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Use of choline analogous anion-exchangers for the extraction of polyphenols from pomegranate juice

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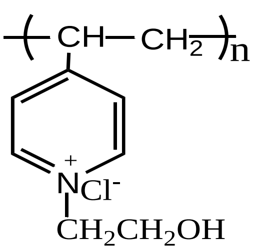
Abstract: *Polyphenols have anti-cancer effects because of antioxidant, anti-inflammatory, anti-proliferative activities and their effects on sub-cellular signaling pathways, induction of cell cycle arrest and apoptosis. Pomegranate juice has high contents of polyphenols such as punicalagin, ellagic acid, ellagitannin, gallotannins, anthocyanins and flavonoids, hence, it has potent anti-cancer effects. But one drawback with pomegranate is high level of calorific value due to presence of fructose; hence extraction of polyphenols can be very useful. Anion-exchangers are very effective materials used in the extraction of polyphenolic compounds. Hence, in present study an attempt has been made to synthesize and use choline analogous poly(4-vinyl pyridine) based anion-exchangers for the extraction of polyphenols from pomegranate juice.*

Key Words: *Polyphenols, pomegranate juice, anion-exchanger, gallic acid equivalents.*

1. INTRODUCTION:

Consumption of fruits and vegetables with high polyphenolic content reduce cardiovascular, cerebrovascular diseases and cancer mortality. Polyphenols have anti-cancer effects due to antioxidant, anti-inflammatory, and anti-proliferative activities as well as effects on sub-cellular signaling pathways, induction of cell cycle arrest and apoptosis (1, 2).Pomegranate juice (PJ) and extracts have been shown to have be *in vitro* antioxidantand *in vivo* anti-atherosclerotic properties due to free radical scavenging activities (3,4).These properties attributed to high contents of polyphenols such as punicalagin, ellagic acid in its free and bound forms (ellagitannin, gallotannins and anthocyanins) and flavonoids (quercetin, kaempferol and luteolin glycosides) (5).In PJ most abundant and bioactive constituent is punicalagin, which is responsible for more than 50% antioxidant activity of the juice (6).Even though PJ has polyphenols that have antioxidant, anti-viral and anti-tumor activity, but one drawback with this juice is high level of calorific value because of present fructose. Hence, extraction of polyphenolic compounds from PJ is very useful, as it reduce the contents of high caloric value.

General methods used for the extraction of polyphenols are associated with large consumption of organic solvents. To avoid this problem ion-exchange methods are very useful and extraction of polyphenolic compounds can be achieved by using anion-exchanger polymers (7, 8). The quaternized polymers have a large potential as anion-exchanger. These can be used in removal of different metal ions from water and also have antimicrobial activities and emulsification properties (9, 10). In present study an attempt has been made to synthesize different types of choline analogous poly(4-vinyl pyridine) based anion-exchangers and use in extraction of polyphenols from PJ. These were prepared by the quaternization of poly(4-VP) with 2-chloroethanol to generate a bioactive polymer with pendant choline analogous group, as shown (10):



Choline has $[(CH_3)_3N^+(CH_2CH_2OH)^-OH]$ structure and is an important constituent of fat metabolism. The counter anion (Cl^-) was replaced with Br^-, OH^-, SH^-, NO_3^-, BF_4^- andCF_3COO^- by simple metathesis reaction to obtain a series of polymers (11, 12).Synthesize polymers have positively charged beads that associated with exchangeable anion, therefore can act as anion exchangers. Total polyphenolic contents in PJ was determined in gallic acid quivalents (GAE) by using Folin-Ciocalteu reagent and gallic acid (13, 14).

2. EXPERIMENTAL:

2.1. MATERIALS:

Pomegranate juice was extracted from the fruits available in market. 4-Vinyl pyridine (Merck, Schuchardt, Germany), Gallic acid (S. D. Fine, Mumbai), Folin–Ciocaleu reagent (S. D. Fine, Mumbai), benzoyl peroxide (S. D. Fine, Mumbai), ethylene glycol dimethacrylate (Merck, Schuchardt, Germany), 2-chloroethanol (S. D. Fine, Mumbai), NaBr, NaOH, NaSH, $NaNO_3$, $NaBF_4$ and CF_3COOK were used as received.

2.2. SYNTHESIS OF ANION-EXCHANGERS:

Anion-exchangers were prepared by taking known amount of 4-VP, 1% (by weight) initiator benzoy peroxide, 2% (by weight) cross linker ethylene glycol dimethacry late was added and polymerization was carried at 70 ºC in water bath for 2.5 h. The cross linked network was washed with ethanol to remove any uncross linked polymer and then dried at 60 ºC. The quaternization reaction on poly(4-VP) network was carried out by taking poly(4-VP) and 2-chloroethanol in 1:5 weight ratio and heated at 50 ºC for 36 h to ensure maximum extent of reaction. The synthesized anion-exchanger was denoted as [Poly(4-VP-*cl*-EGDMA)-$CH_2CH_2OH]^+$ Cl^-. The unreacted 2-chloroethanol from the anion-exchanger was removed by washing with water and then dired at 60 ºC till constant weight was obtained.

To get a series of new anion-exchanger, Cl^- was replaced by simple metathesis reaction using different anions as Br^-,OH^-, SH^-, NO_3^-, BF_4^-, and CF_3COO^-. 7 g each of NaBr, NaOH, NaSH, $NaNO_3$, $NaBF_4$, or CF_3COOK was dissolved in minimum amount of distilled water and 1 g [Poly(4-VP-*cl*-EGDMA)-$CH_2CH_2OH]^+$ $Cl^-$was added to each salts solutions and then was stirred for 6 h (10). Then networks were washed with distilled water and dried at 60 ºC till constant weight was obtained.

2.3. EXTRACTION OF POLYPHENOLIC COMPOUNDS:

Reference curve was prepared by mixing 0.5 mL aliquot of 0.05, 0.1, 0.15, 0 .2, 0.25 and 0.3 mg/mL of ethanolicgallic acid solutions with 2.5 mL Folin–Ciocaleu reagent (diluted ten-fold) and 2 mL (75 g/L) sodium carbonate solution. Then after 1h absorbance was taken on UV spectrophotometer (Varian Cary-300) at 768nm and reference curve was drawn as concentration of gallic acid (mg/ml) versus absorbance.

25 mL of PJ was stirred with 1g of different anion-exchangers for 6h and then filtered. From filtrate, 0.5 mL of PJ was mixed with 2.5 mL Folin–Ciocaleu reagent and 2 mL (75g/L) Na_2CO_3 solution and absorbance was taken by same procedure as discussed above and from reference curve, the gallic acid concentration was found. Polyphenolic content in the filtrate in GAE was calculated by following formula (15).

$$C = c.V/w$$

Where, C is polyphenolic content (mg/g) in PJ, in GAE; c is the concentration of gallic acid (mg/mL) established from reference curve; V is the volume of PJ in mL; w is the weight of PJ in g. Polyphenols initial present in PJ was also determined by following the same procedure as discussed above.

Polyphenolic uptake by the polymer was calculated by following formula.

$$\%\ \text{uptake}\ (P_u) = \frac{\text{Initial polyphenolic content} - \text{polyphenolic content left in filtrate}}{\text{Initial polyphenolic content}} \times 100$$

Polymer, which showed maximum uptake, was reused in repeated cycles. After filtration new allotment of 25 mL of PJ was added each time to the polymer, which was already used. This process was repeated a number of times and the contents of polyphenolic compounds in each filtrate were measured as GAE.

3. RESULTS AND DISCUSSION:

3.1. EXTRACTION OF POLYPHENOLIC COMPOUNDS:

Bioseparation is the process of extraction of bioactive compounds from the extracts of biological origin and the compounds should have maximum activity, i.e., there should be no degradation or inactivation and the extracted compound should have maximum possible purity. Results of the uptake of polyphenolic compounds from PJ by different anion-exchangers are given in Figure 3.1.1. These results correlate with the polymer structure as one having Cl^- showed maximum uptake capacity, while the polymer having OH^- despite of the high swellability of cross linked network it showed the lowest uptake. It may be as polyphenols being of acidic in nature, lose their H^+. Hence, similarity of the polyphenolic ions and OH^- restrict the access of polyphenolic ions to positive charge centre on polymer chains, which is not the case with other polymeric support. Polymer having $Cl^-$was used again and again for the uptake of polyphenolic contents and results are presented in Figure 3.1.2. The repeatability or reusabilities of the polymer support is good upto fourth cycle and thereafter, it decreased, perhaps due to non-leaching of polyphenols on the active sites.

4. CONCLUSION:

From foregone discussion, it can be concluded that the poly(4-VP) based anion exchangers were prepared following by a simple protocol. The counter anion of the polymer can be exchanged by different anions with metathesis reaction. These anion exchangers can be used for the extraction of polyphenolic compounds from the pomegranate juice. A large effect of the counter anions of the different polymers was observed on the uptake of polyphenolic compounds; the polymer having Cl^- anion was shown to have maximum uptake capacity.

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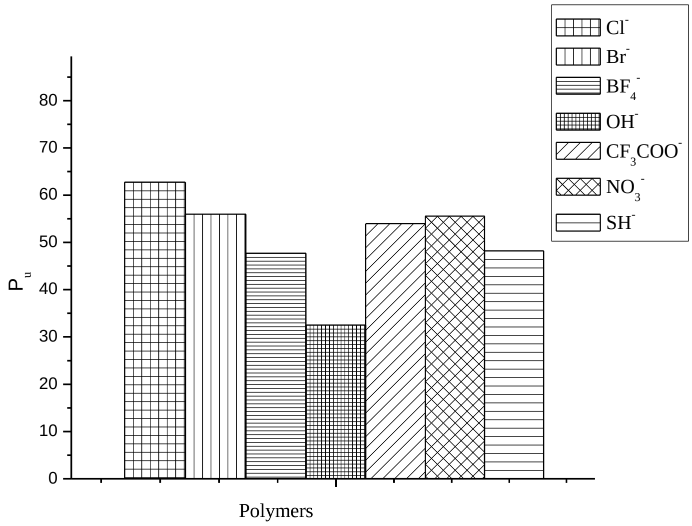

Figure 3.1.1. Uptake of polyphenolic compounds by different polymers

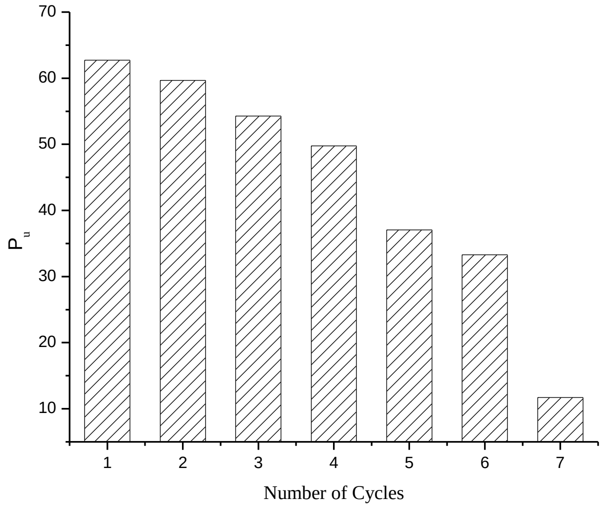

Figure 3.1.2. Polyphenolic uptake as function of number of cycles