Turkish Electronic Journal of Biotechnology
Special Issue, p:6-10, 2002

http://www.biyotekder.hacettepe.edu.tr/dergi.html

IN VITRO ANTIBACTERIAL ACTIVITIES OF THREE *HYPERICUM* SPECIES FROM WEST ANATOLIA

Gözde (Elgin) MERAL[1] and N. Ülkü KARABAY[2]
1.Ege University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Bornova, İzmir.
2.Ege University, Faculty of Science, Department of Biology, Bornova, İzmir.

Özet

Hypericum türleri, tıbbi özelliklere sahip olduğu bilinen otsu bitkilerdir ve bir çok ülkede fitoterapi amaçlı olarak yaygın biçimde kullanılmaktadır. Bazı *Hypericum* türleri, gram-pozitif ve gram-negatif bakterilere karşı antimikrobiyal aktivite göstermektedir. Bu çalışmada, *H. triquetrifolium* Turra., *H. perforatum* L. ve *H. empetrifolium* Willd.' ın total ekstreleri 4 Gram-pozitif (*Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* 6538/P, *Staphylococcus epidermidis* ATCC 12228 ve *Enterococcus faecalis* ATCC 29212) ve 4 Gram-negatif (*Pseudomonas aeruginosae* ATCC 27853, *Enterobacter cloacae* ATCC 13047, *Escherichia coli* ATCC 29998 ve *Escherichia coli* ATCC 25922) organizmaya karşı antibakteriyal aktivite bakımından araştırılmıştır. Toz haline getirilmiş bitkilerin ekstraksiyonu için 80 °C metanol kullanılmıştır. Total ekstraktlar metanolde çözülmüş ve 1 mg/ml. konsantrasyonda test edilmiştir. Kontrol için saf solvan kullanılmıştır. In vitro antibakteriyal çalışmalar disk difüzyon metoduyla gerçekleştirilmiştir. Antibakteriyal çalışma, üç *Hypericum* türünün ekstrelerinin de bakterilerin tamamına karşı aktiviteye sahip olduğunu göstermiştir.
Anahtar kelimeler: *Hypericum*, total ekstract, antibakteriyal aktivite.

Abstract

Hypericum species are herbaceous plants known to have medicinal properties and are widely used in phytotheraphy in many countries. Antimicrobial activities of several species of Hypericum were exhibited against the gram-positive and gram-negative bacteria. In this study, total extract of H. triquetrifolium Turra., H. perforatum L. and H. empetrifolium Willd. were investigated for antibacterial activity against 4 Gram-positive (Staphylococcus aureus ATCC 29213, Staphylococcus aureus 6538/P, Staphylococcus epidermidis ATCC 12228 and Enterococcus faecalis ATCC 29212) and 4 Gram-negative (Pseudomonas aeruginosae ATCC 27853, Enterobacter cloacae ATCC 13047, Escherichia coli ATCC 29998 and Escherichia coli ATCC 8737) organisms. Methanol at 80 °C was used for soxhlation for extraction of the powdered plant. The total extracts were dissolved in methanol and were tested at a concentration of 1 mg/ml. Pure solvent were used for control. In vitro antibacterial studies were carried out by disc diffusion method. The antibacterial studies showed that extracts of three Hypericum species have an activity against all of the bacteria.
Key words: Hypericum, total extract, antibacterial activity.

Introduction

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (1, 2). Plants still continue to be almost the exclusive source of drugs for the majority of the world's population (3, 4, 5).

Hypericum species belonging to *Cluciaceae* (*Hypericaceae*) family are widely found Europe, Asia, Northern Africa and America (6). The genus *Hypericum* comprises more than 400 species in the world but 77 species were found in Turkey (7, 8). Some of these species are well known folk medicines used in Turkey and several countries, where they are employed in various curative treatments (9, 10). The genus *Hypericum* contains shrubs or herbs usually with translucent glands containing essential oils and sometimes red or black glands containing hypericin. Leaves simple, opposite or rarely whorled. Sepals 5, imbricate in bud. Petals 5 free, contorted in bud. Stamens in fascicles or apparently indefinite. Ovary superior with axile or parietal placentation. Seeds without endosperm (7).

Hypericum perforatum L. is a well known medicinal plant for a long period of time and was recognized as "Sarı Kantaron" in Turkey and "St. John's Wort" in Anglo-Saxon folk medicine. Nowadays, the use of *Hypericum* extracts is used mainly as an antidepressive drug (6). *H. perforatum* L. is also used both for therapeutic purposes (in all pulmonary complaints, bladder troubles, in suppression of urine, dysentery, worms, diarrhoea, and hysteria) and as a flavouring material in the preparation of foods and alcoholic beverages (11). The extract of aerial parts is used as an anti-inflammatory and healing agent (6). There are a few studies concerning the medical usage of *H.triquetrifolium* Turra. and *H.empetrifolium* Willd. although many studies exist concerning *H. perforatum* L. (12, 13).

The aim of the present study is to assess the in vitro antibacterial activities of total extracts of *H. triquetrifolium* Turra., *H. perforatum* L. and *H. empetrifolium* Willd. This paper introduces *H. triquetrifolium* Turra., and *H. empetrifolium* Willd. whose antibacterial activities have not been reported before.

Material and Methods

2.1. Plant Material

Fresh plants of *H. triquetrifolium* Turra. from wild collections, collected in October 1999 from Karaali village, Manisa, *H. perforatum* L. collected in June 1999 from Karagöl, İzmir and *H. empetrifolium* Willd. collected in July 1999 from Urla, İzmir were used. Mainly, the aerial parts of the plants that have a high proportion of buds and flowers were selected. The plant was identified by em. Professor E. Sauer,Saarland University, Institute of Botany, Germany. The voucher specimen of the plants used in the present study was kept for record in the herbarium of Ege University, Faculty of Pharmacy, Department of Pharmaceutical Botany, (voucher no. 5435, 5434, 5436, respectively).

2.1. Extraction of *Hypericum* species

The crude drug was dried in shade and fine powder of the plant was obtained by a mill (Brabender OHG, Duisburg). A modified method of Wagner and Bladt (1994) was used for the extraction of the powdered plant (14). Methanol at 80ºC was used for soxhlation, using 750 mL methanol for 100 g crude drug and the extracts were dried in vacuo (yields are 36.2%, 25.94% and 26.36%, respectively). After the lyophilization (Labconco lyophizateur, -50 °C) of the extracts (yields are 76.32%, 87.48% and 80.67%, respectively), they were administered to microorganisms.

It was determined by HPLC method that *H. triquetrifolium* Turra. contains 0.141%, *H. perforatum* L., 0.205% and *H.empetrifolium* Willd., 0.151% hypericine according to the analyses which were performed in Ege University, Center for Drug R&D and Pharmacokinetic Applications, Izmir, TURKEY by Meral, G. (15).

2.2. Antimicrobial Activity

In vitro antibacterial studies were carried out by the disc diffusion method (16, 17) against four Gram-positive (*Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* 6538/P, *Staphylococcus epidermidis* ATCC 12228 and *Enterococcus faecalis* ATCC 29212) and four Gram-negative (*Pseudomonas aeruginosae* ATCC 27853, *Enterobacter cloacae* ATCC 13047, *Escherichia coli* ATCC 29998 and *Escherichia coli* ATCC 25922) organisms. The total extracts were dissolved in methanol and were tested at a concentration of 1 mg/ml. Pure solvent were used for control. Sulbactam/ Ampicillin and Amoxicilin (Oxoid) were used as standard antibiotics for comparison. All blank discs had a diameter of 6 mm. and deposited on the surface of the cultured trypticase soya agar petri dishes. The plates were incubated for 24 h. at 37 °C except the *E. cloacae* ATCC incubated for 24 h. at 30 °C. The experiments were repeated six times and the results were expressed as average values.

Results and Discussion

Results of antibacterial activity of the *Hypericum* species, and standard antibiotics (Sulbactam/Ampicillin and Amoxicilin) were showed in Table 1. Methanol as pure solvent was not showed antibacterial activity.

Table 1. Results of antibacterial activity of *Hypericum* species and standard antibiotics (as mm inhibition zone).

Tested microorganisms	*Hypericum triquetrifolium* Turra.	*Hypericum perforatum* L.	*Hypericum empetrifolium* Willd.	Standard antibiotic discs	
				Sulbactam/ Ampicillin	Amoxicillin
E. coli ATCC 29998	11	12	11	12	12
E. coli ATCC 25922	10	11	11	13	11
S. epidermidis ATCC 12228	16	18	13	18	13
S. aureus 6538/P	21	26	20	19	17
S. aureus ATCC 29213	14	15	14	18	13
E.cloacae ATCC 13047	14	19	13	19	15
P.aeroginosa ATCC 27853	13	15	12	10	10
E.faecalis ATCC 29212	9	10	8	8	8

Several species of *Hypericum* exhibit antimicrobial activity. From these species, *H. perforatum* L. is well known and studied. *H. perforatum* L. contains a hypericin, essential oils, flavonoids, tannin and imanine and novoimanine constituents that shows antibiotic activity (18, 19, 20). Novoimanine is also an antibacterial drug derived from *H. perforatum* L. When used in bacteriostatic concentration (0.5 gamma/ml), it induced release of potassium ions from *Staphylococcus aureus* 209P cells (21). *H. perforatum* L. extracts have a broad-spectrum antimicrobial activity against both gram-negative and gram-positive bacteria. The organisms that are studied are *Staphylococcus aureus*, *Streptococcus mutans*, *Proteus vulgaris*, *Escherichia coli* and *Pseudomonas aeruginosa* (22).

Drummondin C which is an antibiotic isolated from a bio assay-directed fractionation of *H. drummondii* (Grev. & Hook.) T.&G. showed significant activity against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and the acid-fast bacterium *Mycobacterium smegmatis* in an agar well diffusion assay (23, 24, 25). Additionally, some of the isolated xanthones from the roots of *H. roeperanum* exhibited antifungal activity against *Candida albicans* (26). The isolated three xanthones and betulinic acid from a dichloromethane extract of stems and roots of *H. brasiliense* and phloroglucinol derivative isolated from the light petroleum ether extract of the aerial parts of *H. calycinum* showed antifungal activity against *Cladosporium cucumerinum* (27, 28).

The in vitro antibacterial activity of *H. perforatum* extract against *E. faecalis* ATCC 29212 ve *E. cloacea* ATCC 13047 bacteria has not been reported before. Therefore, this may be considered as the first report

on the antibacterial activity of *H. perforatum* L. extract against *E. faecalis* ATCC 29212 ve *E. cloacea* ATCC 13047 bacteria. As mentioned above, the antibacterial effects of *H. triquetrifolium* Turra. and *H. empetrifolium* Willd. extracts were not investigated at all.

In conclusion, total extracts of three *Hypericum* species were active against all of the eight microorganisms.

Acknowledgement

- This study is designed in Ege University, Center for Drug R&D and Pharmacokinetic Applications.
- The authors would like to thank to Prof. Işık TUĞLULAR, M.D. and Dr. Akın DENİZCİ for their help in performing the analysis.

References

1. Czygan, F.C. (1993) Kulturgeschichte und Mystik des Johanniskrautes, Zeitschrift für Phytotherapie, 5, 276-282.
2. Ody, P. (1993) The Complete Medicinal Herbal, New York, Dorling Kindersley Limited, 132-133, 170-171.
3. Hölzl, J. (1993) Inhaltstoffe und Wirkmechanismen des Johanniskrautes, Zeitschrift für. Phytotherapie, 5, 255-264.
4. Öztürk, Y., Aydın, S., Başer, K.H.C., Öztürk, N. (1992) *Hypericum perforatum* L. bitkisinin sıçanlarda koleretik aktivitesi. Anadolu Üniversitesi, IX. Bitkisel İlaç Hammaddeleri Toplantısı Bildiri Kitabı, Eskişehir, 216.
5. Maisenbacher, J., Schmidt, U., Schenk, N. (1995) Therapie mit Hypericum bei Angstzustanden, TW Neurologie Psychiatrie, 9, 65-70.
6. Bombardelli, E., Morazzoni, P. (1995) *Hypericum perforatum*, Fitoterapia, Volume LXVI, 1, 43-65.
7. Davis,P.H. (1978-1988) Flora of Turkey and The East Aegean Islands, II (p.355), X (p.473), Edinburg, Edinburg University Press.
8. Zeybek, N., Zeybek, U. (1994) Farmasötik Botanik, İzmir, E.Ü.Ecz.Fak.Yay. No:2, 201.
9. Baytop, T. (1984) Türkiye'de Bitkiler ile Tedavi, İstanbul, İ.Ü.Yay. No:3255, 185.
10. Tanker, N., Koyuncu, M., Coşkun, M. (1998) Farmasötik Botanik, Ankara, A.Ü. Ecz. Fak. Yay. No:78, 278.
11. Newall, C.A., Anderson, L.A., Phillipson, J.D. (1996) Herbal Medicines. London. 250-252.
12. Apaydın, S., Zeybek, U., İnce, İ., Elgin, G., Karamenderes, C., Öztürk, B. (1999) *Hypericum triquetrifolium* Turra. extract exhibits antinociceptive activity in the mouse. J. of Ethnopharmacology. 67, 307-312.
13. Apaydın, S., Göldeli, E., Karamenderes, C., Meral, G., Zeybek, U. (2001) The inhibitory effect of *Hypericum triquetrifolium* Turra. extract on concentrations of rat aortic smooth muscle. Türkiye Klinikleri J. of Medical Research. 19(1), 24-30.
14. Wagner, I., Bladt, S. (1994) Pharmacetical Quality of *Hypericum* Extracts. Journal of Geriatric Psychiatry and Neurology. 7 (Suppl 1), 65-68.
15. Meral, G. (2000) İzmir ve Çevresinde Doğal Yayılış Gösteren Bazı *Hypericum* L. Türleri Üzerinde Araştırmalar, Ege üniversitesi, Sağlık Bilimleri Enst. Doktora Tezi.
16. Cruickshank, R., Duguid, I.P., Marmion, B.P., Swain, R.H.A. (1975) Medical Microbiology, Vol. II. 12 nd. edn. Churchill Livingstone, Edinburgh, London, New York.
17. National Comittee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. (1990) Approved Standard M7-A2. NCCLS, Villanova, PA.
18. Gaedcke, F. (1997) Herstellung, Qualität, Analytik und Anwendung von Johanniskraut-Extrakten, Z.Arz.Gew.pfl., 2, 63-72.
19. Santos, P.A.G., Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Scheffer, J.J.C. (1999) Composition of the essential oil of *Hypericum foliosum* Aiton. from five Azorean islands, Flavour Fragr.J., 14: 283-286.
20. Weiss, R.F. (1991) Herbal Medicine, England, Beaconsfield Publishers, 295.
21. Avenirova, E.L. (1977) Effects of novoimanine on the cellular permeability indices of staphylococci. Antibiotiki. 22 (7), 630-4.
22. Murrey, M.T. (1995) The Healing Power of Herbs, USA, 294.

23. Jayasuria, H., Baker, J.K., Clark, A.M., McChesney, J.D. (1991) Synthesis and the biological evaluation of the structural units of drummondin C. Pharm. Res. 8 (11), 1372-6.
24. Jayasuria, H., Clark, A.M., McChesney, J.D. (1991) New antimicrobial filicinic acid derivatives from *Hypericum drummondii*. J. Nat. Prod. 54 (5), 1314-20.
25. Jayasuria, H., McChesney, J.D., Swanson, S.M., Pezutto, J.M. (1989) Antimicrobial and cytotoxic activity of rottlerin-type compounds from *Hypericum drummondii*. J. Nat. Prod. 52 (2), 325-31.
26. Rath, G., Potterat, O., Mavi,S., Hostettmann, K. (1996) Xanthones from *Hypericum roeperanum*. Pytochemistry, 43(2), 513-20.
27. Rocha, L., Marston, A., Kaplan, M.A., Stoeckli-Evans, H., Thull, U., Testa, B., Hostettmann, K. (1994) An antifungal gamma-pyrone and xanthones with monoamine oxidase inhibitory activity from *Hypericum brasilience*. Phytochemistry. 36(6),1381-5.
28. Decosterd, L.A., Hoffmann, E., Kyburz, R., Bray, D., Hostettmann, K. (1991) A new phloroglucinol derivative from *Hypericum calycinum* with antifungal and in vitro antimalarial activity. Planta Med. 57(6), 548-51.