

## Chemical Profiling of Selected Plants of Zingiberaceae Used in Ethnomedicine of Koraput, India

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Received December 7, 2019

The present study aims to quantify the phytochemicals present in ten selected species such as *Zingiber roseum* (Roxb.) Roscoe, *Curcuma aromatica* Salisb., *Curcuma amada* Roxb., *Zingiber officinale* Roscoe, *Curcuma angustifolia* Roxb, *Alpinia calcarata* (Haw.) Roscoe, *Hedychium coronarium* J.Koenig, *Kaempferia galanga* L., *Curcuma longa* L., *Curcuma caesia* Roxb of *Zingiberaceae*, which are commonly used in ethnomedicine by the tribal people of Koraput, India. The phenol, flavonoid, alkaloid, saponin, tannin, and ascorbic acid are major pharmaceutical parameters that are responsible for ethnomedicinal values were present in varying proportions. The plant species also contain significant amount of antioxidants that can be exploited as a potential source for herbal remedy for various diseases. Thus, public awareness and community-based programmes need to be encouraged at all levels for ex situ and in situ conservation of such species and will further use in exploration of new drugs for pharmaceutical industries.

*Key words: Antioxidant, Ethno-medicine, phytochemicals, Zingiberaceae*

Plants are rich source of many potent and powerful drugs used in different countries (Gislene *et al.*, 2000). Out of nearly, 2,50,000 plant species on earth, more than 80,000 are medicinal in nature. About 5000 species are extensively used in traditional systems of medicine. Among the various groups of plants, Zingiberaceous plants are quietly used in the ethnomedicinal purposes from time immemorial (Singh *et al.*, 2003). Zingiberaceae or Ginger family is one of the largest monocot family in the order Zingiberales, have a great diversity with 53 genera and over 1300 species (Peter, 2007). Most of the species distributed mainly in tropics and subtropics of south East Asia and Africa (Larsen *et al.* 1999). Zingiberaceae produces aromatic rhizomes that are above the ground or subterranean (Soepadmo, 1976). Most of the species under this family are easily recognized by the aromatic characteristic of the leaves and fleshy rhizome. In South-East Asian region, several species of Zingiberaceae are used as spices, condiment, traditional medicines, flavouring agents and as the source of certain dyes (Burkill, 1996; Larsen *et al.*, 1999). In India some of these plants are underutilized crop of the country and have some role in food security and some are used by various indigenous systems of medicine (Peter, 2004). These plants are well known due to their importance in food, spice, medicinal and ornamental values (Sivarajan and Balachandran, 1994). The important genera coming under Zingiberaceae are *Curcuma*, *Kaempferia*, *Hedychium*, *Amomum*, *Zingiber*, *Alpinia* and *Elettaria*. They are well known for their use in traditional medicine (Prakashi *et al.*, 1995; Hussain *et al.*, 1992; Jain and Prakash, 1995).

Koraput district of Odisha state in India is belongs to the part of Eastern Ghats mountain ranges, one of the centres of diversity for many food crops and forest species (Mishra and Chaudhury, 2012; Mishra *et al.*, 2008). These regions are dominated with tribal populations and is blessed with rich and diverse cultural heritage. The tribal people of this region possess rich knowledge and wisdom regarding plants including their usage for treating common ailments (Mishra and Choudhry, 2012). Previous reports showed nearly seven species of plants under Zingiberaceae family are used

by the tribal communities of Koraput (Padhan and Panda, 2016; Padhan and Panda, 2015; Raut *et al.*, 2013). Apart from that few reports also suggest the ethno-botanical use of Zingiberaceae plants by the tribal communities of the Koraput (Mishra and Chaudhury, 2012; Mohapatra and Panda, 2012; Misra *et al.*, 2012; Misra and Misra, 2013; Misra and Misra, 2014; Padhan and Panda, 2016; Padhan and Panda, 2015; Raut *et al.*, 2013). The rich tribal areas of South Odisha particularly Koraput have given less attention in relation to validation of the ethno-medicinal importance of these species. However, most of these reports are incomplete and inadequate and no report is available about phytochemical and antioxidant profiling of medicinal plants of Zingiberaceae family. Owing to the significance of the above context, phytochemical screening of Zingiberaceae plants for pharmacological activity and bioactive compositional information is necessary to validate the use of these plants in the traditional medicine.

## MATERIALS AND METHODS

### Collection and characterisation of plant samples

The rhizomes of ten selected ethnomedicinal plants namely *Zingiber roseum* (Roxb.) Roscoe, *Curcuma aromatica* Salisb., *Curcuma amada* Roxb., *Zingiber officinale* Roscoe, *Curcuma angustifolia* Roxb, *Alpinia calcarata* (Haw.) Roscoe, *Hedychium coronarium* J.Koenig, *Kaempferia galanga* L., *Curcuma longa* L., *Curcuma caesia* Roxb of Zingiberaceae, were collected from different forest patches of Koraput district (Fig. 1 and Fig. 2). The ethnomedicinal importance and their tribal use was presented in Table 1. The collected plants were carefully examined and identified with the help of Flora of Orissa by Saxena and Brahmam (1994 -1996). The rhizomes were oven dried at 40° C and powdered mechanically, sieved and stored in an airtight container for further phytochemical analysis.

### Phytochemical quantification

The phenol content was determined using the method of Sadasivam and Manickam (2007) with some modifications. The samples (0.1 g) were refluxed in 80% methanol and evaporated and the volume of the crude extract was made up to 10 ml. An aliquot was prepared

with 1 ml extract, 0.5 ml Folin-Ciocalteu's reagent (Himedia) (2:1) and 2 ml 20% Na<sub>2</sub>CO<sub>3</sub> and the mixture was incubated in a water bath at 90 to 95°C for 2 min. The absorbance was measured at 650 nm and the total phenol content was expressed as gallic acid equivalence (GAE) mg g<sup>-1</sup> Dwt. using gallic acid as a standard.

Total Flavonoid content was determined by Aluminium chloride colorimetric assay with some modification according to Chang *et al.*, (2002). The crude extracts (0.5 ml) were diluted with 1.5 ml of methanol. To this 0.1 ml AlCl<sub>3</sub>, 0.1 ml Potassium acetate, 2.5 ml distilled water mixed well and allowed to stand for 30 min. The absorbance was measured at 415 nm with spectrophotometer. Results were expressed as mg flavonoid g<sup>-1</sup> of dry samples using quercetin as standard.

Total tannin content of the samples was determined using the Folin-Dennis spectrophotometric procedure described by Sadasivam and Manickam (2007). The tuber samples (0.1 g) were dissolved in 20 ml distilled water, heated for 20 min and centrifuged at 6000 rpm for 15 min. An aliquot of 0.5 ml extract was diluted to 10 ml and mixed with 0.5 ml Folin Dennis reagent (100 g sodium tungstate, 2 g phosphomolybdic acid with 50 ml phosphoric acid) and followed by addition of 1 ml 10% Na<sub>2</sub>CO<sub>3</sub> solution and incubated for 30 min. The intensity of colour was measured at 760 nm and the tannin content was expressed as mg g<sup>-1</sup> Dwt. using tannic acid as a standard.

Total alkaloid content was estimated using the gravimetric method of Harborne (1973). The samples (1 g) were extracted with 40 ml 10% acetic acid in methanol and incubated for 4 h. The solution was filtered and the filtrate was evaporated in a water bath at 80 to 90°C. To the extract, ammonium hydroxide solution was added drop wise until the precipitation was complete. The solution was incubated for 2-3 h and the precipitate was filtered and the filtrate was washed with 1% ammonia solution and allowed to stand for 30 min. The residue was oven dried and weighed to quantify the alkaloid and the value was calculated as mg 100 g<sup>-1</sup> Dwt.

The saponin content in the samples was determined using the method of Nahapetian and Bassiri (1974). The samples were extracted in 20% ethanol and re-extracted with diethyl ether and n-butanol in a separating funnel.

The mixture was washed twice with 5% aqueous sodium chloride. The upper layer was collected and evaporated. After evaporation the samples were dried in the oven to constant weight. The saponin content was calculated as mg g<sup>-1</sup> Dwt.

The total Ascorbic acid content was determined by (Omaye *et al.*, 1962). To 0.5 ml extracts were taken in the glass vials and then mixed by adding 0.1 ml of DTC reagent solution. After incubation 0.75ml of ice cold 65% H<sub>2</sub>SO<sub>4</sub> was added to the solution and the tube were allowed to stand at room temperature for additional 30 min. Colour was developed and absorbance was measured at 520 nm against blank. The ascorbic acid content was calculated by using the standard and express as % on dry weight.

#### Estimation of total antioxidant

The total antioxidant capacity of the extract was evaluated by the Phospho-molybdenum method with some modification of Prieto *et al.*, 1999. An aliquot of 300 µl of tuber extract was mixed with 3 ml of the reagent solution (0.6 M sulphuric acid (H<sub>2</sub>SO<sub>4</sub>), 28 mM sodium dihydrophosphate dehydrate and 4 mM ammonium hepta-molybdate tetrahydrate) and allowed to incubate in water bath at 90°C of 90 min. Then absorbance was measured at 695 nm against blank after complete cooling. The percentage of antioxidant activity as calculated by following equation.

$$\text{Antioxidant activity (\%)} = \frac{(A_{\text{sample}} - A_{\text{blank}})/A_{\text{ascorbic acid}} - A_{\text{blank}}}{A_{\text{ascorbic acid}} - A_{\text{blank}}} \times 100$$

#### DPPH radical scavenging assay

The free radical scavenging activity of all the sample extracts was evaluated by DPPH according to Blois, 1958 with some modifications. The stock solutions of the tuber extracts were prepared to the concentration of 1 mgml<sup>-1</sup>. Briefly, the solution of DPPH (0.1 mM in methanol) of 1 ml was added to 3 ml of various concentrations (100 to 1000 µg/ml) of tuber extract. The mixtures were shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured at 517 nm using UV-VIS spectrophotometer against a control contains DPPH and methanol. Ascorbic acid was used as reference. The capability of the scavenging the DPPH radical was calculated by using the following formula

$$\text{DPPH radical scavenging activity (\% inhibition)} = (A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \times 100$$

$$= \frac{(A_{\text{control}} - A_{\text{test}})}{A_{\text{control}}} * 100$$

Where,  $A_{\text{control}}$  is the absorbance of the control and  $A_{\text{test}}$  is the absorbance of samples.

#### ABTS radical scavenging assay

ABTS radical scavenging assay was carried out with slight modifications of Re *et al.*, 1999. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12-16 h at room temperature in the dark. The resulting solution was then diluted with ethanol to an absorbance of  $0.706 \pm 0.02$  at 734 nm using the spectrophotometer. Various concentrations (100 to 1000  $\mu\text{gml}^{-1}$ ) of the tuber extracts (1 ml) were allowed to react with 2.5 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min. The ABTS scavenging capacity of the extract was compared with that of trolox and percentage inhibition was calculated as

$$\text{ABTS radical scavenging activity (\%)} = (A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \times 100$$

Where  $A_{\text{control}}$  is the absorbance of ABTS radical+methanol;  $A_{\text{test}}$  is the absorbance of ABTS radical with sample extract as standard.

## RESULTS AND DISCUSSION

### Phytochemical compositions

Phytochemical compositions were compared among 10 selected plants of Zingiberaceae family from Koraput, India. Significant differences ( $P < 0.05$ ) existed in phenol content among the selected plants and it was varied from 2.6 to 41.6  $\text{mg g}^{-1}$  dry sample (Fig. 3). The phenol content was higher in *C. longa* followed by *Z. officinale* and *A. calcarata* compared to the other plants. Similar results also reported for *Curcuma*, *Zingiber* and *Alpinia* species (Chan *et al.*, 2008). The phenolic compounds are one of the largest and most ubiquitous groups of plant secondary metabolites, possess bioactivities such as anti apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and cell proliferation activities (Han *et al.*, 2007). The high potential of phenolics to scavenge free

radicals may be due to many phenolic hydroxyl groups they possess (Atmani *et al.*, 2009). Similarly, the flavonoid content varied from 1.13 to 15.48  $\text{mg g}^{-1}$  among the plants (Fig. 3). The total flavonoid content was significantly higher in *C. longa* followed by *Z. officinale* and *C. caesia* and lowest was observed in *C. aromatica*. The phenol and flavonoid possess diverse biological activities which might be related to their antioxidant activity (Huang *et al.*, 2009).

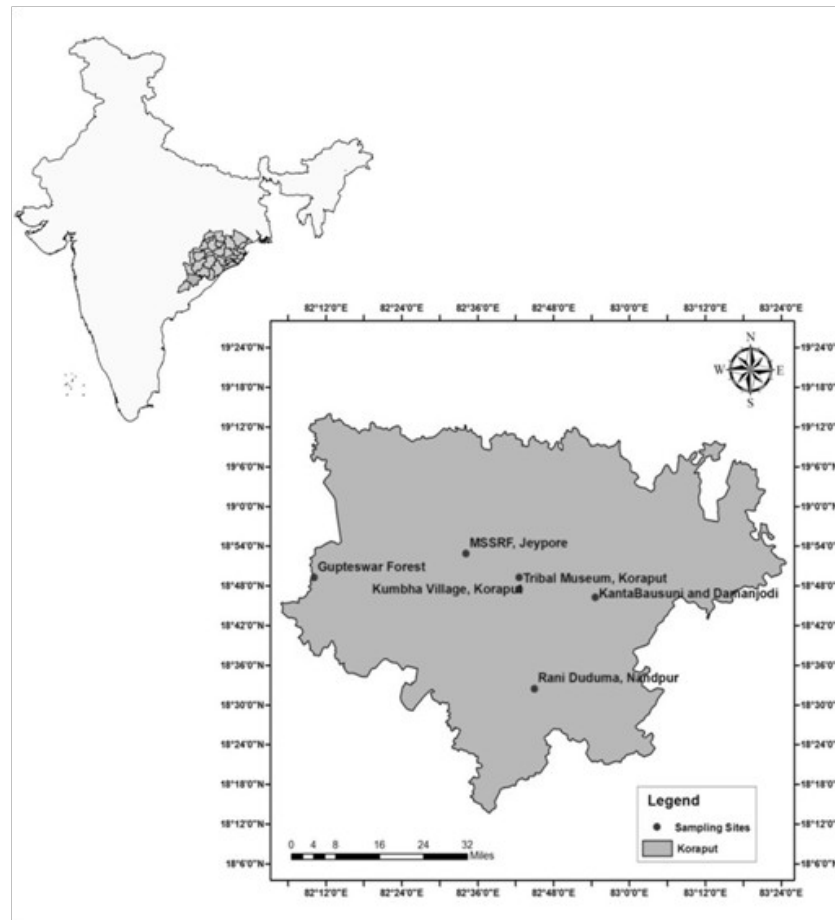
The values of alkaloid and saponin content was varied from 2.09 to 10.62 and 10.31 to 35.85 %, respectively among the studied plants (Fig. 4). The alkaloid content was significantly higher in *C. longa* followed by *H. coronarium* and *C. amada*. Whereas the saponin content was significantly higher in *C. aromatica* followed by *Z. roseum* and *C. caesia*. The lowest saponin content was observed in *C. longa* (10.31%). The presence of alkaloids and saponin in the studied species indicates their uses in medicines as alkaloids and saponin have biological functions with therapeutic efficacy (Sreevidya and Mehrotra, 2003; Okwu and Okwu, 2004). The tannin content in the selected plant samples were varied from 0.65  $\text{mg g}^{-1}$  to 3.63  $\text{mg g}^{-1}$  (Fig. 4). The tannin content was significantly more in *A. calcarata* followed by *H. coronarium* and *Z. officinale*. The plants with tannin-rich have been used for the treatment of diseases like leucorrhoea, rhinorrhoea, healing of wounds and diarrhea (Eleazu *et al.*, 2013).

### Total ascorbic acid and antioxidant capacity

The screening of antioxidant properties in medicinal and food plants have been investigated for finding an efficient remedy for several diseases (Halliwell, 2008). The ascorbic acid and total antioxidant capacity in the selected plant samples are shown in Fig 4. The ascorbic acid content was varied from 0.11 to 0.73  $\text{mg g}^{-1}$  (Fig. 5). The ascorbic acid content has direct effect on medicinal properties in plant part used for treatment of various types of diseases. Among the selected plants *C. longa* had significantly highest ascorbic acid content followed by *A. calcarata* and *C. caesia*. This study suggests that tested plant materials have moderate to potent antioxidant capacity as highest in *C. longa* as it contains more ascorbic acid content as compare to the other species as has been reported earlier (Paramapojn *et al.*, 2009). The total antioxidant capacity or content

(%) of plants varied from 1.2 to 11.4 % (Fig. 4). The more

antioxidant capacity (%) was observed in *C. longa* followed by *Z. officinale* and *Z. roseum*.



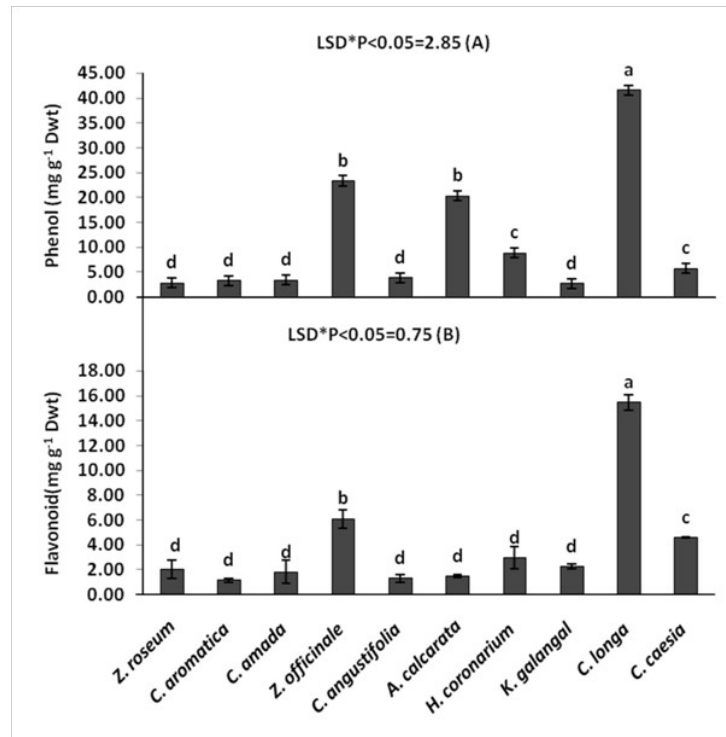
**Figure 1.** Map of the study sites in Koraput district of Odisha, India.

**Table 1** List of selected plants of Zingiberaceae family used by the tribal people of Koraput.

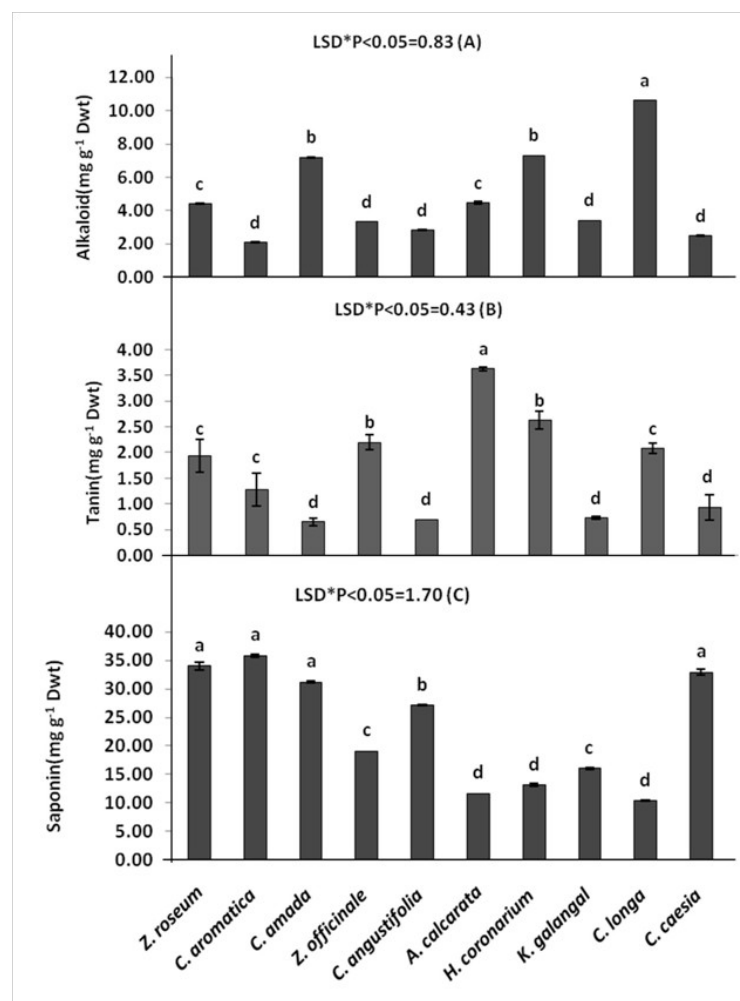
Sl No	Species Name	Local Name	Habitat	Plant part used	Medicinal uses	References
01	<i>Zingiber roseum</i> (Roxb.) Roscoe	Keu Kanda	Herb	Rhizome	Treatment of cancer and inflammation.	Mittal et al. 2014
02	<i>Curcuma aromatica</i> Salisb.	Bana	Herb	Rhizome	Blood purifier, antioxidant, abdominal discomfort and jaundice	Padhan and Panda, 2016
03	<i>Curcuma amada</i> Roxb.	Amba	Herb	Rhizome	carminative, cold, stomach ache and sprain	Padhan and Panda, 2016
04	<i>Zingiber officinale</i> Roscoe	Ada	Herb	Rhizome	Nausea or arthritis pain.	Raut et al. 2013
05	<i>Curcuma angustifolia</i> Roxb.	Palua	Herb	Rhizome	Bronchitis, asthma, cardio tonic, diuretic, indigestion and diarrhoea,	Padhan and Panda, 2016
06	<i>Alpinia calcarata</i> (Haw.) Roscoe	Torani	Herb	Rhizome	Rheumatism, weakness, carminative and appetizer.	Padhan and Panda, 2016
07	<i>Hedychium coronarium</i> J.Koenig	Phulakanda	Herb	Rhizome	antihelmintic indications, anti-inflammatory and analgesic effects.	Padhan and Panda, 2016
08	<i>Kaempferia galanga</i> L.	Bachha	Herb	Rhizome	Indigestion, heart diseases, sore throat, rheumatism, antibacterial and leprosy.	Padhan and Panda, 2016
09	<i>Curcuma longa</i> L.	Haladi	Herb	Rhizome	Jaundice, blood purifier, antioxidant, cough, worm infection, skin diseases and stomach disorder.	Padhan and Panda, 2016
10	<i>Curcuma caesia</i> Roxb.	Kala Haladi	Herb	Rhizome	For the treatment of leucoderma, asthma, tumor and piles	Dhal et al. 2012



**Figure 2.** Rhizomes of different genus of Zingiberaceae family used by the tribal people of Koraput, India



**Figure 3.** Phenol (A) and Flavonoid (B) (mg g<sup>-1</sup> Dwt.) content of different genus of Zingiberaceae family



**Figure 4.** Alkaloid (A), Tannin (B) and Saponin (C) (mg g<sup>-1</sup> Dwt.) content of different genus of Zingiberaceae family

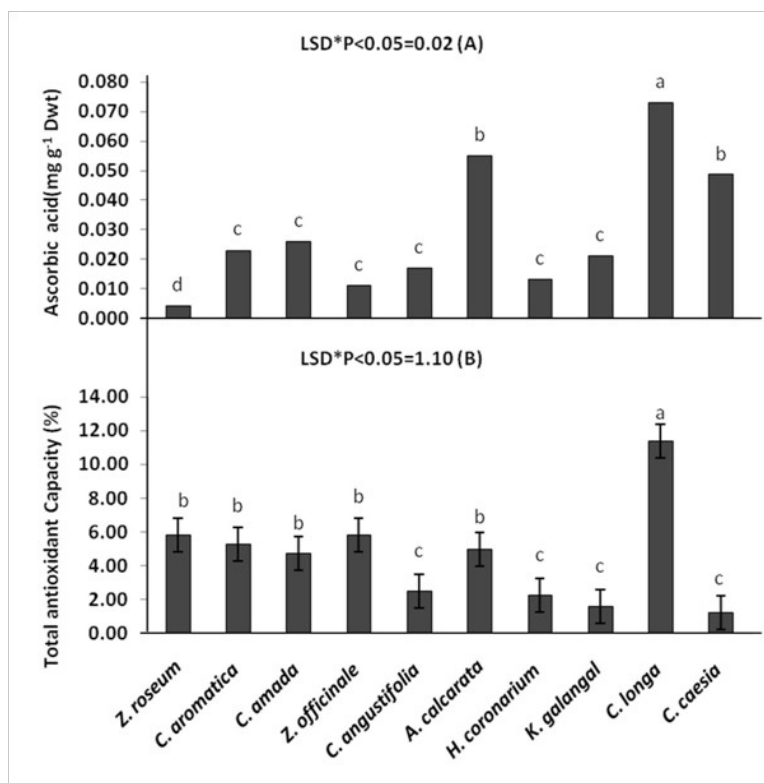


Figure 5. Ascorbic acid (A) and Total antioxidant capacity (B) content of different genus of Zingiberaceae family

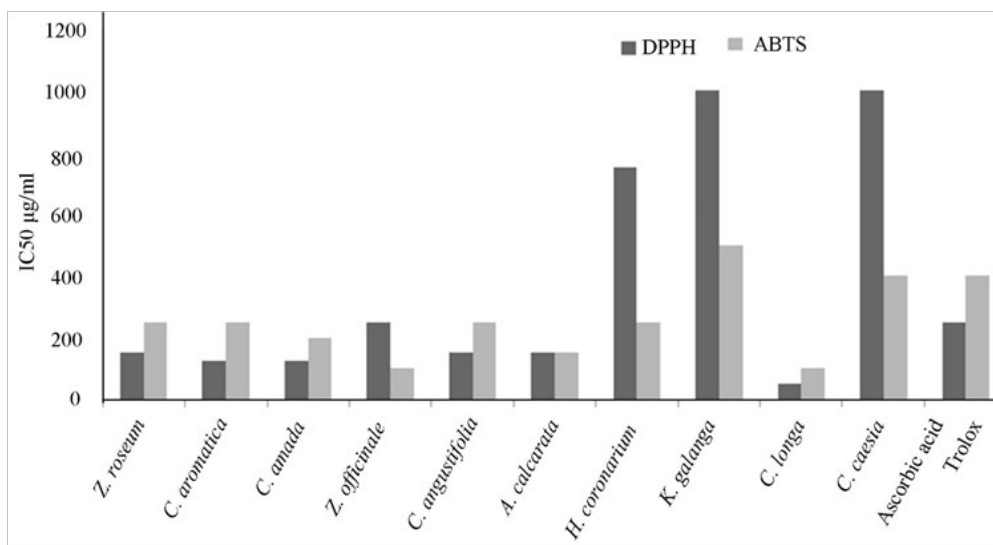


Figure 6. IC<sub>50</sub> values of the rhizome extract of different genus of Zingiberaceae family

#### *In vitro* antioxidant activity

The radical scavenging activity of the selected species of Zingiberaceae extract was measured by determining the ability to scavenge different free radicals such as DPPH and ABTS. The capacity to scavenge these free radicals was compared with the activity of standards such as ascorbic acid and trolox (Fig. 6). The total antioxidant activity was assessed by IC<sub>50</sub> value on using DPPH and ABTS radical scavenging assay. The results of DPPH radical scavenging activities revealed that, six

species had higher scavenging activity than the standard ascorbic acid. Most of the sample extracts showed higher scavenging activity at the concentration between 50 to 250 µg ml<sup>-1</sup> except *H. coronarium*, *K. galanga* and *C. caesia* (Fig. 6). The scavenging effects of the yam species on the basis of IC<sub>50</sub> value were in the following order: *C. longa* > *C. aromatic* and *C. amada* > *C. angustifolia*, *A. Calcarata* and *Z. roseum* > *Z. officinale* and *Ascorbic acid* > *H. coronarium* > *K. galanga* and *C. caesia*. The *C. longa* showed highest



scavenging activity against DPPH radicals with IC<sub>50</sub> value of 50.23 µg ml<sup>-1</sup> whereas, lowest scavenging activity was observed in *K. galanga* and *C. caesia* with the IC<sub>50</sub> of 1000.45 and 1000.87 µg ml<sup>-1</sup> respectively (Fig. 5). Similarly, the ABTS radical scavenging activity was higher in *Z. officinale* and *C. longa* followed by *A. calcarata* > *C. amada* > *Z. roseum*, *C. aromatica*, *C. angustifolia* and *H. coronarium* > *C. caesia* and trolox > *K. galanga* (Fig. 5). The IC<sub>50</sub> values for *Z. officinale* and *C. longa* was 100.57 µg ml<sup>-1</sup> respectively with higher scavenging activity than the standard (Fig. 6). Taken together, phytochemical analysis of these plants revealed that some plants are promising sources of several chemical constituents like alkaloids, flavonoids, phenols and antioxidants with free radical scavenging activity in varying proportions and possess therapeutic substances.

## CONCLUSION

The present study gives the baseline information on phytochemicals present in selected plants of Zingiberaceae used by tribal people of Koraput. Phytochemical compositions of these plants indicates that the phenol, flavonoid, alkaloid, saponin, tannin, ascorbic acid, total antioxidant content and antioxidant activity are major pharmaceutical parameters that are responsible for ethnomedicinal value are present in varying proportions. The plants are also contain significant amounts of antioxidants that can be exploited as a potential source for herbal remedy for various diseases. Thus, public awareness and community-based programmes need to be encouraged at all levels for *ex situ* and *in situ* conservation of such species and will further use in exploration of new drugs for pharmaceutical industries.

## ACKNOWLEDGEMENTS

Authors are grateful to the Head, Department of Biodiversity and Conservation of Natural Resources for providing necessary facilities for the study and also grateful to the University Grant Commission (UGC), New Delhi, India for providing a Non-NET Fellowship.

## CONFLICTS OF INTEREST

All authors have declared that they do not have any conflict of interest for publishing this research.

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