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File: ■ Hibiscus (*Hibiscus sabdariffa*, Malvaceae)
■ Antioxidants
■ Extraction

HC 091423-513

Date: January 30, 2015

RE: Water Extraction Yields the Highest Concentration of Phenolic Compounds from Hibiscus Flowers

Sindi HA, Marshall LJ, Morgan MR. Comparative chemical and biochemical analysis of extracts of *Hibiscus sabdariffa*. *Food Chem*. December 2014;164:23-29.

Hibiscus (*Hibiscus sabdariffa*, Malvaceae) flowers are commonly used for tea and herbal supplements and contain a number of anthocyanins. The four main anthocyanins found in hibiscus flowers are delphinidin 3-*O*-sambubioside, delphinidin 3-*O*-glucoside, cyanidin 3-*O*-sambubioside, and cyanidin 3-*O*-glucoside. Hibiscus extracts have been used in traditional medicine to treat a number of illnesses and have been found to have anticancer, antimicrobial, and antioxidant properties. It is difficult to compare the results among hibiscus studies because of the variability of the source material and extraction techniques. The goal of the current study was to compare the effect of extraction solvent, temperature, and duration on the yield in total phenols and on the antioxidant capacity of dried hibiscus flowers.

The sundried hibiscus flowers used for extraction were obtained from a market in Jeddah, Saudi Arabia. The flowers were originally from Sudan. The dried flowers were ground, and 100 mg of material was extracted with 10 ml of solvent. The solvents used were water, methanol, ethyl acetate, and hexane, all with or without 1% (per volume) formic acid. The tissues were extracted at three temperatures (25°C, 50°C, and solvent boiling point) and three durations (three, five, and ten minutes). The extracts were filtered prior to analysis. Total phenolic concentration was measured with the Folin assay and expressed as gallic acid equivalents. Total anthocyanins were measured by ultraviolet/visible (UV/Vis) spectrophotometry using a pH differential method, and calculated as cyanidin 3-*O*-glucoside equivalents. The following three tests for antioxidant capacity were conducted: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, the ferric reducing antioxidant power (FRAP) assay, and the total equivalent antioxidant capacity (TEAC) assay. High-performance liquid chromatography (HPLC) with UV/Vis detection at 520 nm was used to quantify the four major anthocyanins. Data were compared with Pearson correlation coefficients and analysis of variance.

There was not a single extraction time that was optimal for all of the tests. TEAC was

unaffected by the extraction duration. Total phenolic concentration was highest using an extraction time of ten minutes ($P = 0.02$), while FRAP was lowest at ten minutes ($P < 0.05$). DPPH was highest after extraction for three minutes ($P < 0.01$). Further comparisons of extraction techniques were made using a ten-minute extraction time. With regard to extraction temperature, for both DPPH and FRAP, the solvent boiling point resulted in the highest values ($P = 0.001$ for both). Total anthocyanin concentration was also higher at the solvent boiling point, while total phenolic concentration was highest at 50°C ($P < 0.05$ for both). Extraction with water, with or without formic acid, yielded the best results for total phenolics, DPPH, FRAP, TEAC, and total anthocyanins. Methanol extraction resulted in the second best outcome for all measurements. Using ethyl acetate and hexane resulted in hibiscus extracts with negligible total phenolic and anthocyanin contents and also no activities on the DPPH and TEAC assays. When the extracts were analyzed by HPLC, four anthocyanins were found in the water and methanol extracts. The two major anthocyanins were delphinidin 3-*O*-sambubioside and cyanidin 3-*O*-sambubioside, while delphinidin 3-*O*-glucoside and cyanidin 3-*O*-glucoside were found only in low concentrations. These compounds were not found in either the ethyl acetate or the hexane extract.

Water was considered the best solvent for extracting the four anthocyanins found in the hibiscus flowers used in this study. The addition of formic acid had little effect on extraction efficiency. This is in contrast with another study in which the addition of formic acid increased the extraction efficiency. The optimal duration of the extraction varied with the assay. Total phenolics were highest after ten minutes of extraction, while an extraction time of less than ten minutes resulted in higher FRAP and DPPH values. The authors note that, while no anthocyanins were detected in the ethyl acetate and hexane extracts, other important bioactive compounds not measured were likely present. Other studies have recommended combining extracts made with at least two solvents in order to increase the range of compounds present. Assays appropriate to each solvent must also be used to take into account the polarity of the compounds found within those solvents.

–Cheryl McCutchan, PhD

The American Botanical Council has chosen not to include the original article.