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Metabolite profiling of polyphenols in a *Terminalia chebula* Retzius ayurvedic decoction and evaluation of its chemopreventive activity

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ABSTRACT

Ethnopharmacological relevance: The decoction of *Terminalia chebula* fruit is an ayurvedic remedy whose prolonged oral administration is prized as a generic intestinal and hepatic detoxifying agent. Its administration is suggested also under the perspective of a reduced risk of cancer, metabolic and cardiovascular diseases.
Aim of the study: To evaluate the phytochemical profile and the chemopreventive potential of *Terminalia chebula* fruit decoction prepared according to the ayurvedic decoction recipe.
Materials and methods: The quali- and quantitative metabolite profiling of polyphenols was obtained using HPLC–UV/DAD and HPLC-ESI-MS. The crude decoction and purified compounds were tested for their capability to interact with the EphA2-ephrin-A1 system and for their antimutagenic properties against dietary and environmental mutagens (AA, 2-NF, NaN_3, and heterocyclic amines IQ, MeIQ, MeIQx, Glu-P1, Glu-P2,) in the Ames–Salmonella/microsome assay, with and without enzymatic induction.
Results: The decoction was found to contain 3,4,6-tri-*O*-galloyl-D-glucose (55.87 mg/g), chebulic acid (54.03 mg/g), β-punicalagin (41.25 mg/g), corilagin (40.31 mg/g), α-punicalagin (35.55 mg/g), chebulagic acid (29.09 mg/g), gallic acid (27.96 mg/g) 1,3,4,6-tri-*O*-galloyl-β-D-glucose (24.25 mg/g) chebulinic acid (20.23 mg/g), 1,2,3,4,6-penta-*O*-galloyl-D-glucose (13.53 mg/g), ellagic acid (8.00 mg/g), 1,6-di-*O*-galloyl-D-glucose (4.16 mg/g). An inhibitory effect was recorded in both *Salmonella typhimurium* TA98 and TA100 strains against the mutagenic activity of heterocyclic amines (22–61%), promutagen AA (91–97%) and directly acting mutagen 2-NF (52%) with but not against NaN_3 (7%). Galloyl derivatives allowed an inhibition of mutagenicity induced by MeIQ above 80% at 0.01 mol/plate. Both decoction and purified compounds were able to modulate the EphA2-ephrinA1 system, suggesting a potential multiple chemopreventive mechanism.
Conclusions: The traditional ayurvedic decoction of *Terminalia chebula* may harbour a potential as a safe and low-cost chemopreventive agent at the intestinal level, if administered according to the ayurvedic specifications. Moreover, its recourse may enhance the presence of some polyphenolic constituents.

1. Introduction

As reported by the World Health Organization (WHO), the recourse to plant-based folkloric remedies still represents a benchmark in least-developed regions and a large portion of the global population actually relies on Ayurveda, Traditional Chinese Medicine, Tibetan Medicine or other similar primary health-care systems for their medical needs (Balachandran and Govindarajan, 2005; Khan and Balick, 2001; Mukherjee and Wahile, 2006). The traditional pharmaceutical repositories of these ethnomedical systems comprise powdered plant materials, expressed juices or extracts prepared with different solvents of immediate availability, but hardly ever includes preparations requiring purification or fractionation steps, as a consequence of the limited technological milieu in which these treatments have been developed. As a consequence, decoctions are frequently encountered for various practical reasons, including the availability of water and the concurrent need of sterilization for both herbal drugs and solvent, an hygienic prophylaxis that must not be overlooked when operating in some geographic and socio-cultural contexts (Kumar et al., 2007). However, because of the customary

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research design, most of the scientific information available at present on ethnobotanical remedies is referred to extracts obtained with the best performing solvent or to preparations that differ from those suggested by traditional health-care systems (World Health Organization (WHO), 2000). Notwithstanding its fundamental relevance in drug discovery and in the optimization of phytoterapy treatments, such process offers few reliable data in terms of direct validation of the ethnopharmacological praxis and on the potential enforcement of traditional remedies in low-cost primary healthcare systems. In fact, the local customary preparation is somehow lost by the focus of research and its direct evaluation exits from the stage, with various consequences. Recurring research approaches involving extracts obtained with organic solvents (hereby included hydroalcoholic extracts) or technological devices (i.e. ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction) and even purified bioactive molecules are of utmost relevance in the quest for new active phytochemicals, but may be misleading when the final goal is the backing of the rational enforcement of traditional herbal drugs in least developed countries or their straightforward use as food supplements worldwide. Relevant phytochemical and bioactivity differences may occur as solvent, temperature and extraction time change among laboratory protocols and local traditional recipes, making the results not necessarily sufficient for the actual administration of traditional herbal products. In some cases, bioactivity, toxicology, and even the clinical response observed may not be directly linked to the original preparation, both in terms of efficacy and safety, as emerged in the well-known case of *Piper methysticum* (Bouhlel et al., 2007; Coté et al., 2004). Moreover, in the peculiar case of decoctions a distinct phytochemical pattern emerges, including the chemical modification of relevant constituents during the open-air boiling of the plant material, as recently reported for ginsenosides in different Panax species decocted as per the Chinese pharmacopoeia (Eloff, 1998; Li et al., 2010a; Wang et al., 2008). Thus, data obtained from different solvents or with laboratory-scale protocols of extraction may not be considered as a proof of efficacy for traditional preparations like infusions and decoctions (Li et al., 2010b). This is relevant in particular with the ayurvedic materia medica, in which peculiar decoction recipes are widely used. The Ayurvedic recipe for decoctions (*kvatha*) is distinct from other extraction protocols and the resulting liquid is orally administered several times during the day (Indian Ayurvedic Pharmacopoeia).

Terminalia chebula or Haritaki is a staple ayurvedic remedy and different drugs are prepared from its leaves, roots and bark; alone or in combination with other herbal drugs like *Emblica officinalis* Gaertn. or *Terminalia bellerica* Roxb., it represents an esteemed ayurvedic *rasayana*. The prolonged oral administration of its decoction is considered a generic detoxifying agent at intestinal and hepatic level and is reputed as a way to "restore normal function" also under the perspective of a reduced risk of cancer, metabolic and cardiovascular diseases. This includes well-being maintenance and the prevention of a wide range of degenerative diseases involving inflammation, carcinogenesis and oxidative stress (Govindarajan et al., 2005; Patwardhan et al., 2004). Besides that, traditional uses of *Terminalia chebula* decoction encompasses digestive, tonic, antipyretic, spasmolytic, astringent, expectorant, antiasthmatic, antiviral and hypoglycemic purposes and such bioactivities seem to be related to the presence of polyphenolic compounds like corilagin, chebulagic, chebulic, chebulinic acids and other galloyl derivatives (Pfundstein et al., 2010). However, no reports are available on the phytochemical profile of the traditional pharmaceutical form actually employed in the ayurvedic medicine and on its chemopreventive properties. Following this concept, a decoction of *Terminalia chebula* was obtained from dried fruits according to the instructions of Ayurvedic Pharmacopoeia, a tailored HPLC–UV/DAD and HPLC-ESI-MS method was set-up, and biological assays were performed in order to obtain a first set of data on its chemical composition and on biological activities of interest for disease prevention related to its ayurvedic use.

2. Materials and methods

2.1. Solvents and chemicals

Analytical grade ethyl acetate (EtOAc), HPLC-grade acetonitrile (ACN), *dimethyl sulfoxide* (DMSO), formic acid, anhydrous sodium sulphate (Na_2SO_4), ellagic acid, sodium azide (NaN_3), aminoanthracene (AA), 2-nitrofluorene (2-NF) were from Sigma-Aldrich (Milan, Italy). Gallic acid was from extrasynthese (Genay, France). Punicalagin, corilagin, chebulagic acid and chebulinic acid were from PhytoLab GmbH & Co. (Vestenbergsgreuth, Germany). Water (H_2O) was purified using a Milli-Q Plus185 system from Millipore (Milford, MA, USA). Heterocyclic amines (HCAs), namely the quinolines 2-amino-3-methylimidazo-[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo-[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline (MeIQx), the imidazoles 2-amino-6-methyldipyrido-[1,2-a:3′,2′-d]imidazole (Glu-P-1) and 2-aminodipirydo-[1,2-a:3′,2′-d]imidazole (Glu-P-2) were supplied by Toronto Research Chemicals Inc. (Toronto, Canada). All the microbial culture media were from Oxoid Italia (Garbagnate, Italy). Lyophilized post-mitochondrial supernatant S9 fraction, was purchased from Molecular Toxicology, Inc. (Boone, NC, USA).

2.2. Plant material and extraction

Dried *Terminalia chebula* Retz. fruits, purchased from MAP Italia (Verona), were powdered and extracted by decoction following a typical Ayurvedic medicine protocol: 100 g of the grounded plant material were boiled in 3 L of H_2O until reduction of the volume to 750 mL; then, the crude fruit extract was filtered, freeze-dried and stored in the dark at −20 °C, protected from light and humidity. At the end of the extraction process 11 g (yield 11%) of dry matter were obtained. A voucher specimen of the herbal drug was deposited as TCH01 in the Dipartimento di Biologia ed Evoluzione of the University of Ferrara (Italy).

2.3. Sample preparation for HPLC analysis

The quali- and quantitative analysis of the secondary metabolites in *Terminalia chebula* decoction was performed on a portion of 0.25 g of freeze-dried fruit extract that was dissolved in H_2O, sonicated for 1 min using an ultrasonic bath at room temperature and brought to 50 mL in a volumetric flask. The solution was filtered through a 0.45 μm cellulose acetate filter into a HPLC vial and injected into the HPLC system. Three injections were performed for each sample. The extraction procedure was repeated twice for each method. The quantification data are therefore the mean of six results.

2.4. HPLC–UV/DAD conditions

Chromatography was performed using an Agilent Technologies (Waldbronn, Germany) modular model 1100 system, consisting of a vacuum degasser, a quaternary pump, an autosampler, a thermostatted column compartment and a diode array detector (DAD). The chromatograms were recorded using an Agilent ChemStation for LC and LC-MS systems (Rev. B.01.03). The analyses were carried out on an Ascentis C_{18} column (250 × 4.6 mm I.D., 5 μm, Supelco, Bellefonte, PA, USA). The mobile phase was composed by (A) 0.1% formic acid in H_2O and (B) 0.1% formic acid in ACN. The gradient elution was modified as follows: 0–2 min 5% B, 2–5 from 5 to 10% B, 5–15 min from 10% to 15% B, 15–20 min from 15% to 17% B,