Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods. Analytical Methods

1. Chemicals and reagents

HPLC grade acetonitrile, methanol and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Water was obtained using a milliQ-Gradient system. Ascorbic acid, chicoric acid, caftaric acid, adenosine, folic acid, 6-gingerol, rosmarinic acid, berberine, cyanidin 3-O-sambubioside-5-O-glucoside, isoquercitrin, isorhamnetin 3-O-rutinoside, cyanidin 3-O-sambubioside, cyanidin 3-O-glucoside, chlorogenic acid, rutin, and quercetin were purchased from Sigma (St. Louis, MO, USA).

2. Preparation of dietary supplement samples

The dietary supplements were encountered in the form of either capsules, tablets, powders or liquids. For capsules, 5 items were weighed, opened and their contents were mixed and triturated in a mortar and pestle prior. Each dietary supplement purchased as powders or capsules or tablets, about 1000 mg for powders and average weight in case of capsule content or tablets were weighed into centrifuge tubes, re-suspended with methanol, vortex and sonicated for 30 minutes, following centrifugation for 15 minutes at 959 x g. The procedure was repeated for three times and the clear supernatant was subsequently transferred to a 10 mL volumetric flask. The final volume was adjusted with methanol to 10 mL and mixed thoroughly. Prior to injection, the samples were filtered through a 0.45µm polytetrafluoroethylene (PTFE) membrane filter. For dietary supplements purchased in liquid form, a 1:1 dilution was prepared in methanol, filtered and injected.

3. Instrumentation

Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC-QToF-MS)

The analytical methodology is same as reported elsewhere.\(^1\)\(^2\)\(^3\) The liquid chromatographic system is an Agilent Series 1290 and the mass spectrometric analysis was performed with a QToF-MS/MS (Model #G6530A, Agilent Technologies, Palo Alto, CA, USA) equipped with an ESI source with Jet Stream technology. All the operations, acquisition and analysis of data were controlled by Agilent MassHunter Acquisition Software Ver. A.01.00 and operated under MassHunter Workstation software Ver. B.02.00. Each sample was analyzed in both positive and negative modes to provide abundant information for structural identification. Mass spectra are recorded across the range m/z = 50-1700 with accurate mass measurement of all mass peaks. MassHunter Workstation software, including Qualitative Analysis (version B.07.00), was used for processing both raw MS and MS-MS data, including molecular feature extraction, background subtraction, data filtering, and molecular formula estimation. The raw data were processed using the Find by Molecular Feature (MF) algorithm called Molecular Feature Extractor (MFE) within MassHunter Qualitative Analysis software. Extracted molecular features were processed to create a list of compounds.

A compound search for the non-targeted compounds were characterized by matching the experimental molecular formula in an [a] The Agilent MassHunter Forensics and Toxicology (>9000 components) Personal Compound Database (PCD) [b] In-House generated library for 11,000 components of medicinal plant samples. Other search engines included SciFinder (web-based version), Dictionary of Natural product (CRC, 2021), and google search engines by molecular formulae were used for the identification of “known unknowns.” These approaches have been utilized to identify a wide range of components, including additives, compounds from natural products, etc. In-house library includes the compound name, molecular formula, exact mass, CAS #, and structure of each compound. From the possible positive hits, the results were compared with MS-MS experiments and to those available in literature. All compounds either generated a high-abundance [M-H]\(^-\) or/and [M+HCOO]\(^-\) ion in negative mode or a high-abundance [M+H]\(^+\) or/and [M+Na]\(^+\) ion in positive mode, therefore, the [M-H]\(^-\) or [M+H]\(^+\) ions of each compound were selected as the precursor ions for subsequent MS-MS experiments to give more fragment ions. The generation of diagnostic fragment ions provided information concerning the core skeleton and nature of the substituents.
4. Analysis of Dietary Supplements using LC-QToF

In this study, all dietary supplements were analyzed using LC-QToF method. The operation parameters (fragmentation energies, gas flows and temperatures) of a high-resolution mass spectrometer were designed to deliver high-accuracy qualitative data for the identification of components from botanicals. A full scan MS and MS-MS modes using QToF-MS is effective and sensitive in exploring the identifications of both target and non-target compounds from supplements. The methanolic extracts were subjected to both negative and positive ion modes.

[a] Identification of single component ingredients

The identification of single compound ingredients including water soluble vitamins, quercetin, berberine, melatonin, theanine, biotin, amino acids, beta-hydroxy-beta-methylbutyrate, methylsulfonylmethane, were detected based on the accurate or exact mass and MS-MS spectrum of compounds and in some cases comparing with standard compounds.

Products containing folic acid (C19H19N7O6 m/z 442.147 [M+H]+; [M-H] m/z 440.1324) was detected in 2 products [#9 and 23] and not detected in one product [#7]. Vitamin B12 (C63H88CoN15O14P5 m/z 1355.5747 [M+H]+/678.2913 [M+2H]2+) was detected from product # 7 and 23 but was not detected in product # 21. Similar the case with Zinc carnosine (C21H12N4O6Zn m/z 289.0274 [M+H]+) or carnosine (C21H14N6O3 m/z 227.1139 [M+H]+) which was not detected from product # 13.

[b] Identification of botanical ingredients

The botanical raw materials vary greatly in their chemical compositions due to batch variation, cultivar variation, harvesting, processing etc., but the species-specific compounds of any botanical collected at any geographic region will not differ. The content of the compounds will vary but not the species-specific compounds. We usually look for multiple compounds for any botanical ingredient rather than single compound. Well-developed botanical extracts have a characteristic chemical profile or fingerprint that can be used to both determine identity with a high degree of confidence by looking at the suite of compounds. Most of the compound’s attribution is done based on the reported papers/literature. If botanical ingredient extracts are added in low amount (<5 mg), detection might become difficult.

LC-QToF provides the greatest confidence for ensuring the identity with fewer false positives.

For example, elderberry extract (S. nigra) fruit detection is attributed to the presence of anthocyanins [cyanidin 3-O-sambioside-5-O-glucoside (m/z 743.2036 C23H29O20+), cyanidin 3-O-sambubioside m/z 581.1506 C26H29O15+], cyanidin 3-O-glucoside m/z 449.1084 C21H31O11+] and phenolic compounds [chlorogenic acid C16H18O8 m/z 355.1024, rutin C27H30O16 m/z 611.1607, isoquercitrin C21H20O12 m/z 465.1028, isorhamnetin 3-O-rutinoside C25H2O16 m/z 625.1769, quercetin C21H16O7 m/z 303.0499].

Overall, 3 products [#5, 18, 22] containing elderberry were adulterated with Oryza sativa (black rice), which is based on the detection of peonidin 3-O-glucoside and relatively high concentrations of cyanidin 3-O-glucoside in these samples. One product [#30] containing S. nigra fruit extract, could not detect for its presence.

Another example shown for products [#4 and 14] containing quercetin is also spiked with pure flavonoids including kaempferol, rutin, isoquercitrin, isorhamnetin etc or extracts from other flavonoid-rich plant sources.

All other plants not detected in the analyzed dietary supplements are listed in detail with chemical constituents in eTable

eReferences

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## eTable. Phytochemicals Reported for the Botanical Plant Parts Used in the Study

| Product #’s | Common name/ (Scientific name) | Family | Plant Part | Phytochemicals | References |
|-------------|--------------------------------|--------|------------|----------------|------------|
| 30 | Elderberry (Sambucus nigra) | Adoxaceae | Fruit | **Anthocyanins:** Cyanidin 3-O-sambioside-5-O-glucoside, cyanidin 3-O-sambubioside, cyanidin 3-O-glucoside | [1] [28] |
| | | | | **Phenolic compounds:** Chlorogenic acid, rutin, isouquercitrin, isorhamnetin 3-O-rutinoside, quercetin | | |
| 5, 19, 22, 28, 30 | Ginger (Zingiber officinale L.) | Zingiberaeae | Rhizome | **Phenol compounds:** 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 10-shogaol | [2] [3] [28] |
| 19, 28 | Oregano (Origanum vulgare L.) | Lamiaceae | Leaf | **Phenolic acids and flavonoids:** Sinapic acid, 2-hydroxybenzoic acid, m-coumaric acid, rosmarinic acid, 3,7-dimethylquercetin, dihydrobiochanin A, luteolin 7-O-gluconuride | [4] [28] |
| 11 | Horehound (Marrubium vulgare) | Lamiaceae | Aerial parts | **Phenols and flavonoids:** Marrubin, marruboside, forsynthoside B, samioside, marrubenol, verbascoside, 12-hydroxymarrubin, apigenin 7-(2-glucosyl)lactate, luteolin 7-lactate | [5] [28] |
| 12, 19, 28, 30 | Siberian ginseng (Eleutherococcus senticosus) | Araliaceae | Root | **Eleutherosides, flavonoids and phenolic acids:** Eleutheroside B1, Eleutheroside E2, Eleutheroside D, Eleutheroside A-D, hyperin, rutin, afzelin, quercetin, and kaempferol, gallic, protocatechuic, gentisic, 4-OH-benzoic, 3-OH-benzoic, vanillic, *trans*-caffeic, *cis*-caffeic, syringic, *trans*-p-coumaric, *trans*-ferulic, veratic, salicylic, 3-OH-cinnamic, *trans*-sinapic, and *cis*-sinapic acid | [6] [28] |
| 18 | Solomon’s-Seal (Polygonatum odoratum) | Asparagaceae | Rhizome | **Steroidal sapogenins and glycosides/homoiosflavones:** 3-O-β-D-glucopyranosyl-(1→2)-[β-D-xylo-pyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-galactopyranosyl(25S)-spiror-5(6)-en-3β-ol, 3-O-β-D-glucopyranosyl-(1→2)-[β-D-xylo-pyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-galactopyranosyl-(25S)-spiror-5(6),14(15)-dien-3β-ol and 3-O-β-D-glucopyranosyl-(1→2)-[β-D-xylo-pyranosyl-(1→3)]-β-D-glucopyranosyl(1→4)-galactopyranosyl(25S)-spiror-5(6)-en-3β, 14α-diol, polygodosides A-F, polygodosin A, 5,7-dihydroxy-4′-methoxy-6,8-dimethyl-homoiosoflavone, 4′, 5, 7-trihydroxy-6,8-dimethyl-homoiosoflavone, 4′,5,7-trihydroxy-6-ethyl-homoiosoflavone and 5,7-dihydroxy-4′,8-dimethyl-6-ethyl-homoiosoflavone | [7] [8] [9] [28] |
| 18, 30 | Astragalus (Astragalus membranaceus) | Fabaceae | Root | **Saponins and flavonoids:** Cycloartane- and oleanane-type saponins including Astramembranosides A/B, Astragaloside I, Astragaloside II, Astragaloside A, Astragaloside IV-VII | [10] [28] |
| 10, 17, 18 | Woad (Isatis tinctoria) | Brassicaceae | Leaf/Root | **Alkaloids, phenolic acids and flavonoids:** **Leaf:** p-hydroxybenzoic, *o*-methoxybenzoic, *p*-methoxybenzoic, dihydrocaffeic, ferulic, sinapic, salicylic, vanillic, and 4-hydroxyphenylacetic acids, Isandigotone, Indigotisocoumarin A, Isandigotidione Vicenin-2, Isoscoparine, Luteolin-6-C-glucoside-7-O-glucoside, Luteolin-6-glucuronide | [11] [28] |
| 18 | Pau D’Arco | Bigoniaceae | Bark | **Flavonoids/ iridoids/ lignans/phenolic acids:** | [12] [28] |
| Species | Family | Part | Contents |
|---------|--------|------|----------|
| Goldthread (Coptis) | Ranunculaceae | Rhizome | Protobberine-type alkaloids: Berberine, coptisine, jatrorrhizine, palmatine, columbamine, epiberberine, and magnoflorine |
| Japanese catnip (Schizonepeta tenuifolia) | Lamiaceae | Aerial parts | Terpenes/Phenolics: (-)-pulegone, piperitene, schizonepethoside A, schizonepethoside C, (+)-spatulenol, ursolic acid, 2α,3α,24α-trihydroxyolean-12-en-28-oic acid, 5α,8α-epidioxyergosta-6,22-diol-3β-ol, stigmast-4-en-3-one Rosmarinic acid, apigenin-7-O-β-D-glucopyranoside, luteolin-7-O-β-D-glucuronopyranoside, hesperidin, luteolin, diosmetin |
| Polygala tenuifolia | Polygalaceae | Root | Saponins, xanthones: sibiricose A5, sibiricose A6, glomeratose A, tenuifoliside B, tenuifoliside C, sibiricaxanthone B, and polygalaxanthone III |
| Garlic (Allium sativum L.) | Amaryllidaceae | Bulb | Polyphenols and organosulfur compounds: γ-glutamyl-S-alk(en)yl-L-cysteines and S-alk(en)yl-L-cysteine sulfoxides, allicin and deoxyalliin and particularly L-allii as the major sulfur-containing compound |
| Licorice (Glycyrrhiza uralensis, G. glabra, G. inflata) | Fabaceae | Root | Flavonoids and Triterpene Saponins: Species-specific markers including glabridin, glycybridins, hispaglabridins, glabrol from G. glabra, glycycomarin, licoflavonol, licoisoflavone A/B from G. uralensis, and licochalcones A-E from G. inflata were identified. Liquiritin apioside, 4'-O-glucopyranoside, luteolin, liquiritigenin, naringenin, glycyrrhizin, formononetin were detected in all species of Glycyrrhiza |
| Slippery elm (Ulmus rubra Muhl) | Ulmaceae | Inner Bark | Triterpenes: Oleanolic acid, ursoic acid, uvaol, betulinic acid |
| Aloe vera | Asphodelaceae | Leaf | Anthraquinones: Aloe-emodin, Emodin, Aloin A/B |
| Tasmanian blue gum (Eucalyptus globulus) | Myrtaceae | Leaf | Terpenoids, tannins, flavonoids and phloroglucinol derivatives: gallic acid, eucaglobulin, globulisin B, globulol, euglobal Ia1, euglobal Ib1, euglobal Ic, euglobal Ia2, euglobal Ia, euglobal Ib, 2,4-diformylphloroglucinol, quercetin 3-O-glycoside, quercetin 3-O-rhamnoside, quercetin 3-O-β-D-glucuronide. |
| Goldenseal (Hydrastis canadensis L.) | Ranunculaceae | Root | Isoquinoline alkaloids: β-hydrastine, hydrastine, berberine, berberastine, canadine |

*These plants described in the eTable 1 claimed in some of the dietary supplements were not detected and the product codes of these supplements are listed in column 1 of this table to align with products listed in Table 1 of the main article.

References to eTable 1:

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