



EFFECT OF PRE-TREATMENTS AND DRYING METHODS ON NUTRITIONAL QUALITY OF *RHODODENDRON ARBOREUM*, WEST HIMALAYA

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ABSTRACT

Rhododendron arboreum belong to the family Ericaceae is a medicinally and economically important wild edible tree species from the Himalayan region. The fresh flowers of *R. arboreum* after harvesting were treated at different temperatures separately. The study revealed that a quick-drying process was found for the maximum harnessing of phenolics, flavonoids, flavanols, antioxidant activity (FRAP assay and DPPH assay) and protein content. Likewise, shade drying; a common method for drying was obtained best for nutritional attributes. Correlation analyzed that total phenolic content shows a positive relationship with ABTS and FRAP. However, phenolic content showed non-significant relationship with carbohydrates and protein. The biochemical parameters showed significant variation among drying processing techniques. The report suggested that the quality of a dried product is strongly dependent on the conditions used in the drying process.

Keywords: Wild edible, *Rhododendron arboreum*, Drying and storage process, Nutritional value.

1. INTRODUCTION

Wild edible fruits are considered economically and nutritionally important due to their antioxidant potential, nutritional quality and delicious taste. More than 675 wild edible species were documented from the Himalayas, out of which 340 were exclusively reported from Uttarakhand [1-4]. Today, the demand for natural antioxidants products is increasing exponentially and their regular consumption in diets associated with reducing cancer, cardiovascular, ageing and neuro-degenerative diseases [5-7]. The wild edible fruits were reported as the major component of diet or functional food and provided vitamins, minerals, antioxidants, carbohydrates, proteins, nutritional supplements etc, also, rural communities exploited them for the preparation of value-added products such as energy drinks (juice), jam, jellies, multivitamin pills, flavour, colour etc, which sustain the economic upliftment and encourage rural population for engaging in small-scale and village-level cottage industries [8, 9]. Consumers are now becoming aware of the need to consume a variety of fresh fruit and vegetables to maximize their intake of beneficial antioxidants [10].

Today, deterioration is a major problem with fruits or plants after harvesting, cutting and drying procedures is the oldest method for preservation of the food products which extends their shelf life somehow [11]. Blanching is known to inactivate enzymes that cause browning and deterioration of the quality of plant parts [12]. The process of drying is a technique for the preservation of fruits and vegetables because it stops the biochemical changes in perishable plant materials by reducing the moisture content of the material. Numerous techniques of drying such as infrared drying, freeze-drying, microwave drying have been more popular than oven drying, shade drying, due to shorter drying time, high energy efficiency, higher product quality and temperature uniformity [13].

Pretreatments are usually performed precede drying of fruits in order to minimize the adverse changes occurring during drying and subsequent storage. Pretreatments are recommended techniques used to enhance the quality of fruit because they prevent darkening of the cut fruit surface and cause the destruction of pathogens that could cause foodborne illness. There are numerous different pretreatment

methods that have been developed for fruit drying which include ascorbic acid dip, salt solution, honey dip, steam and water blanching [14], sulfite dip [15], blanching, chilling, freezing [16] etc.

Storage temperature plays an essential role in retaining the good eating quality in peach and nectarine. Also, processing condition, extraction technique, storage conditions, drying methods were important factors that influenced the nutritional and antioxidant quality [17-20]. So, it is essential to assess the stability as well as degradation pattern of these bioactive compounds, before they can be used in the formulation of functional foods.

In the western Himalaya, a total of 87 species, 12 subspecies and 8 varieties of *Rhododendron* (family Ericaceae) were recorded [21] and considered a 'keystone element' [22]. This species has high medicinal, commercial and religious values and is distributed throughout the forest as a pure stand or associated with other trees species [23]. The different parts of *R. arboreum* contain diverse bioactive constituents and significantly showed anti-diabetic, anti-bacterial, anti-inflammatory, cardio-protective and cholinergic activity [24]. The flowers are sweet and sour taste and are utilized for the preparation of health-promoting products like squash, jams, jellies and local brew etc. which are used to cure refreshing appetizers and prevent high altitude sickness, however, fried dried flowers with ghee are also used traditionally for the prevention of diarrhoea, blood dysentery, mental retardation and fresh petals are processed to prepare pickles and jelly [25]. Thus, *Rhododendron arboreum* is regarded as a commercially important wild edible fruit species of west Himalaya.

Bioactive compounds are easily affected by the processing method, therefore there is a need for the minimization of processing loss. The effect of a specific drying method on the preservation of raw quality cannot be predicted, as it is dependent on the type of chemical compounds present and the type of plant [26]. Therefore, considering the commercial importance, the demand of *Rhododendron arboreum* flowers, this research was carried out to study the effect of pre-treatment (initial temperature treatment) and different drying methods on the nutritional and antioxidant content.

2. MATERIAL AND METHODS

Fully fresh flowers of *Rhododendron arboreum* were collected randomly from the different positions of the tree from Oak forest, Pithoragarh district. Flowers was

brought to the laboratory and all flowers of each tree were pooled and processed following the procedure [27] and used for further quantification of nutritional and antioxidant activities.

2.1. Drying and Processing Technique

- i. *Oven-dried*: Total thirty grams of fresh flowers was dried in a hot oven at 110°C for 30 min and subsequently dried at room temperature until a constant weight was achieved.
- ii. *Shade-dried*: A total of thirty grams of fresh flowers was dried at room temperature (25°C) till constant weight was attained and then it was powdered.
- iii. Total thirty grams of fresh flowers was kept at 4°C for 1 day and then kept at 110°C for 25 min followed by drying at room temperature till constant weight attained and then it was powdered.
- iv. Total thirty grams of fresh flowers were kept at 4°C for 1 day and then dried at room temperature till constant weight attained and then it was powdered.
- v. Total thirty grams of fresh flowers was kept at -20°C for 1 day in a deep freeze and then dried at 110°C for 25 min followed by drying at room temperature.
- vi. Total thirty grams of fresh flowers was kept at -20°C in deep freeze for 1 day and subsequently dried at room temperature till constant weight was attained and then it was powdered.

2.2. Extract preparation

The dry and processed flowers of *R. arboreum* were extracted with 80% methanol and centrifuged for 20 min at 12,000 rpm (model REMI-CPR-24 Plus) with rotor number (R-247-n). The supernatant was removed and the extract was again filtered using Whatman filter paper (No. 2). The finalized solution was used for to analysis of polyphenolics, antioxidant and nutrient contents.

2.3. Polyphenolics analysis

2.3.1. Total phenolic content

Total phenolic content in the extract was determined by Folin-Ciocalteu's calorimetric method by [6, 28]. An aliquot of the extract (0.50 ml) was diluted with distilled water (4.50 ml) and mixed with Folin-Ciocalteu's reagent (0.50 ml). This was allowed for standing for reaction ($22 \pm 1^\circ\text{C}$, 5 min) and neutralized

by 5.0 ml of 7% (w/v) sodium carbonate and kept in the dark ($22\pm1^\circ\text{C}$, 90 min). The resulting absorbance was measured at 765 nm using UV-Vis spectrophotometer (U-2001, Hitachi, Japan). The results were expressed as mg gallic acid equivalent per gram of fresh weight (mg GAE/g fw).

2.3.2. Total flavonoids content

Total flavonoid content in the extract was determined by the aluminium chloride calorimetric method [29]. Briefly, 0.50 ml of extract was diluted with distilled water (1.50 ml) and 0.50 ml of 10% (w/v) aluminium chloride was added with 0.10 ml (1 M) potassium acetate and distilled water (2.80 ml) and allowed to stand at room temperature for 30 min. The absorbance of the resulting reaction mixture was measured at 415 nm UV-VIS spectrophotometer and results were expressed in mg quercetin equivalent per gram of fresh weight (mg QE/g fw).

2.3.3. Total flavonol content

Total flavonol was measured using the method as described [30]. Briefly, 2.0 ml of extract and 2.0 ml of 2% (w/v) aluminium chloride in ethanolic solution with 3.0 ml of 50 g/l sodium acetate solution were added and allowed to stand at room temperature for 2.5 h at 20°C . The absorbance of the resulting reaction mixture was measured at a 440 nm UV-VIS spectrophotometer. The results were expressed in mg catechin equivalent per gram of fresh weight (mg CE/g fw).

2.3.4. Total tannin content

Total tannin content was estimated following the procedure [31]. 5.0 ml sample extract was added with 0.5 ml Folin-Dennis reagent and 1.0 ml saturated sodium carbonate solution. The reaction mixture was diluted up to 10.0 ml with distilled water and allowed to stand ($22\pm1^\circ\text{C}$, 20 min) and the absorbance of the resulting reaction mixture was measured at 700 nm. The results were expressed in mg tannic acid equivalent per gram of fresh weight (mg TAE/g fw).

2.4. Antioxidant activity

2.4.1. 2-2-azinobis (3 benzylthiazole)-6- sulphonic acid (ABTS) assay

Total antioxidant activity was measured by improved ABTS (ethylbenzothiazoline 6- sulphonic acid) radical scavenging method [32]. The stock solution was prepared by dissolving 38.41 mg of ABTS in 5.0 ml of deionized water and 6.615 mg potassium persulfate in

5.0 ml of deionized water. A total of 10.0 ml a stock solution of $\text{ABTS}^{\cdot+}$ was prepared and allowed to react (16 h, $22\pm1^\circ\text{C}$) in the dark amber coloured bottle. The working $\text{ABTS}^{\cdot+}$ solution has an absorbance of 0.700 ± 0.05 at 734 nm before use. For analysis, 0.10 ml of extract and 3.90 ml of $\text{ABTS}^{\cdot+}$ solution was allowed to stand ($22\pm1^\circ\text{C}$, 6 min, dark) and the absorbance was recorded at 734 nm with respect to blank. Results were expressed in mM ascorbic acid equivalent per 100 g fresh weight (mM AAE / 100 g fw).

2.4.2. 2-2-diphenyl-1-picrylhydrazyl (DPPH) assay

The free radical scavenging antioxidant activity of the extract was determined with slight modification [33]. An aliquot of 0.80 ml was fixed with 2.40 ml freshly prepared DPPH \cdot solution and allowed to stand in the dark ($22\pm1^\circ\text{C}$, 20 min). The resultant reduction absorbance was measured at 520 nm and expressed in mM ascorbic acid equivalent per 100 g fresh weight (mM AAE / 100 g fw).

2.4.3. Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power (FRAP) assay was performed with slight modifications [34]. The reaction mixture was prepared by adding an aqueous solution of 10 mM TPTZ and 20 mM ferric chloride in 300 mM sodium acetate buffer (pH 3.6) at a ratio of 1:1:10 (v:v:v). For analysis, 1.0 ml of extract and 3.0 ml reaction mixture (pre-warmed at 37°C) were allowed to stand for 8 min and absorbance was recorded at 593 nm. The results were expressed in millimole ascorbic acid equivalent per 100 g fresh weight (mM AAE/100 g fw).

2.4.4. Hydroxyl radical scavenging antioxidant (OH) assay

Hydroxyl radical scavenging activity was measured [35, 36]. Briefly, reaction the mixture was prepared with 500 μl of 2-deoxyribose (2.8 mM) in phosphate buffer (50 mM, pH 7.4), 200 μl of premixed ferric chloride (100 mM) and EDTA (100 mM) solution (1:1; v/v), 100 μl of H_2O_2 (200 mM) with or without the extract solution (100 μl).

The reaction mixture was incubated for 1 h at 37°C , and further heated in a boiling water bath for 15 min after the addition of 1 ml of 2.8 % (w/v) trichloroacetic acid (TCA), and 1 ml of 1 % (w/w) 2- thiobarbituric acid. The solution was mixed well and after cooling, the absorbance was measured at 532 nm against ascorbic

acid results were expressed in millimole ascorbic acid equivalent per 100 g fresh weight (mM AAE/100 g fw).

2.4.5. Nitric oxide scavenging antioxidant (NO) assay

Nitric oxide scavenging antioxidant activity was determined in terms of NO• generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction [37]. In brief, the reaction mixture was prepared by adding 1.0 ml of from sodium nitroprusside (SNP) in phosphate-buffered saline (pH 7.4) with 1.0 ml of extract and incubated (25°C) for 30 min. After that, 1.0 ml of the mixture was added with 1.0 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride) and absorbance was recorded in 546 nm and results were expressed in millimole ascorbic acid equivalent per 100 g fresh weight (mM AAE/100 g fw).

2.5. Nutritional Analysis

2.5.1. Total carbohydrate content

Total carbohydrate content was determined [38]. The fresh fruit sample was hydrolyzed by 2.5N HCl and kept in a boiling water bath for three hours. The mixture was neutralized by sodium carbonate and centrifuged. The supernatant was collected for analysis and absorbance recorded in 590 nm against the standard calibration curve of glucose and results were expressed in mg per g fresh weight (mg/g fw).

2.5.2. Total protein content

The protein was estimated [39]. Briefly, the fresh supernatant solution (10.0 ml) and alkaline copper sulphate reagent (5.0 ml) were mixed thoroughly and allowed to stand for 10 min. Thereafter, Folin's reagent (0.5 ml) was added to develop the colour. After 30 min, absorbance measurements were performed at 660 nm against a blank (1.0 ml of 0.5 NaOH in place of the sample). Bovine serum albumin was used to construct a standard curve and the amount of protein in different samples was estimated. The results were expressed in mg per g fresh weight (mg/g fw).

2.5.3. Total amino acid content

The total free amino acid in the fruit was quantified by ninhydrin test [40]. Briefly, 0.5 g of fresh fruit sample was extracted with 80% ethanol, centrifuged and filtered. The reaction mixture was prepared by adding 0.1 ml sample extract, 0.9 ml distilled water and 1.0 ml

of ninhydrin reagent in test tube. The reaction mixture shaken vigorously and then heated in a boiling water bath for 20 min and 5.0 ml (50% propanol) was added. After cooling, the tube at room temperature, the color intensity was measured at 570 nm. The standard curve was prepared by using leucine as standard amino acid and results were expressed in mg/100g fw.

2.5.4. Total proline content

Standard procedure was used for colorimetric quantification of proline [41]. The reaction mixture was prepared by adding sample extract (1.0 ml), 98% formic acid (1.0 ml), (30g/L) ninhydrin solution (2.0 ml), mixed and placed for 15 min in a bath with boiling water (100°C). After the reaction, 20.0 ml of butyl acetate (99.5%) were added to extract the color into the organic phase, then the solution was filtered and dried using filter paper containing 0.2 g of anhydrous sodium sulfate. After 15 min, the absorbance of the organic phase was measured at 590 nm and proline was used for the preparation of the calibration curve and results were expressed in mg/100g fw.

2.6. Statistical analysis

Phytochemical data were subjected to analysis of variance (ANOVA), Pearson correlation coefficient using SPSS programme (16 version). The significance level was determined at $p < 0.05$ and the means were separated using Duncan's multiple range test (DMRT), if the values were significantly different. Data are presented as mean values \pm standard error (SE) of six replicates for each analysis of polyphenolics (total phenolics, flavonoids, flavonols, tannins, proanthocyanidin); antioxidant activity (ABTS, DPPH, FRAP, OH•, NO• assay); nutritional content (total carbohydrate, protein, amino acid, proline, methionine, and ascorbic acid). Data are presented as mean values \pm the standard error (SE).

3. RESULTS AND DISCUSSION

3.1. Effect of different processing of drying on polyphenolic content

Polyphenolic content varied significantly ($p < 0.05$) among the processes of drying (Table 1). The drying process showed a higher amount of total phenolic content (4.89 mg GAE/g), flavonoid (2.86 mg QE/g) and flavanol (4.00 mg CE/g). However, the lowest amount was exhibited in the process VI. Besides, total tannin content (2.90 mg TAE/g) was found maximum in the process V as compared to others.

3.2. Effect of different processing of drying on antioxidant activity

The antioxidant activity showed significant ($p < 0.05$) variation in different drying processes (Table 2). The maximum reducing antioxidant activity (1.52 mM AAE/100g) and free radical scavenging activity (36.61 mM AAE/100g) was recorded in drying process I respectively. However, hydroxyl ion scavenging antioxidant activity (2.54 mM AAE/100g) and nitric oxide scavenging activity (3.79 mM AAE/100g) were exhibited maximum in the process III. Amongst the processes, total antioxidant activity (31.87 mM

AAE/100g) was recorded higher in process II as compared to others.

3.3. Effect of different processing of drying on nutritional content

Nutritional content varied significantly ($p < 0.05$) in different drying processes (Table 3). The drying process IV was recorded best for harnessing maximum total carbohydrate content (1.07 mg/g) and total amino acid (1.68 µg/g). However, process I was found best for total protein (8.93 µg/g) and total proline (1.26 µg/g) recorded maximum in process III respectively.

Table 1: Effect of different processing in polyphenolics of *Rhododendron arboreum* at Oak Forest

Treatment	Total phenol	Total flavonoid	Total flavonol	Total tannin
I	4.89±0.06 ^a	2.86±0.01 ^a	4.00±0.02 ^a	2.09±0.01 ¹
II	3.92±0.05 ^b	2.36±0.02 ^b	3.61±0.01 ^d	2.32±0.01 ^c
III	2.39±0.06 ^d	2.04±0.04 ^c	3.81±0.02 ^c	2.80±0.01 ^b
IV	3.53±0.11 ^c	2.01±0.02 ^c	3.58±0.01 ^d	2.28±0.02 ^d
V	3.99±0.08 ^b	2.40±0.01 ^b	3.87±0.02 ^b	2.90±0.01 ^a
VI	1.94±0.05 ^e	1.89±0.03 ^d	2.41±0.03 ^c	2.20±0.01 ^e

Values are mean ± standard error; different letters in a column indicate significant variation ($p < 0.05$); total phenolics expressed as mg GAE/g dw; flavonoids expressed as mg QE/g dw; flavonols expressed as mg QE/g dw; tannins expressed as mg TAE/g dw

Table 2: Effect of different processing in antioxidant activity of *Rhododendron arboreum* at Oak Forest

Treatment	ABTS	FRAP	DPPH	NO	OH
I	25.87±0.08 ^c	1.52±0.01 ^a	36.61±0.20 ^a	3.09±0.03 ^b	1.25±0.05 ^c
II	31.87±0.10 ^a	1.32±0.01 ^d	25.70±0.26 ^d	2.55±0.03 ^c	1.78±0.10 ^b
III	24.96±0.12 ^d	1.25±0.01 ^c	24.92±0.01 ^d	3.79±0.03 ^a	2.54±0.15 ^a
IV	28.45±0.20 ^b	1.12±0.01 ¹	31.11±0.58 ^b	1.95±0.03 ^d	1.33±0.04 ^c
V	21.25±0.08 ¹	1.39±0.01 ^b	29.95±0.13 ^c	1.09±0.03 ^c	1.20±0.09 ^c
VI	21.94±0.09 ^c	1.36±0.01 ^c	22.59±0.02 ^c	1.79±0.17 ^d	1.17±0.07 ^c

Values are mean ± standard error; different letters in a column indicate significant variation ($p < 0.05$); ABTS, DPPH, FRAP, NO, OH activity expressed as mM AAE/100 g dw

Table 3: Effect of different processing in nutritional content of *Rhododendron arboreum* at Oak Forest

Treatment	Carbohydrate	Protein	Amino acid	Proline
I	1.01±0.01 ^b	8.93±0.03 ^a	1.28±0.02 ^c	0.99±0.01 ^d
II	0.83±0.01 ^d	3.24±0.02 ^b	1.30±0.01 ^c	0.66±0.01 ¹
III	1.02±0.01 ^b	3.27±0.01 ^b	1.15±0.01 ^d	1.26±0.01 ^b
IV	1.07±0.01 ^a	1.28±0.03 ^c	1.68±0.01 ^a	1.07±0.01 ^c
V	1.02±0.01 ^b	1.31±0.06 ^c	1.47±0.01 ^b	0.85±0.04 ^c
VI	0.93±0.01 ^c	1.23±0.01 ^c	1.06±0.02 ^c	1.40±0.01 ^a

Values are mean ± standard error; different letters in a column indicate significant variation ($p < 0.05$); total protein, carbohydrate, amino acid, proline expressed as mg/100g dw

3.4. Correlation between analyzed parameters

While developing the correlation between analysed parameters, a significant positive relationship was obtained (Table 4). Total phenolic content showed positive ($p < 0.05$) relationship with ABTS activity ($r = 0.339$) and FRAP activity ($r = 0.438$; $p < 0.05$).

Similarly, phenolic content positively correlated with amino acid ($r = 0.502$; $p < 0.05$) (Fig. 1). However, phenol positively correlated ($r = 0.900$) with flavonoid. Phenolic content showed non-significant relationship with carbohydrate ($r = 0.007$) and protein ($r = 0.0317$).

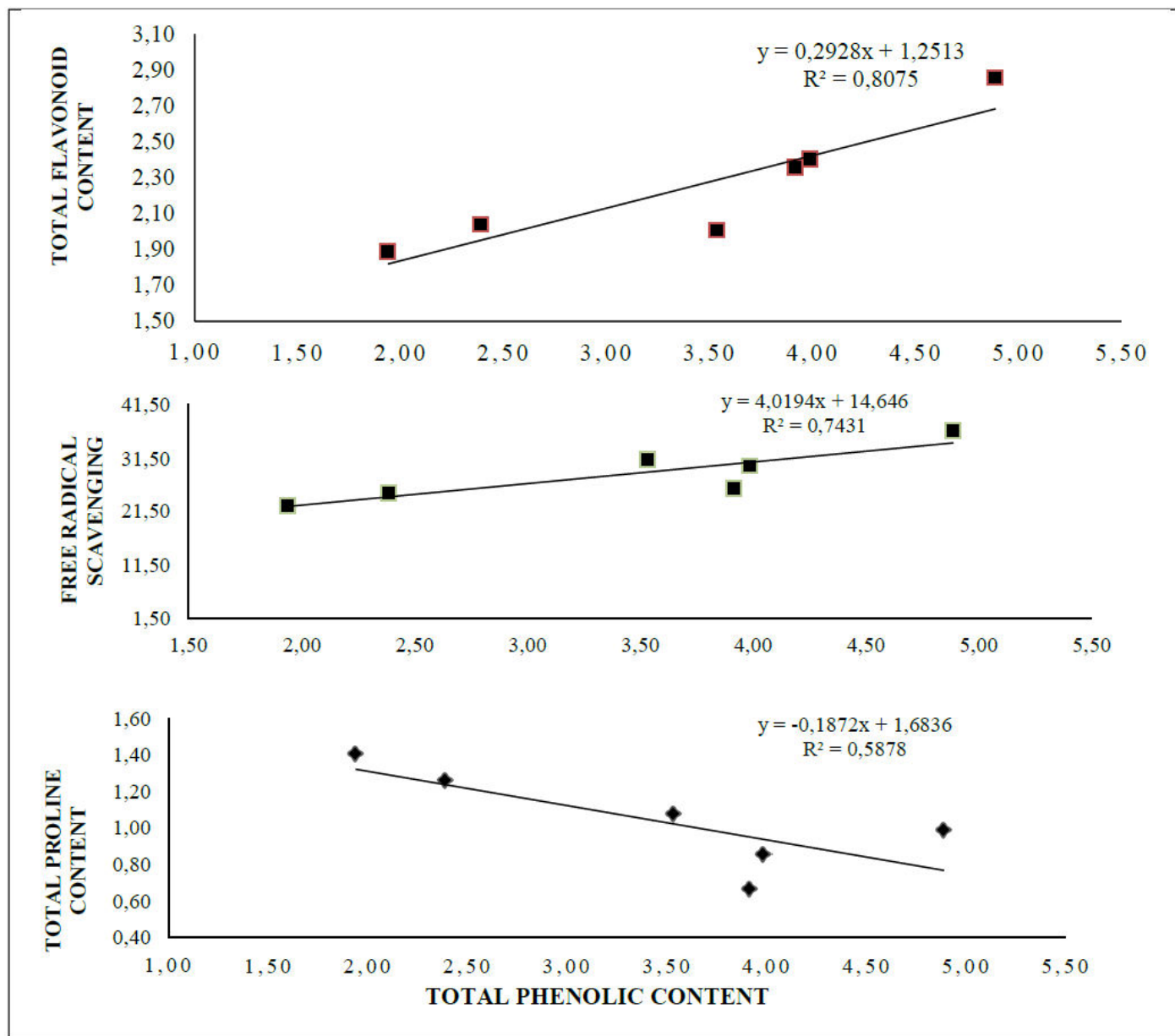


Fig. 1: Relationship between total phenolic content and analysed parameters ($p < 0.05$)

Table 4: Correlation between analyzed parameters

	Phenol	Flavonoid	Flavonol	Tannin	ABTS	FRAP	DPPH	NO	OH	Carbo-hydrate	Protein	Amino Acid	Proline
Phenol	1												
Flavonoid	.900*	1											
Flavonol	.725	.656	1										
Tannin	-.188	-.181	.356	1									
ABTS	.339	.135	.276	-.395	1								
FRAP	.438	.749	.109	-.178	-.343	1							
DPPH	.861*	.780	.665	-.249	.095	.353	1						
NO	.353	.271	.263	-.677	.750	-.146	.428	1					
OH	-.343	-.238	.290	.444	.281	-.345	-.399	.154	1				
Carbohydrate	.073	-.027	.350	.262	-.401	-.240	.504	.011	-.081	1			
Protein	.317	.517	.517	-.089	.494	.313	.162	.535	.623	-.313	1		
Amino Acid	.502	.097	.450	.078	.289	-.451	.531	.166	-.323	.481	-.375	1	
Proline	-.766	-.591	-.589	-.081	-.546	-.166	-.357	-.106	.095	.357	-.286	-.462	1

Note: R-correlation coefficient, * Significant at $p < 0.05$; (whereas- Phenol-PHE; Flavonoid-FLV; Flavonol-FAL; Tannin-TAN)

Generally, in many countries, shade drying is preferred by people as it is the cheapest method after harvesting any part of the plant species. The drying process is carried for food preservation especially from a commercial point of view. Several studies have been reported on the effects of the drying process of edible species on Apple [42], *Carica papaya* [43] and *Daucus carrots* [44]. *Rhododendron arboreum* is one of the most stately and impressive species and contains large quantities of nutritional content but mostly it cannot be consumed fresh. The flower of the species needs processing and due to its health benefits, it is widely used as juices, jam etc. Therefore, the quality of raw materials is very important for the preparation of value-added products and their consumption. In the present study, different drying processing methodology was explored for the quantification of biochemical attributes of the species. Due to their antioxidant properties, bioactive compounds in plants play a crucial role in the prevention of several diseases [4, 5]. The analyzed biochemical parameters showed significant variation among drying processing techniques. The report suggested that the quality of a dried product is strongly dependent on the conditions used in the drying process. As well, the drying process helps to conserve their desirable qualities and extend their shelf life [20]. However, several kinds of research highlighted that nutritional and antioxidant activity was influenced by different processing techniques [9, 18, 19]. The analyzed parameters varied significantly in different drying processes. The results suggest that a quick-drying process (P1) was found for the maximum harnessing of phenolics, flavonoids, flavanols, antioxidant activity (FRAP assay and DPPH assay) and protein content. Likewise, shade dried (P IV), a common method for drying, was obtained best for nutritional attributes (total carbohydrate content and amino acid). Previous reports on *Azadirachta indica* showed a similar kind of variation and indicated that several enzymatic and non-enzymatic processes may affect the composition of bioactive components in fresh plant tissue [20] and these compounds are oxidized when exposed to the atmosphere for a long time [45]. In the previous study, a similar type of trend was also observed in *Rhododendron arboreum* flowers which contain bioactive compounds with good antioxidant activity [46].

4. CONCLUSION

The flowers of *Rhododendron arboreum* extract contain various phytochemical processes which are biologically

important. Bioactive compounds are easily affected by the processing method, therefore there is a need for the minimization of processing loss. The present study concluded that different drying processing methodology was explored for the quantification of biochemical attributes of the species. The quick-drying process was found best for the maximum harnessing of phenolics, flavonoids, flavanols, antioxidant activity (FRAP assay and DPPH assay) and protein content.

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Conflict of interest

None declared

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