



RESEARCH ARTICLE

Nutrients, anti-nutrients composition and antioxidant potentiality of two ethnomedicinally important culinary spices of Tripura, northeast India

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ARTICLE HISTORY

Received: 24 October 2025

Accepted: 05 February 2026

Available online

Version 1.0 : 24 August 2026

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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CITE THIS ARTICLE

Chiranjit P, Kripamoy C, Bimal D. Nutrients, anti-nutrients composition and antioxidant potentiality of two ethnomedicinally important culinary spices of Tripura, northeast India. Trends in Current Biology. 2026; 4(3): 31–37. <https://doi.org/10.14719/tcb.9455>

Abstract

The present study was aimed to estimate the nutrient and anti-nutrient compositions and antioxidant potentiality of two commonly used spices in the different food preparations (curries, guda and meat) to improve the palatability of the food items. The people of Tripura especially the Manipuri community are often using these spices in their diet since time immemorial consciously or unintentionally. Both the spices have significant ethnomedicinal importance although most of the people are not aware of the facts. Proximate composition analysis reveals that these two spices are enriched with nutrients and have strong antioxidant activity. These two comparatively less popular spices can serve as the key future driving force in the province of nutraceutical industries and thus may play a significant role in the livelihood development of the people of Tripura.

Keywords

antioxidant activity; ethnobotany; livelihood; Manipuri community; nutraceutical industries; nutrient analysis

Introduction

Ethnobotany is the scientific study of phytoresources used by the indigenous people in their everyday life (1). Medicinal plants occupy important positions in the socio-cultural, spiritual and medicinal gridiron of rural people of India (2). The preferences of cost-effective and harmless therapeutics over high priced and apprehension on side effects of conventional drugs are augmented which increase the dependency on phytomedicine as a remedial measure of several ailments (3). Culinary spices have been used from primeval times as food preservatives, to improve the palatability of the food stuffs and possess health-enhancing properties (4). Spices are imparting a proficient effect on certain target body functions as well as fulfilled the indispensable nutritional requirements (5). Moreover, they have noticeable pharmacological and medicinal properties (6).

Food spices are utilized in different food cuisine to improve flavour and colour of the dishes used in the daily diet. Different plant parts such as seed, bark, fruit, root and leaves are used as spices. *Allium tuberosum* (*A. tuberosum*) and *Zingiber zerumbet* (*Z. Zerumbet*) are used as spices by the indigenous people especially the people belonging to the Manipuri community of Tripura. They are using these 2 spices to improve the palatability of the traditional dishes although they are not fully aware about the nutritional benefits of these spices. Literature survey concerning their ethnobotanical and pharmacological activities pointed out the fact that both spices acquired enormous importance in both the aspects. The whole plant of *A. tuberosum* is taken as a vegetable for curing various liver disorders as well as gastrointestinal disorders and fever (7); roots can treat dyspepsia and prevent gastric ulcer (8). *Zingiber zerumbet* has important economic properties, as the rhizome can be used as both a spice and a traditional medicine (9). Presence of antioxidant activity among the vegetables, fruits and spices has been postulated globally (10–11) and relate to the presence of antioxidant vitamins (12). Existing literature survey and to best of our

knowledge no study that has been endeavoured directing the estimation of proximate composition and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity along with their respective ethnomedicinal properties of these 2 spices promoted us to carry out this present work.

Materials and Methods

Study sites and plant identification

Allium tuberosum Rottl. ex. Spreng. and *Zingiber zerumbet* (L.) Roscoe ex Sm. were collected from village of Bajalghat (23°56'30" N, 91°18'27" E at 29 masl) and Taltala (23°57'53" N, 91°17'07" E at 20 masl) of West Tripura district. Collection was done during active growth periods of these 2 spices. Plants were identified by using the Flora of Tripura (13).

Ethnobotanical survey

A field survey was conducted during August 2020 to gather the information from the people consuming these spices in their dish preparations on the ethnobotanical importance. Male and female participants (n = 50) with the age ranging from 20–65 and consented to share their respective traditional knowledge were considered for data collection. The informants acquired their unique techniques of plant identification and medicinal preparations to cure the ailments. Information was collected through semi-structured questionnaires and interviewing the individual participants or group discussion. The data collected by interviewing the respondent was cross verified by discussing with the Maiba, the medicine man of Manipuri community.

Sample collection

Healthy and disease-free leaves of *A. tuberosum* and rhizomes of *Z. zerumbet* were harvested from the home gardens of the Manipuri community. The leaves of *A. tuberosum* were collected mechanically by using sharp knives without uprooting the plants whilst rhizomes were collected by uprooting the plants by using trowel. The organic debris and soil particles adhered with the rhizomes were removed. After that, the respective samples were taken into zippered marked polythene bags and transported to the Plant Diversity and Forest Biotechnology Laboratory for further assessment (Fig. 1).

Sample preparation

The shade dried leaf of *A. tuberosum* and rhizome of *Z. zerumbet* were ground in the powdery form by using a mechanical grinder and used for further assessment (Fig. 1). Total protein content was determined from the fresh samples. Phenol and flavonoid contents were determined by using the shade dried powder. For evaluation of the antioxidant activity methanol and petroleum ether extract were used (14).

Estimation of moisture content

Leaf and rhizome samples were chopped into small pieces using sterilized blades. 10 g of the chopped samples were taken in the previously weighed Petri plates. Then the sample was kept in a hot-air oven for overnight at $100 \pm 2^\circ\text{C}$. The dried samples were brought to room temperature and weighed to a constant weight. The loss in weight was considered as the moisture percentage and was calculated using the following formula:

Where, W_1 = weight of the sample (leaf and rhizome) taken, W_2 = weight of the oven-dried samples

Determination of carbohydrate

$$\text{Percentage of moisture content (\%)} = \frac{W_1 - W_2 \times 100}{W_1}$$

Carbohydrate was determined by using the method ascribed (15) from the dried leaf and rhizome samples. 100 mg of the sample was taken into boiling tubes and hydrolyzed with 5 ml of 2.5 N-HCl for 3 hr and cool at room temperature followed by the neutralization with sodium carbonate pellets. The volume is made up to 10 ml and centrifuge at 5000 rpm for 15 min. The supernatant was collected and 1 mL aliquots were taken for analysis. Then 4 mL of 2 % anthrone (w/v in concentrated H_2SO_4) reagent was added and heated in a boiling water bath for 10 min. The absorbance was taken at 630 nm using a spectrophotometer. Glucose was used as a standard.

Determination of protein

The protein content was estimated (16). 100 mg of the sample was ground well with a pestle and mortar in 10 ml of the potassium phosphate buffer (0.1 M, pH 7.5) and centrifuge at 5000 rpm for 15 min. The pellet was discarded and the supernatant was used for protein estimation. From the supernatant 1 mL of sample was taken in dried test tubes and 5 mL of reagent 'C' was added. The reagent 'C' was prepared by mixing reagent 'A' and reagent 'B' in a ratio (50 : 1 v/v). Reagent 'A' is a mixture of 2 % Na_2CO_3 and 0.1 N NaOH and reagent 'B' is a mixture of 0.5 % CuSO_4 and 1 % Na-K tartarate. The solution was shaken vigorously and allowed to stand for 20 min. After that 0.5 ml of Folin–Ciocalteu reagent was added and incubated at room temperature for 30 min. Absorbance was measured at 660 nm using a spectrophotometer. Bovine Serum Albumin (BSA) was used as a standard.

Estimation of total free amino acids

The amount of total free amino acid was determined by adopting the methods prescribed (17). For this, 100 mg of dried samples were homogenized in 10 mL of 50 % aqueous ethanol with a pinch of activated charcoal. The slurry was centrifuged at 5000 rpm for 10 min and the free amino acid was extracted in the form of a clear supernatant which was used for spectrophotometric estimation. The volume of supernatant was raised to 10 mL with aqueous 50 % ethanol. To 1 mL of the supernatant 2 mL of 2 %, Ninhydrin (w/v in dehydrated alcohol) was added. The mixture was kept on a water bath at $75 \pm 2^\circ\text{C}$ for 10 min and after cooling, aqueous alcohol (1 : 1) was added to make up the volume to 3 mL. The absorbance was measured at 570 nm on a spectrophotometer. Glycine was used as a standard.

Determination of fat content

The fat content was determined (18). 2 g of the sample was taken in a dried test tubes and petroleum ether was added on that and allowed to stand for 16 hr. After 16 hr the petroleum ether was evaporated to dryness and weights the flask before after for fat.

Estimation of crude fibre

Crude fibre of the flower samples was estimated (18). 1 g of dried leaf sample was subjected to acid and subsequent alkali digestion for degradation of native cellulose and lignin. The residue obtained after final filtration was weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fibre contents.

Determination of total phenols

Total phenol was determined (19). 200 mg of sample were crushed in 10 % methanol and centrifuged for 20 min at 5000 rpm. 1 mL supernatant was taken and 1 mL Folin Ciocalteu reagent was added flowed by the incubation for 3 min at room temperature. Then, 1 mL of saturated 20 % Na_2CO_3 was added and kept in a water bath for

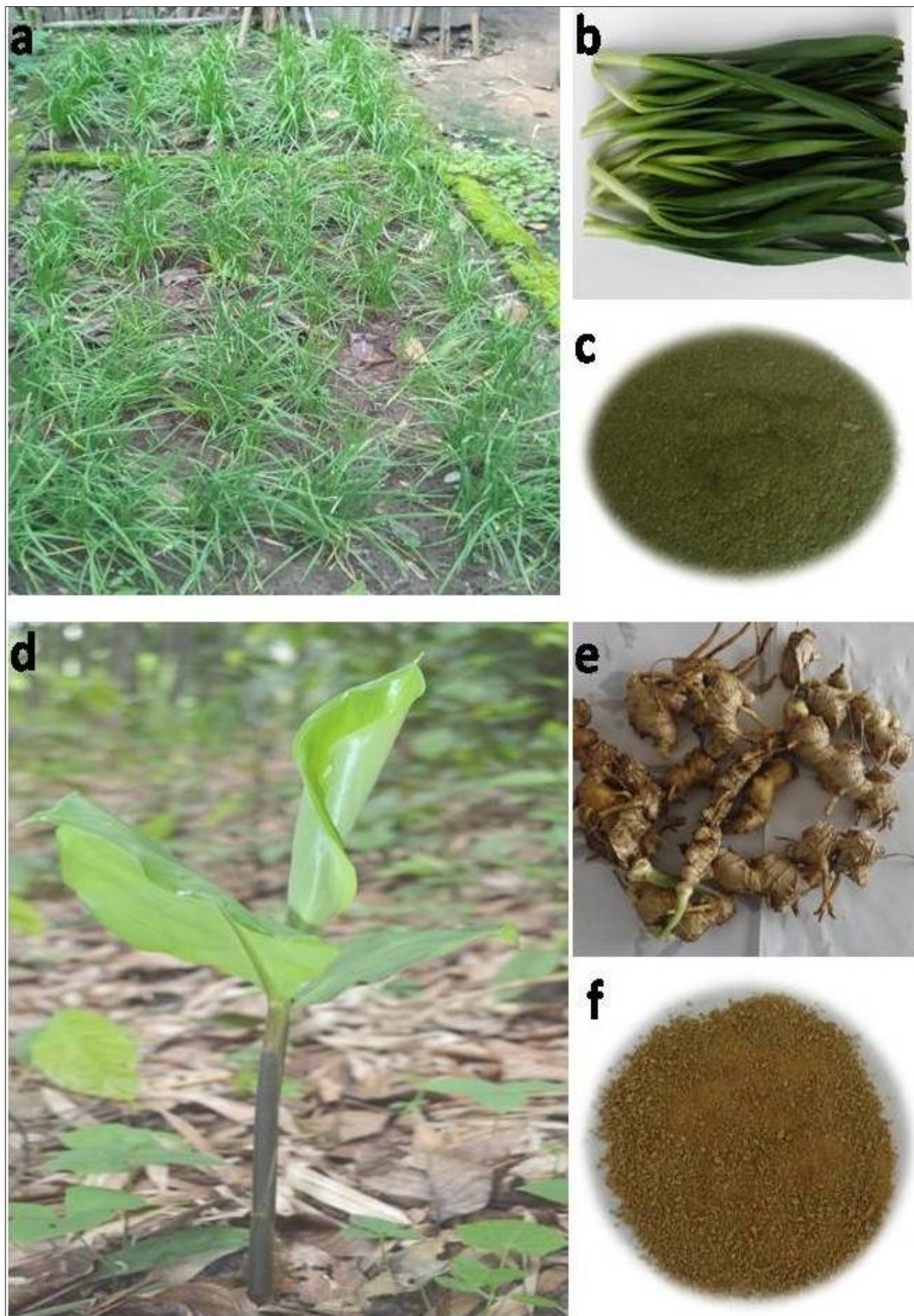


Fig. 1. Spices used by the Manipuri community of Tripura. (a) Habitat of *Allium tuberosum*; (b) & (c) Leaves and powder of *Allium tuberosum*; (d) Habitat of *Zingiber zerumbet*; (e) and (f) Rhizomes and powder of *Zingiber zerumbet*.

1 min. The absorbance was measured at 725 nm. Gallic acid was used as a standard.

Determination of flavonoids

The flavonoid content was estimated (20). 0.5 mL of test samples solution in methanol (5 mg/100 mL) was mixed with 2 mL of distilled water and 150 µL of 5 % sodium nitrate. After 6 min, 150 µL of 10 % aluminium chloride and 2 mL of 1 M sodium hydroxide was added and left at room temperature for 15 min. The absorbance of the mixtures was measured at 510 nm. Catechin was used as a standard.

DPPH radical scavenging activity

The free radical scavenging activities of methanol extract of all the samples were evaluated by DPPH (1, 1-diphenyl-2-picryl-hydrazyl) method as ascribed (21). Different concentration of methanol extracts (100, 200, 400, 600, 800, 1000 µg/mL) were mixed with 300 µL DPPH (0.02mM). The absorbance was measured at 517 nm using a UV-VIS double beam spectrophotometer (Dynamica, DB-20) after 30 min of incubation at dark. Blank is prepared by mixing methanol with DPPH. Ascorbic acid was used as a reference. Scavenging of DPPH was calculated by using the formula:

$$\text{DPPH scavenging activity (\% of inhibition)} = (A_0 - A_1) \times 100 / A_0$$

Where, A_0 is the absorbance of the control reaction and A_1 is the absorbance of the sample.

Data analysis

Each of the analysis was performed in triplicate and expressed as Mean $\pm$ SD. Antioxidant activity was determined and IC_{50} value was calculated using the linear regression curve in Microsoft Excel 2007.

Results

Ethnobotanical importance

The people belonging Manipuri community used these 2 spices as a remedial measure for several ailments like skin infection, diabetes, common cold and cough. The mode of medical preparations differs for both spices. Very few numbers of respondents are aware of the medicinal importance of these 2 spices although they are using these in their dish preparations for improving the palatability of the dishes (Table 1).

Nutrient and antinutrient contents

Nutrient analysis reveals that both the spices contain adequate amounts of essential daily supplements such as total protein,

soluble sugar, total carbohydrate, crude fibre, total free amino acids, and total fat.

Soluble sugar (1.79 ± 0.11) and total carbohydrate (2.89 ± 0.02) content were higher in rhizome *Z. zerumbet* compared to leaves of *A. tuberosum*. Total free amino acid, total protein and fat content was recorded in higher amount in the leaves of *A. tuberosum*. Antinutrient analysis pointed out that total phenol (10.44 ± 0.06) and total flavonoid (6.41 ± 0.47) contents were higher in *A. tuberosum* compared to *Z. zerumbet* (Table 2).

Antioxidant activity and IC_{50} values

Methanolic extracts of *A. tuberosum* leaves exhibited maximum antioxidant activity compared to the rhizomes of *Z. zerumbet* whilst in petroleum ether extracts *Z. zerumbet* rhizomes exhibited maximum antioxidant activity compared to the leaves of *A. tuberosum*. Methanolic extracts usually exhibit higher antioxidant activity than petroleum ether extracts due to higher polar phenolic compounds and flavonoids concentrations. But in some cases petroleum ether extracts showed significant antioxidant activity due to specific compounds. Methanolic extracts of *A. tuberosum* leaves exhibited maximum antioxidant activity due to the presence of high phenol and flavonoid contents conversely *Z. zerumbet* possess tuberosine A, tuberoside A and tuberoside N which may be correlated with the higher antioxidant activities in petroleum ether extracts (Table 3).

Discussion

The leaves of *A. tuberosum* are chopped into small pieces and used in most of the curries for the betterment of the taste and delicacy although sometimes leaves are used to garnish food preparations. The rhizome's paste of *Z. zerumbet* is used in selective food preparations such as in 'gudak' and meat. The food spices selected for this study have noteworthy ethnomedicinal potential (7, 9, 22) which may serve for the improvement of primary health care system of local inhabitant of Tripura especially belonging to Manipuri community. In addition, both these spices are the reservoir of pharmacologically active phytoconstituents having enormous pharmacological potentiality (23–24). In vitro trials of these active constituents are required for the concrete generalization with respect to their possible application in pharmaceutical industries. These 2 spices are enriched with nutrients and have potent antioxidant activity. Literature surveys concerning the nutrient analysis of these 2 spices revealed that meager information are

Table 1. Ethnomedicinal importance of two spices of Tripura

Name of the plant	Vernacular name	Ethnomedicinal importance
<i>Allium tuberosum</i>	Nennam(M)	Leaves are used as remedial measures of stomach disorder and diabetes. Tubers are used to treat skin allergy.
<i>Zingiber zerumbet</i>	Jaindhana(M)	Raw tuber paste used after biting of some poisonous insect. Dried tubers in mixture with honey are used to treat common cough and cold.

Table 2. Nutrients and anti-nutrient contents in two spices of Tripura

Name of the plant	Nutrient contents (mg/g of sample)							Antinutrient content (mg/g of sample)	
	MC	SS	TC	TP	TFA	F	CF	TP	TFL
<i>Allium tuberosum</i>	87.13 $\pm$ 0.32*	1.50 $\pm$ 0.01	2.51 $\pm$ 0.01	0.51 $\pm$ 0.01	1.41 $\pm$ 0.08	0.17 $\pm$ 0.27	1.34 $\pm$ 0.18	10.44 $\pm$ 0.06	6.41 $\pm$ 0.47
<i>Zingiber zerumbet</i>	72.36 $\pm$ 0.40	1.79 $\pm$ 0.11	2.89 $\pm$ 0.02	0.23 $\pm$ 0.01	0.42 $\pm$ 0.04	0.08 $\pm$ 0.21	1.57 $\pm$ 0.21	6.55 $\pm$ 0.38	3.46 $\pm$ 0.31

MC = Moisture content, SS = Soluble sugar, TC = Total carbohydrate, TP = Total protein, TFA = Total free amino acids, F = Fat, CF = Crude fibre, TP = Total phenol, TFL = Total flavonoid *Mean $\pm$ SE

Table 3. Antioxidant activity in methanolic and ethanolic extracts and IC₅₀ values of two spices of Tripura

Name of the plant	Extract	DPPH inhibition percentage (%)						IC ₅₀ µg/mL
		100 µg/mL	200 µg/mL	400 µg/mL	600 µg/mL	800 µg/mL	1000 µg/mL	
<i>Allium tuberosum</i>	M	23.33 ± 2.85*	33.65 ± 1.98	59.84 ± 3.06	63.49 ± 1.92	73.65 ± 1.67	83.17 ± 0.99	420
	P	13.80 ± 1.42	27.93 ± 1.19	45.39 ± 3.17	59.36 ± 0.72	61.74 ± 0.72	72.69 ± 1.19	568
<i>Zingiber zerumbet</i>	M	35.87 ± 0.99	39.04 ± 0.95	50.47 ± 1.25	59.84 ± 1.67	63.01 ± 1.53	73.01 ± 1.80	430
	P	36.66 ± 2.18	44.12 ± 1.67	49.04 ± 1.26	54.92 ± 1.19	56.82 ± 0.54	66.19 ± 0.82	473

M=Methanolic extract; P= Petroleum ether extract *Mean ± SE

Table 4. Major compounds found in two spices of Tripura

Name of the plants	Major compounds
<i>Allium tuberosum</i>	Zerumbone, humulene, caryophyllene, zingiberene, Borneol, α-pinene, linalool, limonene, camphene, sabinene, citral, γ-terpinene, eucalyptol, β-mircene, Kaempferol, kaempferol-3-O-methyl, Kaempferol-3-O-(2,4-di-O-acetyl-α-L-rhamnopyranoside), Kaempferol-3-O-(3,4-di-O-acetyl-α-L-rhamnopyranoside), Kaempferol-3-O-(4-acetyl) rhamnoside, Kaempferol-3,4',7-O-trimethylether [33-46].
<i>Zingiber zerumbet</i>	Daucosterol, adenosine, thymidine, dimethyl disulfide, allyl methyl disulfide, dimethyl trisulfide, allyl methyl trisulfide, methyl 1-propenyl disulfide, diallyl disulfide, tuberosine A, tuberoside A, tuberoside N [24]

available on these 2 spices (25). This present study is a preliminary effort concerning their nutrient composition and antioxidant potential which will offer a suitable baseline for the future research on these 2 species. Reports are available on the existence of direct correlation between the cereal intake and spice consumption among the urban and rural peoples (26) which was reaffirmed in this present study as the people of manipuri community are using the spices in their daily food cuisines.

Moreover, reports are available regarding the nutrition composition of some other spices that are used in the daily diet from other parts from India and abroad (27–28). Both *A. tuberosum* and *Z. zerumbet* are collected from the home gardens and have medicinal properties. *Allium* spp. has potent antioxidant activity (29) which was reaffirmed in this present study. Phenol and flavonoids compounds are the key determinants of the antioxidant activity (30). Antioxidants are an important group of medicinal preventive compounds as well as being food additives inhibiting detrimental changes of easily oxidizable nutrients are extracted from agricultural or horticultural crops, or medicinal plants (30). The foliar phenolic and flavonoid compounds of *A. tuberosum* are higher compared to the rhizomes of *Z. zerumbet* and these compounds are considered as major antioxidant constituents (31–32). The superiority of the leaf's antioxidant activities compared to rhizomes may be explained by the concentration and nature of individual phenolic compound (Table 4).

Conclusion

These two spices consumed by the people of Tripura used in different food item preparations not only improve palatability of the dishes but also enriched in nutrients which in turn can be used as a dietary element in their daily diet. The presence of biologically significant phytoconstituents and potent antioxidant activity along with the perceptible ethnobotanical and pharmacological importance may enable their active participation in nutraceutical industries and thus may play a decisive role in the prevention of various ailments.

Acknowledgements

The authors are thankful to Head, Department of Forestry and Biodiversity for providing the laboratory facilities to carry out the work.

Authors' contributions

BD designed the work. CP and KC did the analysis. KC analysed the data. KC, CP wrote the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no competing interests.

Ethical issues: None

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