İstanbul Ecz. Fak. Mec.
35-36 (2) (2002-2003)

J. Fac. Pharm. Istanbul
35-36 (2) (2002-2003)

INVESTIGATIONS ON *HYPERICUM SCABRUM*

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SUMMARY

In this study the quantitative determination of hypericin and pseudohypericin by HPLC has been performed on *Hypericum scabrum* collected from Konya, Karaman. Antimicrobial activity of the plant has also been investigated.

ÖZET

Bu çalışmada *Hypericum scabrum* türünün Konya'dan toplanan örneğinde YBSK ile hiperisin ve psödohiperisin miktar tayini yapılmış ve ayrıca bitkinin antibakteriyal aktivitesi araştırılmıştır.

Key words: *Hypericum scabrum*; *H. perforatum*; hypericin; pseudohypericin; determination; antimicrobial activity.

INTRODUCTION

Hypericum scabrum L. (Hypericaceae), an herbaceaus perennial plant which grows in inner and eastern Turkey is used in folk medicine for the treatment of hemorroids and diarrohea (1,2). Tannins and volatile oil contents of the plant collected

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from Yozgat were reported by Tanker et al. (3). In a recent paper the antibacterial activity of the plant has also been shown (4). It has been demonstrated that *H. scabrum* of Tajikistan containing 0.17-0.23 % volatile oil, 2.08-2.42 % flavonoid and 4.58-7.96% tannins is used against cronic ulser, cistitis (5).

The present investigation has been carried out on *H. scabrum* collected from Konya Karaman, to evaluate its biological activities by determining the hypericin and pseudohypericin contents and antimicrobial activities of the extracts obtained with several solvents. Phytochemical analysis has also been performed for the identification of the groups of its active constituents. Hypericin and pseudohypericin contents of *H. scabrum* have been compared with those of *H. perforatum*, a popular phytomedicine which is used as antidepressant.

RESULTS AND DISCUSSION

Hypericin and pseudohypericin content of *H. scabrum* have been determined by HPLC comparing with those of *H. perforatum*.The results are shown in Table 1.

Table 1 : Content of hypericin and pseudohypericin in *H. scabrum* and *H. perforatum*

	Hypericin %	**Pseudohypericin%**
H. scabrum	0,00046	0,00035
H. perforatum	0,03	0,011

The poor yield of hypericin and pseudohypericin in *H. scabrum* indicates that this plant can not be used as a source for these compounds. Presence of tannins and flavonoids confirms the traditional use of *H. scabrum* (Table 2).

Table 2 : The result of preliminary phytochemical analysis

	Water	**Petroleum ether**	**Ether**	**Chloroform**	**Methanol**
Flavonoids	++	-	-	+	+
Antraquinons	-				
Saponins	+				
Tannins	+++				
Alkaloids	-				
Sterols	-	+++	+	+	-

Total ethanol extract of the plant was found to be active against *Staphylococcus aureus* (Table 3). Minimum inhibitory concentration of the active extract was determined as 39 μg/ml (Table 4).

Table 3 : The antimicrobial activity of *H. scabrum* in several extracts

	Petroleum ether	Ether	Chloroform	Methanol	Ethanol
Staphylococcus aureus	-	-	-	-	+
Staphylococcus epidermidis	-	-	-	-	-
Escherichia coli	-	-	-	-	-
Klebsiella. pneumoniae	-	-	-	-	-
Pseudomonas. aeuroginosa	-	-	-	-	-
Salmonella typhi	-	-	-	-	-
Shigella flexneri	-	-	-	-	-
Proteus mirabilis	-	-	-	-	-
Candida albicans	-	-	-	-	-

Table 4 : The MIC value of the active extract (MIC) (μg/ml)

	Staphylococcus aureus
H. scabrum (ethanol extract)	39

This result is in accordance with the result of previous workers who showed the antibacterial activity of several *Hypericum* species (6-8).

EXPERIMENTAL

Plant Material

H. scabrum was collected from Konya-Karaman, Taşkent-Ermenek on 14th July, 2001 and identified by Neriman Özhatay. A voucher specimen is deposited at the Herbarium of Faculty of Pharmacy, Istanbul University (ISTE 80455).

Preliminary Phytochemical Analysis

Determination of flavonoids, antraquinons, saponins, tannins and alkaloids were carried out on 10% infusions prepared from the aerial parts of the plant.Determination of sterols and flavonoids were also performed on petroleum ether, ether, chloroform, methanol exract. The result is shown in Table 1.

Determination of hypericin and pseudohypericin by HPLC

A high-performance liquid chromatographic method has been developed for the quantitative determination of hypericin and pseudohypericin. The quantification of naphtodiantrones were determined by an HPLC method modified from Lamaison et all (9). The yield of hypericin and pseudohypericin in *H.scabrum* and *H. perforatum* have been compared.

Materials and Methods

10 g powdered material was extracted with 100 ml methanol at room temperature for 30 minutes using an ultrasonic bath. After filtration the residue was extracted with 100 ml acetone at room temperature for 30 minutes using an ultrasonic bath and filtered. The methanol and acetone filtrates were combined and reduced to dryness under vacuum. The dry material was dissolved in 4 ml methanol and transferred into a volumetric flask and diluted to volume with methanol.

Preparation of the standard solutions

Hypericin : 1 mg of hypericin was dissolved in 4 ml methanol using an ultrasonic bath then transferred into a 10 ml volumetric flask and diluted to volume with methanol. (stock solution, c = 0.1 mg/ml). From the stock solution 2, 4, 6, 8, 10µg/ml solutions were prepared for the calibration curve.

Pseudohypericin: The same way has been followed to prepare the standard of pseudohypericin (stock solution, c = 0.1 mg/ml). From the stock 2.5, 3, 3.5, 4, 4.5µg/ml solutions were prepared.

The standards of purity 98% were purchased from Sigma (Planta Natural Products, Wien).

Apparatus and Chromatographic Conditions of HPLC

The apparatus used was a SCL-10A SHIMADZU equipped with a ECV-10 AL pump, a SIL-10AD injector, and an SPD-M 10A Photodiode array detector. The separations were carried out using a EC 250/4 Nucleosil 100-5 C_{18} column.

The operating conditions of HPLC were: ambient temperature; the flow rate of eluting solvent 1.7 ml/min. ; wavelength of UV detector 590 nm ; the injection volume was 10 µl.

Gradient elution was performed using an A eluent (water: phosphoric acid (250:0.6) v/v), B eluent (asetonitril: water (225:25) v/v) and C eluent (methanol) with the following linear combination: at time 0, 50% A, 50% B, 0% C; at time 14 min, 10%

A, 75% B, 15% C; at time 18 min, 5% A, 85% B, 10% C; at time 30 min, 0% A, 100% B, 0% C. Total run time was 30 minutes.

Determination of the main aglycon flavonoid

The dried and powdered plant material was extracted with ethanol at the Soxhlet apparatus. The extract was evaporated in vacuo to a smaller volume, diluted with water, then transferred into a separating funnel and extracted with toluen, chloroform and ethyl acetate respectively.

Isolation of the main aglycon quercetin from the chloroform extract was performed with preparative paper chromatography (Whatmann No:1) using the solvent system acetic acid: water (15:85). The structure of quercetin was identified with UV and IR spectra and comparing TLC R_f values with those of authentic samples.

Antimicrobial activity of *H. scabrum*

Preparation of extracts

Powdered aerial parts of the plant material was extracted sequantially in petroleum ether, ether, chloroform and methanol. A total ethanol extract from the plant was prepared.

Disk diffusion method

Disk diffusion method was used for antimicrobial activity. Antimicrobial activity against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumaniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 1539, *Salmonella typhi*, *Shigella flexneri*, *Proteus mirabilis* ATCC 14153 and antifungal activity against *Candida albicans* ATCC 10231 were investigated. Mueller-Hinton agar in Petri dishes were inoculated with a bacterial suspension (10^5 cfu/ml). Paper disks were impregnated with test extracts then laid onto the surface of the agar. A control extract disk was also placed onto the surface of the agar. The petri dishes were incubated for 24 h at 37 °C, and zones of inhibiton appeared in the presence of antibacterial substances.

Minimum inhibitory concentration assay

Microdilution broth method has been used.The inoculum was prepared with a 4-6 h broth culture of each bacteria strain adjusted to a turbidity equivalent to 0.5 Mc Farland standart, diluted in broth media to give a final concentration of 5×10^5 cfu/ml in the test tray. The trays were covered and placed in plastic bags to prevent drying. The trays containing Muller-Hinton broth were then incubated at 35 ° C for 24 hrs and

examined for the growth. The MIC was defined as the lowest concentration able to inhibit any visible microorganism growth.

The results of these two experiments are shown in Table 3 and 4.

Acknowledgements

Standards support from Planta Natural Products is also gratefully acknowledged.

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