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SHORT COMMUNICATION

Hypogymnia tubulosa extracts: chemical profile and biological activities

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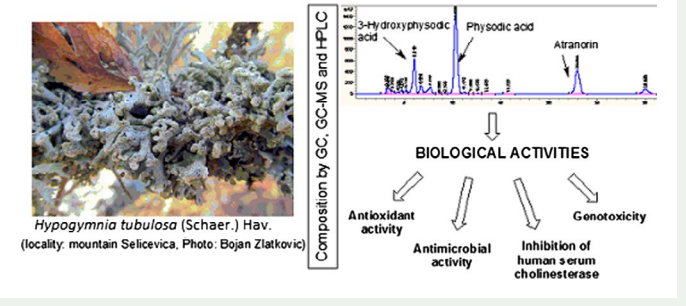
ABSTRACT

This study reports for the first time in the chemical composition of acetone, ether, ethyl acetate and dichloromethane extracts of *Hypogymnia tubulosa* determined by HPLC-UV, GC-FID and GC-MS as well as effect of *H. tubulosa* acetone extract on micronucleus distribution on human lymphocytes and on cholinesterase activity. Additionally, antioxidant (estimated via DPPH, ABTS, TRP, CUPRAC and TPC assays) and antibacterial activity against two Gram-positive and three Gram-negative bacteria were also determined. The HPLC-UV analysis revealed the presence of depsidones, 3-hydroxyphysodic, 4-*O*-methyl physodic acid, physodic and physodalic acid together with two depsides, atranorin and chloroatranorin. GC-FID and GC-MS analyses enabled the identification of atranol, chloroatranol, atraric acid, olivetol, olivetonide and 3-hydroxyolivetonide as the main components. The results of present study show that *H. tubulosa* acetone extract is a promising candidate for *in vivo* experiments considering antioxidant activity.



Hypogymnia tubulosa (Schaer.) Hav.
(locality: mountain Selicevica, Photo: Bojan Zlatkovic)

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Hypogymnia tubulosa; chemical composition; micronucleus test; antioxidant activity; cholinesterase inhibition; antibacterial activity

1. Introduction

Hypogymnia is a genus of lichenised fungi in the phylum Ascomycota within the family Parmeliaceae. Lichen *Hypogymnia tubulosa* (Schaer.) Hav. (common name powder-headed

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tube lichen, synonyms: *Ceratophyllum tubulosum* (Schaer.) M. Choisy, *Hypogymnia physodes* var. *tubulosa* (Schaer.) Walt. Watson, *Hypogymnia tubulosa f. farinosa* (Hillmann) Rass. and *Hypogymnia tubulosa f. rugososorediosa* (Gyeln.) Räsänen) belongs to a foliose type of lichens. Its major secondary metabolite is physodic acid accompanied by 3-hydroxyphysodic acid located in medulla and atranorin and chloroatranorin detected in upper cortex (Nash et al. 2002). Several papers are related to biological activities of *H. tubulosa* extracts and their constituents (Yilmaz et al. 2005; Stojanović et al. 2013, 2014; Ranković et al. 2014). Due to significant biological activities and lack of data on the relative representation of *H. tubulosa* extracts constituents, the aim of present study was to evaluate for the first time the effect of *H. tubulosa* acetone extract on micronucleus distribution on human lymphocytes, effect on cholinesterase activity, antioxidant activity and GC, and HPLC profile of acetone, ether, dichloromethane and ethyl acetate extracts. Additionally, antibacterial activity against two Gram-positive and three Gram-negative bacteria was determined.

2. Results and discussion

2.1. HPLC analysis

Chemical composition of acetone, ether, ethyl acetate and dichloromethane extracts is given in Table S1.

Four lichen acids 3-hydroxyphysodic, physodalic, 4-*O*-methylphysodic and physodic acid together with two depsides atranorin and chloroatranorin, were identified based on HPLC chromatograms (Figure S1) and UV spectra.

Predominant compounds of acetone and ethyl acetate extracts were depsidones: physodic acid (47.3 and 44.3%, respectively) accompanied with 3-hydroxyphysodic acid (14.8 and 15.4%, respectively), 4-*O*-methyl physodic acid (2.8 and 4.4%, respectively) and physodalic acid (2.3 and 3.9%, respectively). The abundance of atranorin and chloroatranorin was 12.8 and 3.7% for acetone extract and 18.1 and 4.3% for ethyl acetate extract, respectively. Dichloromethane extract differs from the others mainly by the content of atranorin, chloroatranorin and physodalic acid (31.1, 9.6 and 10.6%, respectively).

Intensity of HPLC signals (Figure S1) have revealed that extraction ability of solvents have decreased in this order: acetone, ethyl acetate, ether, dichloromethane (HPLC signals intensity 5:3:2:1) favouring acetone as the solvent of choice for lichen substances. Regarding the chemical composition *H. tubulosa* is similar to *H. physodes* composition (Table S1).

2.2. GC analysis

Qualitative composition and relative abundance of the volatile compounds of *H. tubulosa* acetone, ether, ethyl acetate and dichloromethane extracts are given in Table S2.

GC-FID and GC-MS analyses enabled the identification of chloroatranol, atranol, 4-pentylresorcinol, atraric acid, olivetol, olivetonide and 3-hydroxyolivetonide as a dominant components. Olivetol and 3-hydroxyolivetonide (Figure S2) were major compounds identified in acetone (27.7 and 20.4%, respectively) and ether extracts (28.3 and 16.4%, respectively) followed by olivetonide and 4-pentyl-1,3-benzenediol (14.2 and 14.8% in acetone; 17.2 and 15.6% in ether extract). On the other hand, the amount of atraric acid was approximately equal in acetone, ether and ethyl acetate extracts (8.1, 7.4 and 8.5%, respectively). Atranol and chloroatranol were presented in all investigated samples in the considerable amounts,

while orcinol and β-orcinol were detected in lower amounts (Table S2). Atraric acid was a predominant compound of dichloromethane extract (54.6%) followed by atranol and chloroatranol (24.5 and 9.4%, respectively). Previously, olivetonide was identified in *Pseudoevernia furfuracea* and *H. physodes* extracts (Joulain and Tabacchi 2009; Stojanovic et al. 2011; Mitrović et al. 2014), while 3-hydroxyolivetonide was identified only in extract of *P. furfuracea* (Joulain and Tabacchi 2009). The olivetonides are lactones of enol forms of olivetonic and 3-hydroxyolivetonic acid which are products of hydrolysis of physodic and 3-hydroxyphysodic acid (Figure S2). 4-Pentylresorcinol is component by which *H. tubulosa* and *H.physodes* extracts differ from each other.

2.3. Cytokinesis – block micronucleus assay (CBMN)

The acetone extract of *H. tubulosa* was tested for *in vitro* protective effect on chromosome aberrations in peripheral human lymphocytes at concentrations of 1.0, 2.0 and 3.0 µg/mL. The results are presented in Table S3. The treatment with alkylating agent mitomycin C (MMC, negative control) at concentration of 0.1 µg/mL gave an increase in the micronuclei (MN) frequency of 27.0% compared to the control cell cultures. The cell cultures treated with amifostine WR-2721 (positive control) at concentration of 1 µg/mL gave a decrease in the MN frequency of 11.4% compared to the control cell cultures (Table S3). At the concentration of 2.0 µg/mL *H. tubulosa* extract caused a decrease on the MN frequency of 4.2%, while higher concentration (3 µg/mL) gave an increase of 7.2% compared to the control cell cultures. The obtained results are similar to previously published data for *H. physodes* extracts. Namely, treatment of the cell cultures with acetone extract of *H. physodes* at concentration of 1 µg/mL showed a 5.4% decrease in the frequency of MN, while at concentration of 2 µg/mL gave increases in MN frequency of 3.3%. Physodalic, physodic, 3-hydroxyphysodic acid and atranorin at concentration of 1 µg/mL exhibited prominent effect decreasing the frequency of MN by 22.0, 28.2, 30.3 and 21.6%, respectively (Stojanović et al. 2013).

2.4. Total phenolic content and antioxidant activity

TPC analysis showed a high content of phenolic components in the acetone extract of *H. tubulosa* (134.0719 ± 2.7960 µg galic acid equivalents per mg of dry extract). The assessment of the DPPH and ABTS scavenging activity showed that extract reduced the concentration of DPPH and ABTS radicals by 93.5% (IC_{50} – 8.02 ± 0.01 mg/mL) and 99.3% (IC_{50} – 7.55 ± 0.04 mg/mL), respectively. The value of the total reducing power ability was 2.8514 ± 0.0544 µg ascorbic acid equivalents (AAE) per mg of dry extract weight. The results obtained in CUPRAC assay for *H. tubulosa* acetone extract were 46.47 ± 0.2342 µg Trolox equivalents per mg dry extract. Due to significant phenolic content and observed antioxidant activities *H. tubulosa* can be considered as a potential source of antioxidants substances.

2.5. Cholinesterase activity

Acetone extract inhibited cholinesterase to extent of 23.6% at concentration of 10 mg, while at concentration of 1 mg showed a weak activation effect on cholinesterase to an extent of 3.3%. In the conducted experiment, neostigmine bromide (commercial cholinesterase inhibitor) inhibited cholinesterase for 96.6%.

2.6. Antibacterial activity

The results of the antibacterial assays (Table S4) against two Gram-positive *(Bacillus spizizenii* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and three Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NCTC 6017) showed that the acetone extract of *H. tubulosa* had moderate activity against Gram-positive bacteria and no activity against Gram-negative bacteria at concentration of 1 mg per disc. These results were not entirely in accordance with those previously published (Yilmaz et al. 2005). According to Yilmaz et al. (2005) acetone extract of *H. tubulosa* gave high microbicidal (MIC) value for *E. coli* (530 µg per disc) and moderate MIC value for *S. aureus* (66.3 µg per disc). Since the quantitative composition of the extract was not mentioned in the above paper, it may be assumed that the different compositions of the previously and here examined extracts cause observed difference.

3. Conclusion

This is the first report on the chemical profiling of the lichen *H. tubulosa* obtained by GC and HPLC as well as for effect on micronucleus on human lymphocytes, antioxidant and cholinesterase activity. Results show that chemical composition, is similar to that of *H. physodes* composition, to a certain extent. Of all tested biological activities only antioxidant activity is manifested to the extent that qualifies *H. tubulosa* for *in vivo* tests.

Disclosure statement

No potential conflict of interest was reported by the authors.

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