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## Unlisted ingredients in teas and herbal brews revealed in DNA tests by high school students

Take a second look at your iced or steaming tea. Guided by scientific experts, three New York City high school students using tabletop DNA technologies found several herbal brews and a few brands of tea contain ingredients unlisted on the manufacturers' package.

The teen sleuths also demonstrated new-to-science genetic variation between broad-leaf teas from exported from India versus small-leaf teas exported from China.

Guided by DNA "barcoding" experts at The Rockefeller University, an ethno-botanist at Tufts University and a molecular botany expert at The New York Botanical Garden, co-authors Catherine Gamble, 18, Rohan Kirpekar, 18, and Grace Young, 15, of Trinity School in Manhattan, published their findings today in the *Nature* journal *Scientific Reports*.

The unlisted ingredients included weeds such as annual bluegrass and herbal plants such as chamomile. The surprise ingredients are mostly harmless but could affect a tiny minority of consumers with acute allergies. Three (4 percent) of the 70 tea products tested and 21 (35 percent) of 60 herbal products had unlisted ingredients.

For example, DNA testing showed that an herbal infusion labelled "St. John's wort" (*Hypericum perforatum*) included material from a fern in genus *Terpischore*. A DNA "barcode" obtained from another herbal tea labelled "ginger root, linden, lemon peel, blackberry leaves, and lemongrass" matched annual bluegrass (*Poa annua*), a common weed unrelated to lemongrass. Four herbal infusions yielded sequences identical or nearly identical to the tea plant, *C. sinensis* but none listed "tea" as an ingredient. The most common non-label ingredient, found in seven herbal products, was chamomile (*Matricaria recutita*).

Four products yielded barcodes of plants closely matching parsley, but none listed ingredients in that plant family.

Other unlisted ingredients included the common weeds white goosefoot (*Chenopodium album*) and red bartsia (*Odontites vernus*); a garden flower, lantana (*Lantana spp.*); an ornamental tree, Taiwanese cheesewood (*Pittosporum pentandrum*); and herbal plants such as alfalfa (*Medicago sativa*), lemon balm (*Melissa officinalis*), heal-all (*Prunella vulgaris*), blackberry (*Rubus spp.*) and papaya (*Carica papaya*).

"After water, tea and its many herbal variations represent the world's most popular beverage – by far. Literally billions of cups are consumed every day, more than all the coffee, pop and every other drink combined," says Gamble, who begins studies at Harvard in September. "What's in those little bags of tea and herbal tea products is a matter of interest to billions of people."

"It's important to list every ingredient in a product because some people need to be very careful about what they consume," says Kirpekar, who enters Columbia in the fall. "Allergy symptoms might be just watery eyes but some people can get more seriously sick and they'd never know the reason was in their comforting hot drink. We were surprised to find many herbal teas in particular with unlisted ingredients."

"It's a mystery why ingredients are unlisted," adds Young, who can make the rare boast at 15 of having co-authored a peer-reviewed academic paper. "It might just be a weed picked up during harvesting or the residue of a plant used in one product gets passed to the next product in a processing facility."

"Maybe unlisted ingredients like chamomile or parsley are added to provide flavour or color to herbal teas, serving the same purpose as garlic or onion in cooking. Perhaps manufacturers want to sell full looking bags and pad them with filler."

"All of that, though, is speculation; proper answers require a completely different type of investigation."

DNA barcoding technology identifies and distinguishes known and unknown species quickly, cheaply, easily and accurately based on a snippet of genetic code. Experts at various institutions around the world are building an authoritative library of DNA barcodes for both plants and animals.

Teas are made from leaves of the tea plant, *Camellia sinensis*; herbal infusions, which are often called "teas," use the roots, leaves, stems, seeds, or flowers of many different plants. But appearance does not easily identify the bits of dried plants, which sometimes are also cooked or fermented, that are used to prepare infusions and teas. The researchers found the plant DNA extremely resilient and obtained barcodes from 90 percent of the 146 products sampled

The products, half teas and half herbals, from 33 different manufacturers spanning 17 countries, were collected or purchased at 25 locations in New York City including stores, school dining halls, and the homes of the investigators.

Some of the DNA extractions and amplifications were carried out on a dining room table in the apartment of mentor and barcoding expert Mark Stoeckle, an adjunct faculty member with the Program for the Human Environment at The Rockefeller University.

The lab equipment was purchased used on the Internet for about \$5,000, highlighting the viability of public DNA-based plant identification. After extracting and amplifying the DNA in the home lab, the samples were mailed to a commercial DNA sequencing facility. The total cost was about \$15 per sample and took about 24 hours in total.

Most of the DNA analysis was done at The New York Botanical Garden. When the students obtained a DNA sequence, they checked it using the GenBank DNA database maintained by the US National Library of Medicine, that retrieves matching sequences and candidate species names almost instantly.

"These results demonstrate a low-cost approach for plant identification that could be used in educational, regulatory, and research settings to produce practical information and scientific insight," says Stoeckle.

Also mentoring the students were Jesse Ausubel, director of the Program for the Human Environment at Rockefeller University and vice-president of the Alfred P. Sloan Foundation; Damon Little of the Lewis B. and Dorothy Cullman Program for Molecular Systematics, The New York Botanical Garden; and Selena Ahmed of Tufts University. One of the world's top experts on teas, Ahmed co-authored the 2011 book *Tea Horse Road* about tea production, trade, and ancient tea rituals in southwestern China where tea drinking probably originated.

In addition to the unlisted ingredients, the young scientists helped discover that the tea plant includes a genetic difference between broad-leaf assamica variety tea exported from India and small-leaf sinensis variety tea exported from China, the two largest tea-producing countries by far.

"We were excited to make a genetic discovery, particularly in an important crop plant like tea that scientists have scrutinized in detail," says Young.

"This finding will help track commercial shipments and aid research on the geographic origin and diversity of wild and cultivated tea plant resources," says Ahmed.

Says Little: "After tea ingredients are dried and processed into powders it is very difficult to determine which species the ingredients came from. This study demonstrates the power of DNA barcoding to easily and rapidly identify plant materials. I hope that manufacturers will adopt this technology for quality assurance."

The students also helped construct a "Klee diagram," a clever new way to visually represent the genetic relationship between species. Like a heat map, where hot is shown in red and cold in blue, their Klee diagram depicts the genetic relationships among and within the families of plants consumed as teas, using a color scale from red (a close genetic relationship) to blue (distant). Based on the DNA of 39 plants tested, the image is the world's first family portrait of tea and herbal tea plants.

"The Klee diagram creates a genetic 'mood chart' for mint, ginger, linden and other herb teas," says Jesse Ausubel, director of the PHE at Rockefeller University. "The students grouped 39 plants used for herbal tea into six genetic blocks: one anchored by ginseng and parsley, another by Echinacea and the asters, a third large mint block encompassing also jasmine and lavender, a fourth fruity block with hibiscus and orange, a fifth block with rooibos and other beans, and a final block with lemongrass and other grassy plants."

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