Heavy metal contamination and genotoxicity assessment of anthelmintic medicinal plants in Assam, India

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Research Article

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Abstract

Medicinal plants possess several metals obtained naturally or through pollution. The study evaluates the genotoxic potentials and the presence of essential elements and toxic heavy metals in *Sesbania sesban* var. *bicolor* (leaves), *Cyperus compressus* (roots) and *Asparagus racemosus* (roots), which are used as anthelmintics in the traditional medicine of the Santhals in India. Genotoxicity study was performed as per Organization for Economic Co-operation and Development (OECD) guidelines in rats. Element contents were evaluated using inductively coupled plasma-optical emission spectrometry (ICP-OES). Administration of *S. sesban* var. *bicolor* (400 mg/kg), *C. compressus* (700 mg/kg) and *A. racemosus* (500 mg/kg) did not cause any abnormalities in the chromosomes. Elemental analysis revealed the presence of lead (5.00 ± 0.05, 4.98 ± 0.01, 4.99 ± 0.07 ppm), cadmium (4.99 ± 0.04, 4.90 ± 0.03, 5.00 ± 0.08 ppm) and arsenic (0.98 ± 0.03, 0.41 ± 0.05, 0.92 ± 0.03 ppm), respectively. Although genotoxic potentials were lacking, the presence of toxic heavy metals in them is a cause of concern.

Introduction

In recent years a growing interest in herbal medicine has developed due to its long history of usage and belief that they are natural and hence safe (Woo et al. 2012). However, considering medicinal plants to be safe and efficacious because of their long history of traditional use is not only unreliable but also dangerous (De Smet 2004; Kahaliw et al. 2018). Several herbal medicines continue to be sold freely in markets and fairs, and also by herbal vendors in the streets with little or no restriction (Lanini et al. 2012). Several countries like Bulgaria, and Nepal, including India, lack proper quality and control regulations for medicinal plants (FAO 2019).

Toxicity associated with medicinal plants has been established by several workers. Medicinal plants as well as their constituents are capable of causing genotoxicity in their users (Seukep et al. 2014). A study by Nath et al. (2017) revealed that *Acorus calamus*, used in folk medicine in India, caused genotoxic and hepatotoxic effects in its users. Several medicinal plants from Ethiopia, Argentina, Serbia, and parts of Africa have also been shown to produce genotoxic effects (Mattana et al. 2014; Seukep et al. 2014; Kahaliw et al. 2018; Madić et al. 2019). Inspite of their efficacy, medicinal plants can also be a source of toxic elements (Yuan et al. 2011). This has encouraged researchers to investigate medicinal plants for the presence of toxic elements in them (Brima 2017). Elements such as Al, As, Cd, Cu, Pb, Hg, Tl, Sn, and Zn are common adulterants found in medicinal plants (Woo et al. 2012). Toxic potentials of herbal medicine are attributed to two major factors, intrinsic (direct) which include active chemical constituents present in the herbs and extrinsic (indirect) which include contamination, adulteration and misidentification of the medicinal plants (Drew and Myers 1997). A vast majority of herbal medicine still await proper clinical trials and the assessment of their efficacy and safety has become a growing concern. Owing to reports of toxicity caused by usage of traditional herbal medicines, a thorough investigation on safety profiles of medicinal plants seems necessary (Nath et al. 2017).

The leaves of *S. sesban* var. *bicolor*, (Fabaceae) and roots of *Cyperus compressus* (Cyperaceae) and *Asparagus racemosus* (Asparagaceae) are used in the traditional medicine system of the Santhals to treat helminth infections. *A. racemosus* has also been claimed to possess anti-mutagenic properties (Akram et al. 2020). Since these medicinal plants are used frequently, this study was conducted to evaluate their potential genotoxic effects, if any in rodent models and also to evaluate the presence of both essential and toxic elements in them.

Methods

Chemicals

The following chemicals were procured from the respective companies to carry out this study. Colchicine (Sigma-Aldrich), disodium hydrogen phosphate (Na_2HPO_4) (Himedia), DPX (SDFCL), Giemsa stain (Sigma-Aldrich), glacial, acetic acid (Himedia), methanol (Rankem), nitric acid (HNO_3) (Himedia), potassium chloride (KCl) (SDFCL), potassium dihydrogen phosphate (KH_2PO_4) (Rankem), sodium chloride (NaCl) (Sigma-Aldrich), and sulphuric acid (H_2SO_4) (Himedia).

Plant material

Fresh leaves of *S. sesban* var. *bicolor* and roots of *C. compressus*, and *A. racemosus* were collected from natural habitats in Assam with the help of traditional healers. Herbariums were made and identified by a plant taxonomist from the Department of Botany, North-Eastern Hill University (NEHU) and voucher specimen numbers were assigned. Plant materials were washed, shade dried and powdered in a kitchen mixer. The plant materials were then extracted in methanol using Soxhlet apparatus and stored at 4°C in vials. The powdered plant material and plant methanolic extracts were used to carry out further analysis.

Experimental animals

Laboratory inbred Wistar strain rats weighing about 150 to 200 g which were procured from the animal room of the Department of Zoology, NEHU, were used to perform genotoxicity studies. Animals were kept in separate cages with ad libitum access to water and food. Experiments in laboratory animals was performed after due approval from the Institutional Ethics Committee (IEC). All the animals were handled strictly according to the guidelines stated by the IEC and comply with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Genotoxicity studies

Genotoxicity studies were conducted on Wistar rats following the protocol of Guo and Wu (2008) with minor modifications. The animals were dosed according to the dosage administered by traditional healers to their clients. The recommended dose was taken as the median dose and two doses, one exponentially lower and the other higher that the median doses were selected for the study. Accordingly,

animals were dosed 100, 200 and 400 mg/kg b.w. of *S. sesban* var. *bicolor* leaf extract, 175, 350 and 700 mg/kg b.w. of *C. compressus* root extract and 125, 250 and 500 mg/kg b.w. of *A. racemosus* root extract.

Animals were divided into three groups, with five animals in each. Group 1 served as the control and received only the vehicle. Group 2 and 3 received highest and lowest dose respectively. All the animals were dosed for 14 days. At the end of the dosing period, the animals were subjected to mitotic arrest by injecting colchicine intraperitonially (4mg/kg). The animals were then sacrificed after 2 h, according to the prescribed guidelines of IEC (Animal Model) of NEHU.

The femurs were dissected out and flushed with pre warmed (37±1°C) 0.06 M KCl through a 40 µm cell strainer into a 50 ml centrifuge tube. The samples were kept in Carnoy's fixative then centrifuged at 1500 rpm for 15 min (4 times) with KCl. After each centrifugation, the supernatant was discarded and the pellet was used to carry out further examinations. Finally, the cells were resuspended in 2 ml fixative and stored at -20° C for further use.

To make the slides, a few drops of the cell suspension was dropped on grease free, methanol cooled slides from a height of about 1 m. The slides were then warmed in flame for 2 seconds to allow the cells to spread following which they were allowed to dry, and then stained in 5% buffered Giemsa stain, (pH – 7) for 10 minutes. The slides were then washed in distilled water to remove excess stain and allowed to dry. The slides were mounted in DPX and observed in oil at 100 x for various chromosomal aberrations which included chromatid breaks (CB), isochromatid breaks (ICB), chromosomal fragments (CF), exchanges (E) and sister chromatid unions (SCU). To score various chromosomal aberrations, about 100 well spread metaphase plates were examined per animal (Nath et al., 2017).

Elemental analysis

To perform elemental analysis, the powdered plant material (1 g) was subjected to acid digestion using HCl and HNO_3 in the ratio 2:1. The acid mixture was made to evaporate in a hot plate following which distilled water was added and then filtered. The filtrate was made to a final volume of 50 ml then subjected to elemental by inductively coupled plasma-optical emission spectrometry (ICP-OES) using Thermo Fischer iCAP 7600. Essential elements such as chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn) and nickel (Ni) were estimated. Toxic heavy metals arsenic (As), cadmium (Cd), and lead (Pb) were also evaluated. The values were compared with the WHO/FAO permissible limits (ppm) and inferences were drawn (WHO, 1998; FAO/WHO, 1999; WHO, 2005; Valadez-Vega et al., 2011; Shah et al., 2013; Dabanovic et al., 2016).

Statistical analysis

The results obtained in the study were analysed using OriginPro 8. All data are represented as mean ± standard error of mean (SEM). $P \leq 0.05$ was considered to be significantly different.

Results

Genotoxicity studies

Genotoxicity studies of the three test plants did not reveal any abnormalities or deviations from the control group. The photomicrographs of bone marrow metaphase chromosome spreads revealed intact and normal chromosomes (Fig. 1).

Elemental Analysis

Elemental analysis of the studied plants revealed that the levels of essential elements namely, Co, Cr and Ni were found to be above the permissible limits as set by the WHO/FAO in all the three studied plant extracts. Cu, Zn, Fe, and Mn were found to be present in safe concentrations. Fe was however not detected in *C. compressus*. Two of the estimated toxic elements namely, Cd and As were found to be above the permissible limits of WHO/FAO in all the three plant species. Although Pb was detected in all the three plant extracts, it was found to be below the permissible limits (Table 1).

Table 1
Elemental concentration in the test plants' samples

Plants	Essential elements							Potentially toxic elements		
	Cu	Zn	Fe	Co	Mn	Cr	Ni	Pb	Cd	As
S. sesban var. *bicolor*	9.95 ± 0.03	4.82 ± 0.03	5.91 ± 0.01	5.00 ± 0.03	8.15 ± 0.04	9.88 ± 0.05	4.93 ± 0.05	5.00 ± 0.05	4.99 ± 0.04	0.98 ± 0.03
C. compressus	9.06 ± 0.02	4.86 ± 0.02	ND	4.90 ± 0.02	8.46 ± 0.09	9.17 ± 0.01	4.96 ± 0.01	4.98 ± 0.01	4.90 ± 0.03	0.41 ± 0.05
A. racemosus	9.97 ± 0.05	5.00 ± 0.05	7.94 ± 0.07	5.02 ± 0.05	9.97 ± 0.02	9.92 ± 0.07	4.99 ± 0.07	4.99 ± 0.07	5.00 ± 0.08	0.92 ± 0.03
FAO/WHO permissible limits	10	50	20	1.5	200	1.5	1.5	10	0.3	0.1

Data are expressed as mean ± SEM. Values are significantly different at p 0.05. All concentrations in ppm (parts per million).

Discussion

Efficacy and safety profiles of medicinal plants must be based on proper scientific validations (Kahaliw et al. 2018). This study has evaluated the genotoxic potentials of three medicinal plants as well as estimated the content of essential and toxic elements present in them. Genotoxicity study is conducted to observe for any damages caused by long term administration of plant extract and has a significant role

in the identification of toxic effects of any drugs (Turkez et al. 2017). A similar study by Nath et al. (2017), on the toxicity studies of *Acorus calamus*, revealed that the crude extract caused genotoxicity in the form of chromosome breaks, fragments, sister chromatid unions and exchanges. Similar results were reported in a study by Seukep et al. (2014) on African medicinal plants where, 33 plants were found to cause genotoxic effects in animal or bacterial systems. However, Edziri et al. (2013) performed a similar study on *Cleome amblyocarpa* a medicinal plant used in Tunisia and observed that it did not cause any genotoxic effects. Similarly, the finding of this study also revealed that the administration of the plant extracts did not cause abnormalities at the chromosomal level in animals, indicating that that they lack genotoxic potentials.

Metals are essential nutrients required for various biochemical and physiological functions. Essential elements are important for human health for the prevention and control of diseases. Several authors have estimated the element contents of medicinal plants (Pattar et al. 2019; Jyothsna et al. 2020). In the present study, all the studied plants revealed high level of essential elements such as Co, Cr and Ni. All the plants also showed presence of high amounts of toxic elements Cd and As. Pb was also detected in safe limits in all the three studied plants. Since plants absorb minerals from the soil, this accumulation of toxic elements in them can be attributed to this phenomenon (Singh et al. 1997).

Exposure to Pb results in bone marrow and kidney damage, neurotoxicity, and gastrointestinal and neurodevelopmental defects (Tchounwou et al. 2012) whereas the main cause of Cd exposure occurs through ingestion of foods containing Cd (Satarug et al. 2003). It is a pulmonary and gastrointestinal irritant and its ingestion or inhalation could cause death (Baselt and Cravey 1995). It is also carcinogenic and its chronic exposure could result in renal damage and pulmonary fibrosis (Tchounwou et al. 2012). Exposure to As occurs through ingestion, inhalation, dermal contact and parenteral route (Tchounwou et al. 2003) and is known to cause vitamin A deficiency resulting in night blindness (Saha et al. 1999). The exposure of these metals may affect different organ systems, including the cardiovascular, dermatologic, nervous, hepatobiliary, renal, gastro-intestinal, and respiratory systems (Tchounwou et al. 2003).

Cr exposure may cause oxidative stress in the cell resulting into damage of DNA and proteins (Stohs and Bagchi 1995). It possesses mutagenic properties and is categorized as a group 1 human carcinogen (Zhang et al. 2011). On the other hand, Ni induced toxicity occurs by pulmonary absorption and it is a known haematotoxic, immunotoxic, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, hepatotoxic and carcinogenic agent (Das et al. 2008). Excess Co intake is characterized neurological, cardiovascular and endocrine deficits (Leyssens et al. 2017). A study by Tschinkel et al. (2020) revealed similar results where several medicinal plants used in Brazil showed high amounts of Pb and Cd in them. Also, Silva et al. (2016) reported high concentrations of Ba, Cr, Fe, Hg, Se and Ni in *Achillea millefolium, Senna alexandriana* and *Malva sylvestris* used in traditional medicine in Brazil. However, some authors have reported normal levels of elements in medicinal plants in their studies (Ebrahim et al. 2012; Brima 2017).

Conclusions

This is the first study in the region that has detected the presence of toxic elements in medicinal plants. Although the studied plants did not reveal genotoxic effects, the presence of toxic heavy metals in them is a cause of concern since these medicinal plants are used widely. The accumulation of these toxic elements can be attributed to presence of these elements in the soil of the studied area. Their uncontrolled consumption can result in adverse toxic effects in their users and hence their usage in the area should be done with caution.

Declarations

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Competing interests

All the authors declare that they have no competing interests.

Availability of data and materials

All data generated pertaining to this study have been included in this article.

Code availability

Not applicable

Authors' contribution

AKY proposed, conceptualized and supervised the study and interpreted the genotoxicity results of the study. ADS performed the genotoxicity experiments, handled the animals, analysed the data and wrote the first draft. All authors read and approved the final manuscript.

Ethics approval

All animals were procured from the animal room of the Department of Zoology, North-Eastern Hill University (NEHU). Genotoxicity studies were performed after approval and written consent was obtained from the Department of Zoology and IEC (Animal models), NEHU (vide, Member Secretary, IEC, NEHU, letter number 01, dated December 4, 2014). All the experiments on animals were performed strictly as per the guidelines laid by the IEC, NEHU, Shillong and ARRIVE guidelines. Plant specimens were brought to

the Department of Botany, (NEHU) where specimens were identified and allotted voucher numbers (NEHU – 12084: *Sesbania sesban* var. *bicolor*, NEHU – 12085: *Cyperus compressus* and NEHU – 12098: *Asparagus racemosus*). A copy of each plant specimen was retained in the Parasitology and Ethnopharmacology Lab, Department of Zoology, NEHU. The research was approved by the university research committee.

Consent to participate

Not applicable

Consent for publication

Not applicable

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Figures

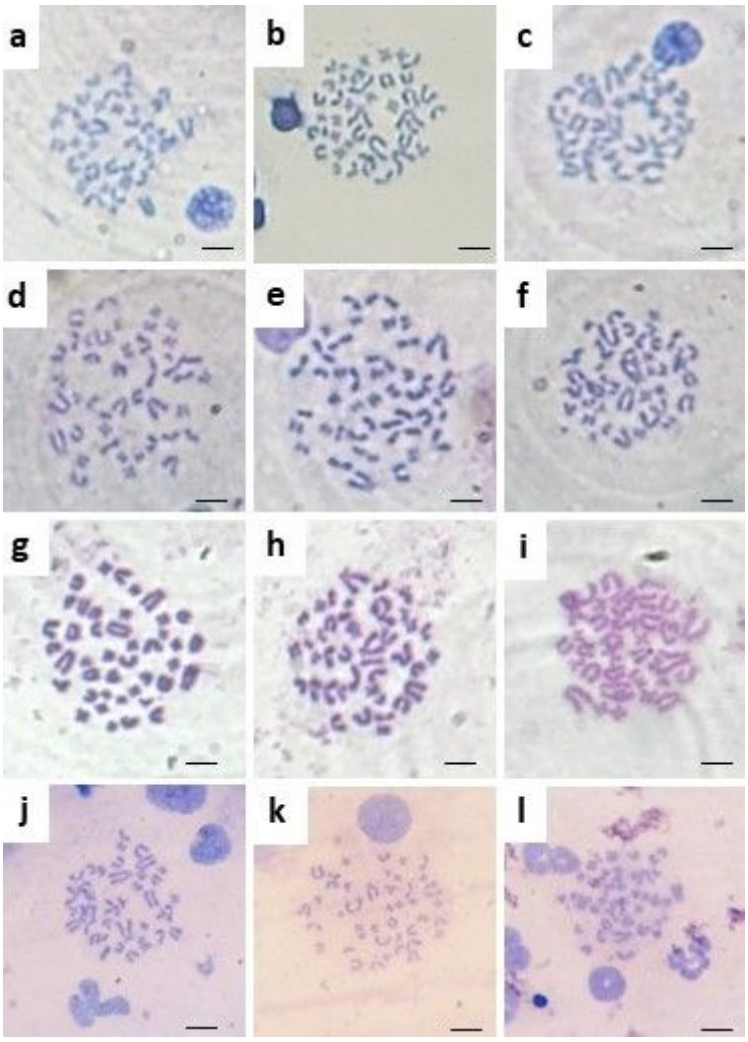

Figure 1

Photomicrographs of bone marrow metaphase chromosome spreads of rats. (a, b, c) – control rat, showing normal set of chromosomes; (d, e, f) rat treated with S. sesban var. bicolor leaf extract; (g, h, i) rat treated with C. compressus root extract; (j, k, l) rat treated with A. racemosus root extract. Chromosome breaks (CB), chromosomal fragments (CF) and sister chromatid unions (SCU), exchanges (E). Scale – 100x, (-) = 5μm

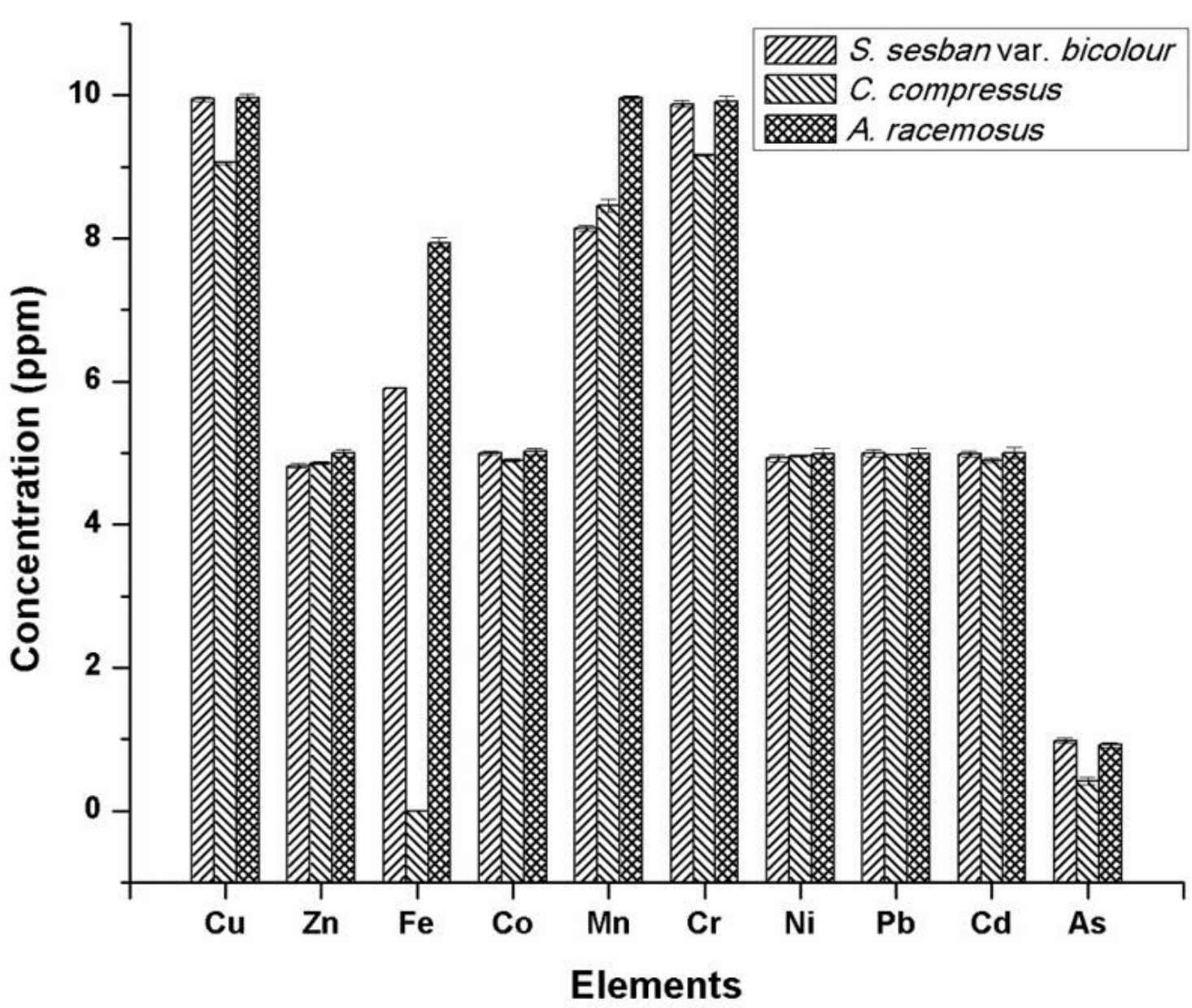

Figure 2

Concentration of various elements in the samples of S. sesban var. bicolor, C. compressus and A. racemosus

Supplementary Files

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