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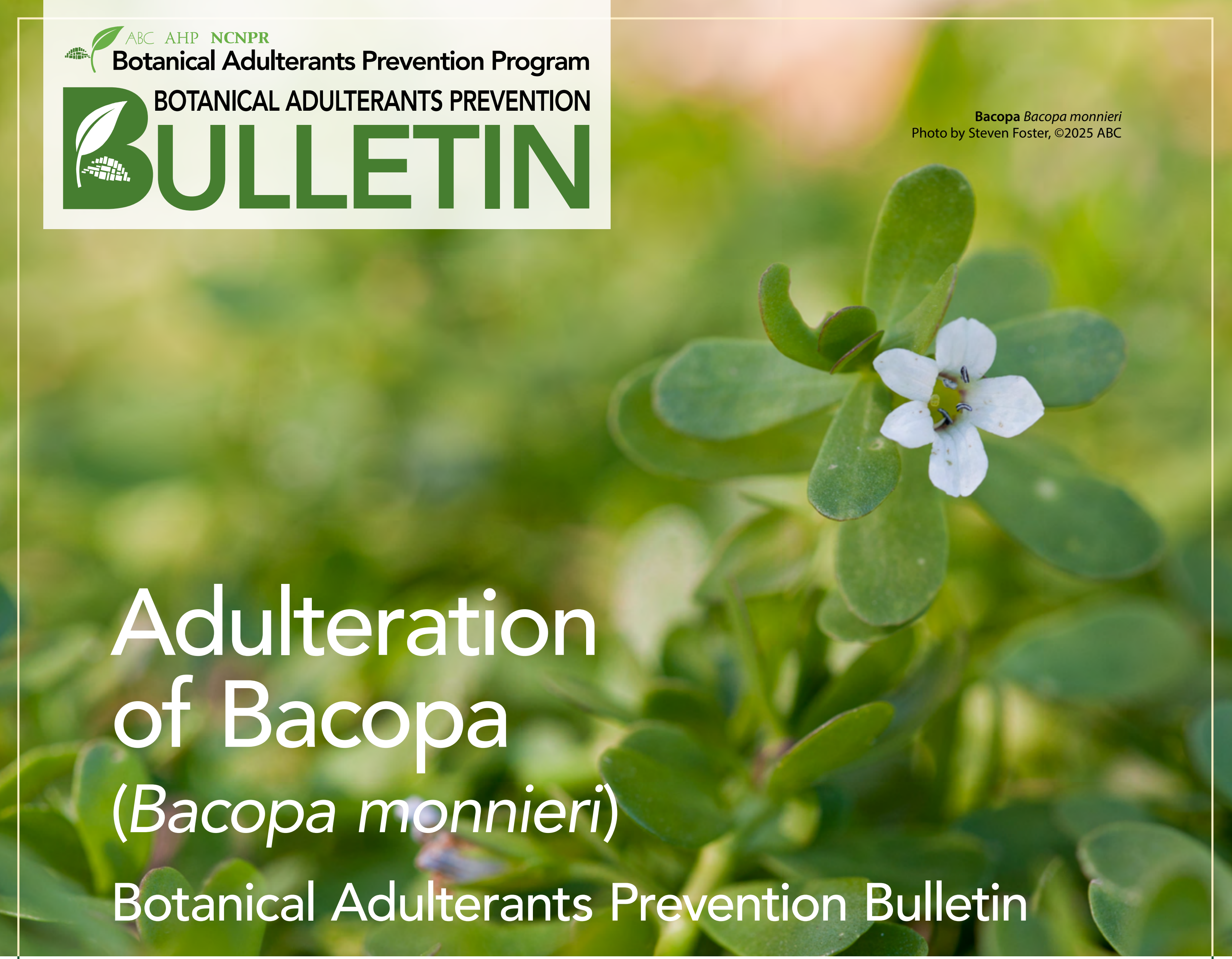
Botanical Adulterants Prevention Program
BOTANICAL ADULTERANTS PREVENTION BULLETIN



Bacopa *Bacopa monnieri*
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Adulteration of Bacopa (*Bacopa monnieri*)

Botanical Adulterants Prevention Bulletin

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Goal: The objective of this bulletin is to give a summary of general information on the aerial parts and extracts of bacopa (*Bacopa monnieri* (L.) Wettst.), including an overview of adulteration and mislabeling of bacopa raw material and its products, with some emphasis on gotu kola (*Centella asiatica*) as the most common potential substitute. Additionally, it aims to describe the trade dynamics and market trends, as well as laboratory techniques useful for detecting mislabeling and adulteration, while also addressing the economic and safety implications for both consumers and industry stakeholders. This bulletin may serve as a useful resource for quality control professionals as well as members of the international phytomedicine and botanical supplement sectors by providing guidance and awareness within the natural products community.

1. General Information

1.1 Common names:

Bacopa is the standardized common name for *Bacopa monnieri* in the United States according to the third edition of the *American Herbal Products Association's Herbs of Commerce.*[1] It is also known by the following English common names: coastal water hyssop, herb of grace, thyme-leafed gratiola, water hyssop, and white hyssop.[2-4] Bacopa shares the common Sanskrit name *brahmi* with another well-known medicinal plant, gotu kola (*Centella asiatica* [L.] Urban, Apiaceae), and similar therapeutic properties are attributed to both in Ayurvedic medicine. These two herbs are traded interchangeably on purpose or inadvertently.[5-7]

Gotu kola is the accepted standardized common name for *C. asiatica* in the United States per *Herbs of Commerce* and in many other countries.[1] Additionally, it is also known by other English common names such as Asiatic pennywort, centella, gotu cola, Indian pennywort, marsh pennywort, pennyweed, sheeprot, and spade leaf.[8,9]

In various parts of India, *B. monnieri* is known by the names *jala brahmi*, *neer brahmi*, *nirubrahmi*, and *nirabrahmi* which mean "water-brahmi" in reference to its marshy and aquatic habitat. Meanwhile, *mandukaparni* is now widely regarded as the correct Sanskrit name of *C. asiatica,* as its leaves resemble the shape of a frog's feet.

To avoid any confusion, the name bacopa is used for *B. monnieri* and gotu kola for *C. asiatica* throughout this document.

1.2 Common names in other languages:

1.2.1 Common names of bacopa in other languages[4,10-16]

Arabic: farfakh

Bengali: birami, brahmisaka

Chinese (Mandarin): jia ma chi xian (假马齿苋)

French: bacopa de monnier, bacopa des herbalists, bacopa médicinale, bramia de saint-paul, petite bacopa

German: Kleines Fettblatt, Brahmi

Hindi: adha-birni, afed chamni, barami, brahmi, farfakh, gundala, indravalli, jalbuti, jalnim, nirbrahmi, mandukaparni, safedchamani

Nepali: medhagiree

Persian: jaranab

Sanskrit: aindri, brahmi, gundala, indravalli, jalasaya, kapotavanka, manduki, matsyaksi, nirabrahmi, sarasvati, tiktalonika, toyavalli, vami, jala-brahmi, jala-primmi, mandukamata, matsyakshi, medhya, sarasvati, sureshta, survarchala, swayambhuvi, vaidhatri, vallari, vara, vira

Swedish: litet tjockblad, brahmi

Tamil: ahazndapoozndu, brahmi vazhukkai, nirabrahmi, nirpirami, piramivazhukkai, piramiyapundu

Telagu: neerisambraanimokka, sambarenu, sambrani, sambraanichettu, sambranichettu, sambrareniaaku, sambrani-aku, sambrani-chettu, sambranichettu

Thai: phrom mi, phak mi

Urdu: brahmi, brahmi buti, jalanim, jal brahmi, nirabrahmi

1.2.2 Common names of gotu kola in other languages[12,13,17,18]

Arabic: artaniyal-hindi, zarnab

Bengali: brahma-manduki, tholkhuri, thulkuri

Chinese (Mandarin): ji xue cao (积雪草)

French: écuelle d'eau, fausse violette, hydrocotyle Asiatique

German: Asiatisches Sumpfpfennigkraut, Indischer Wassernabel

Hindi: brahmamanduki, khulakudi

Nepali: kholachagya

Persian: sard Turkistan

Sanskrit: brahmi, cheka parni, mandukaparni, manduukaparni, manduukaparnikaa, maanduuki

Swedish: sallatsspikblad

Tamil: babassa, vallarai, vellarai

Telagu: brahmakuraku, manduka, saraswathi aku

Thai: buabok, bua-bog

Urdu: brahmi, brahmi buti

1.3 Accepted Latin binomials:

Bacopa: *Bacopa monnieri* (L.) Wettst.[16,19]

Gotu Kola: *Centella asiatica* (L.) Urban[17]

1.4 Synonyms:

Bacopa:

Homotypic Synonyms: *Anisocalyx limnanthiflorus* (L.) Hance, *Bramia monnieri* (L.) Drake, *Gratiola monnieri* (L.) L., *Herpestis monnieri* (L.) Rothm., *Moniera monnieri* (L.) Britton, *Lysimachia monnieri* L.[19]

Gotu Kola:

Homotypic Synonyms: *Hydrocotyle asiatica* L.[17]

The taxonomic resources of the Royal Botanical Garden, Kew include 32 heterotypic synonyms for bacopa and 37 for gotu kola.[17,19]

1.5 Botanical family:

Bacopa: Plantaginaceae[19]

Gotu kola: Apiaceae[17]

1.6 Distribution:

The genus *Bacopa* includes 55 to 70 species of aquatic herbs, most of which grow as amphibious plants and are distributed across tropical and subtropical regions worldwide.[10] *Bacopa monnieri* is native to a wide range of regions across the globe, including parts of North and South America, Asia, the Arabian Peninsula, Africa, Australia, and several islands of the Antilles, lesser Antilles, and the Gilbert and Laccadive islands. It can be found in countries such as Afghanistan, Argentina, India, Brazil, Pakistan, Nepal, Sri Lanka, and the United States, among many others. It is listed as invasive in Japan, Singapore, Spain, and Portugal.[10,12,16,19]

Centella asiatica is a perennial species native to the Caucasus, tropical and subtropical Africa, Asia, eastern Australia, South America, and various Pacific islands (Hawaii, Fiji, Samoa, Vanuatu, etc.).[8,17] Thus, bacopa and gotu kola grow in many of the same countries, as both plants are native to similar tropical and subtropical regions. They both prefer marshy, aquatic environments and grow well in similar conditions.

1.7 Plant part and form:

The aerial parts of bacopa, including stems and leaves, are dried and used either whole or as a fine powder.[20-23] The *Ayurvedic Pharmacopoeia of India* describes bacopa as a dried whole plant.[14] Bacopa is also consumed as fresh juice, infusion, decoction, tincture, syrup, tea, paste, pill, or eaten fresh (leaves).[15] Powdered bacopa and its extracts are marketed as capsules, caplets, tablets, syrups, or drops.[24] It is used as a functional food ingredient in fruit beverages, health-booster energy drinks, flavored granules, breakfast cereals, biscuits, memory enhancer tonics, etc.[25] Additionally, bacopa extracts and oil infusions are used in creams, massage oils, cosmetics, and hair and skincare products. It is consumed alone or with herbs like ginkgo (*Ginkgo biloba*, Ginkgoaceae) leaf or ashwagandha (*Withania somnifera*, Solanaceae) root as an herbal tea. In India, it is added to various foods (ice creams, soups, ghee, cereals, etc.) to enrich their nutritional value.[24] Whole aerial parts of gotu kola are similarly used in the same forms and preparations as bacopa.

Although the US Food and Drug Administration (FDA) does not classify bacopa as "Generally Recognized as Safe" (GRAS) for food, its use is allowed in dietary supplements under the Dietary Supplement Health and Education Act (DSHEA). In Australia, it is regulated as an active ingredient in complementary medicines with approved Ayurvedic use statements. In countries like Bangladesh, India, Malaysia, Pakistan, and Sri Lanka, where traditional Ayurvedic, Siddha, and Unani therapeutic systems are integrated into the country's healthcare system, bacopa is regulated as an active component in pharmaceutical products.[26]

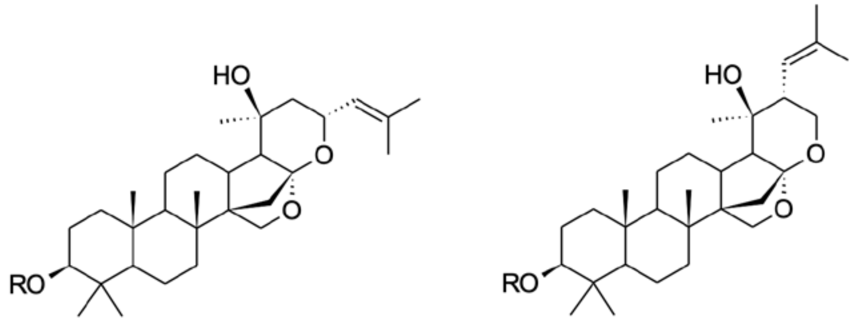
1.8 Key constituents and chemical markers of bacopa and gotu kola:

Bacopa contains alkaloids (brahmine, herpestine, and nicotine), cucurbitacins (bacobitacins A-D and cucurbitacin E), dammarane type triterpenoid saponins (bacopasaponins, bacopasides, and bacosides) with jujubogenin and pseudojujubogenin as the aglycones, pentacyclic triterpenes (betulinic acid and bacosine), sterols (β-sitosterol, stigmastanol, and stigmasterol), flavonoids (apigenin, luteolin, naringenin, oroxindin, quercetin, and wogonin), phenylethanoid glycosides (monnierasides I-III and plantainoside B), phenylpropanoids, and other miscellaneous compounds.[15,24,26,27] Bacoside A is a mixture of four triglycosidic saponins, called bacoside A3, bacopaside II, bacopasaponin C, and the jujubogenin isomer of bacosaponin C (syn: bacopaside X, bacopaside VII), while bacoside B, although shrouded with controversies, has been reported to consist of bacopaside N1, bacopaside N2, bacopaside IV and bacopaside V.[28] Powdered bacopa leaf samples typically contain about 1-3% bacoside A (D. Mundkinajeddu [Natural Remedies] email, December 2, 2024), and the content of the latter is known to have geographical and seasonal variations (4.35-10.50%).[29]

According to the *United States Pharmacopeia* (USP) monograph, bacopa (dried herb or its powder) must contain not less than 2.5% of triterpene glycosides, calculated on the dried basis, as the sum of bacopaside I, bacoside A3, bacopaside II, the jujubogenin isomer of bacopasaponin C, and bacopasaponin C.[20,21] The *British Pharmacopoeia* (BP) mentions that the dried aerial parts of bacopa contain not less than 1% of bacopa saponins, expressed as bacopaside II.[23]

Gotu kola's main chemical constituents are ursane

Figure 1. Representatives of secondary metabolites of *Bacopa monnieri*



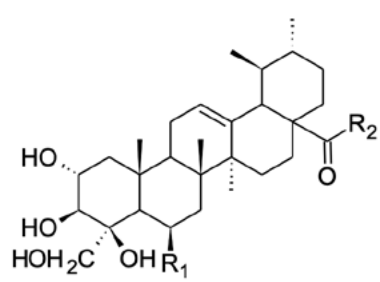
Bacopaside X: R = α-L-arabofuranosyl(1→2)-[β-D-glucopyranosyl-(1→3)]-α-L-arabopyranosyl
Bacoside A3: R = α-L-arabofuranosyl(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl

Bacopaside I: R = α-L-arabofuranosyl(1→2)-[6-*O*-sulfonyl-β-D-glucopyranosyl-(1→3)]-α-L-arabopyranosyl
Bacopaside II: R = α-L-arabofuranosyl(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl
Bacopasaponin C: R = α-L-arabofuranosyl(1→2)-[β-D-glucopyranosyl-(1→3)]-α-L-arabopyranosyl

type triterpenes, including asiatic acid and madecassic acid, as well as their glycoside esters, asiaticoside and madecassoside. Other notable ursane triterpenes found in gotu kola are corosolic acid, euscaphic acid, pomolic acid, and ursolic acid. Additionally, the plant contains oleanane type triterpenes such as bayogenin, centellasapogenol A, 3-epimaslinic acid, and terminolic acid, along with their glycosides, asiaticoside B, centellasaponins, centelloside D, and centelloside F. Dammarane type triterpene glycosides (centelloside A and B, ginsenosides, gypenosides, notoginsenosides) are also present.[30]

In addition to triterpenes, gotu kola contains flavonoids (apigenin, astragalin, castillicetin, castilliferol, catechin, epicatechin, isoquercetin, kaempferol, luteolin, myricetin, naringin, naringenin, petuletin, and quercetin), and a variety of phenolic acids (caffeoylquinic acid derivatives, chlorogenic acid derivatives, rosmarinic acid, and triferulic acid), polyacetylenes, steroids (β-sitosterol, campesterol, castasterone, and stigmasterol) and their glycosides, tannins, and other miscellaneous compounds. Although some researchers have reported the presence of alkaloids such as hydrocotyline and vallerine in gotu kola, the lack of a comprehensive structural elucidation raises doubts about their presence.[30]

Figure 2. Representatives of secondary metabolites of *Centella asiatica*



Asiatic acid: R_1 = H, R_2 = OH
Madecassic acid: R_1 = OH, R_2 = OH
Asiaticoside: R_1 = H, R_2 = α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-O-
Madecassoside: R_1 = OH, R_2 = α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-O-

1.9 General use[s]:

Bacopa and its extracts are used as a brain tonic to enhance cognitive function and improve focus, mental performance, and memory recall. It is also recommended for individuals who suffer from insomnia, nervous disorders, and anxiety.[13,31] The daily recommended dose is 5-13 ml of 1:2 liquid extract and the weekly recommended dose is 35-90 ml of the same extract.[31] For the powdered plant, the suggested dose is 5-10 grams and 8-16 ml for the bacopa infusion.[32] For bacopa extracts standardized to contain 20% bacosides, the dosage is 200-400 mg daily in divided doses for adults, and 100-200 mg in divided doses for children.[25] The *Ayurvedic Pharmacopeia of India* recommends a daily dose of 1-3 g in powder form of dried whole plant.[14]

1.10 Historical use[s]:

Bacopa is a revered herb in Ayurveda, known for its ability to enhance concentration, memory, and the senses.[12] It is also recognized in the *Chinese Materia Medica*.[5] In Ayurveda, it is believed to be a *rasāyana* herb that promotes longevity, strengthens the brain, and supports overall vitality. It aids digestion, alleviates symptoms of hoarseness or sore throat, and is recommended for various conditions such as skin diseases, anemia, diabetes, cough, fever, arthritis, edema, anorexia, urinary issues, and psychological disorders.[12-14,32,33] Bacopa is also used for treating vitiated (imbalanced) *kapha* and *vata* conditions, biliousness, neuralgia, ascites, flatulence, leprosy, preparations for memory enhancement, and included in traditional formulations such as Brahmighrtam, Sarasvataristam, Brahmitailam, and Misrakasneham.[12,14]

In Unani medicine, majun brahmi (a polyherbal formulation containing bacopa) is considered a brain tonic and in Siddha medicine, it is used for constipation, dysuria, and poor memory.[26,34] The leaves are particularly appreciated for conditions like asthenia, nervous breakdown, and threadworms. A poultice made from the plant is used for bronchitis and joint pain, and fresh juice is applied to burns.[12,26,32] In Nepal, bacopa is used to treat burns, while in Rajasthan, boiled leaves are applied topically for postnatal pain relief, and in Maharashtra, eating the leaves is believed to help with stuttering.[15,26]

2. Market

2.1 Importance in the trade:

Trade volumes for bacopa are not as extensively documented as more mainstream botanical commodities, and detailed, country-specific trade data cannot be accessed. However, India is one of the largest producers of bacopa. Out of the estimated 1,178 medicinal plant species used in the production of 1,622 botanical raw drugs traded in India, the annual domestic consumption of 198 species exceeded 100 metric tons (MT) during 2014-2015. Among these plants, bacopa ranked 43rd in volume, with the Indian herbal industry's annual domestic consumption of botanical raw drug estimated at 1,135 MT. In terms of trade and consumption in India, the annual demand was estimated to be between 2,000 and 4,000 MT.[35]

The American Botanical Council's annual Herb Market Reports noted that bacopa dietary supplement sales in the United States are increasing steadily across both the mainstream multi-outlet (MULO, also known as mass market) and natural channels (Table 1) (T. Smith email to S. Gafner, September 9, 2024).[36-45] The natural channel includes natural, health food, and specialty stores such as co-ops, associations, independent stores, and large regional chains, while the mass market channel covers food/grocery and drug stores, mass merchandisers, and convenience stores. In the United States, retail sales for bacopa-based supplements ranked 34th among the top-selling herbal dietary supplements in the natural channel and 35th in the mainstream channel in 2023, showing a 71.7% increase in mainstream channel total sales compared to the previous year.[45]

Supply sources:

Most of the commercial supply of bacopa is harvested from wild populations across its native range (section 1.6), it is also cultivated in India, Nepal, and Sri Lanka. The Asia-Pacific area is the largest trading sector for bacopa, with India and China being major exporters.[7]

In India, bacopa grows as a weed in the rice fields of the Eastern and North-Eastern regions.[46] The International Union for Conservation of Nature (IUCN) threat category of bacopa is "Least Concern" (LC). However, the National Medicinal Plants Board (NMPB) of India prioritized it for conservation along with 31 other medicinal plants.[47] The Central Institute of Medicinal

Table 1. US Sales Data for Bacopa Dietary Supplements (2014–2023)[36-45]

Year	Mass Market Channel Sales Rank	Mass Market Channel Sales (USD)	Natural Channel Sales Rank	Natural Channel Sales (USD)
2023	35	9,997,966	34	3,721,122
2022	n/a	5,824,543	36	3,463,210
2021	n/a	4,682,542	n/a	2,913,308
2020	n/a	2,816,061	n/a	2,151,423
2019	n/a	340,375	n/a	680,057
2018	n/a	21,750	n/a	569,508
2017	146	14,632	116	496,696
2016	163	9,761	108	589,423
2015	170	2,355	105	423,107
2014	n/a	2,615	n/a	395,271

n/a: Not available

and Aromatic Plants of India has developed three bacopa varieties that can be grown as perennials, allowing for at least two harvests per year. Additionally, the Indian Institute of Integrative Medicine (IIIM) in Jammu, India, has produced a bacopa cultivar with a bacoside A content ranging from 1.8% to 2.2%.[46]

Market dynamics:

The NMPB provides market prices for bacopa and gotu kola in its "Market Price of Medicinal Plants" database, sourced from various regional markets. As of January 2023, the average local market price for bacopa (whole plant) was 236 Indian Rupees (INR) (US $2.80) per kilogram, based on data from 13 cities. The highest recorded price was 800 INR (US $9.50) in Bangalore while the lowest was 17.50 INR (US $0.21) in Gaura-Pendra-Marwahi.[48] By comparison, average local market prices for bacopa in early 2011 ranged between 45 to 95 INR/kg (US $1.00–2.10/kg).[26]

For the main confounding species, gotu kola (whole plant), the NMPB reported an average local market price of 111.50 INR/kg (US $1.33), based on data from nine locations in January 2023. The highest price was 120 INR (US $1.43) in the Berhampur, Chennai, and Delhi markets, while the lowest was 75 INR/kg (US $0.89) in Kolkata.[48] However, informal conversations with several bacopa and gotu kola suppliers suggest that prices strongly depend on the quality, including the presence of more leaves and active ingredient content for bacopa, and origin of the materials, with gotu kola generally costing approximately twice as much as bacopa (Bhaumik Darji [Verdure Sciences], Deepak Mundkinajeddu [Natural Remedies], and Aru Mugam [India Glycols, Ltd], oral communications to S. Gafner, October 29 and 30, 2024). Hence, the current price range of gotu kola and bacopa does not provide an argument for a financial incentive in substituting gotu kola for bacopa. This is also supported by literature data suggesting that the adulteration of bacopa with gotu kola is unlikely to be economically motivated.

Adulteration

3.1 Known adulterants:

The brahmi trade chain holds significant value; hence, a high prevalence of substitution, either economically motivated or unintentional, has been observed within the supply chain to meet demand. Bacopa shares the common Sanskrit name of *brahmi* with gotu kola, and Ayurvedic literature acknowledges both these species as legitimate substitutes for each other. Although *B. monnieri* and *C. asiatica* have overlapping therapeutic uses and are commonly substituted for each other, their phytochemical constituents, mode of action, some of their pharmacological effects, and functional groups differ. Regional differences have also contributed to this confusion, with *C. asiatica* prescribed as brahmi, while in Bengal and Bihar, *B. monnieri* is known as brahmi. In Saurashtra and some parts of South India, the plant *Merremia emarginata* (Convolvulaceae) is sold under the name of brahmi and mandukaparni due to some of its morphological similarities with gotu kola.[49-51]

3.2 Sources of information supporting adulteration:

According to Mahapatra et al, there is a historical confusion between brahmi (bacopa) and mandukaparni (gotu kola), and both plants have been used since ancient times, with references dating back to 1000 BCE in the *Atharvaveda*. Ancient texts like the *Charaka Samhita* (estimated to be written between 100 BCE and 200 CE) and *Sushruta Samhita* (likely written between 400 and 300 BCE) mention both plants, but their specific roles in medicine vary, leading to further ambiguity. The 12th-century commentator Dalhana has also mentioned mandukaparni as brahmi and brahmi as mandukaparni, adding to the confusion.[49]

Although the interchangeable use of bacopa and gotu kola for formulations to support brain health is well known and documented, there are only a few published studies on the authentication of commercial products. Genetic methods were employed to investigate adulteration in all the studies[7,52-54] combined with high-performance liquid chromatography and ultraviolet detection (HPLC-UV) in one of them.[7]

Santosh Kumar et al aimed to assess the adulteration rate in the raw herbal trade and collected bacopa samples from South India. Isolated DNA barcode sequences from the nuclear ribosomal DNA internal transcribed spacer (nr-ITS) and *psbA-trnH* regions of the plants in commercial samples were compared with those obtained from authenticated botanical reference materials. Five of eight "brahmi" samples were found to contain bacopa, while the other three were gotu kola.[53] Adulterants and contaminants in two complementary and alternative medicine products labeled as containing bacopa were investigated targeting the *rbcL* genomic region using a next-generation DNA sequencing instrument. Pharmaceutical adulterants were analyzed through liquid and gas chromatography, while heavy metals were assessed via inductively coupled plasma mass spectrometry (ICP-MS). One product was found to contain additional plant DNA and caffeine, while the lead content in the other product exceeded Australia's Therapeutic Goods Administration's (TGA's) guidelines based on a daily dose of 2.2 µg for a 60 kg person.[55]

In another study,[7] the authenticity of 18 commercial samples labeled as brahmi was investigated using DNA-based methods, including sequence characterized amplified region (SCAR) marker-based PCR and metabarcoding alongside HPLC-UV analysis. The results revealed that six products contained only bacopa, three products contained only gotu kola, one product was a mixture of bacopa and gotu kola, and eight products contained neither of the plants. High relative concentrations (more than 35%) of DNA were found from 12 different plant species in bacopa products through metabarcoding analysis. The number of undeclared species detected per sample ranged from two to 28. The most commonly found species were sweet potato (*Ipomea batatas,* Convolvulaceae), false daisy (*Eclipta alba*, Asteraceae), sicklepod (*Senna obtusifolia*, Fabaceae), bitter melon (*Momordica charantia*, Cucurbitaceae), holy basil (*Ocimum tenuiflorum*, Lamiaceae), neem tree (*Azadirachta indica*, Meliaceae), curry tree (*Murraya koenigii*, Rutaceae), Siberian cocklebur (*Xanthium sibiricum*, Asteraceae), white tephrosia (*Tephrosia candida*, Fabaceae), Christ's thorn jujube (*Ziziphus spina-christi*, Rhamnaceae), ajwain (*Trachyspermum ammi*, Apiaceae), and ashwagandha.

Notably, none of the products listed gotu kola as an ingredient on their labels. It is important to note that many of the commercial products were tablets; hence, it is not clear if the absence of bacopa DNA is due to adulteration or due to the processing, which may have removed good quality bacopa DNA from the products. DNA metabarcoding is known to be a sensitive technique that can detect other species at trace levels in herbal products including contaminants from wind-carried species, transport, storage, or unclean equipment. Also, pharmacopeias provide foreign matter limits for each herb material, referring to substances found in the herbal material that are not part of the labeled plant, such as small amounts of other plants, dirt, insects, stones, or different parts of the relevant plant. The foreign matter limit for the aerial parts of bacopa in the BP monograph is <1% while the foreign organic (excluding dirt and stones) matter limit is < 2% according to the USP monograph. Since the DNA method applied cannot reliably measure the concentration of contaminants in the samples, the contaminants found in this study might be at low concentrations and fall within acceptable limits.[7]

In a 2024 study from Portugal, published ahead of peer-review, a quantitative real-time PCR method was used to determine the amount of bacopa DNA in six bacopa products labeled to contain brahmi leaf or whole plant powder, which were obtained in local stores or from online retailers. The results revealed that three out of six tested products labeled as containing bacopa leaf or whole plant powder lacked detectable bacopa DNA, while another sample was determined to contain only 4.19% (w/w) bacopa. Contrarily, the 11 products labeled to contain gotu kola leaves or aerial parts were all found to be authentic.[54]

3.3 Accidental or intentional adulteration:

As discussed in Section 3.1, bacopa is frequently substituted — whether accidentally or intentionally — with gotu kola, and vice-versa under the name brahmi. Such substitution may also lead to rare cases of unintentional adulteration with other *Centella* species or morphologically similar plants mistakenly harvested as gotu kola.

3.4 Frequency of occurrence:

There is no comprehensive published study on the frequency of adulteration for bacopa, and available information is based on a limited number of publications with small sample sizes. In the most extensive investigation to date, Shah et al reported that four of 18 commercial products tested (22.2%) were adulterated with gotu kola, and bacopa DNA was absent in eight of the products (44.4%).[7] Although adulteration rates of 37.5% (3/8)[53] and 50% (1/2)[55] were found in two studies, no adulterants were detected in bacopa products in two other studies, where the sample sizes were six[56] and eight[52], respectively.

3.5 Possible safety/therapeutic issues:

Gotu kola, the main confounding substitute and/or adulterating species of bacopa, is also a well-known and widely used medicinal plant. Based on available data, adulteration with gotu kola does not lead to any safety problems or therapeutic issues.

Bacopa is known to bioaccumulate various pollutants, including heavy metals, pesticides, and toxins from its surrounding environment.[57-59] In India, much of the bacopa is cultivated in the rice paddy fields of Eastern and North-Eastern regions following the rice harvest. During this time, the soil may contain trace residues of herbicides and pesticides commonly used in rice farming, posing a risk of contaminating the bacopa. Therefore, the primary safety concern with bacopa products might be contamination rather than herb adulteration.[59]

3.6 Analytical methods to detect adulteration and substitutions:

The identification and authentication of bacopa herb and powdered material can be done through a combination of macroscopic (when whole or semi-whole), organoleptic, microscopic, chemical, and genetic analyses. The morphological and microscopical characteristics of different parts of bacopa[3,15,24,60,61] and gotu kola[51,62-65] are well described in several sources including pharmacopeial monographs.[14,20,21,23,34,64] Additionally, the key morphological and microscopic differences between the leaves of the two species are summarized in the American Herbal Pharmacopoeia's *Microscopic Characterization of Botanical Medicines*:[5] While trichomes are absent in bacopa, gotu kola leaves feature simple or multicellular trichomes. Bacopa has anisocytic stomata whereas gotu kola may have paracytic, anisocytic, or anomocytic stomata. Both sides of bacopa leaves have capitate glands (glandular trichomes) that have seven or eight cells. Palisade parenchyma cannot be seen in bacopa leaves; however, gotu kola leaves have single or two-rowed palisade parenchyma. Furthermore, large idioblasts are characteristic of bacopa.

Bacopa *Bacopa monnieri*
Photo by Steven Foster, ©2025 ABC

DNA barcoding and other genetic methods have been frequently used in published papers to identify bacopa and/or gotu kola samples and commercial products.[7,52-56,66,67] A multi-locus approach of two barcode regions, namely nr-ITS and *psbA-trnH*, was used to establish the DNA barcode signatures of 30 highly traded medicinal plants in South India. Three of eight products labeled as brahmi were found to contain gotu kola only.[53] Similarly, detection of the substitution of bacopa with gotu kola was successfully achieved by using universal DNA barcode primers (*atpF–atpH*, *trnL*, and *psbA-trnH*) based on differences in amplicon length of bacopa and gotu kola.[67]

In another study, DNA metabarcoding, Randomly Amplified Polymorphic DNA (RAPD)-based SCAR markers, and high-performance liquid chromatography with ultraviolet/visible detection (HPLC-UV/Vis) were combined to analyze 18 commercial samples. Authors noted that DNA metabarcoding is effective for detecting a wide range of adulterants, while HPLC-UV/Vis excels in identifying and monitoring specific bioactive compounds in the products. The RAPD-based SCAR marker assessment was considered by the authors as the most cost-effective and simple method but it is limited in its ability to detect non-target plants.[7] Yadav et al developed RAPD-based SCAR markers for the identification of bacopa and detection of gotu kola, and the possible adulterants false daisy and low mallow (*Malva pusilla*, Malvaceae). While the method was efficient in detecting and distinguishing bacopa and its adulterants, none of the tested commercial samples (n = 8) were found to be adulterated.[52]

A 2023 study aimed to differentiate *Centella asiatica* from its potential adulterants *Hydrocotyle umbellata* (Araliaceae), *Bacopa monnieri*, and *B. caroliniana*. DNA barcoding coupled with high-resolution melting (Bar-HRM) analysis using ITS, *matK,* and *rbcL* loci was employed to identify four plant species. Additionally, HPLC was conducted to obtain the chemical fingerprints of each plant. Beyond distinguishing the species, the Bar-HRM method was able to detect the presence of *B. monnieri* and *H. umbellata* when mixed with *C. asiatica*, although only at concentrations of 75% and higher, which makes this approach impractical for quality control purposes.[66] Bar-HRM analysis also successfully differentiated three *Bacopa* species (*B. monnieri*, *B. caroliniana*, and *B. floribunda*) and its applicability was tested in an investigation into the authenticity of commercial bacopa products. All six single-ingredient bacopa products (including dried leaf, powder, capsule, and tablets) collected in Bangkok, Thailand, were found to be authentic.[56]

Various pharmacopeial monographs[20-23,34,64,68] provide thin layer chromatography (TLC) methods for authenticating bacopa and gotu kola and detecting their adulterants. The BP specifies bacopaside I and II as marker compounds for bacopa in its TLC method.[23] Seasong et al utilized bacoside A3, bacopasaponin C, and bacopasides I, II, and X as reference standards to analyze the fingerprints of 13 bacopa samples by TLC.[23] The HPTLC Association provides a useful HPTLC method that includes the chromatographic fingerprints of bacopa, gotu kola, adulterated bacopa samples, and reference compounds such as asiaticoside, bacopasaponin C, bacopaside I, II, X, bacoside A3, bacosine, madecassic acid, and madecassoside.[61,69] The HPTLC Association's website also contains HPTLC methods from the European Pharmacopoeia, Indian Pharmacopoeia, Pharmeuropa, Siddha Pharmacopoeia, and USP for bacopa.[69] Additionally, several HPTLC methods for the quantitative analysis of bacoside A,[29,70] bacoside B,[70] luteolin,[71] or marker-based standardization of commercial formulations containing bacopa[72] may also be useful in detecting substitutions and adulteration.

High performance liquid chromatography (HPLC) is used primarily for the quantitative analysis of total saponins, bacosides, bacopasides, and bacopasaponin C,[73-76] as well as compounds like apigenin[73] or betulinic acid.[27] However, HPLC can also play a valuable role in detecting substitutions and adulterations. Typically, HPLC is paired with various detectors such as UV, evaporative light scattering (ELSD), photodiode array, and mass spectrometry (MS) detectors, e.g., ion trap time of flight MS. Furthermore, the USP[20-22] and BP[23] provide HPLC methods for assessing the content of triterpene glycosides and total bacopa saponins, respectively.

A study from 2015 used the HPLC method of the BP to analyze the bacopaside content of a commercial product, separating bacosides by an isocratic mobile phase and UV-Vis detection at 205 nm. Bacopaside II served as the standard and selected bacoside peaks were identified by their relative retention time to bacopaside II. The authors concluded that while the method is valid for bacopaside II analysis, it lacks the specificity, robustness, and reproducibility needed for accurate total bacoside quantification.[76]

Moreover, Saini et al used a UV-spectrophotometer to analyze the amount of bacoside A in two batches each of four commercial bacopa formulations. The results of this study revealed significant batch-to-batch variations between formulations.[77] However, this approach is neither specific for bacoside A nor appropriate to detect substitution with gotu kola.

4. Conclusions

Bacopa monnieri (bacopa) and *Centella asiatica* (gotu kola) are widely used herbs in traditional medicine, but they are occasionally adulterated or substituted for one another. Although both herbs are generally considered safe when used at recommended doses due to their long history of use, the use of the vernacular name brahmi, and permissible interchangeable use in certain traditional medicine systems can lead to confusion and possibly substitution of raw materials. Thus, in trade and product specifications, it is essential to specify the correct Latin names and test the materials using validated analytical methods, such as macroscopic, microscopic analysis, genetic methods, or marker-based methods such as HPLC-UV and HPTLC.

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Revision summary

Version #, Author,	Date Revised	Section Revised	List of Changes
Version 1, N. Orhan	N/A	N/A	None