Research Article

Biochemical Screening of Ethanolic Extracts of Five Accessions of *Zingiber Officinale Roscoe*. Rhizomes from Kumaun and Garhwal Region of Uttarakhand, India

Jyotsna Dhanik* and Vivekanand

Department of Chemistry, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, 263145, U.S. Nagar, Uttarakhand

Abstract

In this study, five ginger accessions from Kumaun (Bana, Kapkot, Takula) and Garhwal (Roorkee, Chamoli) region of Uttarakhand were collected and their biochemical screening was evaluated in terms of the total phenolic content, flavonoid content, orthodihydric content, proanthocyanidins content as well as tannin contents in an effort to compare and validate the medicinal & nutritional potential of ginger rhizomes. On the basis of the analysis it was found that the total phenolic content, flavonoid content and orthodihydric content, of ginger sample collected from Bana was highest and least in sample collected from Takula. The maximum tannin content was found in Kapkot and least in Chamoli. The sample collected from Chamoli contains higher proanthocyanidins content while Takula contains the least. The results provided evidence that ginger rhizomes collected from Bana, Pithoragarh District of Uttarakhand are potential source of phenolics and could be serve as basis for future drugs and food materials.

Keywords: Spices, *Zingiber officinale Roscoe*, phenolic, Uttarakhand, antioxidant

***Correspondence**

Author: Jyotsna Dhanik

Email: jyotsna.dhanik@gmail.com

Introduction

Natural bioactive compounds especially from plant sources, including spices have been investigated for their characteristics and health effects. India has been recognized as a land of spices. Spices form an important ingredient of Indian food system. Spices have been known for years as effective therapeutic food [1]. Spices are non-leafy parts (e.g. bud, fruit, seed, bark, rhizome, bulb) of plants used as a flavoring or seasoning, although many can also be used as a herbal medicine. Ginger, *Zingiber officinale Roscoe*, a monocotyledonous herbaceous plant of family Zingiberaceae is widely used around the world in foods as a spice and it has been an important ingredient for the treatment of several diseases [2]. It is cultivated in many tropical and subtropical countries including China, India, Nigeria, Australia, Jamaica and Haiti. Among which, India and China are the world's leading producers of ginger [3]. Uttarakhand state is hub of unique biodiversity comprising different climatic zones with a wide range of plant species. Both Kumaun and Garhwal regions of Uttarakhand represents the reservoir of different medicinal and aromatic plants. Floral diversity in terms of genetic and phytochemical in these regions is due to a wide range of climate, topology and environmental conditions.

The antioxidant compounds or phytochemicals from natural sources like plants, fruits, crops and spices are important in the food industry because of their usefulness in various food preparations and health promoting effects [4]. Therefore, the demand for natural antioxidants has increased due to the growing interest in the food and pharmaceutical industries for drug development with fewer side effects and potent against various diseases. Ginger extract also has long been used in traditional medical practices to decrease inflammation. Ginger rhizome extracts contain specific phenolic compounds like gingerol, zingerone, shogaols and its derivatives with various biological activities specifically; antioxidant and anticancer [5].The total phenolics, flavonoids, orthodihydric phenols, proanthocyanidins and tannins are regarded as nutritional secondary metabolites. These compounds present in plants play an important role in protecting the tissues by reducing oxidative stress and enhance the nutritional values of food material. Phenolics include simple phenols, phenolic acids (benzoic and cinnamic acid derivatives), coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans, and lignins. These compounds are among the most widely occurring secondary metabolites in the plant kingdom, acting mainly as phytoalexins, attractants for pollinators, contributors to plant pigmentation, antioxidants, and protective agents against UV light, among others [6].

Flavonoids are large family of polyphenolic components synthesized by plants. It was found that flavonoids functioned to reduce blood-lipid and glucose and to enhance human immunity [7]. Flavonoids were also a kind of

natural antioxidant capable of scavenging free superoxide radical, anti-aging and reducing the risk of cancer. The objectives of the present study were to determine the total phenolic content, total flavonoid content, orthodihydric content, tannins and proanthocyanidins content in five accessions of *Zingiber officinale Roscoe* collected from Kumaun and Garhwal region of Uttarakhand to compare and validate the medicinal & nutritional potential of ginger rhizomes.

Material and methods

Plant Material

Five accessions of ginger (*Zingiber officinale Roscoe)* rhizomes were collected from 5 districts of Uttarakhand State *viz.* of Kumaun and Garhwal region. Three of these accessions Bana (Pithoragarh district), Kapkot (Bageshwar district) and Takula (Almora district) from Kumaun region (29°36´N, 79°42´E) while two of these Roorkee (Haridwar district) &Chamoli (Chamoli district)) from Garhwal region (30°30´N, 78°30´E).

Extracts Preparation

Ginger rhizomes were washed in running tap water, shade dried at room temperature for at least 15 days. The dried materials were pulverized into fine powder by a grinding machine. The material was extracted by successive soaking for a period of 72 hours each in ethanol. All the extracts were stored in sterilized amber coloured bottles and were kept in refrigerator for further study.

Preliminary phytochemical screening

All the extracts of ginger accessions were tested chemically for the detection of various metabolites viz; alkaloids, phenols, tannins and flavonoids by using standard reported protocols [8].

Metabolite Profiling

Total phenolic assay

The total phenolic content of different ginger rhizome extracts was estimated by using the Folin–Ciocalteu reagent method as reported by Singleton and Rossi (1965) with slight modification [9]. 0.5 mL of the extract solutions were mixed with 0.5 mL of Folin–Ciocalteu reagent, 1.0 mL of aqueous solution of 7% saturated sodium carbonate and 8mL of distilled water. The reaction mixture was mixed thoroughly and was allowed to stand for 1 hour in dark. The absorbance was read at 765 nm. The standard curve was established using various concentrations of gallic acid. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g of dry material.

Estimation of Flavonoids

250 µL of plant extract was mixed with 1.25 mL of distilled water and 75µL of 5% sodium nitrite solution. The solutions were incubated for 5 minutes and then 150µl of 10% aluminium chloride solution was added. After 6 minutes 500µL of 1M sodium hydroxide and 275 µL of distilled water were added, after proper mixing of the solution the intensity of pink colour was obtained at 510 nm. The standard curve was established using various concentrations of catechin. The flavonoid content was expressed as catechin equivalents (CNE) in mg/g of dry material [10].

Estimation of orthodihydric phenols

1mL of the extract solution was taken and mixed with equal volume of 0.5N HCl and 1mL of Arnow's reagent (10gm sodium nitrite in 10 gm sodium molybdate and make upto 100 mL with distilled water), 2mL of 1N NaOH and 4.5 mL of distilled water was added. The solution were mixed thoroughly (pink colour was appeared) and the absorbance at 515nm was measured. The standard curve was established using various concentrations of catechol. The orthodihydric phenols content was expressed as catechol equivalents (CLE) in mg/g of dry material [11].

Estimation of Tannins

0.5 mL of the extract solution was taken and mixed with 0.1 mL Folin–Ciocalteu reagent and kept for 15 mins. 2.5 mL of saturated sodium carbonate solution was added and was allowed to stand for 30 mins. The absorbance at 760

nm was measured. The standard curve was established using various concentrations of tannic acid. The tannin content was expressed as tannic acid equivalents (TAE) in mg/g of dry material [12].

Estimation of proanthocyanidins

The proanthocyanidins content of extracts was determined by method developed by (Sun *et al.*, 1998)with slight modification.0.5 mL of the extract solution was taken and mixed with 3 mL of 4% vanillin ethanol solution and 1 mL of concentrated HCl [13]. The mixture was allowed to stand for 15 mins and absorbance was measured at 500 nm. The standard curve was established using various concentrations of tannic acid. The proanthocyanidins content was expressed as tannic acid equivalents (TAE) in mg/g of dry material.

Statistical analysis

Results are presented as mean±standard deviations. One way analysis of variance (ANOVA), Tukey's test ($p<0.05$) and the correlation between different altitude of collection place (Kumaun and Garhwal region) with all metabolites was evaluated by using SPSS16 Statistical Package for Social Science.

Results and Discussion

The preliminary phytochemical screening of ginger ethanolic extracts showed the presence of various phytoconstituents presented in **Table 1**. In view of present study the above facts i.e. total phenols, flavonoids, orthodihydric phenols, tannins and proanthocyanidins content was determined spectrophotometrically and the maximum content of these for all the parameters was found to be highest in ginger rhizomes of Bana from Pithoragarh district of Kumaun region. The total phenols in different ginger rhizomes were obtained in the range of 46.24±0.105 to 29.88±0.278mg/g GAE (gallic acid equivalents). The level of total phenols in ethanolic extracts of different accessions of ginger rhizomes are presented in Table 2. Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds [14-19]. This activity is believed to be mainly due to their redox properties, which plays an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [20, 21].

The different flavonoid content found in the various accessions of ginger rhizomes are shown in the **Table 2**. The total flavonoid content of the ginger rhizomes varied from 38.87±0.47 to 21.03±0.47mg/g CNE (catechin equivalents) and it was found to be maximum in ginger rhizome collected from Bana of Pithoragarh district. The orthodihydric phenol content found in the various rhizomes of ginger (Table 2) ranged from 31.5±0.866 to 15.5±0.500mg/g CLE (catechol equivalents).Tannins are basically astringent, bitter polyphenolic compounds that binds to precipitates proteins and various other organic compounds including amino acids and alkaloids. This tannin-protein complex provides persistent antioxidant activity [22]. Estimated tannic acid in the present study of all five ginger accessions varied from 7.48±0.323to 3.21±0.128 mg/gTAE (tannic acid equivalents).Similarly, the proanthocyanidins content in all five ginger rhizomes ranges from 30.91±1.325to 6.88±1.096 mg/g TAE (Table 2).

Table 1 Phytochemical specifications of ginger rhizomes

Phytoconstituents	Test	Bana	Kapkot	Takula	Roorkee	Chamoli
Alkaloids	Mayer's Test	+	+	+	+	+
	Wagner's Test	+	+	+	+	+
	Dragendroff's test	+	+	+	+	+
	Hager's test	-	-	-	-	-
Phenols	Ferric Chloride Test	+	+	+	+	+
Tannins	Gelatin Test	+	+	+	+	+
Flavonoids	Alkaline Reagent Test	+	+	+	+	+
	Lead acetate Test	+	+	+	+	+
	Shinoda Test	-	+	-	-	+

+ = presence, - =absence

The results obtained showed that all the five ginger accessions possess high variation in all these metabolites. On the basis of the analysis it was found that the total phenolic content, flavonoid content, orthodihydric content of ginger rhizomes collected from Banaregion was found to be highest followed by Kapkot, Roorkee, Chamoli, and least in Takula. In ginger rhizomes tannin content follows the order Kapkot>Bana>Takula>Roorkee>Chamoli while in, case

of proanthocyanidins content it is different and follows the order Chamoli>Bana>Kapkot>Roorkee>Takula. From the above results it may be concluded that ginger rhizomes from Kumaun region are the richest sources of phenolics than that of Garhwal region.

Table 2 Biochemical screening of different accessions of ginger rhizomes

Ginger accessions	Altitude (mts)	Total phenolics (mg/g GAE)	Flavonoids (mg/g CNE)	Orthodihydric phenols (mg/g CLE)	Tannins (mg/g TAE)	Proanthocyanidins (mg/g TAE)
Bana	520	46.24 ± 0.105^{e}	38.87 ± 0.47^{e}	31.5 ± 0.866^{d}	6.11 ± 0.196^{c}	26.53 ± 1.053^{c}
Kapkot	1104	38.55 ± 0.182^{d}	30.05 ± 0.47^{d}	22 ± 0.5^{c}	7.48 ± 0.323^{d}	22.49 ± 1.993^{b}
Takula	1400	29.88 ± 0.278^{a}	21.03 ± 0.47^{a}	15.5 ± 0.5^{a}	5.6 ± 0.196^{c}	6.88 ± 1.096^{a}
Roorkee	268	34.85 ± 0.278^{c}	28.31 ± 0.308^{c}	20 ± 0.5^{b}	4.02 ± 0.267^{b}	19.86 ± 1.096^{b}
Chamoli	2100	33.03 ± 0.378^{b}	24.92 ± 0.308^{b}	18.83 ± 0.764^{b}	3.21 ± 0.128^{a}	30.91 ± 1.325^{d}

Values are Means of three replicates ± Standard Deviation. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ($p<0.05$).

Correlation analysis

Correlation analysis of all metabolites (**Table 3**) shows moderate to strong correlations. In present investigation, found that total phenols are positively correlated with flavonoids and orthodihydric phenols content at $\alpha=0.01$ level of significance. Flavonoids are positively correlated with orthodihydric phenols content at $\alpha=0.01$. The positive correlation describes high degree of interdependence between these parameters.

Table 3 Correlation coefficients, R, for relationships between the altitude and assays of different accessions of ginger rhizomes

	Altitude	T.P.C	F.L	O.P	T.N	P.C
Altitude	1	-.533	-.633	-.545	.164	.007
T.P.C		1	.992**	.989**	.463	.516
F.L			1	.987**	.381	.533
O.P				1	.364	.528
T.N					1	-.255
P.C						1

Correlation is significantat the 0.01 level; **T.P.C= total phenolic content; F.L= Flavonoids**; O.P = ortho dihydric phenols ; T.N = tannins ; P.C= proanthocyanidins

Conclusion

The results of this study indicated that ginger rhizomes from Pithoragarh district of Kumaun region are the richest sources of phenolics than that of Garhwal region and could be serve as basis for future drugs and food materials. The phytochemical contents in ginger accessions may also be influenced by environmental factors, such as growing region, cultivation methods, climatic conditions. This new investigation of ginger rhizomes from Kumaun and Garhwal region could be a first step to develop a new variety of ginger for further applications.

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