

Quantification and Comparison of Total Phenol Content (TPC) and Total Tannin Content (TTC) of Pomegranate Seeds during Two Different Seasons of Saurashtra Region

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Abstract: *Punica granatum* L. is economically important fruit crop which belongs to Punicaceae family. It is commonly known as Dadam in Saurashtra region. Phytochemical analysis of Pomegranate was performed using two solvents (1) Methanol and (2) Acetone. Present study showed Quantification of Total Phenol Content (TPC) and Total Tannin content (TTC) of Pomegranate seeds collected from Saurashtra region during two different seasons Ambe bahar (Fruit harvest in June-August) and Hasta bahar (Fruit harvest in February- April). These study revealed that TPC were higher in Both extract in Ambe bahar whereas TTC were higher in Hasta bahar for methanolic extract but in acetic extract they were higher in Ambe bahar. From this study it was concluded that variations in quantity of secondary metabolites were observed due to the Seasonal changes in the same region and geographical conditions.

Key words: *Punica granatum* L., Phytochemicals, Phenols, Tannins, Seasons, Saurashtra.

1. INTRODUCTION:

Phytochemicals are certain non-nutritive plant chemicals that occur naturally in plants and they are not required by human body for sustain of life but they protect our body from various diseases [1]. Due to their numerous medicinal uses, these phytochemicals become more popular and they also play important role against various number of diseases like asthma, arthritis and cancer etc. Naturally occurring phytochemicals do not have any side effects and they also cure diseases without causing any harm to mankind so they are known as “man-friendly medicines” [2]. Plants produce an amazing diversity of low molecular weight compounds. Although the structures of close to 50,000 have already been elucidated. There are probably hundreds of thousands of such compounds. Only a few of these are part of ‘primary’ metabolic pathways (those common to all organisms). The rest are termed ‘secondary’ metabolite. The health benefits of fruit and vegetables are mainly from the phytochemicals and a range of polyphenolics [3]. They acts as defense chemicals. Their absence does not cause harmful effects in the plants. They include alkaloids, phenolics, steroids, essential oils, lignins, resins and tannins etc [4]. These phytochemicals are already a part of our diet through various vegetables and fruits. Pomegranate is found to be rich source of phytochemicals [5]

Plant material: *Punica granatum* L.

Family: Punicaceae

Common name: Dadam, anar

Plant part used : Seeds



Figure 1: Pomegranate tree (Visavadar-Saurashtra region)

Punica granatum is popularly known as pomegranate (Anar). It belongs to Punicaceae family, which is a large deciduous shrub or small tree native to Asia. Different part of pomegranate like bark, leaves, immature fruits, and seeds have medicinal properties due to the presence of various phytoconstituents in them [6]. The quality and quantity of these phytoconstituents may vary depending upon the variety, processing, cooking, growing conditions and

climate of the region [7]. Many researchers reported that phytochemicals like tannins, phenols, flavonoids, glycosides are present in the peel, seeds, leaves and pulp of pomegranate [8]. Due to the presence of these phytochemicals they show various biological properties like antioxidant, anti-inflammatory, anti-bacterial, anti-diabetic etc [9]. Pomegranate also traditionally useful in heart problems, dental care, arthritis, digestive and skin disorder etc [10]. In this study, total phenol content and total tannin content of pomegranate seeds were investigated using acetone and methanol extract of seeds which were collected during two different seasons of Saurashtra region.

2. MATERIALS:

Seeds of *Punica granatum* L. were collected from Visavadar (Junagadh district) Saurashtra region. The seeds of *Punica granatum* L. were collected two times during two different seasons of Saurashtra region (Fig.1). Here, season 1 was Ambe bahar (Fruit harvest in June-August) and season 2 was Hasta bahar (Fruit harvest in February-April). The seeds of plant collected during this season and washed and dried at room temperature and then crushed. Dried powder stored in the air tight bottle for extract preparation.

1.1. EXTRACT PREPARATION:

10gm of Pomegranate seeds were extracted using 100ml of organic solvent (Methanol and acetone) for 24 hours and then filtered using Whatmann filter paper No. 1. The extracted solution, (filtrate) was kept overnight to obtain dry extract while the residue after filtration was discarded. The methanol and acetone extracts of each part was stored in a cool and dry place after transferring them into vials.

3. METHODS:

3.1 Quantitative analysis

3.1.1. Estimation of Total phenol content ^[11]

Quantitative analysis of Total Phenol contents (TPC) of *Punica granatum* L. seeds were done by Folin-ciocalteu's method with some modifications. For the preparation of calibration curve, gallic acid used as standard. Gallic acid was prepared in two different solvent such as methanol and acetone with different concentrations. Aliquots of 0.5 ml of each concentration of gallic acid were mixed with 2 ml of (1:10) Folin-ciocalteu's reagent and 2 ml of 7.5% sodium carbonate solution. Then tubes were shaken vigorously and mixed well. The mixture was allowed to incubate for 30 minutes at room temperature before the absorbance was measured at 760 nm. Same as for plant extracts, 0.5 ml of all extracts (1mg/ml) were treated and absorbance was measured.

3.1.2. Estimation of Total Tannin content ^[12]

Quantitative analysis of total Tannin content (TTC) of *Punica granatum* L. seeds were analyzed by Folin-denis method with some modifications. Tannic acid was used as standard. Tannic acid was prepared with different concentrations of different solvents. The methanolic and acetone extract mixed with 0.3 ml of Folin-denis reagent followed by 1 ml of Na₂CO₃ (7.5%) solution and made up to 10 ml with distill water. These mixtures were shaken well and allowed it to 30 minutes for incubation at room temperature and measured the absorbance at 755 nm using UV-visible spectrophotometer. Total tannins in extracts were expressed as equivalent to tannic acid (mg TE/g extract).

4. RESULTS AND DISCUSSION:

Here, **Season 1** was Ambe bahar (Fruit harvest in June- August).

Season 2 was Hasta bahar (Fruit harvest in February-April).

4.1. Total Phenol Content:

Total phenol content of *Punica granatum* L. seeds were estimated by Folin- ciocalteu's method for both season of Saurashtra region of Gujarat. TPC was calculated for methanol extract from standard calibration curve of Gallic acid ($y=0.2088x+0.0751$, $R^2=0.9976$) and for acetone extract from standard calibration curve of Gallic acid ($y=0.1842x+0.064$, $R^2=0.9901$) in terms of mg/ml Gallic acid equivalent of sample (Fig.2). For methanol extract of *Punica granatum* L. seeds, the values of TPC were found between 305mg to 0.0462 mg in first season (Ambe bahar) but in the season 2(Hasta bahar) TPC were found between 0.224 mg to 0.309 mg/ml of Gallic acid equivalent(GAE)(Fig.4) whereas for acetone extract of *Punica granatum* L. seeds, the values of TPC were found between 0.146 mg to 0.291 mg in season 1(Ambe bahar) but during second season (Hasta bahar) TPC were found Between 0.125 mg to 0.241 mg/ml Gallic acid equivalents (GAE)(Fig 5).Among these two season of *Punica granatum* L. seeds, it was concluded that season 1(Ambe bahar) recorded the best result in quantity of phenol content than season 2(Hasta bahar) for the both acetone and methanol extract.

4.2. Total Tannin Content:

Total tannin content of *Punica granatum* L. seeds was calculated for methanol extract from standard calibration curve of Tannic acid($y=0.1704x+0.0913$, $R^2=0.9871$) and for acetone extract from standard calibration curve of Tannic acid($y=0.1545x+0.0253$, $R^2=0.9975$) in terms of mg/ml Tannic acid equivalent of sample (Fig.3). For methanol extract of *Punica granatum* L. seeds, the values of TTC were found between 106mg to 0.148 mg in first season(Ambe bahar) but in the season 2(Hasta bahar) TTC were found between 0.104 mg to 0.160 mg/ml of Gallic acid equivalent(GAE)(Fig.6) whereas for acetone extract of *Punica granatum* L. seeds, the values of TTC were found between 0.048 mg to 0.098 mg in season 1(Ambe bahar) but during second season(Hasta bahar) TTC were found between 0.035 mg to 0.057 mg/ml Gallic acid equivalents(GAE)(Fig.7). From this study it was concluded that methanol extract showed higher tannin content in season 2(Hasta bahar) but in season 1(Ambe bahar) acetone extract showed higher tannin content compare to season 2(Hasta bahar).

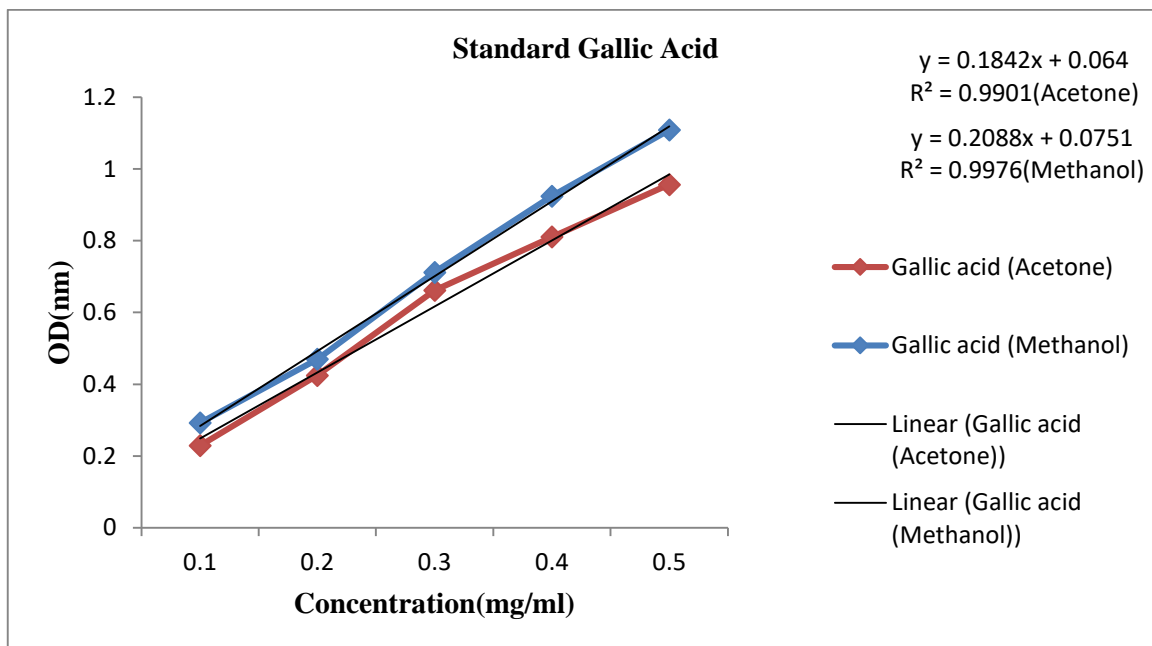


Figure 2: standard graph of Gallic acid (Acetone and Methanol)

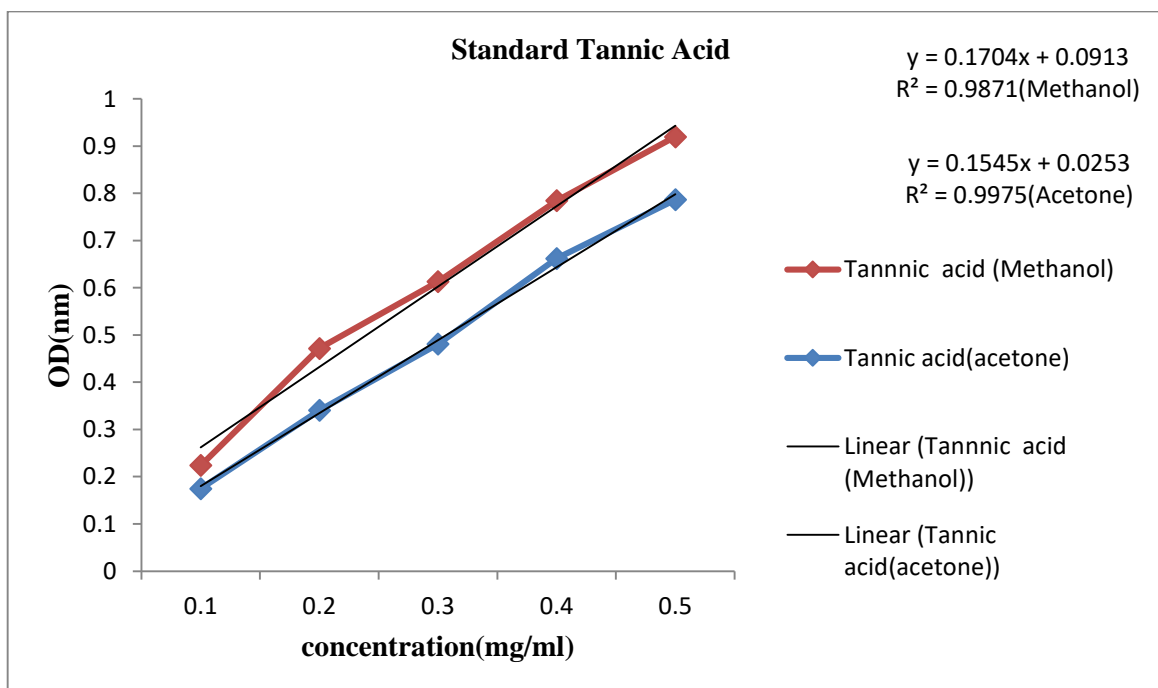


Figure 3: standard graph of Tannic acid (Acetone and Methanol)

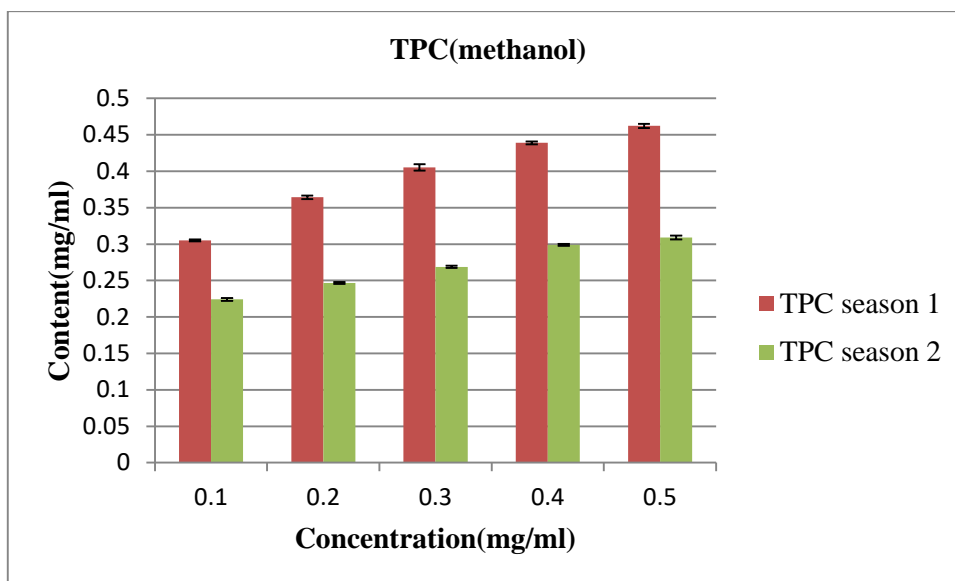


Figure 4: Total phenol content of ME of *Punica granatum* L.seeds from saurashtra region

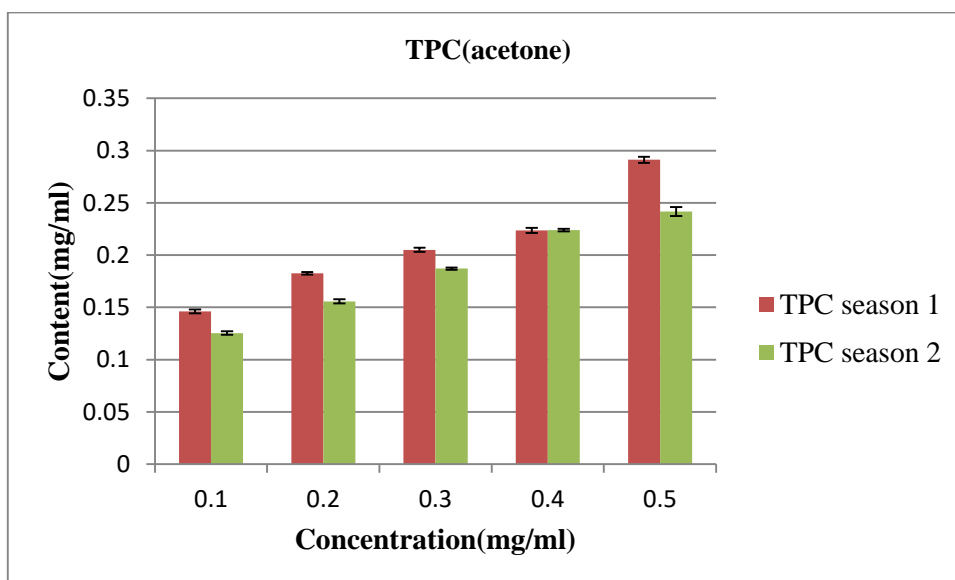


Figure 5: Total phenol content of AE of *Punica granatum* L.seeds from saurashtra region

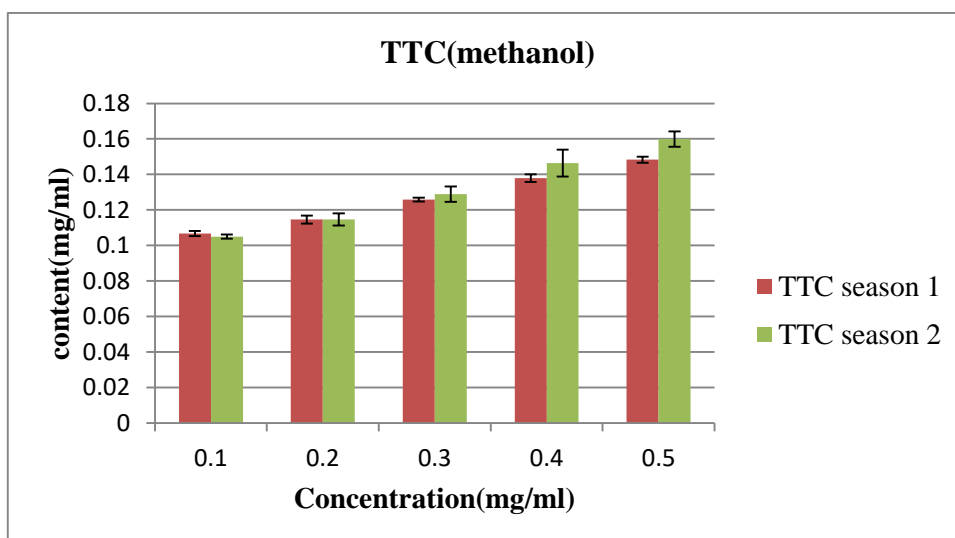


Figure 6: Total tannin content of ME of *Punica granatum* L. seeds from saurashtra region

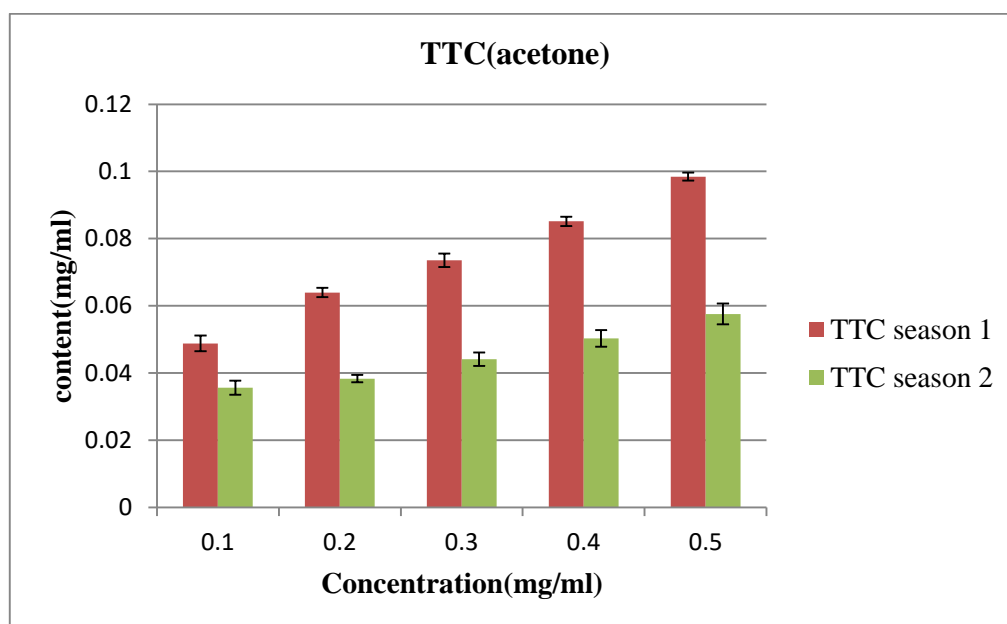


Figure 7: Total tannin content of AE of *Punica granatum* L. seeds from saurashtra region

4.3. Comparison of Total Phenol content of Pomegranate Seeds:

This result revealed that in methanolic extract Total phenol content was higher than acetone extract. The result showed that for the acetone extract and methanolic extract season 1 (Ambe bahar) had high Total phenol content while season 2 (Hasta bahar) had low (Fig.8).

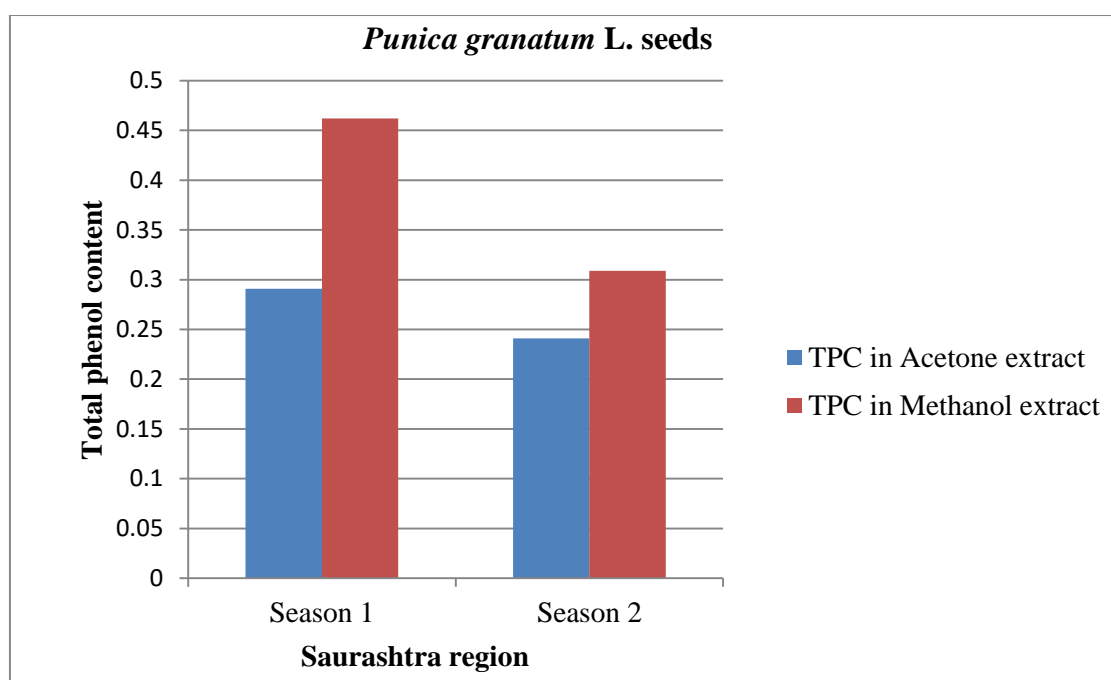


Figure 8: Comparison of Total Phenol Content for Acetone and Methanol extract of *Punica granatum* L. seeds during two different seasons of Saurashtra region

4.4. Comparison of Total Tannin content of Pomegranate Seeds:

This result revealed that in methanolic extract Total phenol content was higher than acetone extract. The result showed that for the acetone extract season 1 (Ambe bahar) had high Total phenol content while season 2 (Hasta bahar) had low. But for the methanolic extract season 2 (Hasta bahar) had high Total tannin content while season 1 (Ambe bahar) had low. This result was totally reversed than the acetonic extract (Fig.9).

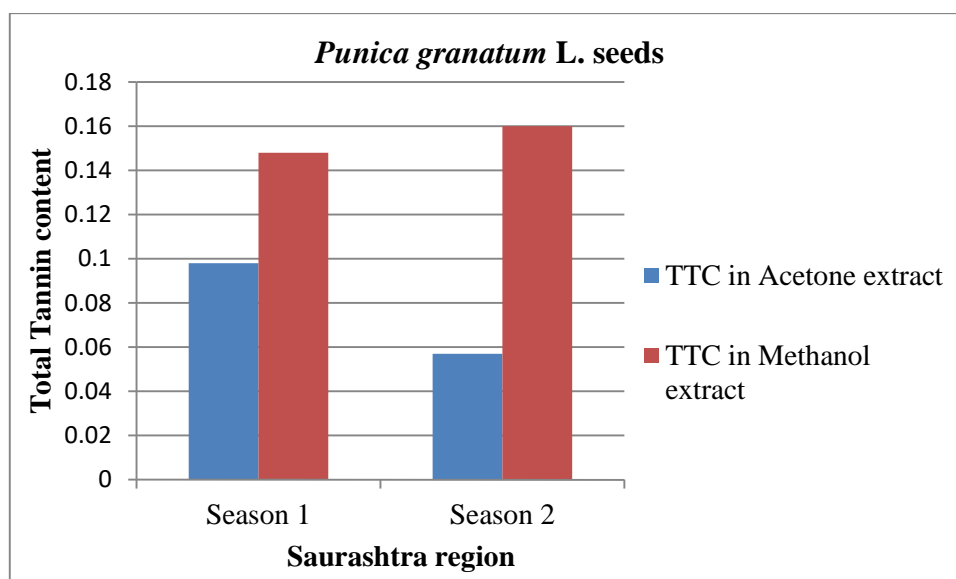


Figure 9: Comparison of Total Tannin Content for Acetone and Methanol extract of *Punica granatum L.* seeds during two different seasons of Saurashtra region.

5. CONCLUSION:

Changes in the climate and various seasons of that particular region have an immense impact on the availability of the active components in medicinal plants. Here, Total Phenol Content were found higher in season 1 (Ambe bahar) in both extract than the season 2 while Total Tannin Content were found higher in season 1 (Ambe bahar) in acetonic extract but in methanolic extract they were found higher in season 2 (Hasta bahar). Thus Ambe bahar (Season 1) is best than the Hasta bahar (Season 2) in terms of quantity of TPC and TTC in the Saurashtra region. So we can say that variations in quantity of secondary metabolites were observed due to the Seasonal changes in the same region and geographical conditions.

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