Phytochemical study of selected Zingiberaceae plant species in the valley district of Manipur

Ajit Kumar Das[1], Ningombam Babyrose Devi[2], P.K. Singh[3]
[1,2]Department of Ecology and Environmental Sciences, Assam University, Silchar, Assam
[3]Centre of Advanced Study in Life Sciences
Department of Life Sciences, Manipur University, Chanchipur, Imphal, Manipur

Abstract:

In the present study methanolic rhizome extract of the three plant species of Zingiberaceae namely *Amomum subulatum* Roxb., *Boesenbergia longiflora* (Wall.) Kuntze and *Curcuma angustifolia* Roxb.were evaluated for determination of total phenol, flavonoid and tannin content. *Amomum subulatum* Roxb. was recorded to have highest phenol content with 5.65 mg/g among the other two species. Flavonoid and tannin content was found highest in *Boesenbergia longiflora* (Wall.) Kuntze (1.8 mg/g) and *Amomum subulatum* Roxb. (3.52 mg/g) respectively. The presence of the high amount of phenol and flavonoid in the plant can be a potential source of antioxidant.

Keywords: flavonoids, phenols, phytochemicals, tannins, Zingiberaceae.

Introduction:

Phytochemicals are the naturally occurring chemical compounds produced by plants. They are biological active compounds which are looking forward as a new source of drugs for controlling several chronic diseases.

Phytochemical screening and estimation of the compounds is very much necessary in the modern world so as to screen the ethnobotanical importance of the plant and its bioactive substances. *Amomum subulatum* Roxb., *Boesenbergia longiflora* (Wall.) Kuntze and *Curcuma angustifolia* Roxb. are utilized as vegetable and food flavoring agent (Devi *et al*., 2014).*Curcuma angustifolia* Roxb. is also recorded as one of the wild species of Manipur which has great economic potential (Devi *et al*., 2016).

Many members of Zingiberaceae plants have been documented as food flavouring agent, dyes, perfumes, and in curing several human ailments (Singh, 1994; Akimpou and Yadava, 2005;Deb *et al.*, 2011;Das *et al*., 2017) and few of them have been cultivated and utilized all over the world as food and ornamentals (Daemei and Kumar, 2011).

Among the phytochemicals, phenolic compounds play a vital role in preventing a number of chronic diseases in human body having anti inflammatory, antioxidants and anticarcinogenic properties (Craig, 1999).

Phenols, flavonoids and tannins are among one of the phenolic compounds. Phenols and flavonoids have been reported to have nutritional properties but tannins are antinutritional. Their beneficial properties depend upon their chemical structure and dosage (Muller-Harvey and Allan, A. B., 1992). They exhibit certain biologically significant functions as protection against oxidative stress and degenerative diseases (Atanassova and Christova-Bagdassarian, 2009).

The Zingiberaceae plant species apart from edible uses has certain properties in curing and preventing common and serious human ailments. These phytochemical compounds may be the cause which imparts medicinal values that are useful in controlling various diseases and disorders (Saddiqui *et al.*, 2017).

Methodology:

Collection of plant samples:

The fresh disease-free rhizomes of the plant samples were collected from the valley districts and washed properly 3-4 times in running tape water and lastly by distilled water. It is then cut into small pieces and shade dried and ground to fine powder. It is then subjected for extraction and testing.

Extraction of plant sample:

The dried powdered sample (20 g) of the selected species were subjected to extraction with 200 ml of 80 % methanol in Soxhlet apparatus for 24 hr and filtered through Whatman paper no. 1 filter paper. The crude methanolic extract of the sample was obtained by evaporating the extract to dryness. The extracts were then kept in a dark coloured reagent bottle and stored in a refrigerator at 4^0 C for further analysis.

Different Tests for Qualitative phytochemical screening:

The different qualitative tests were carried out for understanding the preliminary idea about the chemical composition present in the methanolic extract of the plant species following the procedures of Kokate (1994). The qualitative outcome was expressed as presence (+) of phyto-constituents.

- **Tests for Phenols**
  5 ml of the extract is diluted with distilled water. To this few drops of 5 % ferric chloride solution was added. A green colour indicated the presence of phenolic compounds.
- **Tests for Flavonoids**
  The sample extract is treated with few drops of hydrochloric acid and magnesium turnings were added. The appearance of magneta red colour indicates the presence of flavonoids in the extract.
- **Tests for Tannins:**
  5 ml of the extract is treated with few drops of 1% ferric chloride solution. Development of blue green colour in the extract shows the presence of tannins.

Quantitative estimation of the Phenolic Compound and Antioxidant assay:

Estimation of Total Phenol Content:

Total phenol content was estimated using Folin-Ciocalteu reagent (FCR) (Thimmaiah, 1999).

- **Reagents**
  1. 80 % methanol
  2. Folin-Ciocalteu reagent
  3. 20 % Na_2CO_3
  4. Stock standard Catechol solution: 50 mg catechol / 50 ml of water
  5. Working standard: dilute 10 times the stock solution
- **Method**

The sample (100 µl) was taken and its volume was made upto 3 ml with distilled water. In the test tube containing the sample, 0.5 ml of Folin-ciocalteu reagent was added. Then after 2 min, 2 ml of 20 % Na_2CO_3 was added and mixed thoroughly. The contents were kept in a boiling water bath for 1 min. Then the test tubes were cooled in running tap water. Absorbance of the blue coloured complex was taken against blank at 650 nm with the help of spectrophotometer. The total phenol content was calculated and expressed in mg/g using a standard curve prepared from catechol.

Estimation of Total Flavonoids Content:

Aluminium Chloride Spectrophotometric method was used for flavonoids determination (Chang *et al.*, 2002).

- **Reagents:**
  1. Aluminium chloride
  2. Potassium acetate
- **Method:**

The sample (100 µl) was taken and 100 µl of aluminium chloride (10 %), 0.1 ml of potassium acetate (1M) and 2.7 ml of distilled water to make the volume to 3 ml. The reaction mixture was kept at room temperature for 30 min. The absorbance was measured at 415 nm using spectrophotometer. The calibration curve was prepared using different concentrations of quercetin expressed in mg/g dry weight.

Estimation of Tannins:

Tannins are estimated by using Folin Denis method which is based on the stiochiometric oxidation of the molecules containing a phenolic hydroxyl group (Thimmaiah, 1999).

- **Reagents:**
  1. Folin Denis Reagent
  2. Sodium carbonate solution
  3. Standard tannic acid solution
- **Method:**

The sample (100 µl) was taken and 0.1 ml of Folin Denis Reagent followed by 1 ml of 35 % Na_2CO_3. The final volume is made up to 10 ml with distil water. The blue colour appeared is measured at 700 nm by using UV-VIS Spectrophotometer. The calibration curve was prepared using tannic acid in mg/g dry weight.

Results and Discussion:

Qualitative phytochemical screening:

The presence of the phenolic compounds was screened in the methanol extract in the rhizomes of the plant species (Table 1).

Table 1. Phytoconstituents of Zingiberaceae plant species:

Name of species	**Test for Phenol**	**Test for Flavonoid**	**Test for Tannin**
Amomum subulatum Roxb.	++	+	++
Boesenbergia longiflora (Wall.) Kuntze	++	+++	+
Curcuma angustifolia Roxb.	+	+	+

(+ low concentration; ++ medium concentration; +++ high concentration)

Table 2. Quantitative estimation of Phenolic Compounds of the plant species:

Name of the plant species	**Total Phenol content** (mg/g)	**Total Flavonoid content** (mg/g)	**Total Tannin content** (mg/g)
Amomum subulatum Roxb.	5.65±0.08	0.69± 0.07	3.52± 0.17
Boesenbergia longiflora (Wall.) Kuntze	5.11±0.03	1.80±0.02	2.85±0.64
Curcuma angustifolia Roxb.	4.11±0.06	1.7 ±0.01	3.33±0.32

Key: The values are the mean of three independent replication ± SEM

The present results show that *Amomum subulatum* Roxb. was recorded to have the highest phenol content (5.65 mg/g) among the plant extract and lowest was recorded in *Curcuma angustifolia* Roxb. with 4.11 mg/g (Table-2).

Boesenbergia longiflora (Wall.) Kuntze was found to have the highest flavonoid content with 1.8 mg/g and lowest content was recorded in *Amomum subulatum* Roxb. with 0.69 mg/g.

The highest tannin content in the methanol extracts of plant species was recorded in *Amomum subulatum* Roxb. (3.52 mg/g) and lowest in *Boesenbergia longiflora* (Wall.)Kuntze with 2.85 mg/g.

Presence of phenols plays a vital role in cells which acts as antioxidants thus protecting the human system (Kokate *et al*., 1998). They form the active ingredients of medicinal plants (Wyk and Wink, 2004).

Flavonoids reduce metal cations through chelation and function as free radical scavengers and thus they have antioxidant property by inhibition of free radicals (Jacob and Burri, 1996).

Tannins forms strong complexes with proteins and other molecules where different approaches of bioprospecting of the plant can be reinforced (Horvath, 1981).

There are certain literature which proves that there is correlation between the flavonoid and the tannin content in the plant. The higher the flavonoid content in the plant the lower will be the tannin content and vice versa (Bag *et al.*, 2015)

Members of this plant family have been consumed and utilized as culinary and medicinal purpose. They are important as they have high antioxidant property (Karakaya *et al.*, 2001).

A significant relationship between antioxidant capacity and total phenolic content was found indicating that the higher the phenolic compound, the higher will be the antioxidant properties of the plant species (Dudonne *et al.*, 2009; Narayanaswamy and Balakrishnan, 2011; Bhavesh *et al*., 2013).

The presence of phenolic compounds in the selected species shows that they possess a high antioxidant activity havingsignificant ethnobotanical importance. It is beneficial to consume such food plants that have a high antioxidant compound content which will defend us from certain chronic diseases, viz. diabetes, cancers and cardiovascular diseases and they can be a potential and promising drug for the future pharmacological industries (Lin *et al.*, 2016).

References

Akimpou, G. K. R. and Yadava, P. S. (2005).Traditional dye yielding plants of Manipur, North East India.*Ind. J. Trad.Knowl.*4(1): 33-38.

Atanassova, M. and Christova- Bagdassarian, V (2009).Determination of tannins content by titrimetric method for comparison of different plant species.*J. of the University of Chemical Technology and Metallurgy.* 44(4): 413-415.

Bag, G. C., Devi, P. G. and Bhaigyabati, Th. (2015).Assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three *Hedychium* species of Manipur valley.*Inter. J. of Pharmaceutical Sc. Review and Res*. 30(1): 154-159.

Bhavesh, V. D., Nayak, Y. and Jayashree, B. S. (2013).*In vitro* antioxidant and antiglycation activity of *Zingiber zerumbet* (Wild Zinger) rhizome extract. *Inter. J. of Res. in Pharmaceutical Sc.* 4(4): 482-489.

Chang, C., Yang, M., Wen, H. and Chern, J. (2002). Estimation of total flavonoid content in Propolis by two complementary calorimetric methods. *J. Food Drug Anala*. 10: 178-182.

Craig, W. J. (1999). Health promoting properties of common herbs.*American J. Clinical Nutrition.* 70: 491-499.

Daemei, P. and Kumar, Y. (2011).Occurrence of *Hedychium* Koenig (Zingiberaceae) in Tamenglong District of Manipur, Northeast India.*Pleione* 5(1): 23-31.

Das, A. K., Rajkumari, R., Khatoon, R. and Singh, P. K. (2017).Glimpses of Ethnobotany & Medicinal Plants of Manipur, North East India.Deep publications.14-286.

Devi, N. B., Singh, P. K. and Das, A. K. (2014).Ethnomedicinal Utilization of Zingiberaceae in the Valley Districts of Manipur.IOSR Journal of Environmental Science, Toxicology and Food Technology.Vol. 8 (2).21-23.

Devi, N. B., Singh, P. K. and Das, A. K. (2016).Wilderness and biodiversity of Gingers in the Valley District, Manipur.International Journal of Scintific and Technology Research.286-291.

Deb, L., Singh, K. R., Singh, K. B. and Thongam, B. (2011). Some ethnomedicinal plants used by the native practitioners of Chandel district, Manipur, India. *International Res. J .of Pharmacy*. 199-200.

Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M. and Merillon, J. (2009). Comparative studies of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD and ORAC assays. *J. of Agri. and Food Chem.* 57: 1768-1774.

Horvath, P. J. (1981). The nutritional and ecological significance of acer-tannins and related polyphenols. M. S. Theses, Cornell University, Ithaca, New York.

Jacob, R. A. and Burri, B. J. (1996).Oxidative Damage and Defense.American Journal of Clinical Nutrition.Vol. 63(6).985-990.

Karakaya, S., El, S. N. and Tas, A. A. (2001). Antioxidant activity of some foods containing phenolic compounds. *International J. of Food Sci and Nutri*. 52: 501-508.

Kokate, C.K., Purohit, A.P. and Gokhale, S.B. (1998).Pharmacognosy (23 rd edition) .NiraliPrakashanPune .106 – 114.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H. and Chen, S. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of Type 2 diabetes.*Molecules* 21: 1374-1393.

Mueller, H. and Allan, A. B. (1992).Tannins, their biochemistry and nutritional properties.*AdvancesinPlantcellBiochemistryandBiotechnology*. 1:151-217.

Narayanaswamy, N. and Balakrishnan, K. P. (2011).Evaluation of some medicinal plants for their antioxidant properties.*Inter. J. of Pharmatech Res.* 3(1): 381-385.

Saddiqui, N., Rauf, M. D. A., Latif, M. D. A. and Mahmood, M. D. Z. (2017).Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal unani drug Gul-e-Zoofa (*Nepetabracteata*Benth).*Journal of Taibah University Medical Sciences.*

Singh, P. K. (1994). Ethnomedicine studies on some indigenous medicinal plants of Manipur. Proceeding IV International Congress of Ethnobotany. 6 (5): 56.

Thimmaiah, S. R. (1999). Standard Methods of Biochemical Analysis.Kalyani Publishers, New Delhi, India.

Wyk, B. E. V. and Wink, M. (2004).Medicinal Plants of the World.Times Edition. 371- 425.