

# Polyphenolics, Nutrients and Antioxidant Activity of *Gaultheria Trichophyllaroyale*: A High Value Wild Edible Plant of Trans Himalaya

## Abstract

*Gaultheria trichophylla* Royle (family-*Ericaceae*) is a high value wild edible plant species of Trans Himalaya and used for various ailments in traditional medicinal systems. The present study focusing on to analyzed biochemical attributes of *G. trichophylla* fruits (berries) across attitudinally diverse sampling sites. The polyphenolics, nutrients and antioxidant activity have shown a strong positive correlation ( $p < 0.05$ ) with altitude. Total phenolics have showed significant ( $p < 0.05$ ) relationship with antioxidant activity (ABTS:  $r^2 = -0.8940.799$ ; DPPH:  $r^2 = 0.9550.912$ ; FRAP activity:  $r^2 = 0.9310.867$ ) and nutrients (Sodium:  $r^2 = 0.9500.573$ ; Potassium:  $r^2 = 0.7570.903$ ; carbohydrate:  $r^2 = 0.9290.862$ ) respectively. Overall, *G. trichophylla* was found potential source of natural antioxidant compounds with high nutritious value and large scale plantation of this species will help in rural development programs across Trans Himalayan villages.

**Keywords:** *Gaultheria Trichophylla*; Polyphenolics; Antioxidant Activity; Wild Edible; Trans Himalaya

## Research Article

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**Abbreviations:** GAE: Gallic Acid Equivalent; TAE: Tannic Acid Equivalent; QE: Quercetin Equivalent; AAE: Ascorbic Acid Equivalent; CN: Cyanidin 3-glucoside; ABTS: 2, 2-azinobis (3-Ethylbenzothiazoline-6-Sulphonic Acid); DPPH: 2, 2-diphenyl-1-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power; TPTZ: 2,4,6-tri-2-pyridyl-1,3,5-triazin; FW: Fresh Weight

## Introduction

In spite of tremendous development in the field of allopathic medicines during 20<sup>th</sup> century, plants still remain one of the major sources of natural antioxidants for humans. In human body, reactive oxygen species (ROS) are formed either as an essential mediator in vital processes including neurotransmission and inflammatory reactions or as a by-product that does not have a role in the actual process [1]. The overproduction of free radicals and imbalance of oxidants and antioxidants constituents in the bodies can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA), create several type of syndromes such as cardiovascular dysfunctions, atherosclerosis, inflammation, carcinogenesis, drug toxicity, aging, reperfusion injury and neurodegenerative diseases [2]. Medicinal plant is potent source of natural secondary metabolites, which protect plants from different type of stress and ultra violet radiations [3]. These secondary metabolites maintain the balance of oxidant and antioxidant compound in human body [4]. Indian Himalayan Region (IHR) is one amongst the biodiversity rich regions in the world and contains 675 wild edible plants [5]. The diversity of wild edible plants in Indian Himalayan Region has traditionally been known and consumed as a potent source of nutritionist food, source of medicine and associated with the human health from

time immemorial [6]. Studies suggested that fruits are rich source of phenolics, flavonoids, tannins, anthocyanins, vitamins and antioxidant contents and regular consumption associated with reducing the risk of cancer, cardiovascular syndrome, aging and neurodegenerative diseases [7, 8].

*Gaultheria trichophylla* Royle (family-*Ericaceae*) is high value wild edible fruit commonly known as Himalayan Raspberry. It is decumbent, mat-forming aromatic shrub occurred in higher altitudinal zone 3200-5300 m asl and distributed across the higher Himalayas of India, Bhutan, China, Nepal, Myanmar and Pakistan [9]. Fruits of the species used for various ailments in traditional system of medicine especially treatment for pain and inflammation, leaf oil used for swelling and fracture and dried branches are used in making incense fire during religious ceremonies by the mountainous people of Uttarakhand [10,11]. Presently, use of wild edibles is limited to certain traditional areas and lacking literature about their phytochemicals and nutritional properties. In the above context, present study attempts to;

- i. To estimate polyphenolics and antioxidant activity by different in vitro assays,
- ii. To determine nutritional profile,
- iii. Develop the relationship between polyphenolics and antioxidant activity.

The outcome of the study will help for prioritization of wild edible fruits in the Himalayan region and their nutritional potential explored in local, national and international markets, which contribute for enhancing the acceptability of wild edibles as source of income generating resources of hill communities.

## Material and Methods

### Sample collection

The fresh fruits of *Gaultheria trichophylla* was collected from different selected locations of Johar valley i.e., Martoli Nanda Devi base camp (3475 m asl, 30°20.539'N/80°11.428'E) and Milam bugyal (3546 m asl, 30°26.326'N/80°08.836'E) from Pithoragarh district, Uttarakhand, trans Himalaya. Fruits were stored at 4°C and processed next day.

### Chemicals and reagents

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, gallic acid, ascorbic acid, quercetin and catechin were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium carbonate, 2-(N-morpholino) ferric chloride, potassium persulphate, potassium acetate, sodium acetate, aluminum chloride, acetic acid and hydrochloric acid from Qualigens (Mumbai, India), and 2,4,6-tri-2-pyridyl-1,3,5-triazin (TPTZ), 2,2-Azinobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), methanol and ethanol from Merck Co., (Darmstadt, Germany). All the chemicals purchased were of analytical grade.

### Extract preparation

Fresh fruits of *Gaultheria trichophylla* were cut into four parts than grounded in to a mortar and pestle to a fine texture. Precisely 20 g of each sample was weighed into a conical flask and extracted with 200 ml 80% methanolic solution. The mixture was homogenized for 1 min using an Ultra-sonicator (Toshiba-India) and kept in water-bath 45°C for 1h. After that, mixture was centrifuged at room temperature for 20 min at 10000 rpm and supernatant was removed and filtered by Whatman filter paper no 2 and stored in amber screw-capped glass vials at -20°C till analysis. The experiments were performed in the Biodiversity Conservation and Management Laboratory of GB Pant National Institute of Himalayan Environment and Sustainable Development (GBPNIHESD) Almora, Uttarakhand India.

**Estimation of total phenolic content:** Folin-Ciocalteu's calorimetric method was used for estimation of total phenolic content in the methanolic extract of fruits with slight modification [12]. In brief, 0.50 ml methanolic extract were diluted with 4.50 ml distilled water and added 0.50 ml Folin-Ciocalteu's reagent and allowed to reaction for 5 minutes. This mixture was neutralized by 2.50 ml of 7% sodium carbonate (w/v) and kept in dark at room temperature for 90 minutes. The absorbance of resulting blue colour was measured at 765 nm using UV-VIS spectrophotometer (Hitachi U-2001) and results were expressed in mg gallic acid equivalent (GAE) per gram of fresh weight (FW).

**Estimation of total tannin content:** Total tannin content was estimated following Nwinuka et al. [13] with slight modification. Briefly, reaction mixture was prepared with adding (0.25 ml) methanolic extract, (2.25 ml) distilled water and added (0.50 ml) Folin's Dennis reagent and allowed to reacts for 1 minute. This reaction mixture was neutralized by adding (1.0 ml) 7% sodium carbonate (w/v) and kept in water bath in 25°C for 20 minutes. The absorbance of reaction mixture was measured at 700 nm using UV-VIS spectrophotometer (Hitachi U-2001) and results were expressed in mg tannic acid equivalent (TAE) per gram of fresh weight (FW).

**Estimation of total flavonoid content:** Total flavonoid content in fruit extract was determined by using aluminum chloride calorimetric method with minor modification [14]. The reaction mixture of (0.50 ml) methanolic extract, (1.50 ml) distilled water and (0.50 ml) 10% (w/v) aluminum chloride, and (0.10 ml) 1 M potassium acetate was prepared. This mixture was incubated at room temperature for 30 min and absorbance was measured at 415 nm UV-VIS spectrophotometer. Quantification of total flavonoids was done and results were expressed in mg quercetin equivalent per gram of fresh weight (mg QE/g FW).

**Estimation of total flavonol content:** Total flavonols in fruit extract was determined using the method of Kumaran and Karunakaran [15] with slight changes. Briefly, (2.0 ml) methanolic extract, (2.0 ml) 2% (w/v) aluminum chloride-ethanolic solution and (3.0 ml) 50 g/l sodium acetate solution were mixed and kept it room temperature for 2.5 h. The absorbance of reaction mixture was measured at 440 nm UV-VIS spectrophotometer and results were expressed in mg catechin equivalent per gram of fresh weight (mg CE/g FW).

**Estimation of total carbohydrate content:** Total carbohydrate content was estimated by followed Hedge et al. [16] with minor modification. The fresh fruit sample was hydrolyzing with 2.5N HCL and kept in boiling water bath for three hours and it was neutralized with sodium carbonate. The extract was then centrifuged and the supernatant was collected for analysis and absorbance was taken at 590 nm and result was expressed mg/g FW.

**Estimation of sodium and potassium content:** For quantitative purpose, working standard solution of the elements namely sodium (Na), potassium (K) were prepared from the stock standard solution. Calibration and measurement of Na and K were analyzed on a flame photometer model-Systronics Medi flame 127 [17] and results were expressed mg/g FW.

**Determination of antioxidant activity:** The antioxidant activity was determined using different in vitro methods such as ABTS [2, 2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salts], DPPH (1, 1-diphenyl-2-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) [2]. All the assays were carried out in six replicates and mean values were considered for analysis.

### Statistical analysis

All the measured parameters polyphenolics (i.e., total phenolics, tannins, flavonoids, flavonols), antioxidant activity by (i.e., ABTS, DPPH, FRAP assay) and nutritional parameters (i.e., carbohydrate, sodium and potassium) were measured in six replicates. The value for each sample was calculated as mean of all replicates with  $\pm$  standard error and subjected to analysis of variation (ANOVA) and mean values were separated by Duncan's Multiple Range Test (DMRT) by t-test using SPSS software version 16 (SPSS Inc., Chicago IL, USA). Correlation coefficient (r) was calculated using the same software.

## Results and Discussion

### Polyphenolics and antioxidant activity

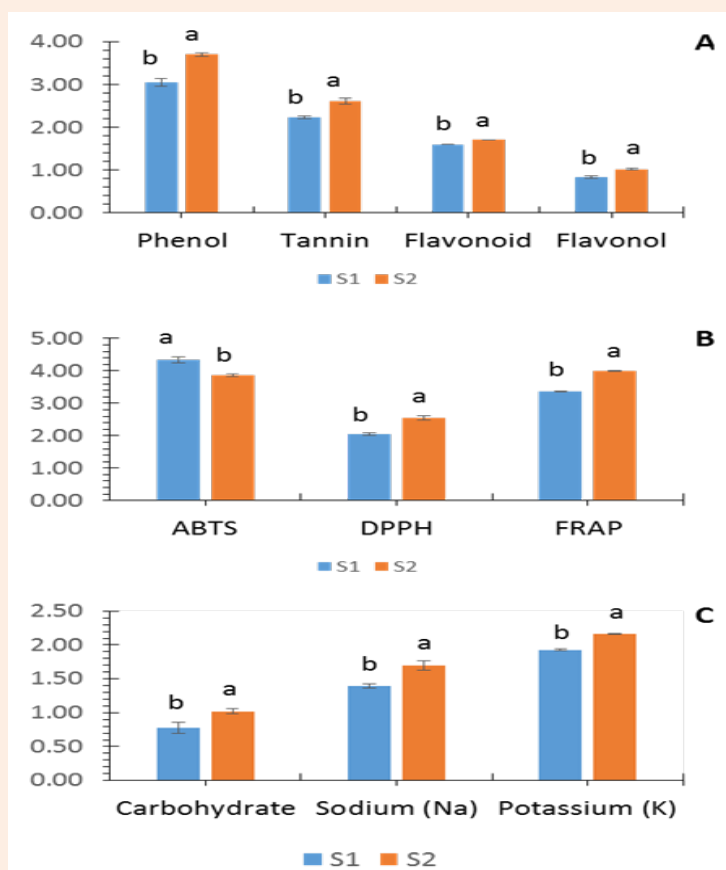
Polyphenolic content and antioxidant activity varied significantly ( $p < 0.05$ ) among the locations. The higher content

of total phenolics (3.71 mg GAE/g FW), tannins (2.62 mg TAE/g FW), flavonoids (1.75 mg QE/g FW) and flavonols (1.03 mg CE/g FW) was recorded in fruits of Milam bugyal (Figure 1). Similarly, fruits of Milam bugyal were reported best sources of free radical scavenging antioxidant activity (DPPH activity)-2.56 mM AAE/100g FW and ferric reducing antioxidant activity (FRAP activity)-3.99 mM AAE/100g FW. However, total antioxidant activity (ABTS activity) - 4.35 mM AAE/100g FW was exhibited in fruits of Martoli bugyal (Figure 1). Researcher reported that polyphenolics such as phenolics, flavonoids, tannins are ubiquitous which showed antioxidant properties and act as reducing agents, hydrogen donors, free radical scavenger, interrupting chain oxidation reaction which prevent oxidative cell damage [18,19] and important for reducing several diseases in human. Present investigation reported that fruits of higher altitude region were major source of polyphenolics and antioxidant contents. Such type of results also reported in fruits of *Myrica esculenta* [7], *Berberis aristata* [20]. Previous studies also suggested that altitudinal gradient, geographic conditions, biotic and abiotic factor along with several environmental conditions such as temperature, rainfall, maturity at harvest, soil, uv radiation,

sunlight, habitat condition etc. were affected the accumulation of secondary metabolites in plants and fruits [21-23]. Increasing altitude accumulation of maximum bioactive compounds also reported in *Hedychium spicatum* [24], *Rosa damascene* [25], *Valeriana jatamansi* [26] and other Himalayan species which was agreement of present results.

### Nutritional content

Nutritional content varied significantly in fruits of *Gaultheria trichophylla* among the locations. The fruits Milam bugyal was exhibited maximum source of carbohydrate (1.02 mg/g FW), sodium (1.70 mg/g FW) and potassium content (2.17 mg/g FW), however, minimum was found in fruits of Martoli bugyal (Figure 1). The nutritional content was essential for structural component and development of the body, proper physiological function and regulation process [27]. Also, important for ionic balance, prevention of chronic diseases [28] and disorders like cramps, irregular heart beat and kidney failure [29]. Previous studies reported that soils, altitude, precipitation, location specific climatic condition was major factor for affecting chemical constituents in fruits [30] and medicinal plant [31].

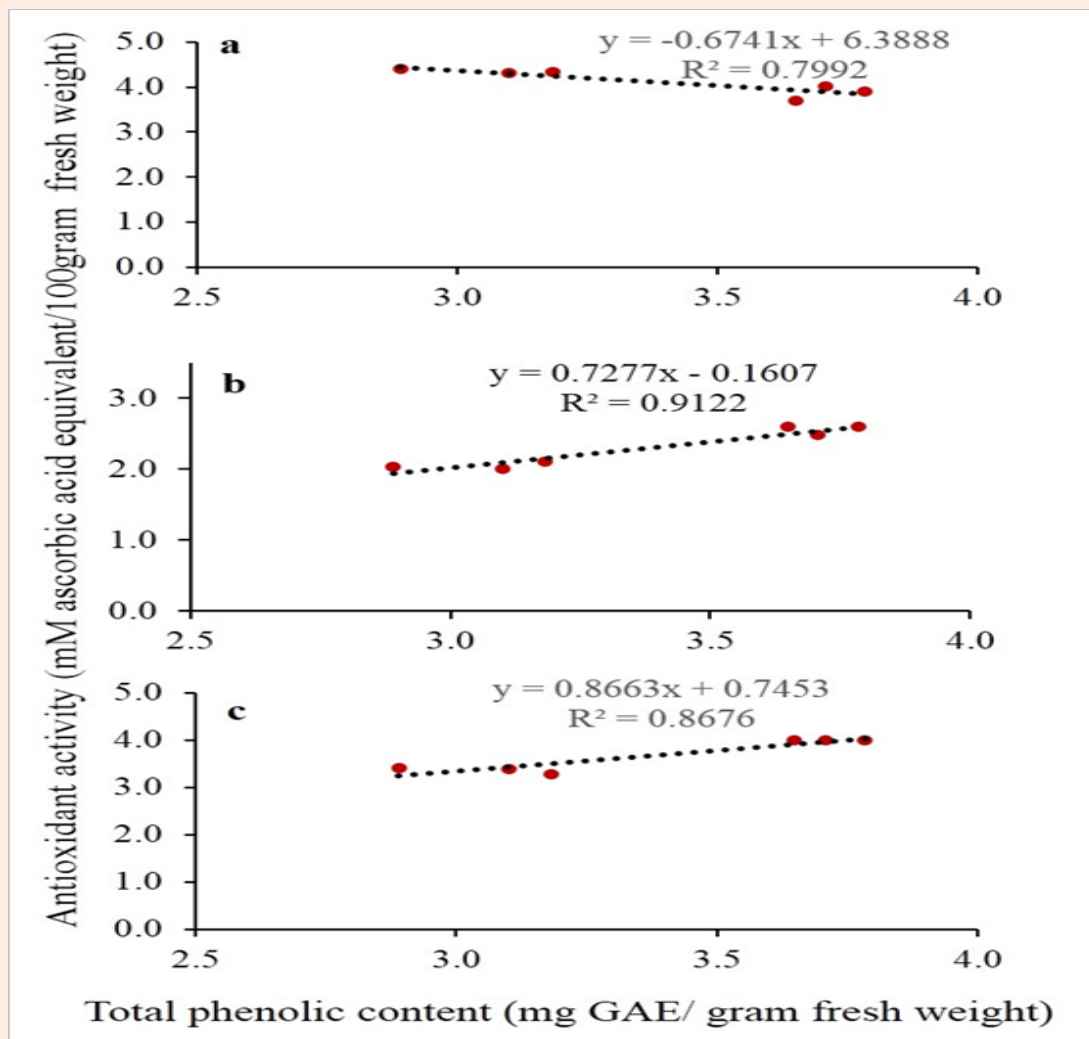


**Figure 1:** Comparison of different parameters across study sites (Aa) phytochemical: Phenol (mg GAE/g FW), Tannin (mg TAE/g FW), Flavonoid (mg QE/g FW), Flavonol (mg CE/g FW); (B) antioxidant activity: ABTS assay AAE/100g FW), DPPH activity (mM AAE/100g FW), FRAP assay (mM AAE/100g FW) and (C) nutrients: Carbohydrate content (mg/g FW), Sodium (mg/ml FW), Potassium (mg/ml FW). S1 and S2 are study sites, GAE: gallic acid equivalent; TAE: Tannic acid equivalent; QE: quercetin equivalent; catechin equivalent; AAE: ascorbic acid equivalent; FW: fresh weight; bars, indicating mean value, capped with same letters within a test are not significantly different from each other and means are separated by Duncan's Multiple Range Test.

### Relationship of altitude with analyzed parameters

While establishing relationship between altitude and analyzed parameters (Supplementary Table 1), altitude showed significant ( $p < 0.01$ ) positive correlation with phenolics ( $r = 0.960$ ), tannins ( $r = 1.00$ ), flavonoids ( $r = 0.975$ ) and flavonol ( $r = 0.953$ ). Likewise, altitude showed strong relationship with DPPH activity ( $r = 0.980$ ) and FRAP activity ( $r = 0.993$ ), however, non-significant ( $r = -0.931$ ) with ABTS activity. Altitude established positive ( $p < 0.01$ ) relationship with carbohydrate ( $r = 0.931$ ) and sodium ( $r = 0.933$ ) content. Similarly, total phenolic content

showed significant ( $p < 0.01$ ) relationship with tannins ( $r = 0.959$ ), flavonoids ( $r = 0.962$ ), flavonols ( $r = 0.952$ ) and antioxidant activity (DPPH and FRAP activity  $r = 0.955$ ;  $0.931$  respectively). As same, phenolics showed significant ( $p < 0.01$ ) liner relationship with antioxidant activity (i.e., DPPH  $r = 0.955$ ; ABTS  $r = 0.894$ ) (Figure 2) and nutritional content (i.e., sodium  $r = 0.903$ ; potassium  $r = 0.862$ ) (Figure 3). Similar kind of trend also reported in Guava [32], *Roscoea procera* [33] by researcher. The earlier studies also indicated that phenolics positively correlated with antioxidant activity [34,35] and responsible for maximum activity.



**Figure 2:** Relationship between total phenolic compounds and antioxidant capacity in *G. trichophylla* berries of different populations. (a) linear correlation between total phenolic content and antioxidant capacity quantified by ABTS assay, (b) linear correlation between total phenolic content and antioxidant capacity quantified by DPPH assay and linear correlation between total phenolic content and (c) antioxidant capacity quantified by FRAP assay.



Supplementary Table 1: Relationship between altitude and analyzed parameter in fruits of *G. trichophylla*.

	Altitude	Phenolics	Tannins	Flavonoids	Flavonols	ABTS	DPPH	FRAP	Carbohydrate	Na	K
Altitude	1										
Phenolics	0.960**	1									
Tannins	1.000**	0.959**	1								
Flavonoids	0.975**	0.962**	0.974**	1							
Flavonols	0.953**	0.952**	0.954**	0.898*	1						
ABTS	-0.931	-0.894	-0.933	-0.909	-0.943	1					
DPPH	0.980**	0.955**	0.981**	0.954**	0.974**	-0.951	1				
FRAP	0.993**	0.931**	0.993**	0.976**	0.918**	-0.927	0.961**	1			
Carbohydrate	0.943**	0.929**	0.944**	0.876*	0.997**	-0.948	0.966**	0.909*	1		
Na	0.933**	0.950**	0.932**	0.948**	0.897*	-0.918	0.896*	0.929**	0.881*	1	
K	0.793	0.757	0.796	0.820*	0.796	-0.943	0.840*	0.805	0.803	0.817*	1

Level of significant \*(p<0.05; \*\*(p<0.01)

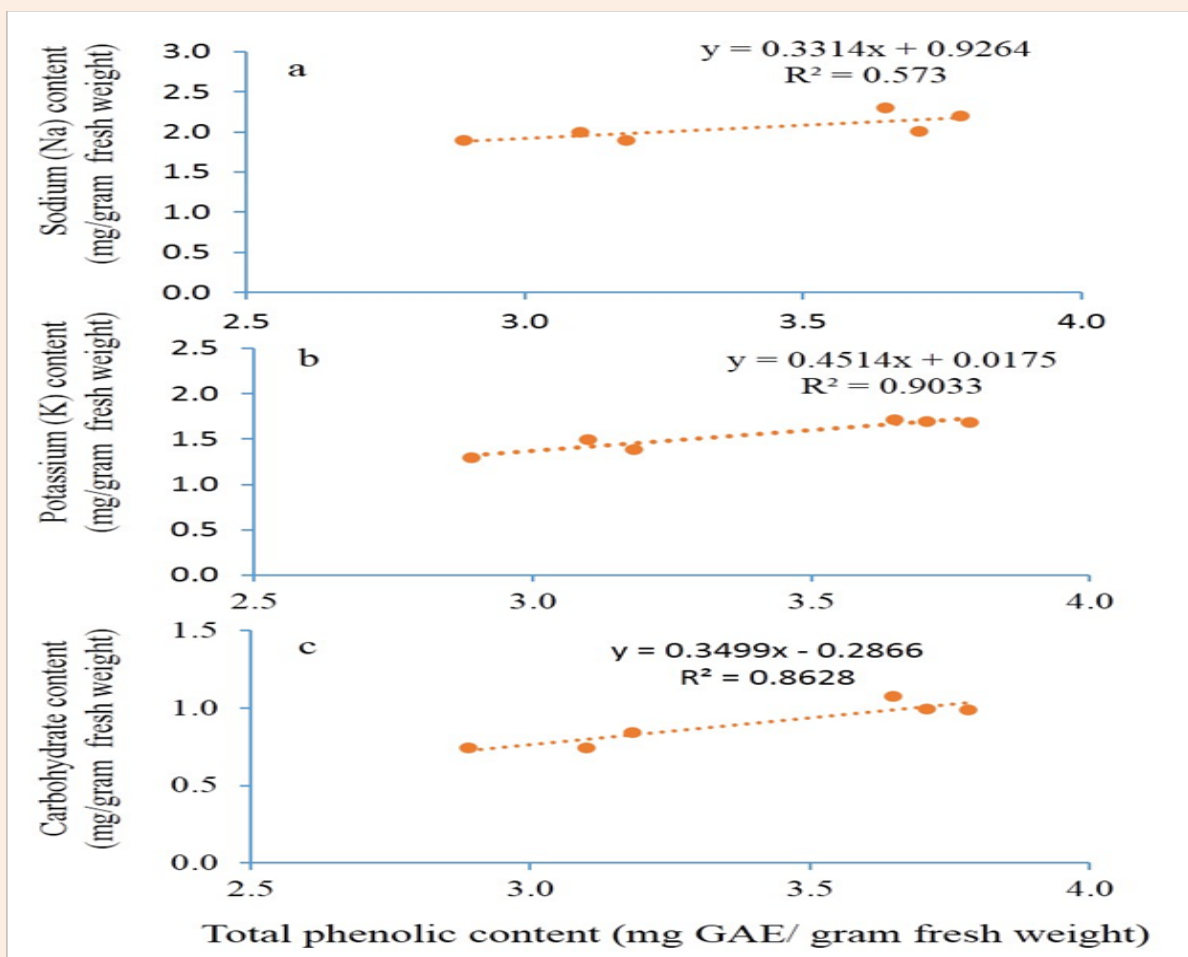


Figure 3: Relationship between total phenolic compounds and nutrients in different natural populations of *G. trichophylla* berries. a-c. Indicating linear correlation between total phenolic contents and sodium, potassium and carbohydrate content respectively.

## Conclusions

Wild edible berries of *Gaultheria trichophylla* have found highly nutritious and rich source of polyphenolics and antioxidants. Therefore, *G. trichophylla* can be used as a potential source of natural antioxidant in pharmaceutical and nutraceutical industries. As well as, berries can be utilized for preparation of nutritious products like juice, jam, sauce and supplement of food stuff by rural inhabitants, which not only contribute for enhancing their economic status but also, reduced the malnutrition. This mechanism will contribute to inhabitants for sustaining in the Trans Himalayan conditions. Furthermore, large scale plantation of the species in suitable location should be promoted for addressing issue of biodiversity conservation, soil erosion, and rural development to fulfill food security.

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## Conflict of Interest

The authors declare no conflicts of interest.

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