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Antioxidant activity of Proanthocyanidin extracted from Pomegranate peels

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Abstract - Phenolics are broadly distributed in the entire plant kingdom and are the most important and rich source of secondary metabolites secreted by plants. Plant flavonoids and polyphenols have drawn increasing attention due to their capable and most potent antioxidant activities and their remarkable effects in prevention of various oxidative stress associated diseases such as cancer.
The recovery of polyphenols and proanthocyanidins from pomegranate peels was done and was highest at 50^0C for 20 minutes in distilled water .The DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging activity was studied and scavenging percentage were determined and found highest yield of antioxidants of 33%Also total proanthocyanidins content was estimated using Acid Butanol Assay in order to yield highest amount of proanthocyanidins and is expected to give highest Antioxidant capacities of proanthocyanidins.

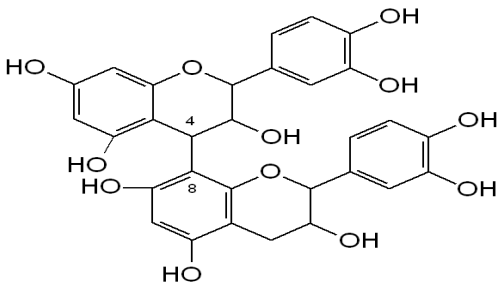
Keywords: Antioxidant activity, plant phenolics, pomegranate peels, proanthocyanidins, extraction, DPPH.

1. INTRODUCTION

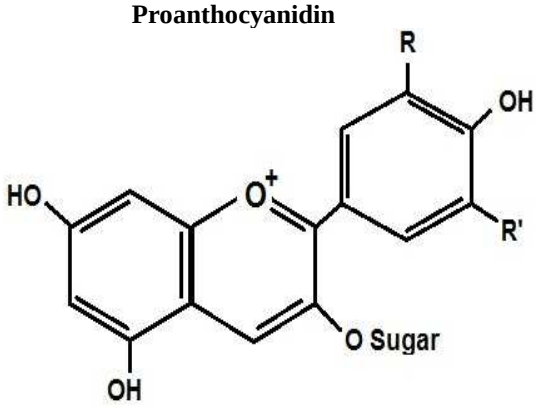
Pomegranate is a fruit bearing plant with red colored seeds. They are highly nutritious and its peels are the nutrition-rich byproduct which gained attention due to its evident anti-bacterial activity, wound-healing properties, anti-cancerous activity and anti-oxidative capacities. [1] [2]

These pomegranate contains tannins which are the major group of polyphenols in our usual diet and are usually divided into two groups A] Hydrolysable tannins and B] Condensed tannins. Hydrolysable tannins are groups containing central core of glucose while condensed tannins are oligomers or polymers of flavan-3-ol linked through an interflavan carbon bonds. They are referred as proanthocyanidins because they are decomposed to anthocyanidins through acid catalyzed oxidation reaction [3]

Proanthocyanids are naturally occurring compounds that are widely found in common foods including cereals, fruits, nuts, pine bark, grape seed and red wines [12]. But, of these pomegranate peels and grape seeds are particularly the rich source of proanthocyanidins. These proanthocyanids are oligomeric and polymeric end products of flavonoid biosynthesis pathway. They are a class of phenolic compounds that take the form of polymers of polyhydroxy flavan-3-ol units like (+)-catechin and (-) – epicatechin [12] [7]. These units are linked mainly through C4→C8 bond. The linkage between C4→C6 also exist which is known as B-type linkages. There is an additional ether bond between C2→C7 resulting in double linkage of flavan-3-ol units which is called as A-type linkage. The proanthocyanidins that consist of epicathechin units are called procyanidins which is considered as most abundant type of proanthocyanidins in plants which is also found in grape seed and pomegranate peels.[4] [12]



Proanthocyanidin



Anthocyanin

A number of plant-derived substances collectively known as phytonutrients or phytochemicals are

becoming increasingly known for their antioxidant activity and hence, the main reason behind using pomegranate peels is due to its high level of proanthocyanidin content and its potential antioxidant activity[5][7][13]. Antioxidants are first line of defense against free radical damage and are critical for maintaining optimum health. These antioxidants are the molecule that inhibits the oxidation of other molecules. Antioxidants terminate chain reactions (reactions that produce free radicals) by removing free radical intermediates and inhibit other oxidation reactions. Antioxidants are capable of stabilizing or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintain optimum cellular and systemic health and well-being [13].In addition to an antioxidant effect, flavonoid compounds may also exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages[6][13].

2. MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu reagent 2N, Gallic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate anhydrous, ferric ammonium sulphate, 2,4,6-Tripyridyl-s-triazine (TPTZ), ferric chloride, acetate buffer, sodium bicarbonate, citric acid, acetone, methanol, ethanol, ethyl acetate, 1-butanol, hydrochloric acid and glacial acetic acid[2] [7] [11].

Equipments

Micropipette 100-1000 µl, sensitive balance, water bath, stirrer, thermometer, centrifuge, conical flask 500 ml, test tubes and UV-Vis spectrophotometer [2].

2.1. METHODS

2.2. *Sample preparation*

Pomegranate was cleaned water and dried with cloth. The peels were manually separated, dried for few days in an open air shade till the peels get completely dry. The dried samples were then powered in blender and were kept until analysis.[2]

2.3. *Extraction procedure*

200 mg of dried and ground peels were kept in water bath with 10 ml distilled water at 50^{o}C for 20 minutes. The liquid extract was separated from solids by centrifugation at 2000 rpm for 10 minute. The supernatant was transferred to a 10 ml flask and distilled water was added to make final volume 10 ml.[2]

2.4. *Total Polyphenol Content*

The total polyphenol content in the extract was determined by the Folin-Ciocalteu method according to the method described by International Organization for Standardization (ISO). 250 µl of the extract was diluted with distilled water to 10 ml. Aliquots of 1 ml samples were mixed with 5 ml of 10-fold Folin-Ciocalteu reagent. After 3 minutes, 4 ml of 7.5% sodium carbonate was added. The mixtures were allowed to stand for 30 minutes at 40^{0}C before absorbance was measured at 734 nm. The total polyphenol content was measured using gallic acid standard curve.[2] [6]

2.5. *Proanthocyanidin content*

The proanthocyanidin content in extract was determined by acid butanol assay. In a 13x100 mm screw cap culture tube 6 ml of acid butanol was added to 1 ml aquilot of sample. Later, 0.2 ml of iron reagent was added and sample was vortex and kept for boiling water bath for 50 minute. Tube was cooled and absorbance was taken at 550 nm. [2] [7] [8]

2.6. *DPPH radical-scavenging effect*

The antioxidant activity was measured in term of hydrogen donating or radical scavenging ability using the stable DPPH method. 250 µl of the extract was diluted using distilled water to 10 ml. aliquots of 200 µl samples were mixed with 100 µM DPPH methanolic solutions. The mixture was placed in dark at room temperature for 60 minute. The absorbance of resulting solution was

then read at 520 nm and the DPPH radical was calculated using the given equation.[2]

2.7. *DPPH radical scavenging effect (%) = [(A_0-A_1)/A_0]*100*

Where A_0 is the absorbance of control at 60 minutes, and A_1 is absorbance of sample at 60 minutes. All samples were analyzed in duplicates. [2] [Fig 1]

2.8. *FRAP Assay*

65 ml of 0.3 M acetate buffer, 6.5 ml of 10 mM TPTZ solution and 6.5 ml 20 mM $Fecl_3$ ware mixed together and are known as FRAP reagent. After preparation of FRAP reagent, 50µl of sample was taken in each test tube and 1ml of FRAP reagent. After additions allow the sample to incubate at RT for 10 minutes carrying the experiment is dark. After 10 minutes of incubation take the absorbance at 595 nm using UV-Vis spectrophotometer.[Fig 2]

3. RESULT AND DISCUSSION

The proanthocyanidin was extracted best in the distilled water at 50^0 C for 20 minutes. The antioxidant capacities was found to be highest which was done using DPPH and FRAP assay whereas total polyphenolic content was estimated by acid butanol assay.[2][7]

The polyphenols extracted from waste pomegranate peels showed beneficial effect for human health. The peels depicted the presence of high amounts of antioxidants [fig 1, fig 2] which are useful to slow down the process ageing. [2]

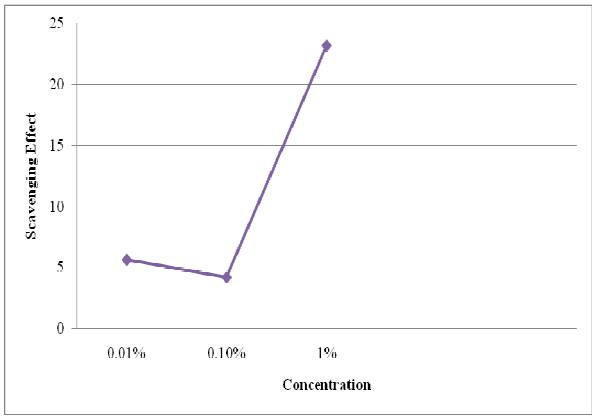


***Fig. 1:** DPPH scavenging activity of different concentrations.*

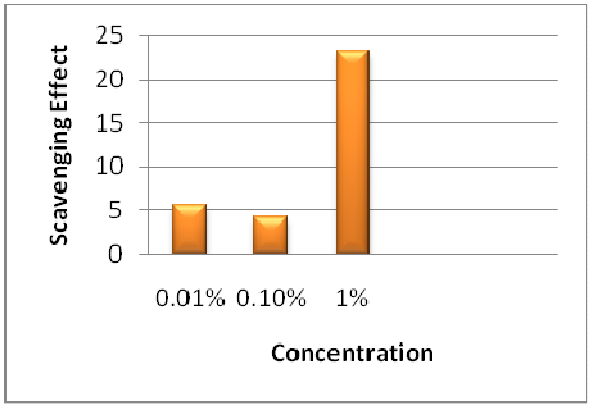


fig 2: Graph showing higher amount of scavenging activity of proanthocyanidins

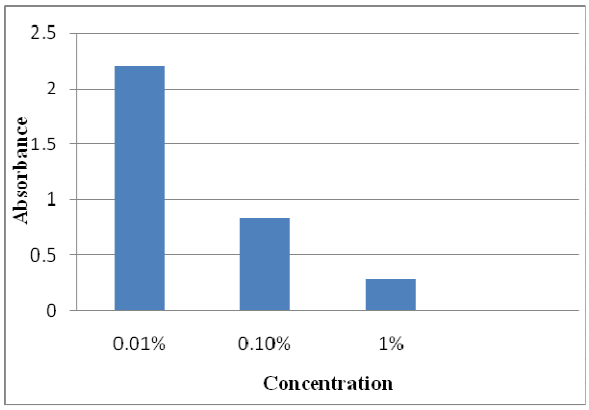


Fig 3: Antioxidant activity of different concentrations using FRAP Assay.

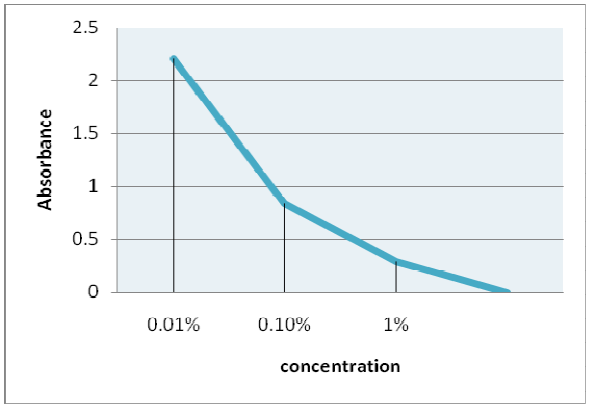


Fig 4: Curved graph showing antioxidant potential of proanthocyanidin

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