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Analysis of Flavonoid and Antimicrobial Activity of Extracts of *Hypericum perforatum*

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Ethyl acetate, *n*-butanol and methanol extracts of *Hypericum perforatum* were analyzed qualitatively and quantitatively for their flavonoid content by RP-HPLC. The components were detected by comparison with authentic standards of five flavonols (quercetin, myricetin, isorhamnetin, rhamnetin and kaempferol) and a flavone (luteolin). Quercetin and myricetin were present in all the three fractions while kaempferol was present only in *n*-butanol fraction. Isorhamnetin, rhamnetin and luteolin were not detected in the extracts. The total sum of detected flavonoids was highest in *n*-butanol fraction (8031.25 mg/kg fresh wt.) followed by ethyl acetate (4805.13 mg/kg fresh wt.) and methanol (4720.00 mg/kg fresh wt.). The extracts were also evaluated for their antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Sarcina lutae* and *Escherichia coli*. The total extracts were tested at concentrations of 1, 10, 25 and 50 µg/µL. *In vitro* antibacterial studies were carried out in 96-well microplates. The *n*-hexane and dichloromethane extracts were inactive against all the tested organisms at all experimental concentrations. The *n*-butanol and ethyl acetate extracts showed activity against all the tested bacterial strains at concentrations of 25 and 50 µg/µL while the methanolic extract was only active against *B. subtilus* at concentrations 25 and 50 µg/µL.

Key Words: *Hypericum perforatum*, Antibacterial activity, Flavonoids.

INTRODUCTION

It is estimated that there are 250,000-500,000 species of plants on earth[1]. A relatively small percentage (1-10 %) of these are used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes[2]. Hippocrates (in the late fifth century B.C.) mentioned 300-400 medicinal plants[3]. In the first century A.D., Dioscorides wrote *De Materia Medica*, a medicinal plant catalog which became the prototype for modern pharmacopoeias. 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[4].

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Hypericums form a large genus comprising of about 370 species distributed worldwide[5]. Many plants of this genus are well known for their medicinal properties and there are a number of reports on the use of these plants to treat infections in local system of medicine[6,7]. *Hypericum perforatum* (St. John's wort), the most important medicinal species of the genus is a herbaceous, aromatic rhizomatous perennial plant with bright yellow flowers[8]. *H. perforatum* keeps its position in the contemporary list of medicinal plants of pharmaceutical importance. The plant contains a wide spectrum of substances out of which anthroglycosides, flavonoids and phloroglucinol derivatives have roles in principle pharmacological effects. Various authorities from the distant past used the herb for treating depression and neuralgic disorders. The herb is astringent, resolutive, detersive, anthelmintic, diuretic and poisonous to horses. The red juice from the plant is reputed as a popular and most curative application in Europe for excoriations, wounds and bruises[9]. Most recent interest in *H. perforatum* has focused on its antidepressant effects[10,11], however, the herb has shown other activities including wound healing, antifungal, antiinflammatory, antivirals and antibacterial[12-17].

The aim of the present study is to determine the flavonoid content of *H. perforatum* extracts in different solvents and to evaluate these extracts for antibacterial activity against a number of bacterial strains.

EXPERIMENTAL

All the solvents used were of analytical grade (Merck, Germany). The standards (quercetin, myricetin, rhamnetin, isorhamnetin, kaempferol and luteolin) were purchased from Sigma Aldrich. The acetonitrile, methanol and water used for HPLC were HPLC grade purchased from Merck (Germany).

Fresh plants of *Hypericum perforatum* were collected from the area between Nathia Gali and Abbotabad, Murree Hills, Pakistan in July, 2007. The plants were identified by Prof. Mir Ajab Ali Khan, Department of Biological Sciences, Quaid-e-Azam University, Islamabad and the specimen deposited in the Prem Madan Herbarium of Lahore College for Women University, Lahore (Specimen Voucher No. PM# 0131).

Extraction procedure: The plant material (1 Kg) was air-dried at room temperature. The dried material (400 g) was grinded into small pieces 2-6 mm by using a crushing machine and was successively extracted with *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol and methanol in a soxhlet apparatus. The fractions were filtered through a paper filter (Whatman, No. 1) and the solvent evaporated under vacuum in a rotary evaporator. Five fractions were obtained in this way, *n*-hexane fraction (18300 mg), dichloromethane fraction (7000 mg), ethyl acetate fraction (7520 mg), *n*-butanol fraction (12500 mg) and methanol fraction (8100 mg).

HPLC Analysis

Sample preparation: A weighed amount of each fraction was dissolved in HPLC grade methanol to give a concentration of 0.1 mg/mL.

Standards: Stock solutions of the standards were prepared as 0.1 mg/mL in HPLC grade methanol. All samples were filtered through a 0.45 μm filter before HPLC analysis.

Apparatus and conditions: The HPLC system (waters) consisted of a pump (1500 series) and a UV detector (2487). The compounds were separated on a prepacked analytical C_{18} column (250 mm × 4.6 mm, 5 μm particle size). The mobile phase used was acetonitrile/water 1:1 acidified with 1 % acetic acid. The flow rate was kept constant at 1.0 mL/min at 25 °C. Throughout the experiment all injection volumes were 10 μL. The peaks were detected at 254 nm. The identity of HPLC peaks was confirmed by injection of authentic standards under similar conditions. Variation of the retention time of each peak was less than 1 %. The flavonoid content was expressed as quercetin equivalent and the quantification was by peak area measurement.

Antibacterial activity assay

Test microorganisms: *Bacillus subtilis* (ATCC 6051), *Pseudomonas aeruginosa* (ATCC 27853), *Sarcina lutae* (ATCC 9341) and *Escherichia coli* (ATCC 25922) were used as test organisms. For microbiological screening of the crude extracts of *H. perforatum*, bacteria were maintained on agar plates for long term storage (37 °C) and prepared for use in the assay by transferring bacteria from the agar into marine bacterial broth 48 h prior to test setup.

Screening of *Hypericum perforatum* extracts for antibacterial activity: The 96-well microplates (non-tissue cultured) were used as the test chambers for the bacterial assay. Extracts of *H. perforatum* were placed in the wells (6 replicates for each treatment) at the final concentration of 1, 10, 25 and 50 mg/mL and then evaporated at room temperature in a fume hood. To start the assay bacteria, growing in the LB broth, were diluted in fresh media until reaching an OD (630 nm) between 0.2 and 0.65. Then, dilutions of bacterial solutions were made according to the method of Amsterdam (1996)[18]. The dilutions were placed in each well (100 μL) under aseptic conditions. Incubation was performed at 37 °C for 48 h. Results were evaluated by comparison of the growth intensity in the control wells and treated wells.

RESULTS AND DISCUSSION

Three flavonoids *viz*, quercetin, myricetin and kaempferol were identified in the extracts of *H. perforatum*. The amount of quercetin (t_R = 2.26 min) was maximum in *n*-butanol fraction followed by ethyl acetate and methanol. The myricetin (t_R = 1.85 min) content was maximum in *n*-butanol followed by methanol and ethyl acetate while kaempferol (t_R = 2.89 min) was detected only in *n*-butanol. The total sum of detected flavonoids was maximum in *n*-butanol fraction followed by ethyl acetate and methanol (Table-1).

In a number of studies, the crude extracts of *H. perforatum* showed antibacterial activity against a number of bacterial strains[16,17]. To assess the extent of activity in

different solvents, the plant was extracted in different solvents and extracts were evaluated for the antibacterial activity. The result of antibacterial activity is summarized in Table-2. In a number of studies flavonoids have shown antibacterial activity against a number of bacterial strains. Some flavonoids, including quercetin and myricetin, were found to be active against resistant bacteria[19-21]. Thus the extracts of *Hypericum perforatum* may find application in diseases caused by MDR bacteria.

TABLE-1
AMOUNT OF FLAVONOIDS (mg/kg FRESH WEIGHT)
IN DIFFERENT EXTRACTS OF *Hypericum perforatum*

Fraction	Q	M	I	R	K	L	Sum total of flavonoids
EtOAc	1457.23	3347.90	nd	nd	nd	nd	4805.13
n-Butanol	2223.75	5645.00	nd	nd	162.50	nd	8031.25
Methanol	508.00	4212.00	nd	nd	nd	nd	4720.00

nd: not detected; Q: quercetin, M: myricetin, I: isorhamnetin, R: rhamnetin, K: kaempferol and L: luteolin.

TABLE-2
ANTIBACTERIAL ACTIVITY OF EXTRACTS OF
H. perforatum AGAINST BACTERIAL STRAINS

Bacterial strain	Concentration of crude extract (mg/L)																			
	n-Hexane				Dichloromethane				*n*-Butanol				Ethyl acetate				Methanol			
	1	10	25	50	1	10	25	50	1	10	25	50	1	10	25	50	1	10	25	50
B. subtilis	–	–	–	–	–	–	–	–	–	+	+	–	–	–	+	+	–	–	+	+
E. coli	–	–	–	–	–	–	–	–	–	+	+	–	–	–	+	+	–	–	–	–
P. aeruginosa	–	–	–	–	–	–	–	–	–	+	+	–	–	–	+	+	–	–	–	–
S. lutae	–	–	–	–	–	–	–	–	–	+	+	–	–	–	+	+	–	–	–	–

–: No activity detected, +: Antibacterial activity detected.

To determine the total activity in active fractions, the quantification method of Eloff was used[22]. The total activity in mL was maximum for the *n*-butanol fraction followed by methanol and ethylacetate (Table-3).

TABLE-3
TOTAL ACTIVITY IN DIFFERENT EXTRACTS OF *H. perforatum*

Fractions	Mass (mg)	MIC (mg/mL)	Total activity (mL)
n-Butanol	12500	10	1250
Ethyl acetate	7520	10	752
Methanol	8100	10	810

Conclusion

Based on total activity, the butanol fraction of *Hypericum perforatum* is most active against bacterial strains and may be used for the extraction and isolation of active substances.

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REFERENCES

1. R.P. Borris, *J. Ethnopharmacol*., **51**, 29 (1996).
2. D.E. Moerman, *J. Ethnopharmacol*., **52**, 1 (1996).
3. R.E. Schultes, The Kingdom of Plants, in ed.: W.A.R. Thomson, Medicines from the Earth, New York, N.Y: McGraw-Hill Book Co. p. 208 (1978).
4. P. Ody, The Complete Medicinal Herbal, New York, Dorling Kindersley Limited, pp. 132-133, 170-171 (1993).
5. D.J. Mabberley, The Plant Book, Cambridge; Cambridge University Press (1987).
6. A. Sokeman, B.M. Jones and M. Erturk, *Phytother. Res*., **13**, 355 (1999).
7. E. Yesilda, I. Gurbuz and H. Shibata, *J. Ethnopharmacol*., **66**, 289 (1999).
8. N.K.B. Robson, *Bull. Brit. Mus. (Nat. Hist.), Bot*., **8**, 55 (1981).
9. S.R. Baquar, Medicinal and Poisonous Plants of Pakistan, edn. 1, pp. 235-236 (1989).
10. V. Kumar, P.N. Singh and S.K. Bhattacharya, *Indian J. Exp. Biol*., **39**, 344 (2001).
11. L. Cervo, M. Rozio, C.B. Ekalle-Sopp, G. Guiso, P. Morazzoni and S. Caccia, *Psychopharmacology*, **164**, 423 (2002).
12. S.G. Rao, A.L. Udupa, S.L. Udupa, P.G. M. Rao, G. Rao and D.R. Kulkarni, *Fitoterapia*, **62**, 508 (1991).
13. A.R. McCutecheon, S.M. Ellis, R.E.W. Hancock and H.N. Tower, *J. Ethnopharmacol*., **44**, 157 (1994).
14. V. Kumar, P.N. Singh and S.K. Bhattacharya, *Indian J. Exp. Biol*., **39**, 339 (2001).
15. C. Zhou and H.C. Chen, *Clin. Med. Pharm. Bull*., **2**, 214 (1996).
16. G. (Elgin) Meral and N.U. Karaby, *Turkish Electro. J. Biotech.,* 6 (2002).
17. T. Milosevic and S. Solujic, *Bull. Chem. Technolo. Macedonia*, **25**, 127 (2006).
18. D. Amsterdam, Susceptibility Testing of Antimicrobials in Liquid Media, In Antibiotics in Laboratory Medicine (V. Lorian, Ed.), Lippincott, Williams & Wilkins, Baltimore, MD, USA, pp. 52-111 (1996).
19. Y. Sato, S. Suzaki, T. Nishikawa, M. Kihara, H. Shibata and T. Higuti, *J. Ethnopharmacol.*, **72**, 483 (2000).
20. T. Higuchi, Y. Sato and S. Murasugi, Use of Flavone Derivatives for Induction of β-Lactam Sensitivity of MRSA, US patent 6, 294, 526 B1 (2001).
21. H. Xu and S.F. Lee, *Phytother. Res*., **15**, 39 (2001).
22. J.N. Eloff, *Phytomedicine*, **11**, 370 (2004).

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