Review of phytochemistry, biological activities and therapeutic potential of *Brachylaena discolor*

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**ABSTRACT**
*Brachylaena discolor* DC. is a shrub or tree widely used as herbal medicine in southern Africa. *Brachylaena discolor* is indigenous to Botswana, Eswatini, Mozambique, South Africa, Zambia and Zimbabwe. This study was aimed at reviewing the phytochemistry, biological activities and therapeutic potential of *B. discolor*. Information on phytochemistry, biological activities and therapeutic potential of *B. discolor* was collected from online sources such as Google Scholar, PubMed and Science Direct, and pre-electronic sources such as books, book chapters, theses and journal articles obtained from the University library. This investigation revealed that the bark, leaf, root, stem and twig infusion or decoction of *B. discolor* are mainly used for magical purposes and as anthelmintic and tonic, and traditional medicine for female infertility, skin infections, renal problems, diabetes, gastro-intestinal problems and respiratory infections. Chemical compounds identified from *B. discolor* include alkaloids, flavonoids, phenolics, phlobatannins, saponins, sesquiterpene lactones, steroids, tannins and terpenoids. Ethnopharmacological review showed that *B. discolor* and phytochemical compounds identified from the species have anticancer, anthelmintic, anti-hyperglycaemic, antibacterial, cytotoxicity, antifungal, antidiabetic, antioxidant and leishmanicidal activities. Advanced ethnopharmacological research on *B. discolor* should focus on the possible biochemical mechanisms of both the crude extracts and identified phytochemical compounds including toxicological, *in vivo* and clinical studies to corroborate the traditional medicinal applications of the species.

**INTRODUCTION**

*Brachylaena discolor* DC. is a shrub or tree belonging to Compositae or Asteraceae family which is commonly referred to as sunflower, aster or daisy family.

The genus name *Brachylaena* R. Br. is a contraction of two Greek words "brachus" meaning "short" and "klaina" meaning "cloak", in reference to the florets which are longer than the bracts surrounding the flower head (Venter and Venter, 2015). The specific name "discolor" which translates to "two-coloured" refers to the leaves, the upper surface of which is darker than the lower (Palmer and Pitman, 1972). The common name of the species "coastal silver-oak", mostly refer to the silver-grey under-surface of the leaves which often gives the tree a silvery appearance (Palmer and Pitman, 1972). *Brachylaena discolor* is distinguished into three infraspecific taxa, namely var. *discolor*, var. *rotundata* (S. Moore) Beentje and var. *transvaalensis* (E. Phillips & Beentje) Beentje. *Brachylaena discolor* is an evergreen or deciduous shrub or tree growing up to...
30 m in height (Beentje, 2000); (Germishuizen and Meyer, 2003). The bark of B. discolor is rough, light black to reddish-brown in colour with lenticelate branches. The leaves of B. discolor are lanceolate to ovate in shape, dull green above and light green to whitish below. The leaf margins of B. discolor are entire or are slightly serrated. The flower heads of B. discolor are grouped into axillate panicles with creamy-white coloured flowers. The fruits of B. discolor are small achenes characterized by apical tuft of creamy brown bristles (Palgrave and Keith, 2002); (Wyk and Wyk, 2013). Brachylaena discolor is indigenous to Zimbabwe, Botswana, Eswatini, Zambia, South Africa and Mozambique (Beentje, 2000). The species is found on termite mounds, sandy soils, secondary bushland, evergreen, dune, kloof, gully forests, forest margins, deciduous woodland, rocky outcrops and hillsides at sea level up to 1900 m above sea level (Germishuizen and Meyer, 2003); (Schmidt et al., 2017). Brachylaena discolor is a valuable medicinal plant species in tropical Africa as the roots and leaves of the species are traded in informal herbal medicine markets in KwaZulu-Natal and Gauteng provinces of South Africa (Cunningham, 1993); (Williams et al., 2001). Thus, the aim of this review is to summarize the phytochemistry, biological activities and therapeutic potential of B. discolor.

**Medicinal uses of Brachylaena discolor**

The twig, stem, bark, root and leaf infusion or decoction of B. discolor are mainly used for magical purposes and as anthelmintic and tonic, and traditional medicine for female infertility, skin infections, renal problems, diabetes, gastro-intestinal problems and as anthelmintic and tonic, and traditional medicine for female infertility, skin infections, renal problems, diabetes, gastro-intestinal problems and respiratory infections (Table 1, Figure 1). The leaves of B. discolor are mixed with those of Erythrophleum lasianthum (Thunb.) Harv. f. ex Benth., Volkameria glabra (E. Mey.) Mabb. & Y.W. Yuan, Zanthoxylum capense (Thunb.) Harv. and roots of Cymbopogon marginatus (Steud.) Stapf. ex Burtt-Davy, Erythrophleum lasianthum Corbishley, Margaritaria discoidea (Baill.) Webster and Hypoxis spp. and used as anthelmintic (Bryant, 1966); (Hutchings et al., 1996). The twigs of B. discolor are mixed with stems of Euphorbia tirucalli L., Hypoxis hemerocaliidea Fisch., C.A. Mey. & Avé-Lall. (corn), Ozoroa engleri R. Fern. & A. Fern. (bark) and Senecio serratuloides DC. (leaves) and applied topically on sores (Wet et al., 2013).

**Phytochemistry of Brachylaena discolor**

Several researchers investigated the phytochemical properties of B. discolor aerial parts and leaves (Table 2). Phytochemical compounds such as alkaloids, flavonoids, phenolics, phlobatannins, saponins, sesquiterpene lactones, steroids, tannins and terpenoids have been identified from B. discolor. Some of the documented chemical compounds could be responsible for the pharmacological properties associated with the species.

**Pharmacological properties of Brachylaena discolor**

The following pharmacological activities have been documented from the aerial parts and leaf extracts of B. discolor and compounds isolated from the species: anthelmintic, antibacterial, antifungal, anticancer, antidiabetic, antioxidant, anti-hyperglycaemic, leishmanicidal and cytotoxicity activities.

**Antihelmintic activities**

(Mcgaw et al., 2000) evaluated the anthelmintic activities of hexane, ethanol and water extracts of B. discolor leaves on the mortality and reproductive ability of the free-living nematode Caenorhabditis elegans in two different assays. All extracts exhibited activities at a concentration of 1.0 mg/ml and 2.0 mg/ml after the two and seven days incubation period (Mcgaw et al., 2000). (Adamu et al., 2013) evaluated the anthelmintic activities of the acetone extract of B. discolor leaves using the egg hatch assay and the larval development tests using Haemonchus contortus with albendazole as positive control. The extract exhibited activities with half maximal effective concentration (EC50) values of 3.6 mg/ml and 17.2 mg/ml for the egg hatch and the larval development assays, respectively (Adamu et al., 2013).

**Antibacterial activities**

(Adamu et al., 2014) evaluated the antibacterial activities of acetone extract of B. discolor leaves against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Enterococcus faecalis using a serial microdilution method with gentamicin as positive control. The extract exhibited activities...
Table 1: Medicinal uses of Brachylaena discolor

| Medicinal use                                      | Part used                                                                 | Reference                                                                 |
|---------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Abdominal pains                                   | Root decoction taken orally                                                | *(Gelfand et al., 1985)*                                                  |
| Anthelmintic                                       | Leaf and root infusion or decoction taken orally                           | *(Thomas and Grant, 2013); *(Constant and Tshisikhawwe, 2018)*            |
| Anthelmintic                                       | Leaves mixed with those of Ekebergia capensis, Clausena anisata (Willd.)  | *(Bryant, 1966); *(Hutchings et al., 1996)*                              |
|                                                   | Hook. f. ex Benth., Volkameria glabra (E. Mey.) Mabb. & Y.W. Yuan, Zanthoxylum capense (Thunb.) Harv. and roots of Cymbopogon marginatus (Steud.) Stapf. ex Burtt-Davy, Erythrophleum lasianthum Corbishley, Margaritaria discoidea (Baill.) Webster and Hypoxis spp. |
| Diabetes                                           | Leaf and root infusion and decoction taken orally                          | *(Watt and Breyer-Brandwijk, 1962); *(Erasto et al., 2005)*               |
| Dysmenorrhoea                                      | Root decoction taken orally                                                | *(Gelfand et al., 1985)*                                                  |
| Female infertility and prevent miscarriage        | Bark and root decoction taken orally                                       | *(Semenya et al., 2013); *(Mhlongo and Wyk, 2019)*                       |
| Fever                                              | Leaf infusion taken orally                                                 | *(Pujol and Naturafrica, 1990)                                            |
| Gastro-intestinal problems (diarrhoea and stomach ache) | Leaf and bark infusion taken orally or anally                              | *(de Wet et al., 2010); *(Monjane et al., 2018)*                         |
| Haemorrhoids                                       | Root infusion applied topically                                            | *(Palmer and Pitman, 1972)*                                               |
| Magical purposes                                   | Leaves, roots and stems                                                    | *(Cunningham, 1988); *(Cunningham and Zondi, 1991)*                     |
| Nervous system                                     | Leaf infusion taken orally                                                 | *(Hutchings, 1989)*                                                      |
| Renal problems                                     | Leaf infusion or decoction taken orally                                    | *(Mellem, 2013), *(Mellem et al., 2015)*                                 |
| Respiratory infections (chest pains, cough, sore throat and tuberculosis) | Leaf and root decoction or infusion taken orally                           | *(York et al., 2011); *(Semenya and Maroyi, 2018)*                      |
| Skin infections (burns, cleaning facial skin and sores) | Leaf and twig infusion applied topically                                   | *(Afolayan et al., 2014); *(Nciki et al., 2016)*                        |
| Sores                                              | Twigs mixed with Euphorbia tirucalli L. (stems), Hypoxis hemerocallidea Fisch., C.A. Mey. & Avé-Lall. (corm), Ozoroa engleri R. Fern. & A. Fern. (bark) and Senecio serratuloides DC. (leaves) | *(Wet et al., 2013)*                                                  |
| Syphilis                                           | Root decoction taken orally                                                | *(Gelfand et al., 1985)*                                                  |
| Tonic                                              | Leaf decoction taken orally                                                | *(Palmer and Pitman, 1972); *(Hutchings, 1989)*                         |
| Ulcers                                             | Leaf infusion taken orally                                                 | *(Chigora et al., 2007)*                                                 |
| Wounds                                             | Leaf infusion applied topically                                            | *(Afolayan et al., 2014)*                                                |
| Ethnoveterinary medicine (anthelmintic)            | Leaf infusion                                                             | *(Hutchings et al., 1996)*                                               |
Table 2: Phytochemical composition of Brachylaena discolor

| Phytochemical compound                        | Plant part                              | Reference                                                      |
|-----------------------------------------------|------------------------------------------|----------------------------------------------------------------|
| 3’-hydroxygenkwanin                           | Leaves                                  | (Monjane et al., 2018)                                         |
| 3'-acetoxy-12-lupene                          | Aerial parts and leaves                  | (Bohlmann and Zdero, 1982); (Adam, 2017)                      |
| 4β,15-Dihydrodehydrozaluzanin C               | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| 6”-O-acetyl homoplantaginin                   | Leaves                                  | (Monjane et al., 2018)                                         |
| 11β,13-Dihydrotubiferin                       | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| 11β,13-Dihydrozaluzanin C                     | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Brachylaenolide                               | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Costunolide                                   | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| α-D-Glucopyranose                             | Leaves                                  | (Adam, 2017)                                                   |
| β-D-Glucopyranose                             | Leaves                                  | (Adam, 2017)                                                   |
| Dehydrobrachylaenolide                        | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Dehydrocostuslactone                          | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Dehydrozaluzanin C                            | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Dihydroxyisopinic acid                        | Leaves                                  | (Monjane et al., 2018)                                         |
| Dihydrodehydrocostuslactone                   | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Eupafolin                                     | Leaves                                  | (Monjane et al., 2018)                                         |
| Furanoheliangolide                            | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Germacranolide                                | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Germacrene D                                  | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Genkwanin5-O-β-D-glucopyranoside              | Leaves                                  | (Adam, 2017)                                                   |
| Germacronolide epoxide                        | Leaves                                  | (Monjane et al., 2018)                                         |
| Hydroxytyrosol                                | Leaves                                  | (Monjane et al., 2018)                                         |
| Linoleic acid                                 | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Linolenic acid                                | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Lupeol acetate                                | Aerial parts and leaves                  | (Bohlmann and Zdero, 1982); (Zdero and Bohlmann, 1987)        |
| Luteolin                                      | Leaves                                  | (Monjane et al., 2018)                                         |
| Onopordopicrin                                | Aerial parts and leaves                  | (Bohlmann and Zdero, 1982); (Zdero and Bohlmann, 1987)        |
| Onoporidin                                    | Leaves                                  | (Monjane et al., 2018)                                         |
| 9-oxo-nerolidol                               | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Quercetin3-Oglucoside-7,3',4’-trimethyl ether  | Leaves                                  | (Monjane et al., 2018)                                         |
| Quercetin-3-O-D-galactopyranoside             | Leaves                                  | (Monjane et al., 2018)                                         |
| Quercetin-7-galactopyranoside                 | Leaves                                  | (Monjane et al., 2018)                                         |
| Salonitenolide                                | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Salonitenolide-8-O-2,3-epoxy isobutyrate      | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| 8-sitosteryl linolenate                       | Leaves                                  | (Adam, 2017)                                                   |
| Tetrahydrodehydrozaluzanin C                 | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| α-tocopherol                                  | Leaves                                  | (Adam, 2017)                                                   |
| Tubiferin                                     | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
| Zaluzanin C                                   | Aerial parts                            | (Bohlmann and Zdero, 1982)                                    |
with minimum inhibitory concentration (MIC) values ranging from 0.2 mg/mL to 1.3 mg/mL in comparison to MIC value of <0.02 mg/mL exhibited by the positive control, and total activity ranging from 52.8 mL/g to 412.5 mL/g (Adamu et al., 2014). (van Vuuren et al., 2015) evaluated the antibacterial activities of dichloromethane : methanol (1:1) and aqueous extracts of B. discolor var. transvaalensis leaves against Staphylococcus aureus, Enterococcus faecalis, Proteus vulgaris, Bacillus cereus, Shigella flexneri, Salmonella typhimurium and Escherichia coli with ciprofloxacin as positive control. The aqueous extract exhibited activities against all tested pathogens with MIC values ranging from 0.3 mg/mL to 2.0 mg/mL while the organic extract exhibited weak activities against Shigella flexneri with MIC value of 4.0 mg/mL. The antibacterial interaction of B. discolor var. transvaalensis used in combination with Psidium guajava L. and Sclerocarya birrea (A. Rich.) Hochst. subsp. caffra (Sond.) Kokwaro was evaluated by calculating the sum of the fractional inhibitory concentrations (\( \Sigma \text{FIC} \)) against Staphylococcus aureus, Enterococcus faecalis, Proteus vulgaris, Bacillus cereus, Shigella flexneri, Salmonella typhimurium and Escherichia coli with ciprofloxacin as positive control. The extracts exhibited activities against tested pathogens with MIC values ranging from 0.02 mg/mL to 12.0 mg/mL while the combination of B. discolor var. transvaalensis with other species resulted in synergistic to additive effects (van Vuuren et al., 2015). (Nciki et al., 2016) evaluated the antibacterial activities of aqueous and dichloromethane : methanol (1:1) extracts of B. discolor var. transvaalensis with 412.5 mL/g to 412.5 mL/g (Adamu et al., 2012). (Nciki et al., 2016) evaluated the antifungal activities of aqueous and dichloromethane : methanol (1:1) extracts of B. discolor twigs against Microsporum canis, Candida albicans and Trichophyton mentagrophytes using the micro-titer plate dilution assay with amphoterin B as positive control. The extract exhibited weak activities against tested pathogens exhibiting MIC values ranging from 250.0 \( \mu g/mL \) to >8000.0 \( \mu g/mL \) which was much higher than MIC values of 0.01 \( \mu g/mL \) to 0.1 \( \mu g/mL \) showed by the positive control (Nciki et al., 2016). (Dikhoba et al., 2019) evaluated the antifungal activities of acetone extract of B. discolor leaves against Fusarium verticilloides, Aspergillus flavus and Aspergillus ochraceus using the microplate dilution method with amphotericin B as positive control. The extract exhibited weak activities against tested pathogens exhibiting MIC values ranging from 0.2 mg/mL to 2.5 mg/mL and total activity of 17.0 mg/L to 271.0 mg/L at both 24 hour and 48 hour incubation periods (Dikhoba et al., 2019).

**Anticancer activities**

(Fouche et al., 2008) evaluated the anticancer activities of dichloromethane extracts of B. discolor var. rotundata leaves against melanoma UACC62, renal TK10 and breast MCF7. The extracts exhibited activities against melanoma UACC62, renal TK10 and breast MCF7 with total growth inhibition (TGI) values of 13.1 \( \mu g/mL \), 15.0 \( \mu g/mL \) and 26.0 \( \mu g/mL \), respectively. The authors also evaluated the anticancer activities of dichloromethane extracts of B. discolor var. rotundata leaves against leukaemia SR, ovarian OVCAR-5 and colon HT 29. The extracts exhibited activities against leukaemia SR, ovarian OVCAR-5 and colon HT 29 with TGI values of 12.3 \( \mu g/mL \), 20.0 \( \mu g/mL \) and 24.6 \( \mu g/mL \), respectively (Fouche et al., 2008).

**Antidiabetic activities**

(De et al., 2008) evaluated the antidiabetic activities of organic and aqueous extracts of B. discolor leaves, roots and stems against 3T3-L1 adipose, C2C12 muscle and Chang liver cells using a glucose utilisation assay with 1.0 \( \mu M \) metformin for Chang liver cells, 1.0 \( \mu M \) insulin for C2C12 and 3T3-L1 cells as positive controls. The extracts demonstrated activities (De et al., 2008). (Mellem et al., 2015) evaluated the antidiabetic activities of the aqueous and methanol extracts of B. discolor leaves by using the \( \alpha \)-amylase inhibition and a-glucosidase inhibition assays with acarbose as positive control.
The extracts exhibited activities on α-amylase and α-glucosidase with half maximal inhibitory concentration (IC$_{50}$) values ranging from 1.8 mg/ml to 11.0 mg/ml in comparison to IC$_{50}$ values of 0.03 mg/ml to 1.2 mg/ml exhibited by the positive control (Mellem et al., 2015).

**Antioxidant activities**

(Adamu et al., 2014) evaluated the antioxidant activities of acetone extract of *B. discolor* leaves using 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assays. The extracts exhibited activities with a trolox equivalent antioxidant capacity (TEAC) value of 0.2 and an EC$_{50}$ value of 2.6, using ABTS and DPPH, respectively (Adamu et al., 2014). (Mellem et al., 2015) evaluated the antioxidant activities of the crude extracts of *B. discolor* leaves by using the DPPH free radical scavenging assay with rutin as positive control. The methanol and aqueous extracts exhibited activities with IC$_{50}$ values of 92.3 µg/ml and 82.8 µg/ml, respectively (Mellem et al., 2015). (Dikhoba et al., 2019) evaluated the antioxidant activities of acetone extract of *B. discolor* leaves using the ABTS and DPPH free radical scavenging assays with ascorbic acid (0.5 mg/ml) as positive control. The extract exhibited activities with IC$_{50}$ values of 0.03 mg/ml and 0.2 mg/ml against ABTS and DPPH, respectively (Dikhoba et al., 2019).

**Anti-hyperglycaemic activities**

(Mellem, 2013) evaluated the anti-hyperglycaemic activities of the methanol extract of *B. discolor* leaves using a streptozotocin-induced diabetic rat model. The doses of 50.0 mg/ml and 150.0 mg/ml were administered daily to both streptozotocin-induced and control rats and the biochemical profile of the rats assessed over 28 days. The extract at both doses caused a significant reduction in the blood glucose levels, and other observed changes included total bilirubin, creatinine, alkaline phosphatase and body weight (Mellem, 2013).

**Leishmanicidal activities**

(Monjane et al., 2018) evaluated the leishmanicidal activities of the compounds onopordopicrin and germacroneolide epoxide isolated from the leaves of *B. discolor* against *Leishmania amazonensis* and *Leishmania braziliensis* using the colorimetric method-XTT with miltefon as positive control. The compound onopordopicrin exhibited activities with IC$_{50}$ values of 39.6 µM and 27.9 µM against *Leishmania amazonensis* and *Leishmania braziliensis*, respectively compared to IC$_{50}$ values of 12.5 µM and 12.0 µM exhibited by the positive control (Monjane et al., 2018).

**Cytotoxicity, toxicity and mutagenicity activities**

(Adamu et al., 2012) evaluated the cytotoxicity activities of acetone extracts of *B. discolor* leaves against Vero monkey kidney cells using the tetrazolium-based colorimetric 3-5-dimethyl thiazol-2-yl-2, 5-diphenyl tetrazolium bromide (MTT) assay. The extract exhibited activities with half maximal lethal dose (LD$_{50}$) value of 0.004 mg/ml (Adamu et al., 2012). (Adamu et al., 2013) evaluated the cytotoxicity activities of acetone extract of *B. discolor* against African Green Monkey kidney (Vero) cells using the tetrazolium-based colorimetric MTT assay. The extract exhibited activities with half maximal lethal concentration (LC$_{50}$) value of 0.008 mg/ml (Adamu et al., 2013). (Mellem et al., 2015) evaluated the cytotoxicity activities of the crude extracts of *B. discolor* leaves against the HeLa cell line using the colorimetric MTT assay. Both extracts stimulated the growth of the HeLa cell line with an increase in cell viability in a concentration dependent manner implying that the extracts are safe for use (Mellem et al., 2015) (Mellem et al., 2015).

(Mellem et al., 2015) Mellem et al. (2015) evaluated the mutagenicity activities of aqueous and methanol extract of *B. discolor* leaves using the *Salmonella typhimurium* TA 100 and TA 98 strains mutagenicity assay. Both extracts exhibited no mutagenic activities up to the highest concentration tested which was 1000.0 µg/ml (Mellem et al., 2015). (Mellem et al., 2015) evaluated the toxicity activities of aqueous and methanol extracts of *B. discolor* leaves using the brine shrimp assay with organophosphate as positive control. Both extracts showed 100% shrimp survival at the highest concentration tested which was 1000.0 µg/ml while the positive control showed 100 % mortality (Mellem et al., 2015).

**CONCLUSIONS**

The present review summarizes the phytochemistry, biological activities and therapeutic potential of *B. discolor*. *Brachylaena discolor* is a variable species, distinguished into three varieties, var. *discolor*, var. *rotundata* and var. *transvaalen-sis* which are deemed as highly potent traditional medicines for various human diseases and ailments. These three varieties have overlapping distributional range in southern Africa and are quite similar in appearance and often confused when growing together. There are similarities and overlaps in terms of ethnomedicinal uses, phytochemistry and biological activities. From a phytochemical and pharmacological point of view, no chemical varia-
tion studies have been conducted on these three varieties. Future studies should try to establish whether there are phytochemical compounds and pharmacological properties that could be used to distinguish these three varieties as this information will complement the taxonomical characters used to distinguish the three varieties.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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