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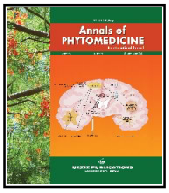

Original Article : Open Access

Phytochemical analysis of Pomegranate (*Punica granatum* L.) mesocarp and Cauliflower (*Brassica oleracea* L. var. *botrytis*) leaves

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Abstract

Pomegranate fruit is a boon for humankind as it has the ability to promote human health due to its antioxidant, anti-inflammatory, antitumoural and antidiabetic activities. Cauliflower leaves are rich source of carotene, iron and calcium but it has higher waste index. However, cauliflower leaves assist in overcoming the health related problems and prevent anemia. The present study provides more evidence on the importance and value of pomegranate fruit, especially pomegranate's mesocarp and cauliflower leaves which are usually considered as a waste product. According to the phytochemical screening, pomegranate mesocarp contains tannins, flavonoids, terpenoids and alkaloids and cauliflower leaves contain alkaloid, terpenoid, sterols, flavonoid and vitamin C. The amounts of extractable components using different solvents (methanol, water, petroleum ether, and chloroform) from pomegranate mesocarp and cauliflower leaves ranged from 0.57-0.02 mg/100 ml and 0.3-0.02 mg/100 ml, respectively. In both methanol and water extract tannin was highly found in methanol extract of pomegranate mesocarp, *i.e.*, 0.35 mg/100 ml. Concentration of flavonoids in pomegranate mesocarp ranged from 0.24 mg/100 ml to 0.02 mg/100 ml, *i.e.,* highest in methanol and water extract, respectively, and lowest in pet ether extract. Concentration of flavonoid in cauliflower leaves was found in methanol extract, *i.e.*, 0.08 mg/100 ml. Concentration of protein in pomegranate mesocarp ranged from 0.42 mg/100 ml to 0.02 mg/100 ml, *i.e.*, highest in methanol extract and lowest in chloroform extract. Concentration of protein in cauliflower leaves ranged from 0.3 mg/100 ml to 0.04 mg/100 ml. Phenolic content in pomegranate mesocarp ranged from 0.57 mg/100 ml to 0.03 mg/100 ml, *i.e.*, highest in water extract and lowest in chloroform. Phenolic content in cauliflower leaves ranged from 0.15 mg/100 ml to 0.03 mg/100 ml, *i.e.*, highest in pet ether and lowest in chloroform and water, respectively. Concentration of antioxidant activity in pomegranate mesocarp ranged from 0.28 mg/100 ml to 0.03 mg/100 ml, *i.e.*, highest in methanol and lowest in chloroform as well as pet ether. Concentration of antioxidant activity in cauliflower leaves ranged from 0.22 mg/100 ml to 0.02 mg/100 ml, *i.e.*, highest in methanol extract and lowest in chloroform extract. The quantitative assays showed that the total content of phenolic compounds flavonoids, concentration of proteins and antioxidant activity in pomegranate mesocarp was higher than that of cauliflower leaves.

1. Introduction

Pomegranate (*Punica granatum* L.) is considered one of the oldest known edible fruits and is termed as a 'Food of Gods' symbolising fertility and prosperity. Just like guava fruit, leaf and other parts of the plant (Edepalli *et al*., 2022), different parts of the pomegranate fruit are also used in folk and traditional medicines. The reason for this increased interest in pomegranate is related to the ability of the juice and fruit extracts to promote human health due to its different properties involving antioxidant, anti-inflammatory, antitumoural and antidiabetic activities similar to *Sida acuta* Burm. f., (SA), a member of the Malvaceae family (Srinivasan and Murali, 2022).

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For local populations, not only the juice but also the typical discarded parts such as mesocarp and exocarp, often cited as peel, are used for medicinal preparations (Ed Stover *et al*., 2009; Khatib *et al*., 2017).

Two Indian cultivars, 'Mridula' ('Arkta') and 'Bhagwa' ('Kesar') are most extensively used for export, particularly to Europe. These cultivars have an appealing red skin and aril color and are soft seeded. Their taste is low-acid, sweet with a relatively small size (200 to 300 g). The rind is relatively thin, which is a weakness, because they are amenable to physical damage. 'Bhagwa' is more prone to physical damage. 'Mridula', 'Bhagwa' and 'Ganesh' are evergreen cultivars. They are exported to Europe usually in January-February (Holland *et al*., 2009).

The pomegranate fruit is berry like with a leathery rind (or husk) enclosing many seeds surrounded by the juicy arils, which comprise the edible portion of the fruit. The aril juice sack is composed of many epidermal cells. According to cultivar, arils range from deep

red to virtually colorless, whereas the enclosed seed varies in content of sclerenchyma tissue, which affects seed softness. The number of locules and arils (and enclosed seeds) varies, but may be as high as 1300 per fruit. The fruit has a prominent calyx, which is maintained to maturity and is a distinctive feature of the pomegranate fruit. The husk is comprised of two parts: the pericarp, is a cuticle layer and fibrous mat; and the mesocarp (known also as the albedo), which is the spongy tissue and inner fruit wall where the arils is attached. Septal membranes are the papery tissue that further compartmentalizes groups of arils, but arils do not attach to this tissue. There is interest in identifying or developing cultivars that have more locules to fill the fruit interior, fewer septal membranes for easier eating, and a thinner mesocarp (Holland *et al*., 2009).

Cauliflower (*Brassica oleracea* L. var. *botrytis*) is one of the most important winter vegetable grown throughout the country. India is the largest producer of cauliflower in the world. The major cauliflower producing states are West Bengal (26%), Bihar (17%), Madhya Pradesh (10%), Orissa (10%), Gujarat (8%), Haryana (7%), Assam (6%) and Maharashtra (3%). The total area of cauliflower in India is 402,000 ha, production 7887,000 MT and productivity 19.6 MT/ha. In Uttarakhand, total area of cauliflower is 2.76 '000 ha and production is 36.72 '000 MT (Savita *et al.,* 2019*).*

Cauliflower leaves are rich source of carotene, iron and calcium however, it has higher waste index. The nutritional value of cauliflower leaves is shown in Table 1 (Pankar and Bornare, 2018). Cauliflower leaves can be utilized in value added products for treating anemia disease and people suffering from micronutrients deficiency. Cauliflower leaves contain antioxidants and other bioactive compounds, just like plants belonging to the genus *Rumex* which are perennial herbs with strong taproots (Eshbakowa *et al.*, 2022), and have been strongly associated with cardiovascular diseases and chronic diseases. Reactive oxygen can cause lipid and protein oxidation, DNA damage and transition of gene expression in the body. They play important role in etiopathology of many diseases like stroke, heart attack and liver injury. Imbalance between ROS and antioxidants, reason of oxidative stress, may be caused by antioxidant defect in dieter increased production of free radicals by stress, smoking, environmental corruption which move into food and water (Prasad *et al.,* 2015).

Table 1: Nutritional composition of cauliflower leaves per 100 g

S.No.	Content	Quantity
1.	Protein	5.9 g
2.	Fat	1.3 g
3.	Carbohydrate	7.6 g
4.	Crude fibre	2 g
5.	Beta carotene	49.526 mg
6.	Iron	41 mg
7.	Energy	66 kcal

The leaves of cauliflower have a high waste index during post harvesting thus creating a foul odour on decomposition. Disposal of the non edible portion of cauliflower (cauliflower waste), which contributes to about 45-60% of the total weight of the vegetable, remains a crucial problem. Classically, the outer layers and extremities of fruits and vegetables are removed during processing, mainly by peeling and pressing. They comprise essentially stalks, peels, seeds and crashed pulp which still contain large amounts of bioactive molecules and biopolymers, resulting in a considerable nutritional loss. The medicinal value of plants has assumed a more important dimension in the past decades owing largely to the discovery that their extracts contain not only minerals but also a diverse array of secondary metabolites with antioxidant potentials. There is enough research conducted on the floral parts of cauliflower whereas the leaves and stalks of the vegetable are highly ignored and discarded as waste (Prasad *et al.*, 2015; Sharmilan *et al.*, 2016).

The health promoting effect of pomegranates has also been mentioned in the traditional medicines just like *T. ammi* have been used as ayurvedic plants to treat various traditional and chronic diseases (Debnath and Sharma, 2022*)*. Fruits, seeds, peel and leaves of pomegranate contain numerous important ingredients and such ingredients show therapeutics importance in the disease cure. For local populations, not only the juice but also the typical discarded parts such as mesocarp and exocarp, often cited as peel, are used for traditional preparations as well as being rich in essential micronutrients. The green leafy vegetables can be utilized for the purpose of enrichment of nutritional deficient products. Cauliflower leaves can be used in traditional products for value addition purpose to overcome the health related problems and prevent anemia. Thus, the characterization of pomegranate mesocarp and cauliflower leaves was done (Rahmani *et al.*, 2017; Prabhavathi *et al.*, 2016).

2. Materials and Methods

2.1 Chemicals

Ferric chloride ($FeCl_3$), chloroform, picric acid, lead acetate, conc. sulphuric acid (H_2SO_4), glacial acetic acid, methanol, water, pet ether, catechin (1 mg/ml), 60% methanol, 5% sodium nitrite, 10% aluminium chloride, 1 M sodium hydroxide, gallic acid (1 mg/ml), FC reagent, 1 M sodium carbonate, 2% sodium carbonate (Na_2CO_3) in 0.1 N NaOH, 1% NaK tartrate in H_2O, 0.5% $CuSO_4.5\ H_2O$ in H_2O, reagent I: 48 ml of A, 1 ml of B, 1 ml C, reagent II: 1 part folin-phenol [2 N]: 1 part water, BSA standard-1 mg/ml, standard-catechin (1 mg/ml), 5% sodium nitrite, 10% $AlCl_3$, 1 M sodium hydroxide, potassium buffer (pH 3.5), potassium ferricynide ($K_3Fe(CN)_6$), trichloroacetic acid (TCA) (10%), ferric chloride ($FeCl_3$) (1%).

2.2 Plant materials and extraction procedure

The mature fruits of pomegranate (bhagawa) were procured from farm and cauliflowers (hybrid) were procured from Mandai, Pune. After washing, pomegranate fruits were allowed to separate the mesocarp from fruit. Then, it was washed again for further procedure. After taking weight (300 g), it was ground in a blender to make fine paste. Samples were lyophilized in a lyophilizator at a pressure of 5 mm Hg at – 50°C. After washing, cauliflower leaves were weighed and ground in mixture to make paste. After 48 h lyophilization, wet powder was obtained. Each sample weighed 100 g. Then, it was dried under shade overnight.

2.3 Methodology

2.3.1 Qualitative test for phytochemicals

1 g of lyophilized sample was taken and mixed with 25 ml of methanol solvent. This mixture was kept on magnetic stirrer for approximately 1 h. Then the mixture was differentiated in two layers which was then filtered with the help of funnel and filter paper. Then this extract was used against various reagents and results were obtained. Remaining solvent could be extracted by using above procedure.

2.3.1.1 Phytochemical tests

2.3.1.1.1 Test for saponins

2-4 ml extract was mixed with 8-10 ml distilled water. On shaking well, foam was formed, therefore saponins were present (Edeoga, 2016; Gowri and Manimegalai, 2017).

2.3.1.1.2 Test for tannins

After adding 2-3 drops of $FeCl_3$ in extract, dark blue coloured ring was formed, therefore tannins were present. (Edeoga *et al.*, 2016).

2.3.1.1.3 Test for flavonoids

After adding few drops of lead acetate solution in extract, yellow precipitate were formed, therefore flavonoids were present (Sofowara, 1993; Harborne, 1973).

2.3.1.1.4 Test for alkaloids

Few drops of picric acid and 5% HCl were added in extract. Yellow or creamy precipitate were formed, therefore alkaloids were present (Gupta *et al.*, 2021).

2.3.1.1.5 Test for terpenoids

1 ml chloroform and 2 ml conc. H_2SO_4 were added in 2 ml extract. Reddish brown ring was formed, therefore terpenoids were present (Edeoga, 2016).

2.3.1.1.6 Wagner's test

I_2 solution was added in 2 ml extract. Blue colour was observed, therefore alkaloids were present (Sofowara, 1993; Harborne, 1973).

2.3.1.1.7 Test for sterol

1 ml $CHCl_3$ (warm), 2 ml glacial acetic acid and 1-2 drops of H_2SO_4 were added in extract. Blue green colour was formed, therefore sterol was present (Ahmed *et al.*, 2015).

2.3.1.1.8 Test for vitamin C

Conc. H_2SO_4 and DNPH were added in extract. Brown ring was observed, therefore vitamin C was present (Farag *et al.*, 2014).

2.3.2 Quantitative test for tannins

Standard catechin (1 mg/ml) was used as a stock and made working standard in 0.01 g in 10 ml 60% methanol (0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1 ml) in 5 test tubes and made the volume to 2 ml of distilled water. Test tube with 2.0 ml distilled water (0 µg catechin per ml) served as a blank. 2.5 ml FC reagent was added in blank as well as 5 test tubes. 0.5 ml of 1 M sodium hydroxide was added in blank as well as 5 test tubes. Then, 0.25 ml of distilled water was added in blank as well as in 5 test tubes. The absorbance was measured at 700 nm and the standard curve was plotted. All the steps were repeated thrice with sample (0.2 ml) and O.D was taken at 700 nm (Edeoga, 2016).

2.3.3 Quantitative test for flavonoids

Standard catechin (1 mg/ml) was used as a stock and made working standard in 0.01 g in 10 ml 60% methanol (0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1 ml) in 5 test tubes and made the volume to 2 ml of distilled water. Test tube with 2.0 ml distilled water (0 µg catechin per ml) served as a blank. 0.3 ml of 5% sodium nitrite was added and the solutions were vortexed and kept to stand for 6 min. After 6 min, 0.3 ml of 10% aluminium chloride was added to the solutions and vortexed. After 5 min, 0.6 ml of 1 M sodium hydroxide was added and the solutions were vortexed. After standing for 10 min, the absorbance of the samples was measured at 510 nm using a UV/VIS spectrophotometer (Perkin Elmer, Lambda XLS, USA). Triplicate tests were done for each sample. Quartz cell was used for measuring the absorbance of samples by UV/VIS spectrophotometer (Ahmed *et al.,* 2015).

2.3.4 Protein estimation by lowery method

Standard (known) protein (BSA) (1 mg/ml) solutions (*e.g.,* 0.2 ml, 0.4 ml , 0.6 ml, 0.8 ml, 1 ml) were prepared in 5 test tubes and made the volume to 1.0 ml using distilled water. Test tube with 1.0 ml distilled water (0 µg BSA per ml) served as a blank. 4.5 ml of reagent I was added and incubated for 10 min. After incubation, 0.5 ml of reagent II was added and incubated for 30 min. The absorbance was measured at 660 nm, and the standard curve was plotted. To estimate the amount of protein present in the given samples, 200 µl of the protein extract was taken and made the volume to 1.0 ml using distilled water. The steps were followed and the amount of protein present in the sample (s) was found using the standard curve (Waterborg and Mathews, 1994).

2.3.5 Total phenolic method

Standard gallic acid (1 mg/ml) was used as a stock and made working standard in 0.01 g in 10 ml distilled water (0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1 ml) in 5 test tubes and made the volume to (2.4 ml, 2.2 ml, 2.0 ml, 1.8 ml, 1.6 ml) of distilled water. Test tube with 2.6 ml distilled water (0 µg gallic acid per ml) served as a blank. 0.1 ml of 10-fold diluted Folin-Ciocalteu reagent was added and vortexed. After 6 min, 0.3 ml of 7.5% sodium carbonate was added and vortexed. After 30 min, the absorbance of each test sample was measured at 760 nm using a UV/VIS spectrophotometer. Triplicate tests were done for each sample. Quartz cell was used for measuring the absorbance of samples by UV/VIS spectrophotometer. The amount of phenolic compounds in each extract was reported as gallic acid equivalents milligram per gram (Ambigaipalan *et al.,* 2016; Sankhalkar and Vernekar, 2016; Al-Rawahil *et al.*, 2014).

2.3.6 Antioxidant activity by K-Frap method

Standard ascorbic acid (1 mg/ml) was used stock and made working standard in 0.01 g in 10 ml distilled water (0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1 ml) in 5 test tubes and made the volume to 2 ml of buffer. Test tube with 2.0 ml buffer served as a blank. 1 ml of $K_3Fe(CN)_6$ was added in blank as well as in 5 test tubes. After two to three min, 1 ml of TCA was added in blank as well as 5 test tubes. 2 ml of distilled water was added in blank as well as in 5 test tubes. 0.5 ml of $FeCl_3$ was added in blank as well as in 5 test tubes. The antioxidant activity present in the given samples was estimated by taking

200 µl of the protein extract and making the volume to 2.0 ml using buffer. The steps were followed and the antioxidant activity present in the sample(s) was found using the standard curve (Koksal and Gula, 2007; Hasan *et al.,* 2018).

3. Results

Estimation of phytochemicals, concentration of phytochemicals, protein concentration, total phenolic content, antioxidant activity were done for pomegranate mesocarp and cauliflower leaves.

3.1 Qualitative test for phytochemicals

The values of phytochemicals for cauliflower leaves and pomegranate mesocarp are summarized in Table 2. Table 2 shows that, saponins and tannins were absent in cauliflower leaves. Flavonoid (methanol), alkaloid (methanol and pet ether), terpenoids (water), sterols (chloroform), vitamin C (pet ether) were present in hybrid cauliflower. Alkaloid (water), terpenoid (methanol) and sterols (methanol) were in abundant quantity present in cauliflower leaves. Table 2 shows that, saponins, sterols and vitamin C were absent in pomegranate mesocarp. Tannin (water and methanol), flavonoid (water and methanol), alkaloid (methanol) were present in abundant quantity in pomegranate mesocarp. Flavonoid (chloroform), alkaloid (water and pet ether) and terpenoid (water and methanol) were present in pomegranate mesocarp.

Table 2: Qualitative test for phytochemicals

	S.No.	Test	Saponins	Tannins	Flavonoids	Alkaloids	Terpenoids	Sterols	Vitamin C
Samples		Solvent							
Cauliflower	1	Water	-	-	-	++	+	-	-
	2	Methanol	-	-	+	+	++	++	-
	3	Pet ether	-	-	-	+	-	-	+
	4	Chloroform	-	-	-	-	-	+	-
Pomegranate	1	Water	-	++	++	+	+	-	-
	2	Methanol	-	++	++	++	+	-	-
	3	Pet ether	-	-	-	+	-	-	-
	4	Chloroform	-	-	+	-	-	-	-

+ = Present, - = Absent, ++ =Abundant

3.2 Quantitative test for tannins

The values of tannin for pomegranate mesocarp are summarized in Figure 1. Figure 1 depicts the tannin activity of different concentration and the standard concentration. For measuring tannin activity, the different concentration and standard concentration transformation was investigated by using standard tannin concentration method. The tannin activity in pomegranate mesocarp increased with increased in sample concentration.

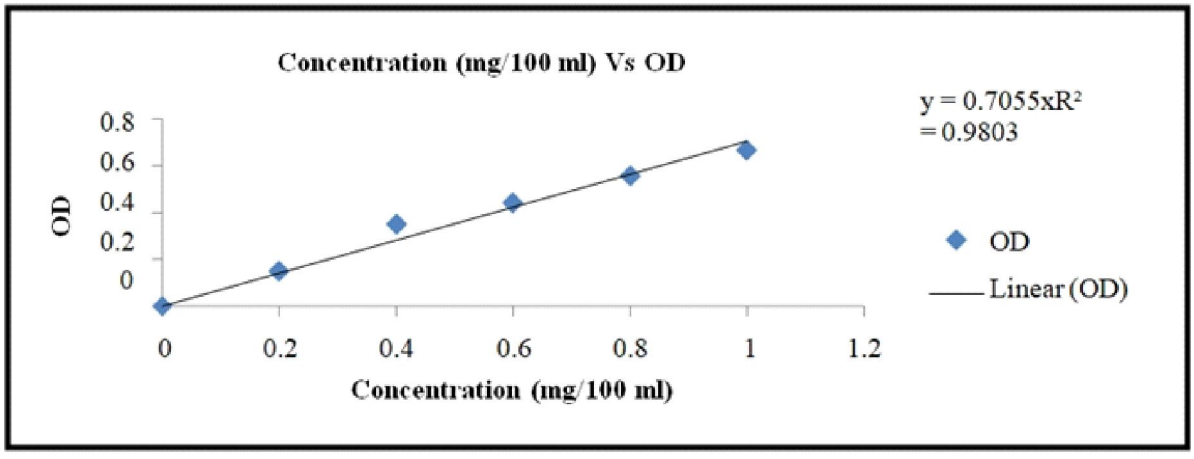

Figure 1: Graph of tannins in pomegranate mesocarp.

Table 3 shows that the tannins in pomegranate mesocarp are only present in methanol and water extract. Further, study was carried out and comparison was done between methanol and water extract. Table 3 also shows that concentration of tannins in pomegranate methanol extract was more as compare to concentration of tannins in pomegranate water extract.

Table 3: Concentration of tannins (mg/100 ml)

Sample	Concentration of tannins (mg/100 ml)
Pomegranate (Methanol)	0.35
Pomegranate (Water)	0.32

Figure 2 also shows that tannin was found in abundant in pomegranate mesocarp (methanol and water extract). In both methanol and water extract, tannin was highly found in methanol extract of pomegranate mesocarp, *i.e.*, 0.35 mg/100 ml.

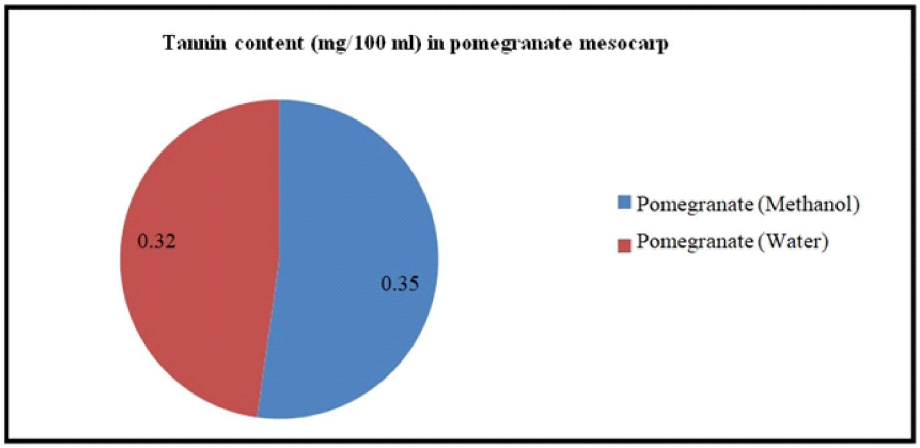

Figure 2: Pie chart of tannin content (mg/100 ml) in pomegranate mesocarp.

3.3 Quantitative test for flavonoids

The values of flavonoids for pomegranate mesocarp and cauliflower leaves are summarized in Figure 3. Figure 3 depicts the flavonoid activity of different concentration and the standard concentration. For measuring flavonoids activity, the different concentration and standard concentration transformation was investigated by using aluminium chloride colorimetric method. The flavonoid activity in different extract increased with increase in standard concentration.

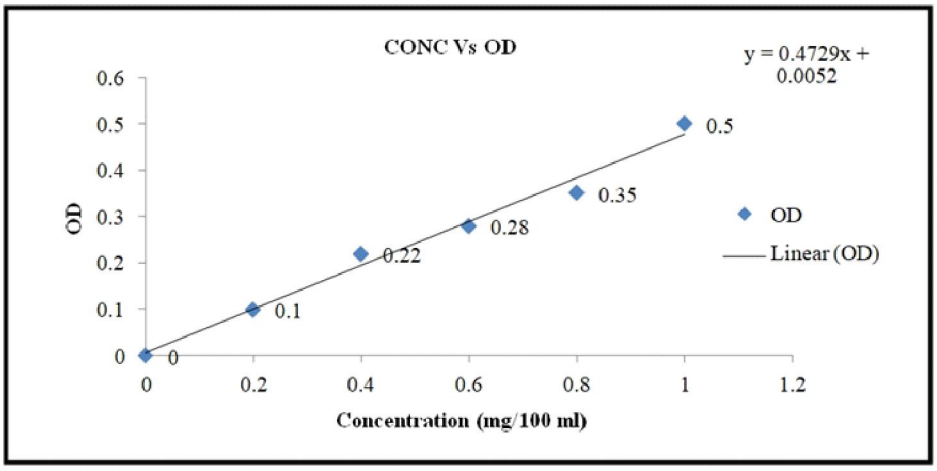

Figure 3: Standard graph for flavonoids.

Table 4 shows that the flavonoid in pomegranate mesocarp was present in methanol, water, pet ether and chloroform extract. Further study showed that the concentration of flavonoid in methanol and water extract was present in equal quantity. Concentration of flavonoids in chloroform and pet ether extract were present in least quantity. Table 4 shows that concentration of flavonoid in pomegranate methanol and water extract (0.24 mg/100 ml) was more as compared to concentration of flavonoid in pomegranate pet ether and chloroform extract (less than 0.05 mg/100 ml).

Table 4: Concentration of flavonoid (mg/100 ml) in pomegranate mesocarp

Sample	Concentration (mg/100 ml)
Pomegranate (Methanol)	0.24
Pomegranate (Water)	0.24
Pomegranate (Pet ether)	0.02
Pomegranate (Chloroform)	0.05

Figure 4 also shows that concentration of flavonoids was found in equal quantity in methanol and water extract of pomegranate mesocarp. In both methanol and water extract, concentration of flavonoid was high as compared to pet ether and chloroform extract. Flavonoid was found abundant in pomegranate mesocarp in methanol and water extract, *i.e.*, 0.24 mg/100 ml and low in pet ether extract, *i.e.*, 0.02 mg/100 ml.

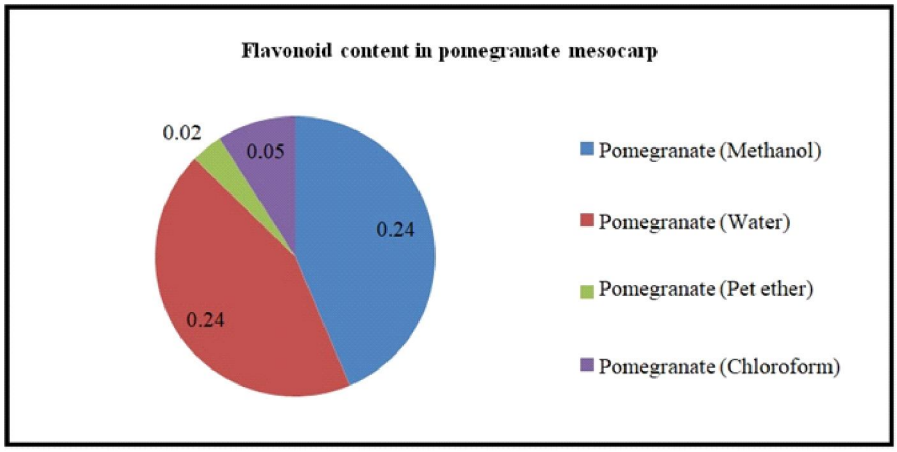

Figure 4: Pie chart for concentration of flavonoids in pomegranate mesocarp.

Table 5 shows that the flavonoid in cauliflower leaves was present in methanol extract. Further study showed that the concentration of flavonoid in methanol extract was found in least quantity. Table 5 also shows that concentration of flavonoid in cauliflower leaves methanol extract was 0.08 mg/100 ml.

Table 5: Concentration of flavonoids in cauliflower leaves (mg/100 ml)

Sample	Concentration (mg/100 ml)
Cauliflower (Methanol)	0.08

3.4 Lowry method

The values of concentration of protein for pomegranate mesocarp and cauliflower leaves are summarized in Figure 5. Figure 5 depicts the protein activity of different concentration and the standard concentration. For measuring protein activity, the different concentration and standard concentration transformation was investigated by using lowry method. The protein activity in different extract increased with increase in standard concentration. Table 6 shows that concentration of protein in pomegranate mesocarp was present in methanol, water, pet ether and chloroform extract. Further study showed that concentration of protein in methanol extract was found more, *i.e.*, 0.42 mg/100 ml as compared to other extracts. Concentration of protein in chloroform extract was also found in least quantity, *i.e.*, 0.02 mg/100 ml.

Table 6: Concentration of protein (mg/100 ml) in pomegranate mesocarp

Sample	Concentration (mg/100 ml)
Pomegranate (Methanol)	0.42
Pomegranate (Water)	0.3
Pomegranate (Pet ether)	0.04
Pomegranate (Chloroform)	0.02

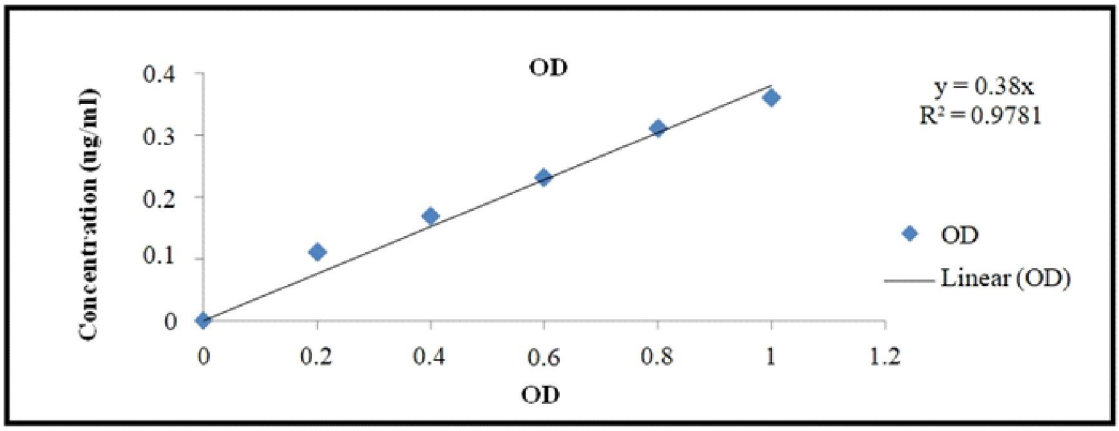

Figure 5: Standard graph for Lowry method.

Figure 6 shows different concentration of proteins graphically. Comparison was done with four type of extracts, *i.e.*, methanol, water, pet ether and chloroform. Pie chart shows that quantity of concentration of proteins in pomegranate mesocarp in methanol extract was higher (0.42 mg/100 ml) and in chloroform extract is lower (0.02 mg/100 ml).

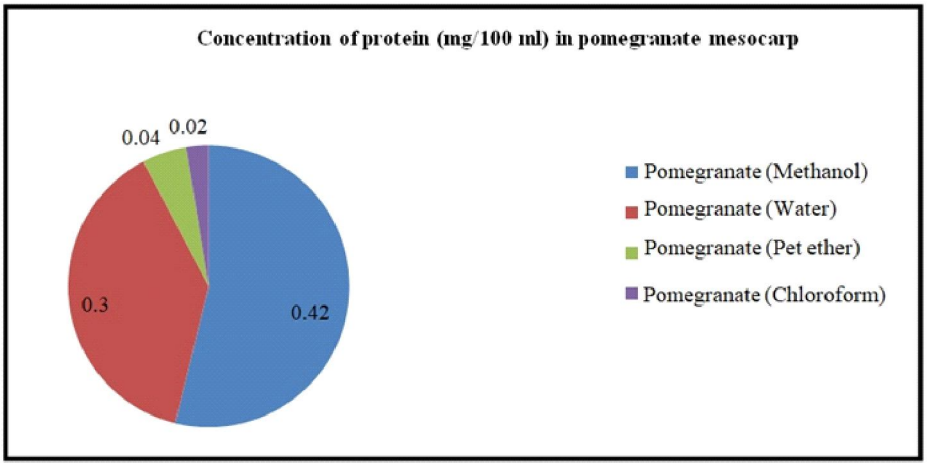

Figure 6: Pie chart of protein (mg/100 ml) in pomegranate mesocarp.

Table 7 shows that concentration of protein in cauliflower leaves was present in methanol, water, pet ether and chloroform extract. Further study showed that concentration of proteins in cauliflower leaves in methanol extract was in more (0.3 mg/100 ml) quantity and concentration of proteins in cauliflower leaves in chloroform extract was found in least quantity, *i.e.*, 0.04 mg/100 ml.

Figure 7 shows different concentration of proteins in cauliflower leaves in methanol, water, pet ether and chloroform extract graphically. Piechart shows that quantity of concentration of proteins in cauliflower leaves in methanol extract was higher (0.3 mg/100 ml) and in chloroform extract was lower (0.04 mg/100 ml).

Table 7: Concentration of protein (mg/100 ml) in cauliflower leaves

Sample	Mean
Cauliflower (Methanol)	0.3
Cauliflower (Water)	0.27
Cauliflower (Pet ether)	0.15
Cauliflower (Chloroform)	0.04

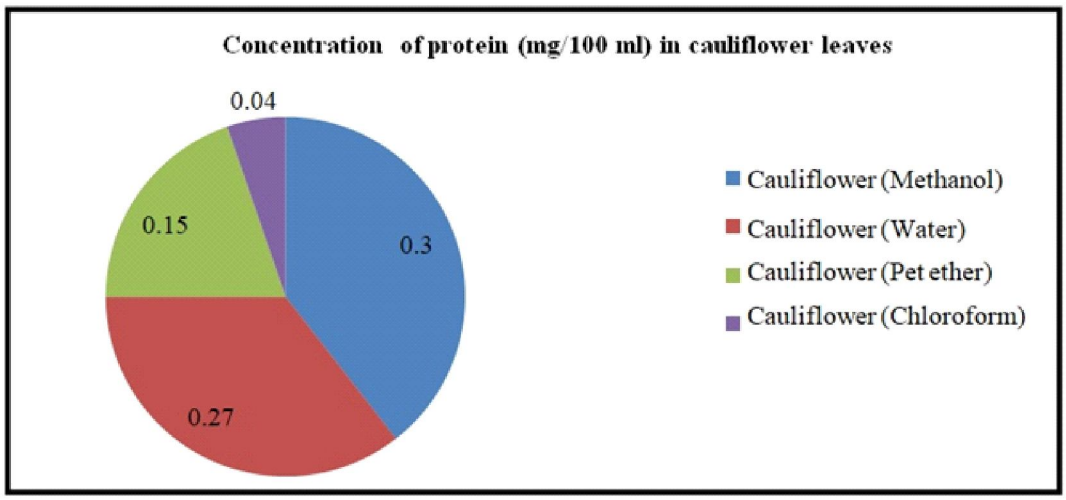

Figure 7: Pie chart of protein (mg/100 ml) in cauliflower leaves.

3.5 Total phenolic method

The values of concentration of phenolic for pomegranate mesocarp and cauliflower leaves are summarized in Figure 8. Figure 8 depicts total phenolic content of different concentration and the standard concentration. For measuring total phenolic content, the different concentration and standard concentration transformation was investigated by Folin-Ciocalteu method, a colourimetric method, based on oxidation-reduction reaction of Folin-Cicocalteu reagent with phenolic compounds. The phenolic content in different extract increased with increase in standard concentration.

Table 8: Concentration of phenolic content (mg/100 ml) in pomegranate mesocarp

Sample	Concentration (mg/100 ml)
Pomegranate (Methanol)	0.38
Pomegranate (Water)	0.57
Pomegranate (Pet ether)	0.05
Pomegranate (Chloroform)	0.03

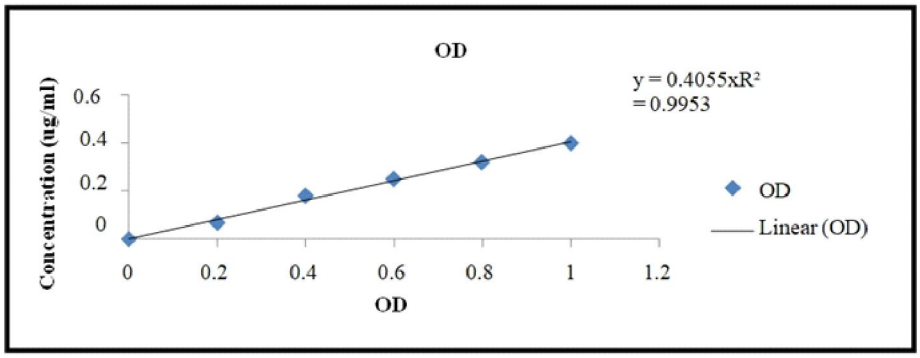

Figure 8: Standard graph of total phenolic method.

Table 8 shows the concentration of phenolic content in pomegranate mesocarp in different extracts, *i.e.*, methanol, water, pet ether and chloroform. Further study showed that concentration of phenolic content in water extract was found more, *i.e.*, 0.57 mg/100 ml as compared to other extracts. The concentration of protein in chloroform extract was found in least quantity, *i.e.*, 0.03 mg/100 ml.

Figure 9 shows different concentration of phenolic content in pomegranate mesocarp in methanol, water, pet ether and chloroform graphically. Pie chart shows that the quantity of concentration of phenolic content in pomegranate mesocarp in water extract was found higher (0.57 mg/100 ml) and in chloroform extract was found lower (0.03 mg/100 ml).

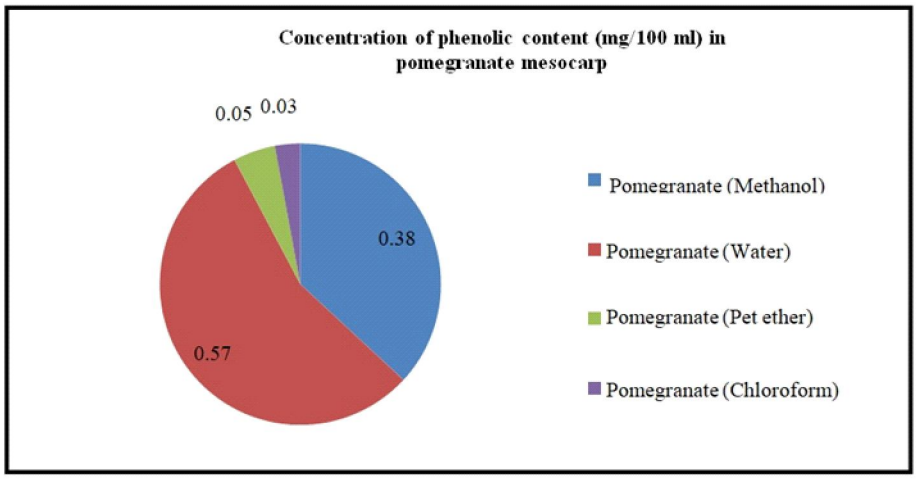

Figure 9: Pie chart of concentration of phenolic content (mg/100 ml) in pomegranate mesocarp.

Table 9 shows that concentration of phenolic content in cauliflower leaves was found in methanol, water, pet ether and chloroform extract. Further study showed that concentration of phenolic content in cauliflower leaves in pet ether extract was more. Concentration of phenolic in cauliflower leaves in chloroform extract as well as water extract was found in least quantity equally, *i.e.*, 0.03 mg/100 ml.

Table 9: Concentration of phenolic content (mg/100 ml) in cauliflower leaves

Sample	Concentration (mg/100 ml)
Cauliflower (Methanol)	0.13
Cauliflower (Water)	0.03
Cauliflower (Pet ether)	0.15
Cauliflower (Chloroform)	0.03

Figure 10 shows different concentration of phenolic content in cauliflower leaves in methanol, water, pet ether and chloroform extract graphically. Piechart shows that quantity of concentration of phenolic content in cauliflower leaves in pet ether extract is higher, *i.e.*, 0.15 mg/100 ml and in water and chloroform extract is lower equally, *i.e.*, 0.03 mg/100 ml.

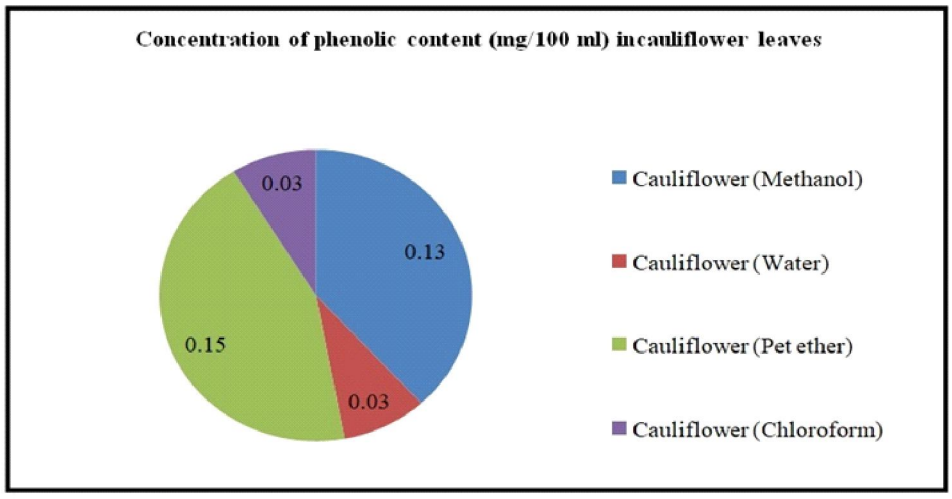

Figure 10: Pie chart of concentration of phenolic content (mg/100 ml) in cauliflower leaves.

3.6 Antioxidant activity by K-Frap method

The values of antioxidant activity for pomegranate mesocarp and cauliflower leaves are summarized in Figure 11. Figure 11 depicts the antioxidant activity of different concentration and the standard concentration. For measuring antioxidant activity, the different concentration and standard concentration transformation was investigated by using K-Frap method. Graph shows that antioxidant activity in different extract increased with increase in standard concentration.

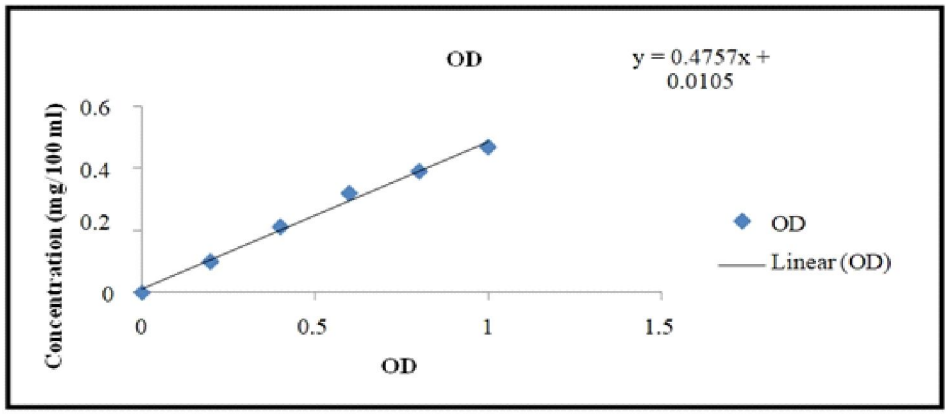

Figure 11: Standard graph for antioxidant activity.

Table 10 : Concentration of antioxidant in pomegranate mesocarp

Sample	Concentration (mg/100 ml)
Pomegranate (Methanol)	0.28
Pomegranate (Water)	0.23
Pomegranate (Pet ether)	0.03
Pomegranate (Chloroform)	0.03

Table 10 shows the concentration of antioxidant activity in pomegranate mesocarp in different extracts, *i.e.*, methanol, water, pet ether and chloroform. Further study showed that concentration of antioxidant activity in methanol extract was found more as compared to other extracts. Concentration of antioxidant in chloroform and pet ether extract was found in least quantity equally, *i.e.*, 0.03 mg/100 ml.

Figure 12 shows different concentration of antioxidant in pomegranate mesocarp with methanol, water, pet ether and chloroform graphically. Pie chart shows that quantity of concentration of antioxidant in pomegranate mesocarp in methanol extract was higher, *i.e.*, 0.28 mg/100 ml which was slightly higher than the water extract, *i.e.*, 0.23 mg/100 ml. Concentration of antioxidant activity pomegranate mesocarp in chloroform as well as pet ether extract was found equally lower, *i.e.*, 0.03 mg/100 ml.

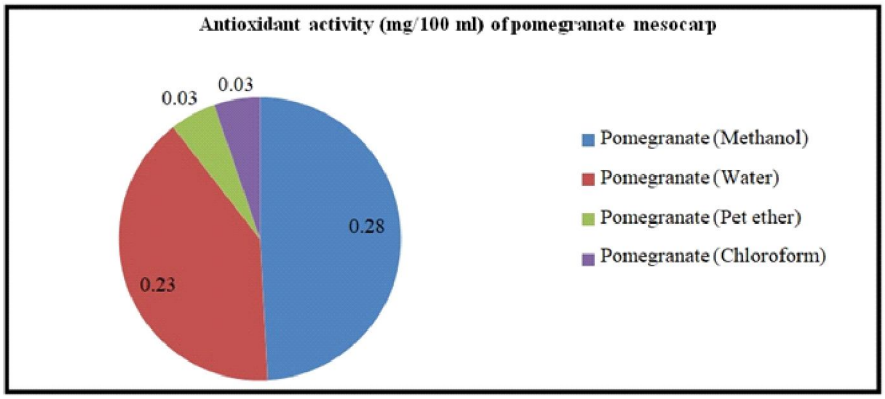

Figure 12: Pie chart of antioxidant activity (mg/100 ml) of pomegranate mesocarp.

Table 11: Concentration of antioxidants (mg/100 ml) in cauliflower leaves

Sample	Concentration (mg/100 ml)
Cauliflower (Methanol)	0.22
Cauliflower (Water)	0.14
Cauliflower (Pet ether)	0.03
Cauliflower (Chloroform)	0.02

Table 11 shows the concentration of antioxidant in cauliflower leaves found in methanol, water, pet ether and chloroform extract.

Further study showed that concentration of antioxidants in cauliflower leaves in methanol extract was found more. Concentration of antioxidants in cauliflower leaves in chloroform extract was found in least quantity.

Figure 13 shows different concentration of antioxidant activity in cauliflower leaves in methanol, water, pet ether and chloroform extract graphically. Pie chart shows that quantity of concentration of antioxidant activity in cauliflower leaves in methanol extract was found higher, *i.e.*, 0.22 mg/100 ml and in chloroform extract was found lower, *i.e.*, 0.02 mg/100 ml.

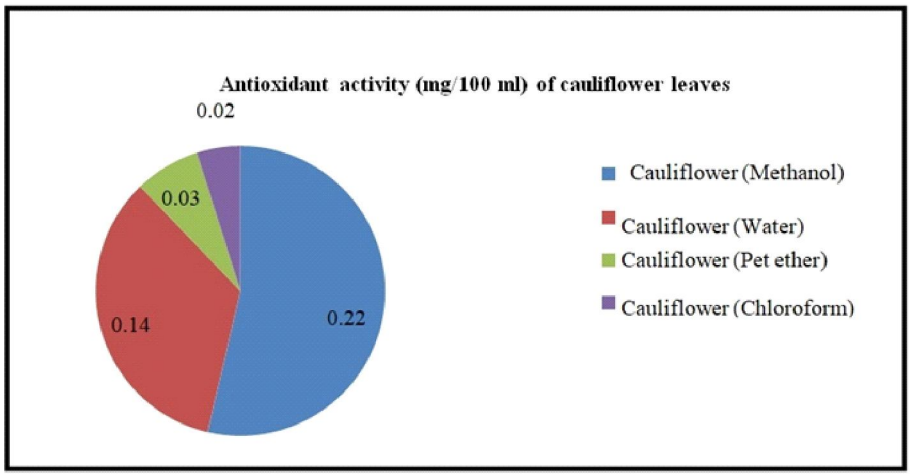

Figure 13: Pie chart of antioxidant activity (mg/100 ml) of cauliflower leaves.

4. Discussion

Pomegranate mesocarp and cauliflower leaves characterized under different objectives to determine phytochemicals, concentration of phytochemicals, protein concentration, total phenolic content and antioxidant activity. Further studies were done on pomegranate mesocarp and cauliflower leaves lyophilized powder.

The amounts of extractable components using different solvents (methanol, water, petroleum ether, and chloroform) from pomegranate mesocarp and cauliflower leaves ranged from 0.57-0.02 mg/100 ml and 0.3-0.02 mg/100 ml, respectively. The identification of phytochemicals in pomegranate mesocarp and cauliflower leaf powder was a crucial starting point for assessing their nutritional, biological and technological aspects. Table 2 shows the qualitative phytochemical screenings of pomegranate mesocarp and cauliflower leaves powder. Each powder was screened for the presence of key families of phytochemicals, *i.e.*, proteins, phenolic compounds, tannins, alkaloids, flavonoids, saponins, sterols, terpenoids and vitamin C. In general, there was a great difference of the phytochemicals between the botanical parts (mesocarp and leaves). Pomegranate mesocarp powder contained tannin (water and methanol), flavonoid (water and methanol), and alkaloid

(methanol) compounds as major constituents. Flavonoid (chloroform), alkaloid (water and pet ether) and terpenoid (water and methanol) were found in pomegranate mesocarp powder as minor components whilst saponins, sterols and vitamin C occurred as trace substances. The levels of pomegranate phytochemicals differed according to the solvents used to extract these compounds. In addition, the composition of pomegranate juice depends on cultivar type, environmental, post harvest and processing factors (Farag *et al.,* 2014). Cauliflower leaves powder contained alkaloid (water), terpenoid (methanol) and sterols (methanol) compounds as major constituents. Flavonoid (methanol), alkaloid (methanol and pet ether), terpenoids (water), sterols (chloroform), vitamin C (pet ether) were found in cauliflower leaves powder as minor components whilst saponins and tannins occurred as trace substances.

The interest in the phenolic had increased outstandingly due to their prominent free radical scavenging activity and other antioxidant attributes. Phenolic compounds are generally classified as simple phenols, a single aromatic ring bearing with atleast one hydroxyl group, and polyphenols with atleast two phenol subunits like flavonoids or three and more phenol subunits called tannins. Based on primary phytochemical screening done, pomegranate mesocarp contained tannins, flavonoids, terpenoids and alkaloids and cauliflower leaves contained flavonoids, terpenoids, alkaloids, steroids and vitamin C (Table 2). For measuring tannins activity, the different concentration and standard concentration transformation was investigated by using standard tannins concentration method. The tannins activity in pomegranate mesocarp, increased with increased in sample concentration (Figure 1). Table 2 shows that tannins in pomegranate mesocarp were only found in methanol and water extract. Table 3 shows that, concentration of tannins in pomegranate methanol extract was more as compare to concentration of tannins in pomegranate water extract. Concentration of tannins in pomegranate methanol extract was 0.35 mg/100 ml and concentration of tannins in pomegranate water extract 0.32 mg/100 ml. Figure 2 also shows that tannin was found in abundant in pomegranate mesocarp (methanol and water extract). In both methanol and water extract tannin was highly found in methanol extract of pomegranate mesocarp, *i.e.*, 0.35 mg/100 ml.

Total flavonoid content of pomegranate mesocarp and cauliflower leaves extracts was estimated by aluminium chloride colorimetric method. The principle of this colorimetric method is based on the formation of acid stable aluminium chloride complexes with hydroxyl groups and keto group of flavanols or flavones. The flavonoids activity in different extract increased with increased in standard concentration (Figure 2). Table 2 shows, flavonoid in pomegranate mesocarp was found in methanol, water and chloroform extract and in cauliflower leaves was found in methanol extract. Table 4 shows that, the flavonoid in pomegranate mesocarp was found in methanol (0.24 mg/100 ml), water (0.24 mg/100 ml), pet ether (0.02 mg/100 ml) and chloroform (0.05 mg/100 ml) extract. Concentration of flavonoid in pomegranate mesocarp methanol and water extract was more as compare to concentration of flavonoid in pomegranate pet ether and chloroform extract. Flavonoids were abundant in pomegranate mesocarp in methanol and water extracts, *i.e.*, 0.24 mg/100 ml and low in pet ether extract as well as chloroform extract (Figure 4). However, in quantitative phytochemical test, flavonoids were found absent in pet ether extract. Table 5 shows that, the flavonoid in cauliflower leaves were present in methanol extract (0.08 mg/100 ml). Pomegranate mesocarp (water and methanol) found highest quantity flavonoids as compared to cauliflower leaves (methanol).

For measuring proteins activity, the different concentration and standard concentration transformation was investigated by using lowry method. The proteins activity in different extract increased with increased in standard concentration. Concentration of protein in methanol extract found more, *i.e.*, 0.42 mg/100 ml as compared to other extracts. As well as concentration of protein in chloroform extract found least quantity, *i.e.*, 0.02 mg/100 ml (Table 6). Concentration of proteins in cauliflower leaves in methanol extract found more (0.3 mg/100 ml) quantity. Concentration of proteins in cauliflower leaves in chloroform extracts found least quantity, *i.e.*, 0.04 mg/100 ml (Table 7). Concentration of protein in pomegranate mesocarp in water and methanol extract found more as compare to concentration of protein cauliflower leaves in water and methanol extract.

Total phenolic contents (TPC) in the present work were determined using Folin-Ciocalteu reagent (FCR) method and were expressed as gallic acid equivalents (GAE). The FCR based assay, commonly known as the total phenols (or phenolic) assay involves the reduction of a phosphor-molybadic-phosphotungstic acid to a blue colored complex under basic conditions, the intensity of which is measured at about 750 nm. The total phenol assay was frequently used due to its convenience, simplicity, and reproducibility regardless of undefined chemical nature of FCR. Concentration of phenolic content in pomegranate mesocarp was found in different extract, *i.e.*, methanol, water, pet ether and chloroform extract (Table 8). Phenolic content in water extract found more, *i.e.*, 0.57 mg/100 ml as compare other extract. As well as concentration of phenolic content in chloroform extract found least quantity, *i.e.*, 0.03 mg/100 ml. Concentration of phenolic content in cauliflower leaves in pet ether extract found more, *i.e.*, 0.15 mg/100 ml. As well as concentration of phenolic content in cauliflower leaves in chloroform extract as well as water extract equally found least quantity, *i.e.*, 0.03 mg/100 ml (Table 9). Pomegranate mesocarp in water and methanol extract of concentration of phenolic content found more as compared to cauliflower leaves in water and methanol extract.

Generally, the samples of fresh vegetables exhibited higher phenolic contents and antioxidant activity. For measuring antioxidant activity, the different concentration and standard concentration transformation was investigated by using K-Frap method. Concentration of antioxidant activity in methanol extract found more as compare other extracts. As well as concentration of antioxidant in chloroform and pet ether extract found least quantity, *i.e.*, 0.03 mg/100 ml, respectively (Table 10). Concentration of antioxidant in pomegranate mesocarp in methanol extract found higher, *i.e.*, 0.28 mg/100 ml which is slightly higher than the water extract, *i.e.*, 0.23 mg/100 ml. Concentration of antioxidant activity pomegranate mesocarp in chloroform as well as pet ether extract found equally lower, *i.e.*, 0.03 mg/100 ml (Figure 12). Concentration of antioxidants in cauliflower leaves in methanol extract was found more. Concentration of antioxidants in cauliflower leaves in chloroform extract found least quantity (Table 11). Concentration of antioxidant activity in cauliflower leaves in methanol extract found higher, *i.e.*, 0.22 mg/100 ml and in chloroform extract found lower, *i.e.*, 0.02 mg/100 ml (Figure 13).

5. Conclusion

The present study provides more evidence on the importance and value of pomegranate fruit, especially pomegranate's mesocarp and cauliflower leaves which are usually considered as a waste product. According to the phytochemical screening, pomegranate mesocarp contains tannins, flavonoids, terpenoids and alkaloids and cauliflower leaves contain alkaloid, terpenoid, sterols, flavonoid and vitamin C. The amounts of extractable components using different solvents (methanol, water, pet ether, and chloroform) from pomegranate mesocarp and cauliflower leaves ranged from 0.57-0.02 mg/100 ml and 0.3-0.02 mg/100 ml, respectively. In both methanol and water extract, tannin was highly found in methanol extract of pomegranate mesocarp, *i.e.*, 0.35 mg/100 ml. Concentration of flavonoids in pomegranate mesocarp ranged from 0.24 mg/100 ml to 0.02 mg/100 ml, *i.e.*, highest in methanol and water extract, respectively, and lowest in pet ether extract. Concentration of flavonoid in cauliflower leaves was found in methanol extract, *i.e.*, 0.08 mg/100 ml. Concentration of protein in pomegranate mesocarp ranged from 0.42 mg/100 ml to 0.02 mg/100 ml, *i.e.*, highest in methanol extract and lowest in chloroform extract. Concentration of protein in cauliflower leaves ranged from 0.3 mg/100 ml to 0.04 mg/100 ml. Phenolic content in pomegranate mesocarp ranged from 0.57 mg/100 ml to 0.03 mg/100 ml, *i.e.*, highest in water extract and lowest in chloroform. Phenolic content in cauliflower leaves ranged from 0.15 mg/100 ml to 0.03 mg/100 ml, *i.e.*, highest in pet ether and lowest in chloroform and water, respectively. Concentration of antioxidant activity in pomegranate mesocarp ranged from 0.28 mg/100 ml to 0.03 mg/100 ml, *i.e.*, highest in methanol and lowest in chloroform as well as pet ether. Concentration of antioxidant activity in cauliflower leaves ranged from 0.22 mg/100 ml to 0.02 mg/100 ml, *i.e.*, highest in methanol extract and lowest in chloroform extract. The quantitative assays showed that the total content of phenolic compounds flavonoids, concentration of proteins and antioxidant activity in pomegranate mesocarp was higher than that of cauliflower leaves.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Ahmed, D.; Khan, M. M. and Saeed, R.(2015). Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from *Adiantum caudatum* leaves. Antioxidants, **4**:394-409.

Al-Rawahil, A. S.; Edwards, G.; Al-Sibani, M.; Al-Thani, G.; Al-Harrasi A. S. and Rahman M. S.(2014). Phenolic constituents of Pomegranate peels (*Punica granatum* L.) cultivated in Oman. European Journal of Medicinal Plant, **4**(3):315-331.

Ambigaipalan, P.; Camargo, A. C. and Shahidi, F. (2016). Phenolic compounds of Pomegranatebyproducts (outer skin, mesocarp, divider membrane) and their antioxidant activities. Journal of Agricultural and Food Chemistry, **64**:6584-6604.

Debnath, S. and Sharma, A.(2022). An insight of *Trachyspermum ammi* L.: A comprehensive review on its aromatic and medicinal potential. Ann. Phytomed., **11**(2):197-204.

Edeoga, H. O. (2016). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., **4**:685-688.

Edepalli, V. S. L.; Kumar, S.; Dalal, A. S.; Patel, S. J.; Singh, S. and Jangir, S.(2022). A review on 24 nutritional and medicinal properties of Guava (*Psidium guajava* L.). Ann. Phytomed., **11**(2):240-244.

Eshbakova, K.; Ashirmatova, N.; Mamarasulov, B.; Khasanova, K.; Komilov, B. and Davranov, K.(2022). Total phenol and flavonoid content, antibacterial and antioxidant activity of extract and fractions of medicinal plants of *Rumex* (Polygonaceae) family in the flora of Uzbekistan. Ann. Phytomed., **11**(2):463-472.

Farag, R. S.; Latif, M. S. A.; Emam, S. S.; Layla and Tawfeek, S.(2014). Phytochemical screening and polyphenol constituents of Pomegranate peels and leave juices. Agriculture and Soil Sciences (LRJASS), **1**(6):086-093.

Gowri, G. and Manimegalai, K. (2017). Phytochemical and xrd analysis of Cauliflower leaf (*Brassica oleracea* var *botrytis*). World Journal of Pharmacy and Pharmaceutical Sciences, **6**(7):1277-1282.

Goyal, B.R.; Agarwal, B.B.; Goyal, R.K. and Mehta, A.A.(2007). Pyto pharmacology of Moringa oleifera Lam: An overview. Nat. Prod. Radiance, **4**:347-353.

Gupta, S.; Acharya, R.; Gamit, R. V. and Shukla, V. J.(2021). Quantitative analysis of tannins, alkaloids, phenols, and flavonoids in Ficus semicordata leaf, stem, stem bark, root, and fruit powder. J. Indian Sys. Medicine, **9**:171-174.

Harborne, J.B.(1973). Phytochemical Methods. London: Chapman and Hall.

Hasan, A. M.; Redha, A. A. and Mandeel, Q.(2018). Phytochemical investigations of Pomegranate (*Punica granatum*) rind and aril extracts and their antioxidant, antidiabetic and antibacterial activity. Natural Products Chemistry and Research, **6**(4):2329-6836.

Holland, D.; Hatib, K. and Bar-Ya'akov, I. (2009). Pomegranate: Botany, horticulture, breeding. Hortic. Rev., **35**:127-191.

Khatib, M.; Innocenti, M..; Giuliani, C.; Al-Tamimi, A.; Romani, A. and Mulinacci, N. (2017). Mesocarp and exocarp of Laffan and wonderful Pomegranate varieties: By-products as a source of Ellagitannins. International Journal of Food Sciences and Nutrition, **4**(2):1-7.

Khasnabis, J.; Rai, C. and Roy, A.(2015). Determination of tannin content by titrimetric method from different types of tea. J. Chem. Pharm. Res., **7**:238-241

Koksal, E. and Gulc, L.(2007). Antioxidant activity of Cauliflower (*Brassica oleracea* L.). Turkish Journal of Agriculture and Forestry, **32**:65-78.

Pankar, S.A. and Bornare, D. T.(2018). Studies on cauliflower leaves powder and its waste utilization in traditional product. Int. J. Agric. Eng., **11**:95-98.

Prabhavathi, R. M.; Prasad, M. P. and Jayaramu, M.(2016). Studies on qualitative and quantitative phytochemical analysis of *Cissus quadrangularis*. Advances in Applied Science Research, **7**(4):11-17.

Prasad, M. S.; Joshi, D. S. D. S.; Narendra, K.; Nadiya, S. K.; Masthani, S. K.; Phani, N. P. and Satya, A. K.(2015). A comparative study of phytochemical analysis and invitro antimicrobial activity of three important vegetables from brassicaceae family. International Journal Research Ayurveda Pharma, **6**(6):767-772.

Rahmani, A. H.; Alsahli, M. A. and Almatroodi, S. A.(2017). Active constituents of Pomegranates (*Punica granatum*) as potential candidates in the management of health through modulation of biological activities. Pharmacogn Journal, **9**(5):689-695.

Rajesh, B. R.; Potty, V. P. and Sreelekshmy, S. G. (2016). Study of total phenol, flavonoids, tannin contents and phytochemical screening. Int. J. Appl. Pure. Sci. Agric., **2**:291-296.

Safeena, M. and Kalinga J.(2020). Qualitative and quantitative screening of secondary metabolites in selected medicinal plants of Sri Lanka. J. Med. Plants Stud., **8**:94-100.

Sankhalkar, S. and Vernekar, V. (2016). Quantitative and qualitative analysis of phenolic and flavonoid content in *Moringa oleifera* L. and *Ocimum tenuiflorum* L. Phcog. Res., **8**:16-21.

Sharmilan, M. J. P. and Jaganathan, D.(2016). Bioactive compounds in Cauliflower leaves (*Brassica oleracea* var. *botrytis*) using GCMS. International Journal of Recent Scientific Research, **7**(4):10459-10463.

Shazia, T.; Swati, K. and Kirti, J. (2016). Spectrophotometric quantification of total phenolic, flavonoid, and alkaloid contents of *Abrus precatorius* L. seeds. Asian J. Pharm. Clin. Res., **9**:371-374.

Sofowara, A. (1993). Medicinal Plants and Traditional Medicine in Africa. Ibadan, Nigeria, Spectrum Books Ltd. pp:289.

Stover, E. (2007). The Pomegranate: A new look at the fruit of Paradise. Horticulture Science, **42**(5):1088-1092.

Srinivasan, N. and Murali, R. (2022). An overview of the traditional importance, phytochemistry, and pharmacological properties of *Sida acuta burm. f.*.Ann. Phytomed., **11**(2):245-254.

Waterborg, J.H. and Matthews, H.R. (1994). The lowry method for protein quantitation. Methods in Molecular Biology Book Series, **32**:1-4.

Zhishen, J.; Mengcheng, T. and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., **64**:555-59.

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