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CONTRIBUTION TO THE CHEMICAL CONSTITUENTS OF BALKAN BRYOPHYTES: PHENOLIC ACIDS, FLAVONOIDS, TRITERPENES AND ALKALOIDS

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Synopsis

In this paper preliminary study of chemical constituents of three bryophyte species will be presented. Gametophytes of *Polytrichum formosum, Eurhynchium hians* (mosses) and *Pellia endiviifolia* (liverworth) have been collected in the native habitats in Petnica near Valjevo (W. Serbia). TLC technique was applied for the preliminary study of petroleum ether and methanol extracts of three investigated species.

According to this assay the presence of flavonoids (aglycones and glycosides) in all examined extracts was confirmed. In extracts of two species phenolic acids were detected. Besides, in the petroleum ether extract of all three species triterpenes were detected. Alkaloids were absent in the extracts of investigated bryophyte species.

Ključne reči:
hemija briofita,
mahovine,
jetrenjače,
fenolne kiseline,
flavonoidi,
triterpeni,
alkaloidi

Sinopsis

PRILOG POZNAVANJU HEMIJSKOG SASTAVA BALKANSKIH BRIOFITA: FENOLNE KISELINE, FLAVONOIDI, TRITERPENI I ALKALOIDI

U ovom radu su dati preliminarni rezultati hemijskih konstituenata tri vrste briofita. Gametofiti *Polytrichum formosum, Eurhynchium hians* (mahovine) i *Pellia endiviifolia* (jetrenjača) su sakupljeni u prirodnim staništima okoline Valjeva (Zap. Srbija). Primenjena je TLC tehnika (tankoslojna hromatografija) u karakterisanju petroleimskog i metanolnog ekstrakta tri odabrane vrste.

Prisustvo flavonoida (aglikona i glikozida) je potvrdjeno u svim analiziranim ekstraktima. U ekstraktima dve vrste detektovane su fenolne kiseline. Osim toga u petroleumskom ekstraktu sve tri vrste detektovani su triterpeni. Alkaloidi nisi pronadjeni kod ni jedne od tri istraživane vrste.

INTRODUCTION

Chemical studies of the bryophytes were neglected for a long time. They have now been shown to be a storehouse of naturally occurring materials, including some with novel chemical structures. Many of these materials display considerable biological activity. Investigations are hampered frequently by too small amounts of plant material. The resulting low yields of components are then generally inadequate to permit testing for biological activity. *In vitro* culture and appropriate chemical synthesis on a preparative scale are being undertaken to overcome this difficulty.

However, bryophytes are the second biggest group of land plants after flowering plants and a source of chemically new and unknown compounds (e.g. Asakawa, 1994, 2001; Sabovljević & al., 2001; Zinsmeister & al., 2003.). Studies of chemical constituents of bryophytes are recently being performed, but still inadequate and neglected (Asakawa, 1995; Toyota & al., 1998; Edelmann & al., 1998; Speicher & al., 2000, 2001; Klink & al., 2002; Popper & Fry, 2003; Hertewich & al., 2003). These data help in systematic of hardly morphologically classified bryophytes (e.g. Asakawa, 2004). The data on biological activities of bryophyte extracts and/or chemical constituents are even hard to find (e.g. Basile & al., 1998a, b, 1999; Dulger & al., 2005; Sabovljević & al., 2006, Sabovljević & Sabovljević, 2007)

MATERIAL AND METHODS

Three selected bryophyte species were used for phytochemical screening using TLC technique in order to show the presence of phenolic components, triterpenes and alkaloids in the their extracts. One liverwort and two mosses, *Pellia endiviifolia* (Dicks.) Dumort. (talous liverwort), *Eurhynchium hians* (Hedw.) Sande Lac. (pleurocarpous moss) and *Polytrichum formosum* Hedw. (acrocarpous moss) were analysed. Only the gametophytes were used for the chemical analyses. The specimens were collected in the native habitats in Petnica near Valjevo (W. Serbia) in August 2002, dried at the room temperature and stored in paper bags at 4°C till the beginning of the experiments.

Preparation of extracts

3.0 g of ground material was extracted by maceration with 30 ml of petroleum ether during 48h at room temperature, the extract was filtered and taken into dryness under reduced pressure. The dry residue was dissolved in 0.2 ml of petroleum ether. The same material used in previous step was left to dry, and extracted afterward with 30 ml of methanol at the sonic bath during 30 minutes. The extract was filtered, the filtrate concentrated under reduced pressure and residue taken up in 0.2 ml of methanol.

Thin layer chromatography

Thin layer chromatography was performed on silica gel 60 F_{254} plates (DC Alufolien, Merck, Germany). The mobile phases used were: toluene-ethyl acetate (70:30, *v/v*) for flavonoid aglycones and triterpenes, ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26, *v/v/v/v*) for phenolic acids and flavonoid glycosides and toluene-ethyl acetate-diethylamine (70:20:10, *v/v/v*) for alkaloids.

Chromatograms were evaluated under UV light at 254 and 365 nm before and after spraying with NP-reagent for flavonoids, after spraying with vanillin-sulphuric acid reagent (VS) for triterpenes and Dragendorff reagent for alkaloids (Wagner & Bladt, 1996).

RESULTS

The lipophilic compounds of *Eurhynchium hians*, *Pellia endiviifolia* and *Polytrichum formosum* were isolated by petroleum ether and analysed by TLC in toluene-ethyl acetate (70:30, *v/v*). The chromatogram of petroleum ether extract of *Eurhynchium hians*, *Pellia endiviifolia* and *Polytrichum formosum* showed the presence of several zones that correspond to flavonoid aglycones. They were detected by quenching fluorescence under UV-245 nm and by yellow fluorescence under UV light at 365 nm before and after spraying with NP-reagent.

Comparing the positions of the zones of yellow fluorescence of all three bryophyte species on the chromatogram, it may be concluded that they contain some substances in common, having at least one substance present in all three petroleum ether extracts (Fig. 1)

After developing the chromatogram in the mobile phase toluene-ethyl acetate (70:30, *v/v*), and spraying with vanillin-sulphuric acid reagent triterpenes were shown as bluish-violet zones in the petroleum ether extracts of the examined bryophyte species (Fig. 2).

The zones in extracts of *Pellia endiviifolia* and *Polytrichum formosum* that are the closest to the start line might derive of the same compound. Similarly, the zones in *Eurhynchium hians* and *Polytrichum formosum* that are the closest to the solvent front are likely to represent the same substance. Regarding the zones at the half of the chromatogram, it may be concluded that petroleum ether extracts of all three bryophytes have one compound in common from triterpene group (Fig. 2).

Methanolic extracts of *Eurhynchium hians*, *Pellia endiviifolia* and *Polytrichum formosum* were analysed by TLC in ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26, *v/v/ v/v*) as developing solvent. Flavonoid glycosides were detected in the chromatogram of methanol extracts of all three species as yellow fluorescing zones (UV-365 nm) before and after spraying with NP reagent. Chromatogram of methanol extracts of *Pellia endiviifolia* and *Polytrichum formosum*, near the solvent front showed the presence of blue fluorescing zones in UV light at 365 nm which correspond to the phenolic acids.

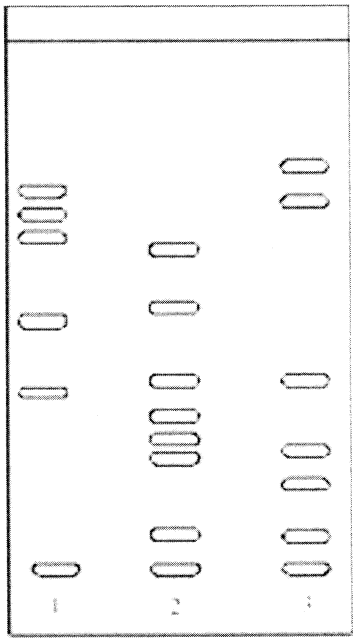

Figure 1. Chromatogram of petroleum ether extracts of *Eurhynchium hians* (1), *Pellia endiviifolia* (2) and *Polytrichum formosum* (3), obtained in toluene-ethylacetate (70:30, *v/v*), after spraying with NP reagent, under UV light at 365 nm.

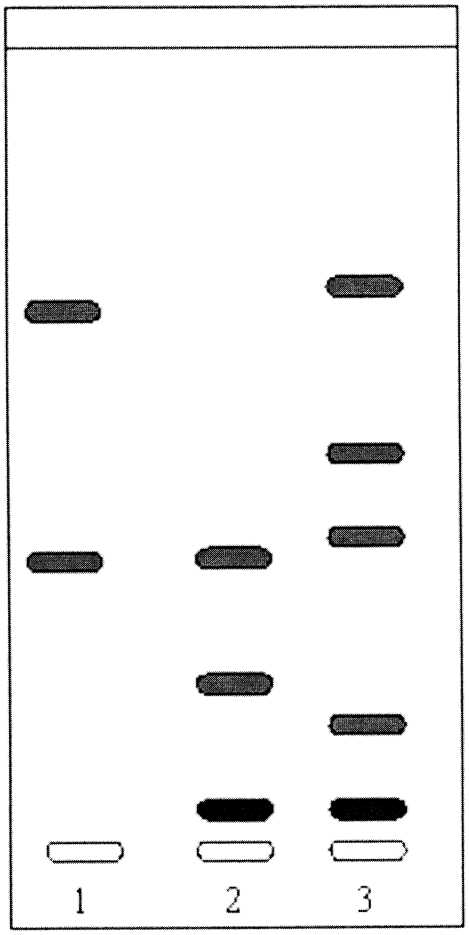

Figure 2. Chromatogram of petroleum ether extract of *Eurhynchium hians* (1), *Pellia endiviifolia* (2) and *Polytrichum formosum* (3), obtained in toluene-ethyl acetate (70:30, *v/v*), after spraying with vanillin-sulphuric acid reagent.

In order to screen methanolic extracts of analysed bryophyte species for presence of alkaloids, the chromatograms were developed in toluene-ethyl acetate-diethylamine (70:20:10, *v/v/v*), and sprayed with Dragendorff reagent. Under given conditions of analysis, no orange-brown or brownish zones that correspond to alkaloid compounds could be noticed.

CONCLUSION

The study gives first insight into chemistry of bryophytes from the Balkan Peninsula. The compounds detected are already known among bryophytes. Generally, liverworts are more often used for chemical studies due to their oil bodies and so other species of *Pellia* was subject of chemical content studies. However, it remains unknown to the authors whether there have been previously chemical analyses of selected moss species for this study at all.

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