Mar. Üniv. Ecz. Der.,
11 (1 - 2), 351 - 355 (1995)

J. Pharm. Univ. Mar.,
11 (1 - 2), 351 - 355 (1995)

PHYTOCHEMICAL STUDIES ON *PLATYCODON GRANDIFLORUM* (JACQ.) A. INTRODUCED IN BULGARIA

Nguyen THUAN* – Stefan NIKOLOV*

SUMMARY

Platycodon grandiflorum (Fam. *Campanulaceae*) is widely used as an expectorant and antiinflammatory drug in the traditional and folk medicine of the East-Asian subcontinent of which it is native, whereas it is not well known in Europe. It has now been introduced in Bulgaria, and therefore, became the object of our phytochemical investigations mainly for the saponins and flavonoids. All the investigated parts of the plant (roots, stems and leaves) contain saponins with HI >1000 . By the TLC methods 5 pentacyclic triterpenoid saponins were established in the roots. In the herb of the plant, 8 flavonoids and 4 phenolic acids were established by PC. By CC method and preparative PC, 3 flavonoids and one phenolic acid have been isolated in pure state. They are: - luteolin -7-O-D - glycoside (Cinerazin), apigenin -7-O-D-glucoside; luteolin and chlorogenic acid. They were identified by the determination of their m.p.s, UV spectras, acid hydrolysis and PC comparison with authentic samples in different solvent systems. The last three compaunds have identified for the first time from the species.

KEY WORDS

Platycodon grandiflorum, saponins, flavonoids, flavones, flavone glycosides

* Department of Pharmacognosy, Pharmaceutical Faculty, Higher Medical School, Sofia 1000 BULGARIA.

INTRODUCTION

Platycodon grandiflorum (Jacq.) A. DC is well known more as a decorative than a medicinal plant in Europe. The species has wide application in the traditional and folk medicine of the oriental countries, where it has its native habitat. The roots contain saponins and has expressed expectorant activity similar to Radix Primulae. Further, sedative, analgetic, antipyretic, anti-ulcerative and tonic effects /1/ has been elucidated. The object of our investigation is to find out the chemical constituents, mainly saponins and flavonoids of *Platycodon grandiflorum* which has been introduced in Bulgaria. No information of its flavonoidal constituents in the herb was available in our survey of literature. In this work we describe the results of the quantitative and TLC studies on the roots of the species for the presence of saponins and sapogenins, and investigation of the herb for flavonoids.

MATERIAL AND METHODS

The plant material was obtained from the introduced samples, cultivated during 1993 in the Institute of Botany, Bulgarian Academy of Sciences, Sofia. A voucher sample has been deposited in the herbarium of the Botanical Institute, Sofia (SOM), Co-298. TLC investigations were carried out on Kieselgel G Merck plates in systems: Chloroform - Methanol - Water (60:36:2) [S_1]; Benzene - Chloroform - Methanol (3:3:1) [S_2], Benzene - Ethyl acetate (6:3) [S_3]. PC was conducted on the paper Filtrak FN_1, and the preparative PC was conducted on Filtrak FN_7 in the systems n-Butanol - Acetic acid - Water (4:1:2.2) [S_4], 15% Acetic acid [S_5] and 60% Acetic acid [S_6]. The saponins and sapogenins were detected by p-Dimethylaminobenzaldehyde [R_1] /2/, after warming the chromatograms at 110° C for 7-8 minutes, and the flavonoids by 3% ethanolic Aluminium hydrochloride solution [R_2]. The acid hydrolysis of the saponins was conducted using 5 % H_2SO_4 for 8 hours, and for the

flavonoids with 5% HCl for 2 hours. UV spectroscopy was carried out on the apparatus SPECORD UV-VIS in methanol and standard diagnostic shift reagents.

EXPERIMENTAL

I. STUDIES ON THE CONTENTS OF SAPONINS AND SAPOGENINS

Froth Index (FI) and Haemolytic Index (HI) were determined for the roots, stems and leaves by established methods. 150 g of the studied plant material, after size reduction were extracted 6 times each, with methanol for 1 hour each. After filtration, the methanolic extracts were combined and concentrated to a thick mass which was dissolved in hot water. This aqueous solution was treated with chloroform, ethyl acetate and n-butanol consecutively. The object of our further work became the butanolic extract which was obtained in the yield of 3.87 g. The saponin contents in the butanolic extract was studied by two-dimensional TLC in the systems S_1 and S_2 and development by R_1. After acid hydrolysis of the butanolic extract, the sapogenins were extracted twice with ether and characterised by TLC in the system S_3 by development with R_1.,

II. STUDIES ON THE CONTENTS OF FLAVONOIDS

220 g herb was extracted five times with 80% ethanol for 1 hour each. After filtration the combined ethanolic extracts were concentrated and the obtained aqueous extract was treated with chloroform and ethyl acetate consecutively. The ethyl acetate extract showed the presence of flavonoids and was further studied for the flavonoidal contents by two-dimensional PC in the systems S_4 and S_5 by development with R_2. 7.0 g dry extract was separated by CC over 40 g polyamide by elution with increasing concentration of ethanol up to 90% ethanol. 200 fractions, each of 100 ml, were collected. From the combined fractions, after rechromatography over column, separation by preparative PC in the systems S_4,S_5 & S_6 and

recrysttalization from methanol, 3 flavonoidal compounds and one phenolic acid were obtained in pure state.

Flavonoid F_1(Luteolin-7-0-glucoside). M.P. 207-210 °C (MeOH); Rf = 0.42 in S_4 and 0.12 in S_5; λ max (MeOH) nm 255,268 sh,350; (+NaOMe)265, 300 sh, 400; (+NaOAc) 269, 266 sh, 370 sh, 400; (+NaOAc +H_3BO_3) 260, 375; (+$AlCl_3$) 275, 300 sh, 330, 430; ($AlCl_3$+ HCl) 270, 300 sh, 358, 390.,

Flavonoid F_2 (Apigenin-7-O-glucoside). M.P. 207-210°C (MeOH), Rf = 0.39 in S_4 and 0.28 in S_5; λ max (MeOH) nm 268,335; (+ NaOMe) 245, 268, 300sh, 385, 390; (+NaOAc) 269, 355, 385, 395; (+NaOAC +H_3BO_3) 269, 340; (+$AlCl_3$) 276, 300, 350, 390; (+$AlCl_3$+HCl) 278, 300, 341, 380.

Flavonoid F_3 (Luteolin). M.P. 293-295°C (MeOH); Rf = 0.84 in S_4 and 0.67 in S_6; λ max (MeOH) nm 256, 351; (+ NaOMe) 268,405;(+ NaOAc) 372, 403; (+ NaOAc +H_3BO_3) 269, 371; (+ $AlCl_3$) 273, 422, (+ $AlCl_3$+ HCl) 275, 357, 380.

Compound F_4 (Chlorogenic acid). Rf = 0.70 in S_4, 0.72 in S_5 and 0.84 in S_6; λ max (MeOH) nm 305, 325.

RESULTS AND DISCUSSION

The phytochemical investigations carried out were targetted towards establishment and characterisation of the saponins and flavonoids in the species *Platycodon grandiflorum* introduced in Bulgaria. It is well known that the expectorant, antiinflammatory, anti-ulcerative and tonic properties are due to these two groups of the biologically active compounds. Besides, the saponin and the flavonoidal contents can be expressed by qualitative and quantitative characters for the standardisation of the crude drugs, extracts, and the phytopreparations which could be obtained from this species.

The results of the determination of the FI<100 and HI>1000 show that saponins with high haemoltyic index is present in all the organs of the species. By two-dimensional TLC of the

purified extracts, it was found that the roots of the plant contain at least 5 pentacyclic triterpenoid compounds. After acid hydrolysis of the purified saponin mixture, 2 sapogenins of the group Δ^{12} - oleanan type were characterised by TLC. These data are confirmed by the literature, which prove that from this species contain sapogenins of the oleanan type platycogenins and platycogenic acid which have been chracterised structuraly /3, 4/. From the herbal part of the plant was obtained a flavonoidal mixture, which by two-dimensional PC studies exhibited the presence of at least 8 flavonoids and 4 polyphenolic acids. By CC and preparative PC separation, 3 flavonoidal compounds and 1 phenolic acid were isolated. By determination of their melting points, PC comparison with authentic samples in different systems and UV - spectral analysis in methanol and other diagnostic shift reagents these compounds were identified as - luteolin-7-O-glucoside, apigenin-7-O-glucoside, luteolin and chlorogenic acid. Further phytochemical investigations on the species *Platycodon grandiflorum*, introduced in Bulgaria are in continuation.

REFERENCES

1. Do Tat Loi Vietnames medicin plants. 1977; 717-718

2. Nikolov SD, Joneidi M, Panova DI. Quatative determination of ruscogenin in *Ruscus* species by densitometry TLC. *Pharmazie* 1976; 31: 611-612

3. Toshiyuki A, Yoichi I, Osamu T. Structure of platycodigenin, a sapogenin of Platycodon grandiflorum. *Tetrahedron Lett* 1968; 53: 5577-80.

4. Tokio K, Hisaco R, Hiroshi H. Structure of platicogenic acids A,B and C, triterpenoid constituents of Platycodon grandiflorum. J. Chem.Soc. 1969 ; 22 : 1313-14