ISGC 2019
May 13th - 17th

N°1091 / PC
TOPIC(s) : Waste valorization

Phytochemical profile and bioactivity of walnut green husks

AUTHORS

Olga FERREIRA / CENTRO DE INVESTIGAÇÃO DE MONTANHA (CIMO) AND LABORATORY OF SEPARATION AND REACTION ENGINEERING - LABORATORY OF CATALYSIS AND MATERIALS (LSRE-LCM), INSTITUTO POLITÉCNICO DE BRAGANÇA, CAMPUS DE SANTA APOLÓNIA, BRAGANÇA
Vanessa VIEIRA / CICECO - AVEIRO INSTITUTE OF MATERIALS, COMPLEXO DE LABORATÓRIOS TECNOLÓGICOS, AVEIRO UNIVERSITY, CAMPUS UNIVERSITÁRIO DE SANTIAGO, AVEIRO
Carla PEREIRA / CENTRO DE INVESTIGAÇÃO DE MONTANHA (CIMO), INSTITUTO POLITÉCNICO DE BRAGANÇA, CAMPUS DE SANTA APOLÓNIA, BRAGANÇA
Rui M.V. ABREU / CENTRO DE INVESTIGAÇÃO DE MONTANHA (CIMO), INSTITUTO POLITÉCNICO DE BRAGANÇA, CAMPUS DE SANTA APOLÓNIA, BRAGANÇA
Ricardo C. CALHELHA / CENTRO DE INVESTIGAÇÃO DE MONTANHA (CIMO), INSTITUTO POLITÉCNICO DE BRAGANÇA, CAMPUS DE SANTA APOLÓNIA, BRAGANÇA
Maria José ALVES / CENTRO DE INVESTIGAÇÃO DE MONTANHA (CIMO), INSTITUTO POLITÉCNICO DE BRAGANÇA, CAMPUS DE SANTA APOLÓNIA, BRAGANÇA
João A.P. COUTINHO / CICECO - AVEIRO INSTITUTE OF MATERIALS, COMPLEXO DE LABORATÓRIOS TECNOLÓGICOS, AVEIRO UNIVERSITY, CAMPUS UNIVERSITÁRIO DE SANTIAGO, AVEIRO
Lillian BARROS / CENTRO DE INVESTIGAÇÃO DE MONTANHA (CIMO), INSTITUTO POLITÉCNICO DE BRAGANÇA, CAMPUS DE SANTA APOLÓNIA, BRAGANÇA
Corresponding author : Isabel C.F.R. FERREIRA / iferreira@ipb.pt

PURPOSE OF THE ABSTRACT

Natural products are widely recognized as a source of high-value compounds that can act as antioxidant, antimicrobial, anti-inflammatory, and natural colouring agents, among other functions [1]. In this work, the green husks of walnuts (Juglans regia L.) were selected (Figure 1), aiming to valorise these bio-residues by studying their chemical profile and bioactive properties.

First, the phytochemical composition of the hydroethanolic extract of green husks was evaluated by liquid chromatography combined with a diode array detector and electrospray ionization mass spectrometer (LC-DAD-ESI/MSn). Sixteen compounds were identified: one organic acid, two hydroxycinnamic acids, six tetralone derivatives, three naphthalene derivatives and four quercetin derivatives, with a total yield of 27.6 ± 0.2 mg/g dry extract (Figure 2).

Then, the bioactivity of the extract was assessed in four dimensions: citotoxicity, anti-inflammatory, antioxidant and antibacterial activities. The cytotoxicity was evaluated using the following tumor cell lines: non-small lung cancer (NCI-H460), breast (MCF-7), cervical (HeLa) and hepatocellular (HepG2) carcinomas. Similar activity against all tested tumor cell lines was obtained, with higher cytotoxic potential against HepG2 (IC50 = 24 ± 2 µg/mL) and MCF-7 (IC50 = 26 ± 1 µg/mL). Regarding the results using non-tumor porcine liver primary cells (PLP2), the walnut green husks extract also revealed to be toxic but at higher concentrations, with GI50 = 87 ± 4 µg/mL.

The anti-inflammatory activity was evaluated using a methodology based on the inhibition of the nitric oxide production by macrophages. An EC50 value of 56 ± 3 µg/mL was obtained, higher than the positive control value using dexamethasone (EC50 = 16 ± 1 µg/mL). The antioxidant activity was also evaluated. To this end, the thiobarbituric acid reactive substances (TBARS) and the oxidative haemolysis inhibition (OxHLIA) assays were

applied. In this case, IC50 values of 101 ± 4 µg/mL and 80 ± 4 µg/mL were found for TBARS and OxHLIA assays, respectively.
Finally, considering the antimicrobial activity, the extract was able to inhibit both Gram-negative (Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, and Morganella morganii) and Gram-positive bacteria (Enterococcus faecalis, Listeria monocytogenes, methicillin-resistant Staphylococcus aureus - MRSA), with higher impact against the MRSA strain (minimum inhibitory concentration MIC = 5 mg/mL).
This comprehensive study, using in vitro cell models, revealed that the hydroethanolic extract of green husks presented significant antioxidant, anti-inflammatory, cytotoxic and antibacterial activities, representing a stepping stone for the development of future applications.

Acknowledgements:
The authors thank the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UID/AGR/00690/2019), L. Barros, R. Calhelha and Carla Pereira contracts and V. Vieira grant (SFRH/BD/108487/2015). To UID/CTM/50011/2019 (CICECO), financed by national funds through the FCT/MCTES. To POCI-01-0145-FEDER-006984 (LA LSRE-LCM), funded by ERDF, through POCI-COMPETE2020 and FCT. To project AllNat - POCI-01-0145-FEDER-030463, financed by national funds through the FCT, and by COMPETE and Portugal2020, and to FEDER-Interreg España-Portugal programme for financial support through the project 0377_Iberphenol_6_E.

FIGURES

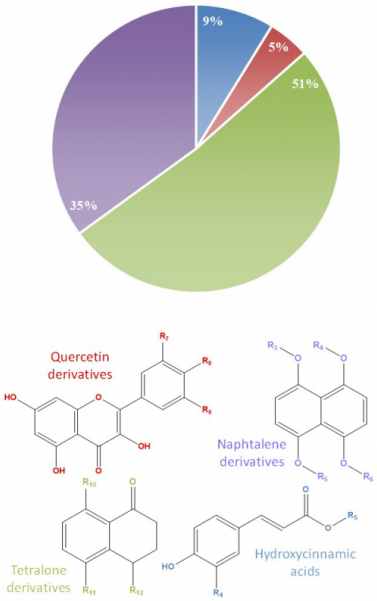

FIGURE 1
The green husks of walnuts.

FIGURE 2
Main families of compounds found in the hydroethanolic extract of green husks.

KEYWORDS
Bio-residues | Walnut green husks | Bioactive compounds | Phytochemical profile

BIBLIOGRAPHY

[1] Rostagno, M.A.; Prado, J.M. Natural Product Extraction; Green Chemistry Series; The Royal Society of Chemistry, 2013.