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Preliminary reports

NEUROCHEMICAL *IN VITRO* ACTIVITY OF XANTHONES FROM *GENTIANELLA AUSTRIACA*

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Austrian gentian, *Gentianella austriaca* (A. KERN. & JOS. KERN), Gentianaceae [syn. *Gentiana germanica* Willd. subsp. *Austriaca*] is endemic alpine plant populated at altitudes above 1500 m and up to 2800 m (STRUWE *et al.* 2002). It may be also found in central mountains of Serbia, over 2000m. Although a rare mountain plant *G. austriaca* is used in traditional medicine to stimulate appetite and to treat digestive complaints, like the other bitter gentians. It is poorly pharmacologically explored, albeit it contains yellow pigments - xanthones, a group of plant secondary metabolites. Xanthones originally evinced taxonomic

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importance, while their pharmacological properties have aroused great interest during the last two decades. Several *in vitro* and *in vivo* studies performed on naturally occurring xanthones (reviewed by PERES and NAGEM, 1997, and PERES *et al.*, 2000) detected a number of their different pharmacological effects (*e.g.* hypoglucemic, antitumor, antioxidant, antihepathotoxic, CNS depressant or stimulant). Some of the simple tri- and tetra-oxygenated xanthones, which were found in many of *Gentiana* species, show strong MAO inhibiting potency (SUZUKI *et al.*, 1981; SCHAUFELBERGER and HOSTETTMANN, 1988; THULL and TESTA, 1994; OHISHI *et al.*, 2000; TOMIĆ *et al.*, 2005). Two tetraoxigenated xanthones of *Gentiana lactea*: bellidifolin (1,5,8-trihydroxy-3-methoxy-.xanthone) and demethylbellidifolin (1,3,5,8-tetrahydroxyxanthone) have been already reported for a strong MAO A inhibition (SCHAUFELBERGER and HOSTETTMANN, 1988), but their eventual *in vivo* effects have not been clarified yet. These two xanthones were also discovered in *G. austriaca*, together with pentaoxigenated xanthone - corimbiferine (MENKOVIĆ *et al.*, 2005). We found an interest to reevaluate and extend this research by exploring *in vitro* interaction of the diethyl-ether (Et_2O) extract of *G. austriaca*, as also of its three isolated and purified xanthones, with some components of the central monoaminergic neurotransmission, in the light of their possible antidepressive potential.

Table 1 . - The effects of the Et_2O extract of Gentianella austriaca and its three xanthones on in vitro DA and 5-HT receptor binding, 5-HT uptake and MAO activity. Radioassays were performed on rat brain synaptosomal and hepatic microsomal (MAO) preparations. The result, are mean IC_{50} values obtained from 2-3 experiments. NS, not significant(IC_{50}>> 1 mg/ml or 1mM

Compounds	IC_{50}							
	D_1	D_2	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	5-HT uptake	MAO A	MAO B
Et_2O extract (μg/ml)	NS	NS	NS	NS	NS	-	3.40	260
Bellidifolin (μM)	NS	NS	NS	NS	NS	1120	1.10	2490
Demethylbellidifolin (μM)	NS	NS	NS	NS	1080	212	2.14	368
Corimbiferine (μM)	-	-	-	-	-	NS	NS	NS

Herba of *G. austriaca* was collected on the Kopaonik mt., Serbia, at altitude of about 2100 m. Dried extract used in experiments was obtained by vacuum evaporation of the Et_2O extraction product of the methanol herbal extract (1:10, w:v). Xanthones, that constituted more than 90% of the extract, were isolated by column chromatography on silica gel and their purity was tested by HPLC (MENKOVIĆ *et al.*, 2005). The range of concentrations of Et_2O extract used in experiments was from 0.1μg/ml to 1mg/ml, and for the xanthones: bellidifolin, demethylbellidifolin and corimbiferine (1,3,8-trihydroxy-4,5-dimethoxy-xanthone), they were 0.1μM-1mM. The radioligands ^{3}H-SCH2339 (specific activity 91Ci/mmol; concentration in radioassays for D_1 receptor binding: 0.4 nM),

^{3}H-spiperone (25 Ci/mmol; D_2 receptors: 0.2 nM), ^{3}H-8OH-DPAT (129 Ci/mmol; $5HT_{1A}$: 1.0nM), ^{3}H-ketanserine (88 Ci/mmol; $5HT_{2A}$: 1.0 nM), ^{3}H-mesulergine (86 Ci/mmol; $5HT_{2C}$: 1.0 nM), ^{3}H-serotonine (146 Ci/mmol; 5-HT uptake: 50 nM) and ^{14}C-tyramine (specific activity 55mCi/mmol; MAO) were purchased from Amersham Life Science, USA; PerkinElmer Life Sciences, USA; or American Radiolab Chemicals, USA. The drugs: serotonin, butaclamol, clorgyline, pargyline, tyramine, amitriptyline were of analitical grade purity obtained from Sigma Chemical, USA or ICN Biomedicals, USA.

Brains of adult male Mill-Hill hooded rats were dissected and used for synaptosomal preparation, while rat livers were used to isolate MAO. The procedures of preparation and a methodology of receptor competitive binding and synaptosomal 5-HT reuptake radioassays are described in details elsewhere (Vogel and Vogel, 1997; TOMIĆ *et al.*, 2005). The potency of extracts to inhibit MAO enzymes isolated from rat liver microsomal fraction was estimated by rating the level of *in vitro* ^{14}C-tyramine degradation. (TOMIĆ *et al.*, 2005). Competition curves were constructed and analyzed by "GraphPad Prism" (v. 4.0.) software.

The results of *in vitro* assays are presented in Table 1 by mean IC_{50} values (from 2-3 experiments) for MAO A and MAO B inhibition. It is obvious that the Et_2O extract of *G.austriaca* and two of the isolated xanthones, bellidifolin and demethylbellidifolin, significantly inhibited MAO enzymes. They are more potent inhibitors of MAO A than of MAO B, and this is partly in line with the other study (SCHAUFELBERGER and HOSTETTMANN, 1988), although it presented quite stronger MAO A inhibiting potential of bellidifolin. The third investigated xanthone, corimbiferine, didn't show similar inhibiting effect. A possible additional influence of the xanthones on the other elements of monoaminergic neurotransmission, that may be connected to the potential antidepressant action, was not found by this study. The extract and xanthones did not show appreciable influence on the *in vitro* radioligand binding to any of the tested DA and 5-HT receptors (IC_{50}> 1mM). Also, the xanthones have certain, but insufficient power to significantly inhibit synaptosomal 5-HT reuptake (IC_{50} = 1.12 and 0.212 mM, for bellidifolin and demethylbellidifolin, respectively).

In conclusion, the present neuropharmacological *in vitro* screening of *G. austriaca* and its xanthones supports their influence on MAO enzymes, but does not suggest any other important monoaminergic effect. However, the marked MAO A blocking potency of bellidifolin and demethylbellidifolin could be sufficient itself to induce some behavioral or psycho-modulation, as it was found after the substantial MAO A blockade generated by gentiacauleine, a xanthone of *Gentiana kochiana* (TOMIĆ *et al.*, 2005). In any case, for the certain conclusion on the subject of the antidepressant potential of *G. austriaca* and their xanthones, an additional *in vivo* studies are needed.

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