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SHORT COMMUNICATION

Secondary metabolites of *Hypericum monogynum* from Pakistan

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Abstract: 4-Chlorobenzoic acid (**1**), quercitrin (**2**), astilbin (**3**), along with β -sitosterol, γ -sitosterol, friedelin and β -amyrin were isolated from the aerial parts of *Hypericum monogynum*. Whereas compound **1** was isolated for the first time from natural sources, flavanonol **3** was not found before in these species.

Keywords: *Hypericum monogynum*; Clusiaceae; 4-chlorobenzoic acid; quercitrin; astilbin.

INTRODUCTION

The genus *Hypericum* L. (family Clusiaceae) comprises about 460 species distributed worldwide in temperate regions. *Hypericum* species were already known to ancient communities as useful medicinal plants. The best known among them is *Hypericum perforatum* (St. John's wort), the use of which as a remedy was described and recommended throughout the Middle Ages. Several groups of bioactive natural products, e.g., naphthodianthrones, phloroglucinols, phenylpropanoids, flavonol glycosides, biflavones, tannins, proanthocyanidins and xanthenes have been identified from the genus. Biological tests of the flavonoids isolated from the genus *Hypericum* demonstrated inhibition of lipoxigenase,¹ antidepressant² and scavenging³ activity, while the flavonoid glycoside exhibited antifungal activity.⁴ In Pakistan, twenty species of this genus are known. *Hypericum monogynum* Miller (Syn. *Hypericum chinense* L.) is distributed mainly in S.E. China and Taiwan. It is cultivated in many parts of the world, including the plains of W. Pakistan. This species is used in traditional medicine as an alternative, antidote and astringent, as well as in the treatment of miasmatic diseases.⁵

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Continuing our chemical examination of the flora of the North-West region of Pakistan and the search for compounds of pharmacological interest, an examination of the aerial parts of *H. monogynum* Miller, originating from Peshawar is now reported.

Previous examinations of *H. monogynum* involved the constituents of the aerial parts⁶ and volatiles.⁷ In a sample from China, Wang *et al.*⁶ identified 10 compounds: quercetin, quercitrin, hyperoside, rutin, (–)-epicatechin, 3,5-dihydroxy-1-methoxyxanthone, 3,4-*O*-isopropylidene shikimic acid, shikimic acid, daucosterol and oleanolic acid.

RESULTS AND DISCUSSION

A combination of different preparative chromatographic techniques and selective extractions with different solvents applied on the MeOH extract of the aerial parts of *H. monogynum* afforded seven compounds: 4-chlorobenzoic acid (**1**),⁸ quercitrin (**2**),⁹ astilbin (**3**) (Fig. 1),¹⁰ β -sitosterol,¹¹ γ -sitosterol,¹² friedelin and β -amyryn.¹³ The occurrence of **2**, also found in the same species before,⁶ as well as flavanonol rhamnoside **3**, detected previously in several species of the genus, among them *H. perforatum*,¹⁴ is not unexpected. Quercitrin (**2**) was demonstrated to be a potent antileishmanial compound, with a low toxicity profile and exhibited antioxidant activity in the DPPH test.¹⁵ The co-occurring flavanonol **3** was shown to exhibit diverse biological activities, such as lipolytic, anti-oxidative, anti-allergic, anti-inflammatory and insecticidal.¹⁶ To the best of our knowledge, *p*-chlorobenzoic acid has not been found from plant sources before. As shown in an investigation of the plant-growth activity of substituted benzoic and phenoxyacetic acids, including **1**,¹⁰ benzoic acids substituted with an electronegative atom capable of displacement by an electron-rich plant substrate exhibit plant-growth activity.¹⁷

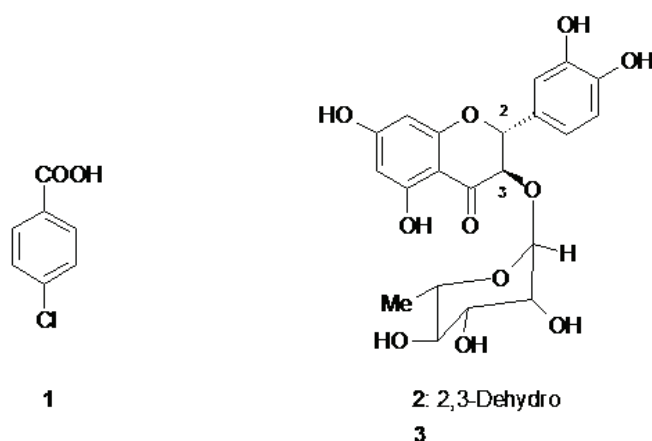


Fig. 1. Structures of 4-chlorobenzoic acid (**1**), quercitrin (**2**) and astilbin (**3**).

EXPERIMENTAL

General

The spectra were recorded with the following instruments: UV, Cintra 40, GBC UV-Vis spectrometer; IR, Perkin-Elmer FT-IR spectrometer 1725X; ^1H - and ^{13}C -NMR, Bruker DMX 600 (600 MHz for ^1H) in CD_3OD with TMS as the internal reference; DCI MS (150 eV, isobutane), Finnigan MAT mass spectrometer 8230, double focusing (BE geometry). Silica gel 60 and polyamide SC_6 were used for column chromatography. Silica gel 60 F₂₅₄ and RP-8 F_{254s} precoated aluminum sheets (0.25 mm, Merck) were used for TLC control.

Plant material

The aerial parts of *Hypericum monogynum* were collected at the University of Peshawar campus during the flowering stage in July 2004 and the taxonomic identification was performed by Professor Abdul Rasheed. A voucher specimen (No. 29304-PUP) is deposited in the Herbarium of the Department of Botany, University of Peshawar.

Extraction and isolation

Air dried, powdered aerial parts (1 kg) were extracted by percolation with methanol at room temperature. The residue (180 g) obtained after solvent removal under reduced pressure was successively extracted with *n*-hexane and ethyl acetate to yield the HE-extract (40 g) and EA-extract (12 g) after evaporation of the solvents. Aliquot of the EA-extract (12 g) was chromatographed on silica gel column with chloroform, gradually increasing polarity by successive addition of methanol. The fraction eluted with chloroform/methanol = 7/1, after recrystallization from methanol/water gave *p*-chlorobenzoic acid (**1**, 60 mg), identified by the identity of its spectral data to those from the SDBS library.⁸ The fraction eluted with chloroform/methanol = 7/2, upon crystallization from methanol/water afforded a mixture of two flavonoids (FF, 1.5 g).

An aliquot of FF (122 mg) was chromatographed on polyamide SC_6 with $\text{MeOH}/\text{H}_2\text{O}$ = 1:1 to afford astilbin (**3**, 41 mg) and quercitrin (**2**, 56 mg). The progress of the elution was monitored by TLC (RP-8 F_{254s}); the developing system was $\text{MeOH}/\text{H}_2\text{O}$ = 1:1.

Repeated silica gel chromatography of the *n*-hexane extract residue (40 g) yielded β -sitosterol (20 mg), γ -sitosterol (17 mg), friedelin (24 mg) and β -amyrin (25 mg).

CONCLUSIONS

The plant constituents quercitrin and astilbin make a different flavonol profile of the *Hypericum monogynum* from Pakistan, compared with the *H. monogynum* from China, which contains quercitrin and its aglycone quercetin. Substituted phenoxyacetic and benzoic acids, such as *p*-chlorobenzoic acid, capable of displacement by an electron-rich plant substrate, could exhibit plant-growth activity. This is the first time *p*-chlorobenzoic acid has been found from plant sources.

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ИЗВОД

СЕКУНДАРНИ МЕТАБОЛИТИ БИЉНЕ ВРСТЕ *Hypericum monogynum* ИЗ ПАКИСТАНА

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4-Хлоробензоева киселина (**1**), кверцитрин (**2**), астилбин (**3**), као и β -ситостерол, γ -ситостерол, фриделин и β -амирин изоловани су из надземних делова биљне врсте *Hypericum monogynum*. По први пут једињење **1** је изоловано из природних извора, док флаванол **3** до сада није изолован из ове биљне врсте.

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