

Higher amount of steviol detected in the leaves of a non-toxic endangered medicinal herb, *Aconitum heterophyllum*

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Received: 26 November 2015 / Accepted: 20 April 2016 / Published online: 5 May 2016
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Abstract *Aconitum heterophyllum* is the only non-toxic species of *Aconitum* genus having immense therapeutic potential. To date, only roots of this plant species are used for the preparation of various herbal formulations while leaves are discarded due to lack of information on the presence of any therapeutically active compounds. To the best of our knowledge, this is the first report on the detection and quantification of diterpene steviol in the leaves of *A. heterophyllum*. Interestingly, the level of steviol is equivalent to *Stevia rebaudiana* leaves which is the richest source reported so far. Thus, *A. heterophyllum* leaves will not only provide a novel source for extraction of steviol but also benefit the harvesters to get additional economic returns on leaf biomass for this high-value non-toxic plant species.

Keywords Steviol · *A. heterophyllum* · *S. rebaudiana* · RP-HPLC · Non-toxic

For centuries, *Aconitum* species have been considered worldwide as a vital source of both poisons and medicines. Despite a total of 300 *Aconitum* species distributed worldwide, the presence of toxic aconites pose a major concern for their use in herbal preparations. The only non-toxic species of *Aconitum* is *A. heterophyllum*

which is commonly known as Atis, and found between 2400 and 3600 m altitude in the Himalayan region of India. The non-toxicity is attributed to the presence of marker compound, atisine, found merely in the roots of *A. heterophyllum* (Malhotra et al. 2014). Apart from non-toxic significance, *A. heterophyllum* has huge applications to mankind in terms of providing medicinal services against chronic fever, throat infection, indigestion, flatulence, diarrhoea, arthritis, abdominal distension, dyspepsia, stomachache and coughs (Tai et al. 2015). These immense therapeutic applications led to the overexploitation of *A. heterophyllum* which has reduced its population in the natural habitat and subsequently listed as “critically endangered medicinal herb” by International Union for Conservation of Nature and Natural Resources (IUCN 1993). Generally, the mature plants of *A. heterophyllum* are uprooted and segregated into root and shoot tissues. The roots are traded for the preparation of herbal formulations against various disorders but the leaves are thrown away. Keeping in view the massive cost of *A. heterophyllum* roots (~Rs. 8000/kg) and difficulty in seed germination, it will be highly beneficial for the farmers if leaves are also exploited for the presence of therapeutically active compounds. The observation will not only benefit the harvesters for harvesting the aerial parts but also expands its utility as a source of broad pharmaceutical agents. To aid in the detection of pharmaceutically active compound, we have detected the diterpene steviol in the leaves of *A. heterophyllum* plants for the first time.

Steviol is the active ingredient of *Stevia rebaudiana*. It is the notorious aglycone backbone of the steviol glycosides viz. stevioside, rebaudioside A–F and dulcoside but is not accountable for their sweetening property. Steviol is

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Table 1 Pharmacological properties of steviol

Pharmacological activity	References
Inhibit glucose absorption	Toskulkao et al. (1995)
Insulinotropic/anti-hyperglycemic	Jeppesen et al. (2000)
Anti-inflammatory	Boonkaewwan et al. (2007)
Anti-diarrheal	Pariwat et al. (2008)
Diuretic	Melis (1997)
Anti-cancer	Takasaki et al. (2009)
Anti-microbial	Patra and Mohanta (2014)

likely to be associated with various therapeutic properties (Table 1) and in combination with a bioactive molecule of maple syrup *i.e.* abscisic acid, it is proposed to play a role in pain management, oxidative stress, anti-inflammation and energy modulation by acting as TRPV1 regulator (Chabot 2013). This indicates that steviol has the potential to be developed as pharmaceutical drug. Currently, *S. rebaudiana* leaves are used as the richest source for the extraction of steviol.

Since information of any pharmaceutically active compound in leaves of *A. heterophyllum* is lacking, this article is, to best of our knowledge, first study for detecting and quantifying steviol in leaves of *A. heterophyllum* plants and comparing the same with *S. rebaudiana*.

A. heterophyllum plants of 2 years age group were collected from the Himalayan Forest Research Institute, Himachal Pradesh, India (2450 m altitude, 31°23'N, 77°44'E) and *Stevia rebaudiana* plants were purchased from IHBT, Palampur. All the plants were maintained at Jaypee University of Information Technology (Waknaghat, Himachal Pradesh; 31°59'N, 77°13'E; 1700 m altitude).

The leaves of *A. heterophyllum* and *S. rebaudiana* were homogenized in a prechilled pestle and mortar using liquid nitrogen. Each powdered sample (1 g) was used for the extraction of steviol with 10 mL of 50 % ethanol leading to 1:10 solid to liquid ratio. The samples were then subjected to sonication at 30 °C for 30 min and filtered through 0.22 µm filter (Millipore). Each filtered sample was diluted 100-fold with 50 % ethanol and injected (20 µL) into HPLC for quantification of steviol. The extraction was performed in triplicates.

Reverse phase HPLC (Waters 515) fitted with C18 (5 µm) 4.6 × 250 mm Waters Symmetry Column was used for the quantification of steviol through PDA detector (Waters 2996). Gradient elution was used for the quantification of steviol which was started with isocratic 5 % B for 5 min, augmented to 95 % B over 20 min, returned to 5 % B for 5 min and holded at 95 % B for 5 min. The flow rate was 1 mL/min and the cycle time of analysis was 30 min at 50 °C. The solvent A was 0.1 % Trifluoroacetic acid in Milli-Q water and solvent B was acetonitrile. The steviol was detected at an absorbance of 200 ± 4 nm wavelength. The identification of steviol was carried out by comparing the retention time and UV spectra of samples with the steviol standard (Chromadex, USA). The HPLC analysis was performed in triplicates.

The detection and quantification of steviol content in leaves of *A. heterophyllum* and *S. rebaudiana* revealed 5.40 and 5.43 %, respectively, indicating no significant variation between two plant species. The HPLC chromatograms and UV spectra of the steviol standard and the different samples are provided in Fig. 1. It was thus inferred from the results that *A. heterophyllum* leaves could be used as a potential source for extraction of steviol. So far only paucity of reports have been published which described the quantification of free steviol in *S. rebaudiana* leaves (0.3–30 µg/g dry wt), leaves of *Rubus suavissimus* (4 µg/g dry wt) and the roots of Indian mangroves (7–43 µg/g dry wt) (Ceunen and Geuns 2013; Gardana et al. 2010; Minne et al. 2004; Subrahmanyam et al. 1999; Wang and Lu 2007). Compared with these studies, this work documented higher steviol levels in leaves of *A. heterophyllum*. In *S. rebaudiana* leaves, the higher content of steviol is likely to be associated with more production of steviol glycosides and thereby providing the sweetness to leaves (Ceunen and Geuns 2013). Conversely, the *A. heterophyllum* leaves being tasteless indicated that higher level of steviol might get accumulated in the leaves and is not converted into steviol glycosides. Keeping in view that *S. rebaudiana* leaves are preferably used for the extraction of steviol glycosides (stevioside and rebaudioside A) not for steviol, the equivalent level of steviol in *A. heterophyllum* leaves provides a novel source for extraction of steviol. Being a critically endangered medicinal herb and concern for shedding of leaves during harvesting, our recommen-

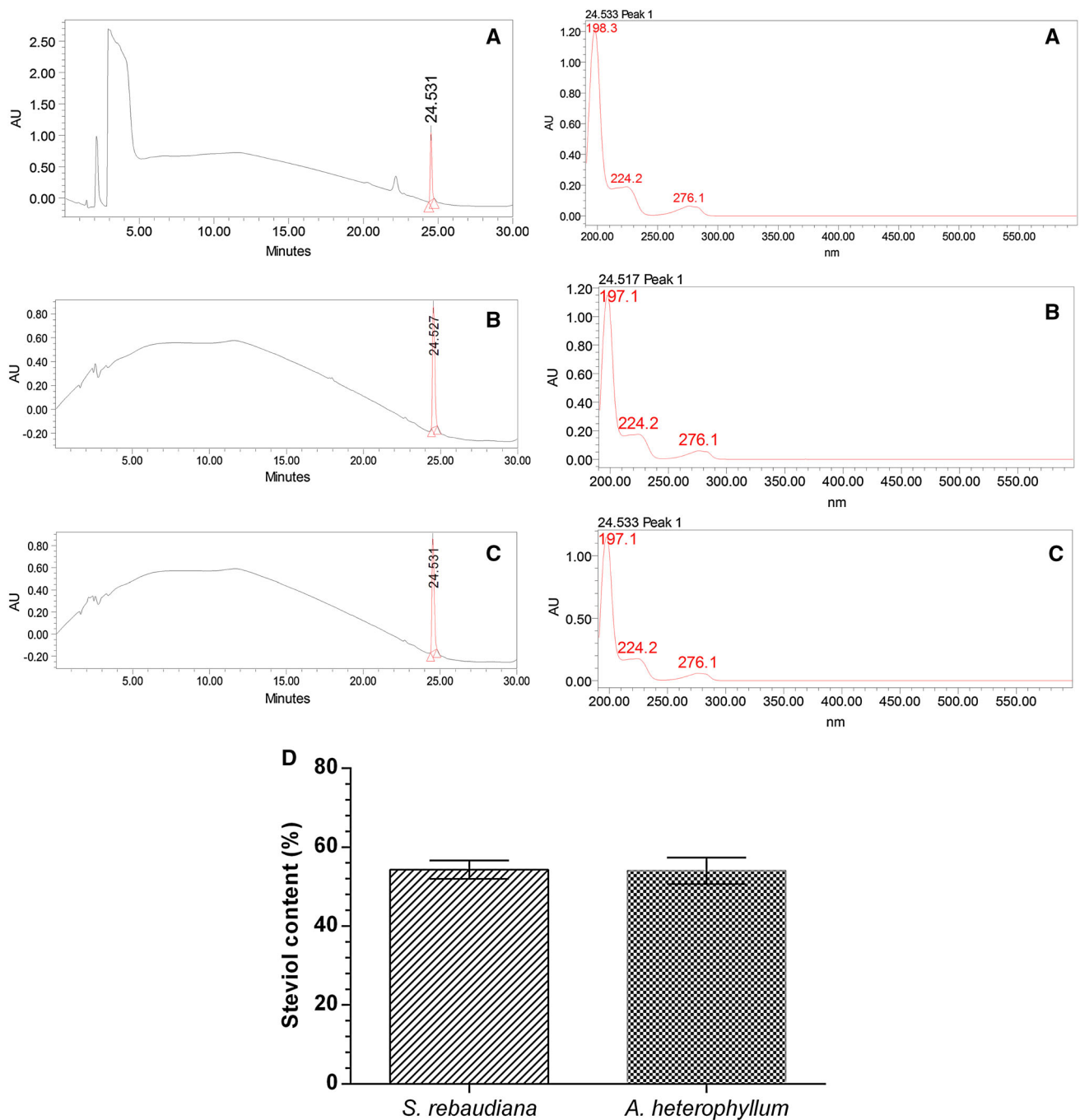


Fig. 1 Steviol content in *S. rebaudiana* and *A. heterophyllum*. **a** HPLC chromatogram and UV spectra of steviol standard, **b** HPLC chromatogram and UV spectra of steviol in *S. rebaudiana* leaves, **c** HPLC chromatogram and UV spectra of steviol in *A.*

heterophyllum leaves, **d** Bar graph showing % steviol content [mean \pm SD (n = 3)] in leaves of *A. heterophyllum* and *S. rebaudiana*

dation for steviol extraction use will give rise to novel source and step forward for broader utilization of this non-toxic species in near future.

Acknowledgments The authors are thankful to the Department of Biotechnology, Ministry of Science and Technology, Government of India for providing financial support to RSC in the form of a Programme Support on high-value medicinal plants.

Compliance with ethical standards

Conflict of interest The authors declare that they do not have any conflict of interest.

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