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File: ■ Nettle (*Urtica* spp., Urticaceae)
■ Antioxidant
■ Lab Analysis

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RE: Safety, Antioxidant, and Anti-inflammatory Profile and Phenolic Composition of Nettle Species

Carvalho AR, Costa G, Figueirinha A, et al. *Urtica* spp.: Phenolic composition, safety, antioxidant and anti-inflammatory activities. *Food Res Int*. September 2017;99(Pt 1):485-494.

Nettle (*Urtica* spp., Urticaceae) is a midsized, stinging plant with serrated leaves, and is used in the textile, paper, cosmetic, and food industries, as well as in the medical world. Protein, fiber, and vitamins are all present in nettle, as are anti-inflammatory compounds such as phenols. The purpose of this study was to evaluate the phenolic composition, antioxidant and anti-inflammatory properties, and safety of the following three species: stinging nettle (*U. dioica*), membranous nettle (*U. membranacea*), and burning nettle (*U. urens*).

Confraria da Urtiga, Portugal (Fornos de Algodres, Portugal), provided aerial parts for the three species. Plant collection occurred in March 2012 in Serra da Estrela, a Portuguese mountain range. To prepare the nettles for study, extracts were obtained by macerating 10 g of the powdered aerial parts in 50% aqueous ethanol (per volume) (200 ml) for 24 hours, under magnetic stirring. The extracts were then vacuum-filtered, concentrated in a rotavapor, frozen, and freeze-dried and stored in the dark at −20°C until use.

The authors sought to identify any phenolic compounds, total phenolic content (TPC), total flavonoid content (TFC), and total hydroxycinnamic acid content (THC). They also evaluated antioxidant activity with DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity, ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity at a pH of 4.5, ABTS radical scavenging activity at a pH of 7.4, and the ferric reducing antioxidant power (FRAP) assay.

Anti-inflammatory activity was assessed through nitrite accumulation, as nitric oxide is an inflammatory mediator. Macrophages were cultured, pre-incubated with different concentrations of nettle extract for one hour, then activated with lipopolysaccharide

(LPS) for 24 hours. (LPS can stimulate many cells to produce nitric oxide.) Nitrite production (% of control) was then measured.

To test the safety of nettle, the authors used hydroalcoholic extracts from the aerial parts in an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. The MTT assay assesses cell metabolic activity; under the right conditions, it can indicate cell viability. Raw 264.7, a mouse leukemic monocyte macrophage cell line, and HepG2, a human hepatic carcinoma cell line, were used in the assay.

HPLC-PDA-ESI/MS^n (high-performance liquid chromatography with photodiode array and electrospray ionization mass spectrometry) and published data were used to analyze the extracts' phenolic composition. The following polyphenols were found: caffeic and *p*-coumaric acid esters, ferulic and sinapic acid derivatives, *C*-glycosylflavonoids, *O*-glycosylflavonoids, and hydroxymethylglutaryl flavonoid glycosides. The identified polyphenols, their ultraviolet (UV) maximum absorption, and MS^n fragmentation pattern in negative electrospray ionization conditions are found in Table 1.

Stinging nettle had the highest TPC, 7.9 ± 1.1 (expressed in g/100 g lyophilized). Next was membranous nettle (2.8 ± 1.2), then burning nettle (0.8 ± 1.3). Membranous nettle had the highest TFC, 2.14 ± 0.4; second was stinging nettle (0.22 ± 0.08), then burning nettle (level not detected). Stinging nettle also had the highest THC, 2.54 ± 0.14, followed by membranous nettle (0.82 ± 0.09), then burning nettle (0.66 ± 0.04).

Antioxidant activity corresponded with TPC. Stinging nettle had the highest antioxidant activity as assessed by DPPH, ABTS (pH 4.5), ABTS (pH 7.4), and FRAP. Membranous nettle had the second highest antioxidant activity (and phenolic content), and burning nettle had the least antioxidant activity (and phenolic content).

Spearman's rank-order correlation coefficient (Spearman's) determines how strong the monotonic association is between two variables and the direction of that association. The authors used Spearman's to evaluate the relationships between TPC, TFC, THC, and the extracts' antiradical and antioxidant powers. The strong correlations between TPC and DPPH, ABTS, and FRAP provide evidence that antiradical and antioxidant capacity are due to the phenolic component. There was also a strong correlation between TPC and THC, indicating that the extracts' antioxidant and antiradical potential are from hydroxycinnamic acid.

All extracts inhibited macrophage production of nitric oxide. Burning nettle, at a concentration of 350 µg/mL, decreased it the most, by 41%, relative to control ($P<0.001$).

Safety results were reported as percentage of MTT reduction compared to control. HepG2 cells treated with stinging nettle 350 µg/mL had the lowest outcome (74.60 ± 18.94); Raw 264.7 treated with membranous nettle 350 µg/mL had the highest (127.13 ± 13.30). The authors assert these results indicate "all the extracts are clearly devoid of toxicity … ." They explain that the increase in MTT reduction could be from increased metabolic activity or cell proliferation and should be studied more.

In conclusion, the authors identified polyphenols and assessed the biological activity and safety of the nettle extracts. Among the identified compounds were statin-like

polyphenols that could have cholesterol-lowering activity and should be further studied. Their characterization of membranous nettle showed for the first time its difference from the other two nettles, in particular its high flavonoid content. Stinging nettle had a higher antioxidant potential, while burning nettle had greater in vitro anti-inflammatory action. Furthermore, the authors note additional research is warranted in order to elucidate the specific mechanism by which the various constituents exert their anti-inflammatory activity, as well as to confirm toxicity in in vivo models. Regarding safety, the authors conclude the extracts were not cytotoxic as studied. The authors claim their findings open new ways for using nettles nutritionally and pharmaceutically.

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—Heather Anderson, MD

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