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Partial characterization of indigo (*Polygonum tinctorium Ait.*) plant seeds and leaves

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ABSTRACT

The aim of this study was to assess the contents of indigo's bioactive compounds, its antioxidant and anticancer activities in acetone, hexane and DMSO extracts and to compare the overall bioactivity with another more used medicinal plant named prolipid. It was found that the contents of the bioactive compounds in the studied extracts from different parts of indigo plant varied ($P < 0.05$): the significantly highest content of polyphenols and flavonoids was in DMSO extract of prolipid, flavanols – in acetone extract of brown seeds ($P < 0.05$ in both cases) and tannins – in DMSO extract of green leaves, but not significantly ($P > 0.05$). Also the level of antioxidant activity was different: the highest antioxidant activity of all studied samples was in prolipid: according to ABTS, FRAP and CUPRAC tests in DMSO extract ($P < 0.05$ in all 3 cases), and only in acetone extract according to DPPH was not significant ($P > 0.05$). The correlations between polyphenol compounds and the antioxidant activities were relatively high. DPPH kinetic measurements were used to compare and distinguish the antiradical activity among indigo extracts by multivariate analysis. The FT-IR spectroscopy evaluated the presence of polyphenols. The interaction between DMSO polyphenol extracts of indigo plant and BSA showed that indigo has a strong ability as other medicinal plants such as prolipid to quench the intrinsic fluorescence of BSA by forming complexes and was measured by 3-dimensional fluorescence (3D-FL). The highest anticancer activity was in prolipid in concentrations of 800 μg/mL against Calu-6, following by indigo brown leaves. In conclusion, organic extracts of indigo brown leaves were analyzed for their antioxidant and anticancer activities and compared with prolipid, using polyphenols composition, antioxidant activities and fluorescence properties. The indigo ability to quench the intrinsic fluorescence of BSA, relatively high content of phenolic compounds and anticancer properties can be used as medicinal plant.

1. Introduction

Medicinal plants are sources of important therapeutic aids for alleviating human ailments. Medicinal herbs are known to contain a variety of antioxidants (Bener et al., 2010; Boo et al., 2012; Nirmaladevi et al., 2010). The successful clinical utilization of cancer chemotherapeutic agents from higher plants has been evident for about half a century (Lau et al., 2004; Pan et al., 2010). Indigo (*Polygonum tinctorium Ait.*) is less known as a natural healing and medicine (Hamburger, 2002). However, there is a scientific basis which can explain the successful use of indigo in traditional medicine (Wei et al., 2005). So, above cited authors isolated from

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[1] This article was written in memory of my dear brother Prof. Simon Trakhtenberg, who died in November 2011, who encouraged me and our research group during all his life.

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the ethanol extract of this Chinese medicinal herb a new indigoid derivative, bisindigotin (1), with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-antagonistic activity. Medicinal plants are effective in treatment of wide range of ailments: from gastrointestinal disorders (Neves et al., 2009) to diabetes (Andrade-Cetto and Heinrich, 2005), respiratory conditions (Pallant and Steenkamp, 2008) and wound healing (Khalil et al., 2007; Ghasemi and Koohpyeh, 2011). Neves et al. (2009) found that the most dominant family for the treatment of gastrointestinal ailments is *Lamiaceae* (18%) and the most frequently used part of the plant is leaves (37.9%). The largest number is used to treat gastrointestinal disorders (73.9%). Organic extracts of *Cymbopogon schoenanthus* L. Spreng (lemon grass) shoots from three different locations in South Tunisia were screened for their antioxidant, acetylcholinesterase and antimicrobial activities (Khadri et al., 2010). Methanol extracts from the leaves, bark and roots of four Cameroonian medicinal plants, *Bersama engleriana*, *Cupressus lusitanica*, *Vitellaria paradoxa* and *Guibourtia tessmannii* were tested for their *in vitro* cytotoxicity, antigonorrheal and antireverse transcriptase activities (Mbaveng et al., 2011). It was of interest to know if also the extracts of indigo have the same properties as some other medicinal plants. Our previous recent reports showed the indigo properties in different solvents (Jang et al., 2011). Therefore, in addition to the study of the contents of the bioactive compounds and antioxidant activity of indigo also its anticancer properties were investigated. As far as we know, there are no published results of such investigations.

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox); quercetin; Tris, tris(hydroxymethy1) aminomethane; bovine serum albumin, 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS); 1,1-diphenyl-2-picrylhydrazyl (DPPH); Folin-Ciocalteu reagent (FCR); lanthanum (III) chloride heptahydrate; $FeCl_3{\cdot}6H_2O$; $CuCl_2{\cdot}2H_2O$; 2,9-dimethyl-1,10-phenanthroline (neocuproine) and bovine serum albumin (BSA) were purchased from Sigma Chemical Co., St. Louis, MO, USA. 2,4,6-Tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionized and distilled water was used throughout.

2.2. Samples and preparation

The following parts of indigo plant (*P. tinctorium*): seeds, and two probes of leaves were investigated: the leaves with slight green color (immature leaves) and the leaves with green brown color (mature leaves) were harvested on April 10, 2010 and on July 20, 2010, from the same place, respectively (Fig. 1). The leaves were dried for 5 days under sunlight. The leaves were pulverized in the laboratory conditions. The particle size was 200 mesh. For comparison was used prolipid (Jastrzebski et al., 2007). Prolipid is a mixture of the following plants: Sonchus 532 Z. *arvensis* L. from the Compositae (*Asteraceae*) family, *Guazuma ulmifolia* L. from the *Sterculiaceae* family and *Murraya paniculata* L. from the *Rutaceae* family. Prolipid contains extracts of *G. ulmifolia* [20 g/100 g dry weight (dw)], *M. paniculata* (10 g/100 g dw) and *S. arvensis* (10 g/100 g dw). Prolipid capsules were obtained as a gift from the drug importer COWIK (Warsaw, Poland).

2.3. Determination of the contents of the main bioactive compounds

The extracts from seeds and leaves were prepared by the same way for all tests (bioactive compounds, antioxidant and anticancer assays). The phenols were extracted with DMSO, acetone and hexane from either the indigo powder, seeds or the prolipid (concentration 25 mg/mL) at room temperature twice during 3 h (Kim et al., 2012). The prolipid capsules were opened and the content was dissolved in water at the same conditions. The polyphenols were determined by Folin-Ciocalteu method (Singleton et al., 1999) with measurement at 750 nm with spectrophotometer (Hewlett–Packard, model 8452A, Rockvile, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g dw. Total flavonoid content was determined by an aluminum chloride colorimetric method. Briefly, 0.25 mL of the indigo or prolipid sample extract was diluted with 1.25 mL of distilled water. Then 75 μL of a 5 g/100 g $NaNO_2$ solution was added to the mixture. After 6 min, 150 μL of a 10 g/100 g $AlCl_3{\cdot}6H_2O$ solution was added, and the mixture was allowed to stand for another 5 min. Half of a milliliter of 1 mol/L NaOH was added, and the total was made up to 2.5 mL with distilled water. The solution was well mixed. The absorbance was measured immediately against the blank at 510 nm in comparison with the standards prepared similarly with known (+)-catechin concentrations. The results are expressed as milligrams of catechin equivalents. The total flavanols amount was estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read. To ensure the presence of flavanols on the nuclei subsequent staining with the DMACA reagent resulted in an intense blue coloration in plant extract. As it was mentioned previously, (+)-catechin served as a standard for flavonoids, and flavanols, and the results were expressed as catechin equivalents (CE).

2.4. Extraction of phenolic compounds for MS

The lyophilized samples of seeds and leaves (1 g) were extracted with 100 mL of methanol/water (1:1) at room temperature and in darkness for 24 h. The extracts were filtered in a Buchner funnel. After removal of the methanol in a rotary evaporator at a temperature below 40 °C, the aqueous solution was then freeze-dried. These extracts were used for MS.

2.5. MS analysis

A mass spectrometer, a TSQ Quantum Access Max (Thermo Fisher Scientific, Basel, Switzerland) was used. Analytes were ionized by electrospray ionization (ESI) in positive mode. Vaporizer temperature was kept at 100 °C. Settings for the ion source were as follows: spray voltage 3000 V, sheath gas pressure 35 AU; ion sweep gas pressure 0 AU; auxiliary gas pressure at 30 AU; capillary temperature at 200 °C, skimmer offset 0 V (Nirmaladevi et al., 2010).

2.6. Determination of antioxidant activity

The AA was determined by four complementary assays:

(1) 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) method for the screening of antioxidant activity is reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids, and plasma antioxidants. The pre-formed radical monocation ABTS is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. The influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption are taken into account when determining the antioxidant activity. $ABTS^{\bullet+}$ radical cation was generated by the interaction of ABTS (7 mM/L) and $K_2S_2O_8$ (2.45 mM/L). This solution was diluted with methanol until the