, 75 (1993).
4. H.M. Lawrence, The Mc Millan Company, p. 823 (1971).
5. IUCN, IUCN Red List Categories Version 3.1 (2001).
6. H.L. Pu, X. Huang and J.H. Zhao, *Planta Med*., **4**, 372 (2002).
7. S.A. Dahanuka, R.A. Kulkarni and N.N. Rege, *Indian J. Pharmacol.*, **32**, 81 (2000).
8. B.T. Li, *Zhongguo Zhiwu Zhi*, **44**, 68 (1994).
9. G. Li-She, F. Cheng-Qi and Y.S. Heng-Ping, *Org. Lett*., **8**, 2285 (2006).
10. B. Prithiviraj, U.P. Singh and M. Manickam, *Plant Patho.*, **46**, 224 (1997).
11. M.L. Cohen, *Science*, **257**, 1050 (1992).
12. M. Ikram and H. Inamul, *Fitoterapia*, **55**, 62 (1984).
13. A.Z. Almagboul, A.K. Bashir and A. Farouk, *Fitoterapia*, **55**, 331 (1985).
14. M. Sousa, C. Pinheiro, M.E.O. Matos, F.J. Matos, M.I. Lacerda and A.A. Craveiro, *Foryaleza*, pp. 385-388 (1991).
15. L. Kubo, H. Muroi and M. Himajima, *Agric. Food Chem*., **41**, 1016 (1993).
16. E.E.S. Shapoval, S.M. Silveira and M.L. Miranda, *J. Ethnopharmacol.*, **44**, 136 (1994).
17. A.A. Izzo, G. Di Carlo and D. Biscardi, *Phytoter. Res.*, **9**, 281 (1995).
18. M. Digrak, M.H. Alma and A. Ilcim, *Pharm. Biol.*, **39**, 346 (2001).
19. A.M. Jansen, J.J.C. Cheffer and A.B. Svendsen, *Aspects Test Methods Planta Med.*, **40**, 395 (1987).
20. G. Saxena, A.R. McCutheon and S. Farmer, *J. Ethnopharmacol.*, **42**, 95 (1994).
21. F.B. Holetz, G.L. Pessini and N.R. Sanches, *Mem. Inst. Oswaldo. Cruz*., **97**, 1027 (2002).
22. N.S. Ryder, S. Wagner and I. Leitner, *Antimicrob. Agents Chemother.*, **42**, 1057 (1998).
23. J. Uno, M.L. Shigematsu and T. Arai, *Antimicrob. Agents Chemother.*, **21**, 912 (1982).
24. Z. Uyar, N. Boke, E. Tukay, O. Koz, I. Yasa and S. Kirmizigül, *Nat. Prod. Res.*, **20**, 999 (2006).
25. R.F. Chandler, S.N. Hooper and M. Harvey, *J. Econ. Bot*., **36**, 203 (1982).
26. G.G.F. Nascimento, J. Locatelli, P.C. Freitas and G.L. Silva, *Brazil. J. Microbiob.*, **31**, 1 (2000).
27. S. Kumar, M. Sahai and A.B. Ray, *Planta Med.*, **59**, 466 (1985).
28. M.M. Cowan, *Clin. Microbiol. Rev*., **12**, 564 (1999).
29. G.A. Jones. T.A. Mc Allister, A.D. Murr and K.J. Cheng, *Appl. Environ. Microbial.*, **60**, 1374 (1994).

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