**Investigations on methods of drying and storage on anthocyanin content and anti-oxidant potentialof hibiscus tea infusions**


## Abstract

*Hibiscus rosa-sinensis* is one of the traditional flowers used in tropics for ornamental gardens and for its curative health benefits. All parts of hibiscus are reported to possess significant medicinal values and the flowers especially are rich source of anthocyanin pigments. Anthocyanins serve as natural food colourants and also contribute to health benefits as a rich source of antioxidants. The major anthocyanin present in the red flowers of *Hibiscus rosa-sinensis* is cyanidin-3-sophoroside (Lowry, 1976). Investigations were carried out to explore the drying method of petals, antioxidant potential and nutritional benefits of hibiscus floral tea and squash from the cv. Red single of hibiscus. The hibiscus petals were subjected to drying by three different methods such as shade, sun and oven. It was observed that highest recovery of dried petals (19.66 %) and anthocyanin content (59.05 mg/l) was attained by shade drying of petals. The dried flower petals were quantified for cyanidin-3-glucoside using HPLC. In another experiment, tea infusions prepared from Green tea, Hibiscus tea and their combination proved significance and highest anthocyanin content was registered in freshly prepared Hibiscus tea (72.13 c3g eq.mg/l). However, highest ABTS (249.19 µg/ml), DPPH radical scavenging activity (733.22 µg/ml) in terms of $IC_{50}$ values, highest total phenol content (82.57 mg GAE/g) and high organoleptic scoring were recorded in freshly prepared Green tea + Hibiscus tea [1:1]. The tea samples were tested for their qualities by storing upto 90 days. The above investigation proved the potential uses and anti-oxidant benefits of hibiscus petals in the preparation of hibiscus tea infusions.



**Keywords:** Hibiscus flowers – shade drying – anthocyanin content – anti-oxidant activity


## Introduction

Flowers are unique in their colour, shape, aesthetic appeal and fragrance. Among the different commercial flower crops, one of the traditionally underutilised crops with several health benefits is *Hibiscus rosa-sinensis*. *Hibiscus rosa-sinensis* has a significant amount of bioactive components such as glycosides, terpenoids, saponins, and flavonoids and so it is recommended as a herbal alternative to cure many diseases (Obi, 1998). The anti-oxidant potential of hibiscus petals is attributed to the presence of red coloured anthocyanin pigment. The food industry has shown an increased interest in red pigments due to their restricted supply (Lauro and Francis, 2000) and hence during the past two decades, research into natural sources of red pigments has expanded widely.

Flower petals of *Hibiscus rosa-sinensis* had the property of antidiabetic, anti-inflammatory, antifertility effects *etc*. **(**Sanadheera *et al.*, 2021),thus it has the capacity to cure many health disorders.

Hibiscus is popularly used as a herbal tea as it has an additive antioxidant interaction when combined with green tea (Farooq and Sehgal, 2019). The red coloured anthocyanin pigment acts as a natural

food colourant and yields an appealing visual quality to processed products. The flower petals of hibiscus can also be utilised for preparation of herbal tea and ready to use processed products such as squash. However, the scientific data are lacking on the methods of drying of hibiscus flower petals and the nutritional qualities of the processed products such as hibiscus tea and squash. The present study was thus formulated to scrutinize the potential benefits of Hibiscus tea,green tea and their combination as well as to investigate the effects of methods of drying and storage on the anti-oxidant properties and nutritional qualities of processed hibiscus products.

**Materials and methods**

**Collection of hibiscus samples**

Freshly harvested Hibiscus flowers (Red Single Cultivar) were collected from the Botanical Garden of the Department of Floriculture, TNAU, Coimbatore for the entire studyand the petals were separated from calyx, stamen and pistil.The investigation was divided in to two experiments laid out in CRD under laboratory conditions.

**Experiment 1**

The first experiment comprised of three drying methods of the petals*viz*.,shade drying for four days($T_1$), sun drying for 6 hours($T_2$) and oven drying at 60°C for 4 hours($T_3$). The duration of drying was fixed based on certain preliminary investigations. The petals were placed over trays in open sun and under ambient laboratory conditions for shade drying. Hot air oven was used for drying the sample and also for the estimation of moisture content. The optimal method of dryingwas decided based on the moisture content (%), percent recovery of dried petals and monomeric anthocyanin content of dried petals. Moisture content was calculated based on initial and final weight after drying and percent recovery was arrived at deducting the moisture loss from cent percent. The Monomeric anthocyanin content was measured using a spectrophotometric pH differential protocol based on Lee *et al*. (2005) and cyanidin 3 glucoside in shade dried of hibiscus petal samples was estimated in HPLC using the method followed by Idham*et al*., 2012. The colour of dried petals was compared with RHS colour chart.

**Experiment 2**

The second experiment was conducted to study the anti-oxidant potential of pure hibiscus tea as well as in combination with green tea. This experiment was laid out with seven treatments comprising ofGreen tea (control), Hibiscus tea (100%), Green tea + hibiscus tea (50:50) and analysed for 0,45 and 90 days of storage.

**Preparation of tea infusions**

Petals were separated from freshly harvested *Hibiscus* flowers, cleaned and shade dried (as fixed as best drying method of experiment 1). Tea infusions were made by boiling precisely weighed (3 g) samples in 200 ml of water for 10 minutes at 90 °C. Green tea, Hibiscus tea, and Hibiscus + green tea infusions were prepared based on the treatment combinations. 1.5 g of green tea leaves and

1.5 g of dried Hibiscus flower petals were used to make the Hibiscus + green tea. The tea infusions were prepared fresh and utilised for further analysis.Tea samples were analysed for the quality parameters after 0 days, 45 days and 90 days of storage. The observations on monomeric anthocyanin content of herbal tea infusions was measured as described above. The antioxidant potential of tea infusions were estimated by performing ABTS radicle scavenging activity assay in accordance with the method published by Re *et al.*, 1999and the DPPH assay was performed using the method of Brand-Williams *et al.*, (1995). The total phenol content was determined using the Folin-Ciocalteu (FC) technique of Singleton and Rossi (1965). UV-Vis spectrophotometer (M/s. Systronics – Model 2201) was used to estimate the monomeric anthocyanin content, total antioxidant activity and total phenol content.

**Colour and Sensory analysis**

The tea infusions were subjected to colour analysis by using chromometer (3 nh spectrophotometer). Tea infusions were placed individually in specimen cell for measurements on L*, a*, b* values. The tea infusions were subjected to sensory evaluation. Each of 10 panelists were presented with tea infusions and a questionnaire was given to evaluate product attributes (colour, flavour, taste and overall acceptability) with a 5-point hedonic scale (Larmond, 1977)ranging from 1, being not acceptable to 5, highly acceptable and the highly desired flavour was identified. The tea infusions were freshly prepared before serving.

**Statistical analysis**

The results obtained from the experiments were carried out in triplicates and the values are expressed in Mean ± SD. The critical difference was worked out at five percent (0.05) probability level. The obtained data were subjected to statistical analysis which was carried out with AGRES software package and MS Excel® spreadsheet. $IC_{50}$ values were identified using software, Graph pad prism 5.0.Correlation matrix using Pearson's correlation coefficient was analysedusing R software package, between anthocyanin content and antioxidant activity $IC_{50}$and total phenol content. Based on 'r'value, the correlation was interpreted as, negative value indicating a negative linear correlation, positive values indicating a positive linear correlation, 0 indicating no linear correlation, While the r values in the range 0-0.3 as weak linear correlation, 0.3–0.7 as moderate linear correlation, and 0.7-1as a strong correlation.

**Results and discussion**

The results of thetwo experiments are presented in Tables 1 to 3 as well as interpreted in Figures 1 to 4. In experiment 1, the observations revealed a significant difference in the moisture content, percentage recovery of petals and anthocyanin content among the different methods and the results are summarised in Table.1

**Drying methods of hibiscus flowers**

Drying methods decrease the water content in the product and inhibit the growth of microorganisms thereby decreasing rate of biochemical action and extending the shelf life of the

products at ambient temperature (Hamrouni et al., 2013). Among the different methods of drying, least drying with was achieved in shade drying with a moisture content of 80.34% when compared to sun (85.36 %) and oven drying (87.04%).The duration of drying was reduced at higher temperature both under sun drying and oven drying, the moisture removal was also the highest. Whereas under shade, there was steady drying for longer duration and hence least moisture was removed. The petals dried at ambient room temperature removes only the moisture whereas other essential bioactive components like anthocyanin content, total phenols etc. degrade slowly when compared to other drying methods. Hence shade drying of petals are considered the best in aspect of withholding the essential bioactive compounds.Ashaye (2013)reported that, oven drying was the best method to dry *Hibiscus sabdariffa* than sun drying but there was a loss of nutrients due to high temperature in oven dried samples.

**Anthocyanin content in dried petals**

The monomeric anthocyanin content of Hibiscus petals subjected to different drying techniques varied significantly according to the method used, ranging from 48.78 ± 0.57 c3g eq.mg/l (sun drying) to 59.05 ± 0.88 c3g eq.mg/l (shade drying) compared to 69.87 c3g eq.mg/l in fresh petals (Table 1). Drying the plant tissues usually results in a reduction of anthocyanin pigment which was highly dependent on the conditions of drying (Zamani and Nazoori, 2022). Temperature is the major factor affecting the content of anthocyanins. Shade dried petals recorded the highest anthocyanin content compared to other drying methods since exposure to high temperature and direct light are avoided. Therefore, these factors promoted to preserve the anthocyanin contents in this method. Similar results were reported by Fernandes *et al.* (2018) in *Centaurea* petals.

**Quantification of Cyanidin-3-glucoside**

The chromatogram representing the cyanidin 3 glucoside is presented in Figs. 1 and2.. Based on retention time and area, the cyanidin 3glucoside in hibiscus aqueous extract was quantified as 309.35mg/100g. Anthocyanin are classified into 17 anthocyanidin or aglycones that occur naturally in plant systems, but only six are common in higher plants *viz*., cyanidin , peonidin, pelargonidin, malvidin, delphinidin and petunidin (Kong et al., 2003). Among them Cyanidin 3 glucoside was found to be the major anthocyanin compound found in red colour petals of hibiscus (Puckhaber *et al*., 2002). The pharmaceutical importance of cyanidin glucosides and its antioxidant property in inhibition of cervical tumour cell developmenthas been earlier reported byRugină*et al.*, 2012.

**Anti-oxidant potential of hibiscus tea infusions**

The results of the experiment in scrutinizing the potential benefits like antioxidant potential, total phenols, anthocyanin and sensory analysis of Green tea, Hibiscus tea and their combination (1:1) at an interval of $0^{th}$ day, $45^{th}$ day and $90^{th}$ day of dried petals are presented in Table 2. Green tea (GT) is one of the beverages consumed all over the world due to its various health promoting factors. The medicinal qualities and flavour of Green tea can be enhanced by combining with any of the plants rich in antioxidant quality(Farooq and Sehgal, 2019).

The antioxidant capacity of aqueous tea infusions to stable, non-biological radicals were investigated *via* DPPH and ABTS assays.The highest ABTS radical scavenging activity with lowest effective concentration of 201.56 was recorded in Green tea followed by the treatments freshly prepared Green tea + Hibiscus tea with $IC_{50}$ values of 249.19 ;the lowest radical scavenging activity (828.82) by ABTS was recorded in the treatment Hibiscus tea stored for 90 days.

DPPH radical scavenging efficacy of tea infusions proved highest activity in Green tea with an $IC_{50}$ of 395.68 followed by freshly prepared Green tea + Hibiscus tea (733.22). ABTS is an unstable, coloured, free radical and it has the ability to dissolve in both aqueous and organic phase to investigate the antioxidant activities of both hydrophobic and hydrophilic antioxidants of food extracts(Kim et al., 2002).The highest ABTS and DPPH radical scavenging activity was recorded in control (Green tea).

Green tea as pure infusion exhibited significant ABTS and DPPH radical scavenging assays and it was succeeded by treatment $T_3$ which comprised of Green tea + Hibiscus tea [1:1] (freshly prepared, zero-day storage). Similar results were reported by Jiji Allen *et al*., 2018who observed that Green tea recorded highest antioxidant potential succeeded by Green tea + Hibiscus tea. This finding is also in accordance with that of Farooq and Sehgal (2019)who reported that combination of Green tea + Hibiscus tea infusions exhibited highest antioxidant potential than the infusions of green tea with *Ocimum* and *Cymbopogon* sp. Previous reports confirmed that there was an additive antioxidant interaction between Green tea + Hibiscus tea extracts in ABTS and DPPH assays. The strength of combination of herbs is increased not only by increase in concentration of individual herbs but also by the interaction between various herbs with different pharmacological properties (Jia *et al*., 2004).

**Total phenol content of tea infusions**

The total phenol content was calculated on the basis of gallic acid standard curve ($R^2$= 0.991). Among the seven treatments, total phenol content was significantly higher in Green tea infusion (120.72 mg GAE/g). This was followed by Green tea + Hibiscus tea (freshly prepared, zero day storage) with the value 82.57 mg GAE/g (Table 2). Freshly prepared extracts exhibited higher total phenol content when compared to stored samples for up to 90 days. There was a gradual decline in phenol content with respect to storage days. Similar result was produced by Farooq and Sehgal (2019)with the manifestation of highest total phenol content by Green tea + hibiscus tea than Green tea + cymbopogon tea. The aqueous extract of *Hibiscus rosa-sinensis* exhibited the highest total phenol content of 54.36 mg/g than ethanol extract of *H. rosa-sinensis* (45.98 mg/g).

The total phenol content of the tea infusions increased with decreased $IC_{50}$ value of ABTS and DPPH, which eventually resulted in an intensified antioxidant radical scavenging activity. The combined effects of Green tea and Hibiscus tea extracts enhanced the total phenolic contents and thereby increased the antioxidant potential in freshly dried petals of Green tea + Hibiscus tea infusions.

**Total anthocyanin content of tea infusions**

The anthocyanin pigment was widely present in petals of Hibiscus, which provides not only the red colour but also has an array of health-promoting benefits. Among the seven treatments, Hibiscus tea freshly prepared, zero day storage ($T_2$), exhibited the highest anthocyanin content of 72.13 mg c3g eq./l respectively and it was followed by the treatments Hibiscus tea stored for 45 days ($T_4$) and Hibiscus tea stored for 90 days ($T_6$) with 69.11mg c3g eq./l and 62.42 mg c3g eq./l respectively (Table 2). The anthocyanin content was found to be decrease gradually when dried petals were stored longer. Hibiscus tea exhibited the highest anthocyanin content thereby protecting the cells against variety of oxidants by exhibiting variety of mechanisms. Similar results were obtained by Carolina *et al.,* 2021 who observed that Hibiscus tea provides more antioxidants when prepared in hot condition (7min at 75°C). The anthocyanin content was found to decrease gradually when dried petals were stored longer. The petals of *Hibiscus rosa-sinensis* is rich in anthocyanin pigment which provides the antioxidant activity (Mak et al., 2013). In addition, they are natural bio-pigments with anti-bacterial properties as well and can be used as natural food colorants (Naz et al., 2007).

The colours obtained on tea infusions were visually observed and their corresponding colour values (L*, a*, b*). Among the different tea infusions, treatment $T_2$ which contains Hibiscus tea (freshly prepared, zero day storage) resulted in the lowest L* values (18.60) which indicates more darkness compared to other infusions (Table 2). The tea infusions were freshly prepared and their organoleptic scoring were observed based on the scores given by 10 panellists. The preference for flavour and taste were highest for Green tea + Hibiscus tea freshly prepared, zero day storage ($T_3$) with scores of 4.2 and 4.3 respectively (Table 4).

**Correlation between anthocyanin content and anti-oxidant potential**

Correlation coefficient was analysed and interpreted in Table 4 andFig. 3. The Correlation matrix proved a positive and strong correlation between anthocyanin content and anti-oxidant potential of hibiscus tea infusions. ($R^2$ =0.971** for DPPH and $R^2$=0.907** for ABTS) was arrived and represented using R package. Similar positive correlation has been reported by Wenkai*et al*., (2015) in Chinese Bayberry and Kumari *et al.* (2022) in Rose varieties.

**Conclusion**

The present investigation has led to the finding that shade drying is the best method for hibiscus petals and has also explored the potential uses of hibiscus petals in the preparation of floral tea infusions. Shade drying of Hibiscus petals yielded high recovery and higher anthocyanin pigment in petals. Green tea + Hibiscus tea (1:1) is a potential health drink with good consumer acceptability owing to the colour, flavour and taste.

**Table 1. Effect of different drying methods on moisture content, % recovery and monomeric anthocyanin content and colour values of Hibiscus petals**

| Treatment details | | Total moisture content (%) | Percentage recovery | Monomeric anthocyanin content (c3g eq.mg/l) | RHS colour chart code | Dried hibiscus petals |
|---|---|---|---|---|---|---|
| $T_1$ | Shade drying | 80.34 ± 2.74 | 19.66 ± 0.55 | 59.05 ± 0.88 | Purple group N77 (C) |  |
| $T_2$ | Sun drying | 85.36 ± 2.83 | 14.64 ± 0.14 | 48.78 ± 0.57 | Purple group N77 (D) |  |
| $T_3$ | Oven drying-60°C | 87.04 ± 1.97 | 12.96 ± 0.08 | 52.06 ± 0.19 | Purple group N77 (A) |  |
| | **Mean** | 84.24 | 15.7 | 53.29 | | |
| | **SE(D)** | 0.693 | 0.230 | 1.292 | | |
| | **CD (0.05)** | 1.730** | 0.574** | 3.224** | | |

**Table 2. Effect of different storage duration of Hibiscus tea and Green tea on antioxidant potential of tea infusions**

| Treatment details | | ABTS ($IC_{50}$µg/ml) | DPPH ($IC_{50}$µg/ml) | Monomeric anthocyanin content (c3g eq.mg/l) | Total phenol content (mg GAE/g) | L* | a* | b* |
|---|---|---|---|---|---|---|---|---|
| $T_1$ | Green tea (control) | $201.56 \pm 3.28^{g}$ | $395.68 \pm 0.99^{g}$ | $1.0 \pm 0.01^{g}$ | $120.72 \pm 3.03^{a}$ | 26.84 | - 0.28 | 0.71 |
| $T_2$ | Hibiscus tea (freshly prepared, zero day storage) | $757.88 \pm 13.66^{c}$ | $1354.79 \pm 17.0^{c}$ | $72.13 \pm 1.30^{a}$ | $64.79 \pm 0.66^{e}$ | 18.60 | 0.67 | -0.45 |
| $T_3$ | Green tea + Hibiscus tea [1:1] (freshly prepared, zero day storage) | $249.19 \pm 4.26^{f}$ | $733.22 \pm 29.74^{f}$ | $37.14 \pm 0.60^{d}$ | $82.57 \pm 1.51^{b}$ | 19.59 | 0.58 | -0.38 |
| $T_4$ | Hibiscus tea (stored for 45 days) | $798.90 \pm 7.92^{b}$ | $1428.74 \pm 24.46^{b}$ | $69.11 \pm 0.83^{b}$ | $60.35 \pm 0.99^{f}$ | 18.89 | 0.66 | -0.44 |
| $T_5$ | Green tea + Hibiscus tea [1:1] (stored for 45 days) | $292.77 \pm 7.91^{e}$ | $802.25 \pm 5.78^{e}$ | $33.63 \pm 1.07^{e}$ | $78.72 \pm 0.57^{c}$ | 20.02 | 0.55 | -0.37 |
| $T_6$ | Hibiscus tea (stored for 90 days) | $828.82 \pm 19.42^{a}$ | $1507.52 \pm 36.68^{a}$ | $62.42 \pm 1.95^{c}$ | $55.08 \pm 0.31^{g}$ | 18.95 | 0.66 | -0.43 |
| $T_7$ | Green tea + Hibiscus tea [1:1] (stored for 90 days) | $335.80 \pm 0.30^{d}$ | $875.87 \pm 15.00^{d}$ | $30.43 \pm 0.008^{f}$ | $72.12 \pm 0.42^{d}$ | 21.34 | 0.54 | -0.35 |
| | **Mean** | 494.99 | 1014.01 | 43.70 | 76.33714 | | | |
| | **SE(d)** | 8.272 | 17.944 | 0.859 | 1.743 | | | |
| | **CD (0.05)** | 17.914** | 38.860** | 1.860** | 3.776** | | | |

**Table 3. Organoleptic scoring of Hibiscus tea and Green tea infusions at different storage duration**

| Treatment details | | Colour | Flavour | Taste | Overall Acceptability |
|---|---|---|---|---|---|
| $T_1$ | Green tea (control) | 3.6 | 3.7 | 3.5 | 3.7 |
| $T_2$ | Hibiscus tea (freshly prepared,zero day storage) | 4.5 | 3.8 | 3.8 | 3.9 |
| $T_3$ | Green tea + Hibiscus [1:1] (freshly prepared, zero day storage) | 4.2 | 4.2 | 4.3 | 4.2 |
| $T_4$ | Hibiscus tea (stored for 45 days) | 4.4 | 3.7 | 3.7 | 3.9 |
| $T_5$ | Green tea + Hibiscus [1:1] (stored for 45 days) | 4.0 | 4.0 | 4.2 | 4.0 |
| $T_6$ | Hibiscus tea (stored for 90 days) | 4.3 | 3.7 | 3.7 | 3.8 |
| $T_7$ | Green tea + Hibiscus (90d) [1:1] (stored for 90 days) | 4.0 | 4.0 | 4.2 | 4.0 |
| | **Mean** | 4.1 | 3.8 | 3.9 | 3.9 |

**List 1 :Scoring Details:**

| SCALE | SCORE RANGE | ACCEPTANCE LEVEL |
|---|---|---|
| 1 | 4.50 – 5.00 | Highly acceptable |
| 2 | 3.50 – 4.49 | Acceptable |
| 3 | 2.50 – 3.49 | Moderately acceptable |
| 4 | 1.50 – 2.49 | Slightly acceptable |
| 5 | 1.00 – 1.49 | Not acceptable |

**Figure 1. HPLC Chromatogram of Cyanidin 3 glucoside (Standard)**

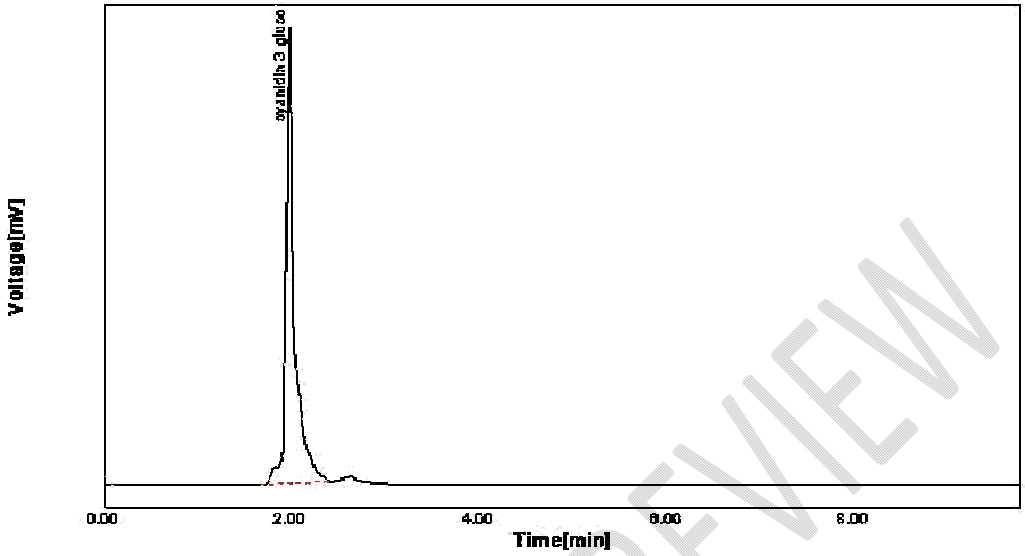



**Figure 2. Chromatogram of Cyanidin 3 glucoside quantified in hibiscus extract**

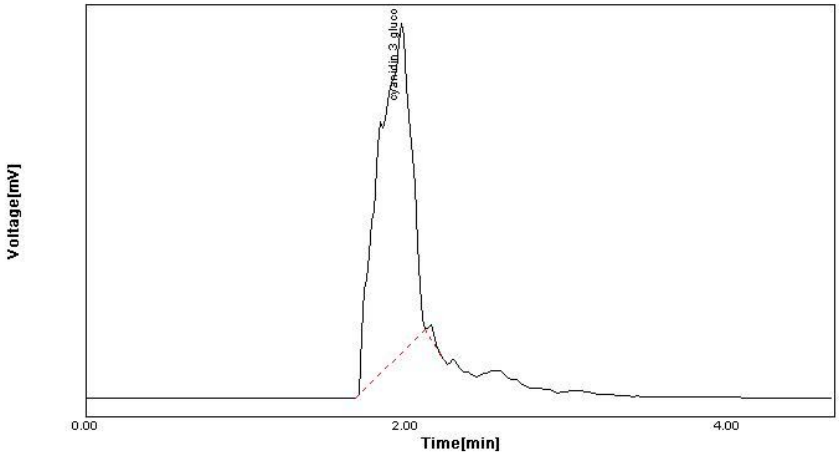
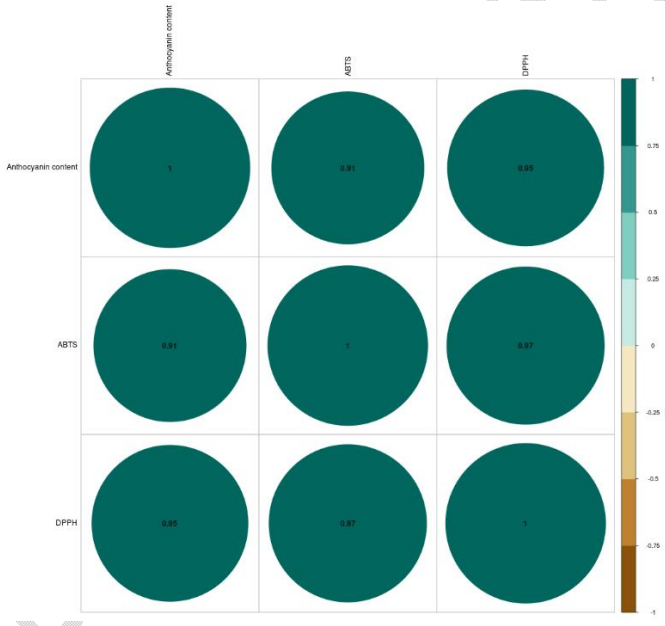


**Table 4. Correlation between anthocyanin content and total anti-oxidant potential of hibiscus tea (Pearsons Correlation coefficient)**

| | **Anthocyanin content** | **ABTS** | **DPPH** |
|---|---|---|---|
| **Anthocyanin content** | 1.000 | 0.907** | 0.955** |
| **ABTS** | 0.907** | 1.000 | 0.971** |
| **DPPH** | 0.955** | 0.971** | 1.000 |

**Fig 3. Correlogram of anthocyanin content and anti-oxidant potential of hibiscus tea**



**Disclaimer (Artificial Intelligence):** Authors declare that No AI technologies have been used during writing or editing this manuscript.

Competing InterestsAuthors have declared that no competing interests exist.

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