

Evaluation of Antioxidant Activity of *Punica granatum*

Ashwini Kasliwal¹, Farha Quadri²

PG Student, Department of Biotechnology, Shivchhatrapati College, N3 Cidco, Aurangabad, Maharashtra, India

Lecturer, Department of Biotechnology, Shivchhatrapati College, N3 Cidco, Aurangabad, Maharashtra, India

ABSTRACT: Antioxidant plays an important role as health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Synthetic Antioxidants (BHA) give rise to many side effects, so it is necessary to look forward for natural antioxidants. Pomegranate content high amount of phenols and these are responsible for the Antioxidant activity. Present study aimed to find how pomegranate peels are potent in its Antioxidant activity. To do the same DPPH and TBA assay was performed Gallic acid was taken as standard. A different concentration (5mg/ml, 10mg/ml, 15 mg/ml, 20 mg/ml, and 25 mg/ml) was assayed. DPPH assay showed percent inhibition from 85.90% to 51.21% with respect to range of concentrations with solvent Methanol. Ethanolic extract of peel showed percent inhibition from 74.25% to 45.52% with the concentration rang of 5mg/ml to 25 mg/ml. TBA assay clarify that Methanolic extract of peel have percent inhibition from 91.27% to 57.38% with respect to concentrations (5mg/ml, 10mg/ml, 15 mg/ml, 20 mg/ml, and 25 mg/ml) and ethanolic extract of peel clearly indicates percent free radical scavenging activities from 89.9% to 48.99% in selected range of concentration(5mg/ml to 25 mg/ml). Linear Regression Plot of different concentration, IC_{50} Value 20mg/ml and 30mg/ml by DPPH and TBA assay indicates Methanolic extract is more efficient and potent than Ethanolic extract and reference Ascorbic acid Thus Pomegranate peels which are considered as waste can be utilized as potent Antioxidant agent.

KEYWORDS: Antioxidant activity, DPPH, IC_{50} value, *Punica granatum*, TBA.

I. INTRODUCTION

Antioxidants play an important role as health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced antioxidants like vitamin C, vitamin E; carotenes, phenolic acids have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties [1].

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reaction or from exogenous factors. *In vivo*, some of these ROS play an important role in cell metabolism including energy production, phagocytosis and intercellular signaling. However, these ROS produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects such as DNA damage, carcinogenesis and various degenerative disorders such as cardiovascular diseases, aging and neuro-degenerative diseases . A potent broad spectrum scavenger of these species may serve as a possible preventive intervention for free radical mediated cellular damage and diseases. Antioxidant based drugs and formulations for the prevention and treatment of complex diseases like Alzheimer's disease and cancer have appeared during last three decades. Recent studies have shown that a number of plant products including polyphenols, terpenes and various plant extracts exerted an antioxidant action. There is also a considerable amount of evidence revealing an association between individuals who have a diet rich in fresh fruits and vegetables and the decreased risk of cardiovascular diseases and certain forms of cancer. There is currently immense interest in natural antioxidants and their role in human health and nutrition. Considerable amount of data have been generated on antioxidant properties of food plants around the globe. However, traditionally used medicinal plants await such

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screening. On the other hand, the medicinal properties of plants have also been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. Several medicinal plants (Rasayana) have also been extensively used in the Indian traditional (Ayurveda) system of medicine for the treatment of number of diseases. Some of these plants have shown potent antioxidant activity. However, majority of plants have not yet been screened for such activity [2].

The importance of oxidation in the body and in foodstuffs has been widely recognized. Oxidative metabolism is essential for survival of cells. A side effect of this dependence is the production of free radicals and other reactive oxygen species that cause oxidative changes. There is increasing evidence for the involvement of such species in a variety of normal *in vivo* regulatory systems. When an excess of free radicals is formed, they can overwhelm protective enzymes such as superoxide dismutase, catalase and peroxidase and cause destructive and lethal cellular effects (*e.g.*, apoptosis) by oxidizing membrane lipids, cellular proteins, DNA and enzymes, thus shutting down cellular respiration. Oxidation can also affect foods, where it is one of the major causes of chemical spoilage, resulting in rancidity and/or deterioration of the nutritional quality, color, flavor, texture and safety of foods. It is estimated that half of the world's fruit and vegetable crops are lost due to postharvest deteriorative reactions. Defense mechanisms against the effects of excessive oxidations are provided by the action of various antioxidants and the need to measure antioxidant activity is well documented [3].

Epidemiological studies show that consumption of fruits and vegetables with high phenolic content correlate with reduced cardio-vascular diseases and cancer mortality [4] Phenolic compounds may produce their beneficial effects by scavenging free radicals. In the past few years there has been an increasing interest in determining relevant dietary sources of antioxidant phenolics. Thus, red fruit juices such as grape and different berry juices have received attention due to their antioxidant activity. Pomegranate juice has become more popular because of the attribution of important biological actions [5]. Thus antioxidant and antitumoral activity of pomegranate bark tannins (punicalcortin) [6] and the antioxidant activity of fermented pomegranate juice [7] have been reported. However, detailed investigations of the phenolic compounds and the antioxidant activity of the juice have not yet been carried out. Pomegranate juice is an important source of anthocyanins, and the 3-glucosides and 3, 5-diglucosides of delphinidin, cyanidin, and pelargonidin have been reported [8]. It also contains 1 g/L citric acid and only 7 mg/L Ascorbic acid In addition; pomegranate bark leaf [9] and the fruit husk [10] are very rich in ellagitannins and gallotannins. Several apigenin and luteolin glycosides from pomegranate leaves [11] and the hydrolyzable tannins punicalagin and punicalin from pomegranate husk have previously been identified [12].

Free radicals, the partially reduced metabolites of oxygen and nitrogen, are highly toxic and reactive. The most common reactive oxygen species are superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), peroxy radical (ROO) and highly reactive hydroxyl radical (OH). Oxidation process is one of the most vital routes for producing free radicals in food, drugs and living systems. Antioxidants are the substances that when present in low concentration significantly delay or reduce the oxidation of the substrate. Antioxidants protect the body from damaging oxidation reactions by reacting with free radicals and other reactive oxygen species within the body and hindering the process of oxidation. Hence, diseases associated with free radicals can be avoided by antioxidant therapy which gained an immense importance [13].

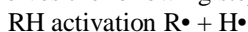
The antioxidant activity of plants might be due to their phenolic compounds. Flavonoids are a group of polyphenolic compounds with known properties like free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory actions [14].

One such protective mechanism is radical-scavenging activity [15] for a compound to be defined as an antioxidant it must satisfy two basic conditions:

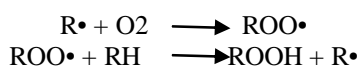
1. When present at low concentration relative to the substrate to be oxidized, it can delay or prevent autoxidation or free radical-mediated oxidation.
2. The resulting radical formed after scavenging must be stable in order to interrupt the oxidation chain reaction [16]

The auto-oxidation involves a free-radical chain process that can be initiated by the action of external agents such as heat, light or ionizing radiation [17] and involves the following steps:

1) *Initiation*



2) *Propagation*



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3) Chain-breaking termination (Inhibition)



Where, RH is the organic substrate; ROO• the corresponding peroxy radical; ROOH the hydro peroxide; AH the antioxidant; A• the stable, comparatively unreactive radical. Phytochemicals are the secondary metabolites produced by plants as a defence mechanism against environmental threats such as harmful ultraviolet (UV) radiation, pathogens, and herbivorous predators. Plant polyphenols are a major group of phytochemicals and an important class of antioxidants. These compounds are widespread virtually in all plant foods, often at high levels (Pietta, 2000). Phenolic compounds are excellent oxygen radical scavengers because the electron reduction potential of the phenolic radical is lower than that of oxygen radicals. Therefore, phenolic compounds can scavenge reactive oxygen intermediates thus preventing further oxidative reactions [18].

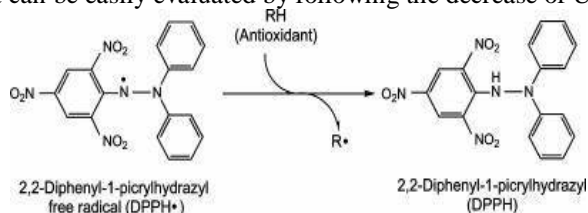
Methods for measurement of antioxidant activity-

1. The ABTS (2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) di ammonium salt) method,
2. The ORAC (oxygen radical absorbing capacity) method,
3. The DPPH (2,2 diphenyl-1-picrylhydrazyl) method,
4. The DMPD (N,N-dimethyl-p-phenylenediamine dihydrochloride) method and
5. The FRAP (ferric reducing ability of plasma) method [19].

The DPPH (2, 2 diphenyl-1-picrylhydrazyl) method-

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity. The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple color). When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical scavenging antioxidant) and is reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH. Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured. More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Diphenylpicrylhydrazine; non radical) with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present) [1].

The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. Figure 1, below, shows the mechanism by which DPPH accepts hydrogen from an antioxidant. DPPH is one of the few stable and commercially available organic nitrogen radicals. The antioxidant effect is proportional to the disappearance of DPPH in test samples. Monitoring DPPH with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm.



A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. It has also been used to quantify antioxidants in complex biological systems in recent years. The DPPH method can be used for solid or liquid samples

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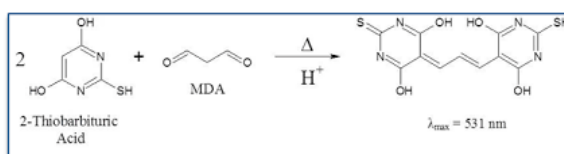
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and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity helps understand the functional properties of foods [20].

The TBA (Thiobarbituric acid)

The TBA assay was proposed over 40 years ago and is now the most commonly used method to detect lipid oxidation. Thiobarbituric acid (TBA) test is used to measure the second product of peroxide oxidation such as aldehyde and ketones (Farag *et al.*, 1989). During the linoleic acid oxidation, peroxides are formed and react with ferrous chloride to form ferric ion. The ferric ion then combines with ammonium thiocyanate and produce ferric thiocyanate. Thiocyanate gave red colour. The higher the colour intensity indicated the higher linoleic acid emulsion oxidation as well as the absorbance. One such compound is malonaldehyde which is measured by the TBA method [21]



Scope of herbal antioxidant

The health benefits attributed to the consumption of fruits, vegetables and cereals are related, at least in part, to their antioxidant activity. Many constituents of these dietary components may contribute to their protective properties, including: vitamins C and E, Selenium and other trace minerals and micronutrients, carotenoids, phytoestrogens, allium compounds, glucosinolates and indoles, dithiolthiones, isothiocyanates, protease inhibitors, fiber and folic acid. These compounds may act independently or in combination as anti-cancer or cardioprotective agents by a variety of mechanisms.

Synthetic Antioxidant BHA, BHT and gallic esters have been suspected to be carcinogenic. In addition, BHT at 200ppm induced significant increase in enzyme activities of rat's liver and kidney and severely altered the features of these organ tissues [22]. Furthermore, WHO recommends the use of natural antioxidants that can delay or inhibit the lipids or other molecules by inhibiting the initiation or proration steps of oxidative chain reactions are used nowadays. Strong limitations have been placed on the use of synthetic antioxidants and trend nowadays is to replace them with naturally occurring antioxidants. Pomegranates are rich source of polyphenol and are potent natural antioxidant work without any side effects [22].

Numbers of fruits are good antioxidants but only the edible part of these gives an antioxidant activity as in Fig, Berries. Pomegranate's peel has potent antioxidant activity. Peel being non-edible portion, generally thrown as a waste. That's why; present studies focuses on the assessment of antioxidant activity of peels of pomegranate, and explore it as a potent natural antioxidant agent.

II. EXPERIMENTAL DESIGN

Percentage of antioxidant activity of *Punica granatum* peel (methanolic and Ethanolic extract) was carried out by using two different methods as DPPH (2, 2 diphenyl-1-picrylhydrazyl) method and TBA (Thiobarbituric acid). The extract of Punica Granatum peel was prepared in 5% concentration (5gm of weighed *Punica granatum* peel added in 100ml of organic solvent). Ethanolic and Methanolic extraction was carried out by using soxhlet (Bio-tech India-15946) apparatus. Soxhlet extraction was carried out using solvent ethanol and methanol. The filtrates were evaporated to get concentrated extract. Dried extract further use for assessment of Antioxidant activity [23].

2% Ascorbic acid was used as a reference. 0.1gm of Ascorbic acid was dissolved in 5ml of distilled water and was allowed to dissolve. And from stock solution of 2% Ascorbic acid various concentrations (5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml, and 25mg/ml) were prepared.

In DPPH assay 1ml of 0.1mM Ethanolic DPPH solution was mixed with 3ml of different concentration (5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and 25mg/ml) of respective extract (Methanolic extract, Ethanolic extract and aqueous Ascorbic acid). The mixture was vigorously shaken and incubated at room temperature for 30minutes followed by taking absorbance at 517nm on UV-VIS Spectrophotometer (BL198 ELico). Control was made up of 1ml respective solvent (Methanol, Ethanol and distilled water) with 1ml of Ethanolic 0.1mM DPPH solution and pure solvent (Methanol, Ethanol and distilled water) taken as a blank [1].

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In TBA assay 1ml 20% TCA with 2ml aqueous Thiobarbituric acid (TBA) mixed with 2ml of different concentration (5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml, and 25mg/ml) respective extract (Methanolic extract, Ethanolic extract and aqueous Ascorbic acid) and kept in boiling water bath for 10minutes followed by centrifugation at 3000rpm for 20minutes, absorbance of Supernatant taken at 532nm on UV-VIS Spectrophotometer (BL198 ELico). Control was made up of 1ml respective solvent (Methanol, Ethanol and distilled water) with 1ml of 20% TCA and 2ml of aqueous TBA solution and pure solvent (Methanol, Ethanol and distilled water) taken as a blank [5].

Statistical analysis was carried out by calculation of percent inhibition that is scavenging effect of obtained respective optical density of each concentration of all extracts obtained by DPPH and TBA methods by following formula-

$$\text{Scavenging effect (\% or Percent inhibition) = } \frac{A_0 - A_1}{A_0} \times 100$$

Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample [24].

Linear Regression plot was plotted as per reading obtained for each extract by both methods.

III. METHODOLOGY

Collection of plant sample-

Plant sample was collected from Botanical Garden of Shivchhatrapati College N-3, cidco Aurangabad.

Authentication of plant sample

Plant sample was authenticated from Department of Botany of Dr. Babasaheb Ambedkar Marathwada University Aurangabad, with **Accession No. - 0554**.

Preparation of *Punica granatum* peel extract-

The extract of *Punica Granatum* peel was prepared in 5% concentration (5gm of weighed *Punica granatum* peel added in 100ml of organic solvent). Ethanolic and Methanolic extraction was carried out by using soxhlet (Bio-tech India-15946) apparatus. Soxhlet extraction was carried out using solvent ethanol and methanol. The filtrates were evaporated to get concentrated extract [24]. Dried extract further use for assessment of Antioxidant activity [26].

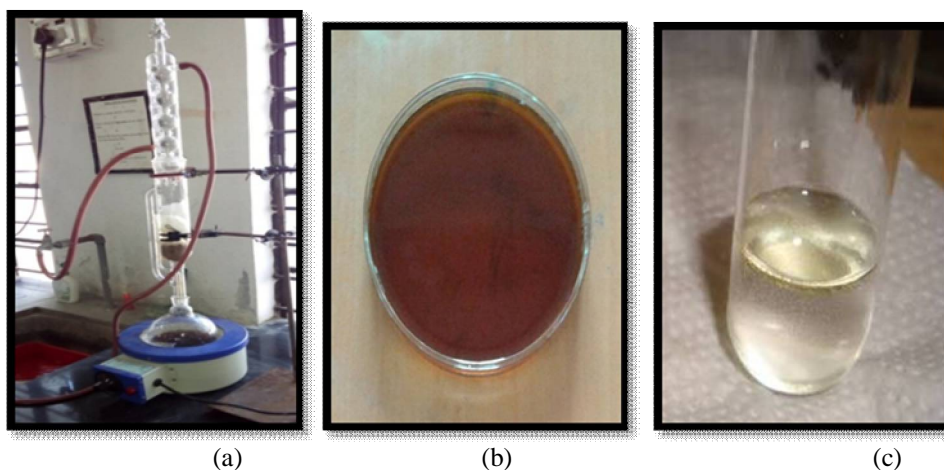


Fig. 1 Extraction - (a) shows the Extraction of *Punica garanatum* peel using Soxhlet, (b) Image of Methanolic extract of peel obtained after Soxhlet extraction and (c) Stock solution of Ascorbic acid (Reference).

Ascorbic acid was taken as reference. Stock solution was made by dissolving 0.1gm of Ascorbic acid (code- 54006) in 5ml of distilled water. From stock solution different concentrations (5mg/ml, 10mg/ml, 15 mg/ml, 20 mg/ml, and 25 mg/ml) were prepared. These concentrations were further assisted for testing scavenging activity [1].

Assessment of antioxidant activity of *Punica granatum* peel-

Percentage of antioxidant activity of *Punica granatum* peel (methanolic and Ethanolic extract) and Ascorbic acid were carried out by using two different methods as DPPH (2,2 diphenyl-1-picrylhydrazyl) method and TBA (Thiobarbituric acid) method were carried out in triplet.

2, 2 diphenyl-1-picrylhydrazyl [DPPH] assay-

In DPPH assay 1ml of 0.1mM Ethanolic DPPH solution was mixed with 3ml of different concentration (5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml, and 25mg/ml) of respective extract (Methanolic peel extract, Ethanolic peel extract and aqueous Ascorbic acid). The mixture was vigorously shaken and incubated at room temperature for 30minutes followed by taking absorbance at 517nm on UV-VIS Spectrophotometer (BL198 ELico). Control was made up of 1ml respective solvent (Methanol, Ethanol and distilled water) with 1ml of Ethanolic 0.1mM DPPH solution. Pure solvent (Methanol, Ethanol and distilled water) taken as a blank [1].

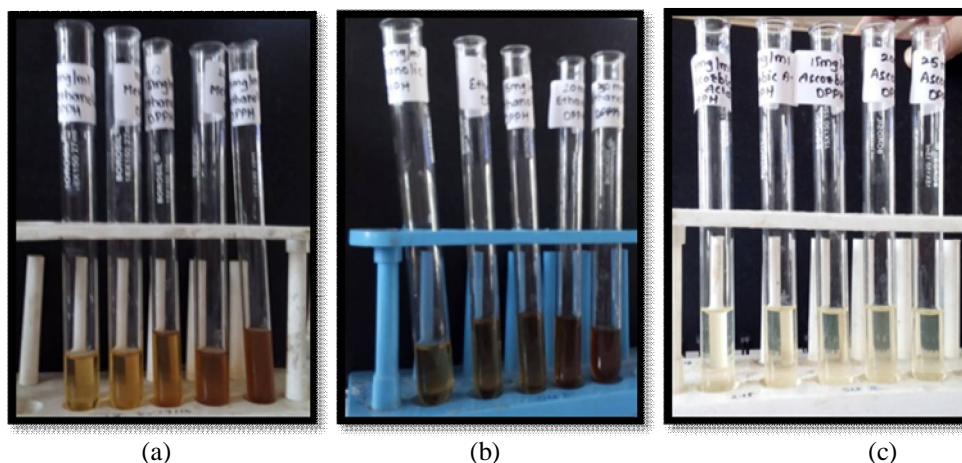


Fig. 2 DPPH Assay- (a) Image of DPPH Assay of Methanolic peel extract pomegranate, (b) It gives DPPH Assay of Ethanolic peel extract of pomegranate, (c) DPPH Assay of Reference (Ascorbic Acid).

Thiobarbituric acid [TBA] assay-

In TBA assay 1ml 20% TCA with 2ml aqueous Thiobarbituric acid (TBA) mixed with 2ml of different concentration (5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml, and 25mg/ml) respective extract (Methanolic peel extract, Ethanolic peel extract and aqueous Ascorbic acid) and kept in boiling water bath for 10minutes followed by centrifugation at 3000rpm for 20minutes, absorbance of Supernatant taken at 532nm on UV-VIS Spectrophotometer (BL198 ELico). Control was made up of 1ml respective solvent (Methanol, Ethanol and distilled water) with 1ml of 20% TCA and 2ml of aqueous TBA solution and pure solvent (Methanol, Ethanol and distilled water) taken as a blank [5].

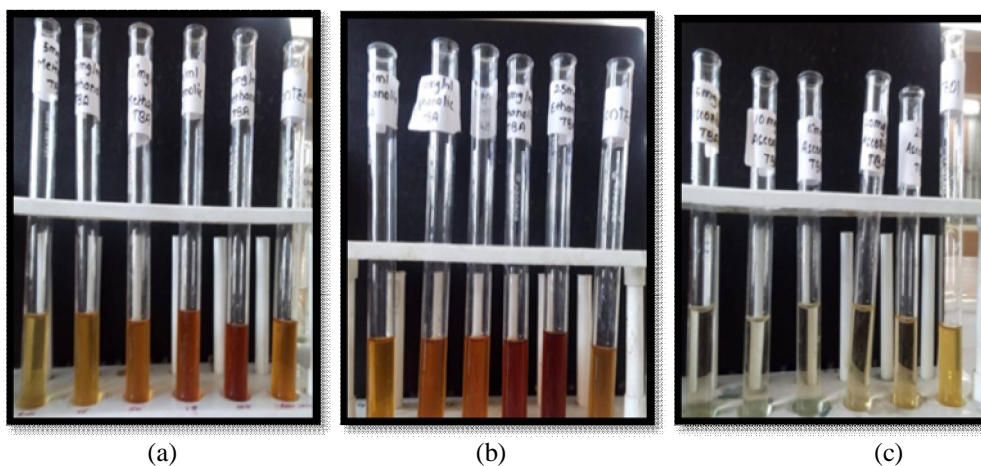


Fig. 3 TBA Assay- (a) TBA Assay of Methanolic peel extract pomegranate, (b) TBA Assay of Ethanolic peel extract of pomegranate, (c) TBA Assay of Reference (Ascorbic Acid).

**Statistical analysis of the obtained data-
Percentage scavenging activity**

Percent inhibition was calculated by following formula-

$$\text{Scavenging effect (\%)} \text{ or Percent inhibition} = \frac{A_2 - A_1}{A_2} \times 100$$

Where A_2 was the Absorbance of control reaction and A_1 was the Absorbance in presence of test or standard sample [26].

IV. RESULT AND DISCUSSION

Some evidence suggests that the biological action of the polyphenols possess antioxidant activity. In investigation, phytochemical screening of peel of pomegranate revealed the presence of phenolics compounds [26]. Hence present study was designed to evaluate the antioxidant activity of peels. The free radicals scavenging activity determined by DPPH and TBA method and were expressed as IC_{50} value (50% inhibition concentration) with Linear Regression plot.

For the Evaluation of Antioxidant activity of *Punica granatum* Plant sample was collected from Botanical garden of Shivchhatrapati College, N-3 and Aurangabad and it was authenticated from Department of Botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad. **Accession Number is 0554.**

**Assessment of antioxidant activity of *Punica granatum* peel-
2, 2-Diphenyl-1-picrylhydrazyl [DPPH] assay-**

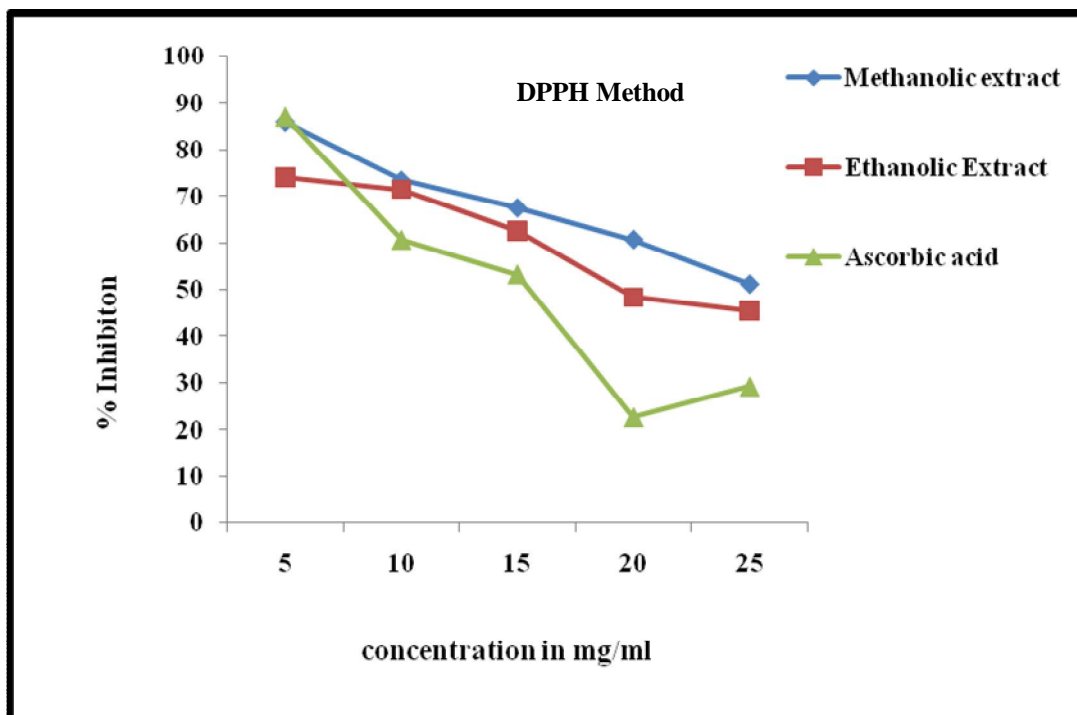
The free radical scavenging activity of pomegranate peel was determined by DPPH assay. Higher free radical scavenging activities of samples indicated by lower absorbance at 517nm [15]. With this point pomegranate peel have very potent antioxidant activity. Here different concentration (5mg/ml, 10mg/ml, 15 mg/ml, 20 mg/ml, and 25 mg/ml) of peel in organic solvent Methanol gave percent inhibition from 85.90% to 51.21% with respect to range of concentrations. Data obtained from Ethanolic extract explore that it gave percent inhibition from 74.25% to 45.52% with the range of concentration.

Ascorbic acid was taken as a reference [26]. DPPH assay of reference were showed percent inhibition in range of 87% to 22.76% with respect with concentration. Linear Regression plot indicates the percent inhibition for each concentration of Methanolic, Ethanolic extract and reference Ascorbic acid as shown in Graph no.1.

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Graph no.-1: The Linear Regression plot of DPPH assay

Linear Regression plot (Graph no. 1) clearly indicates that methanolic extract have highest free radical scavenging ability than Ethanolic extract and reference Ascorbic acid.

Ascorbic acid showed low percent inhibition from not only methanolic extract but also with ethanolic extract.

Calculations for IC_{50} Value-

IC_{50} Value is a concentration required for 50% scavenging of free radicals. EC_{50} Value gave effective concentration required for 50% inhibition [19]. There is no significant difference between IC_{50} Value and EC_{50} Value. Both are calculated by plotting slope on Linear Regression plot [19].

From valuation of obtained figure of Linear Regression plot (Graph no.1) IC_{50} Value of methanolic extract of pomegranate is 20mg/ml. similar work was done by Farag (1989), states that methanolic extract required 22mg/ml concentration for 50% inhibition [26]. Analysis of data obtained from Linear Regression plot (Graph no.1) indicated IC_{50} Value of ethanolic extract as 17mg/ml. Ascorbic acid showed IC_{50} Value at 18mg/ml from obtained statistical data.

Thiobarbituric acid [TBA] assay-

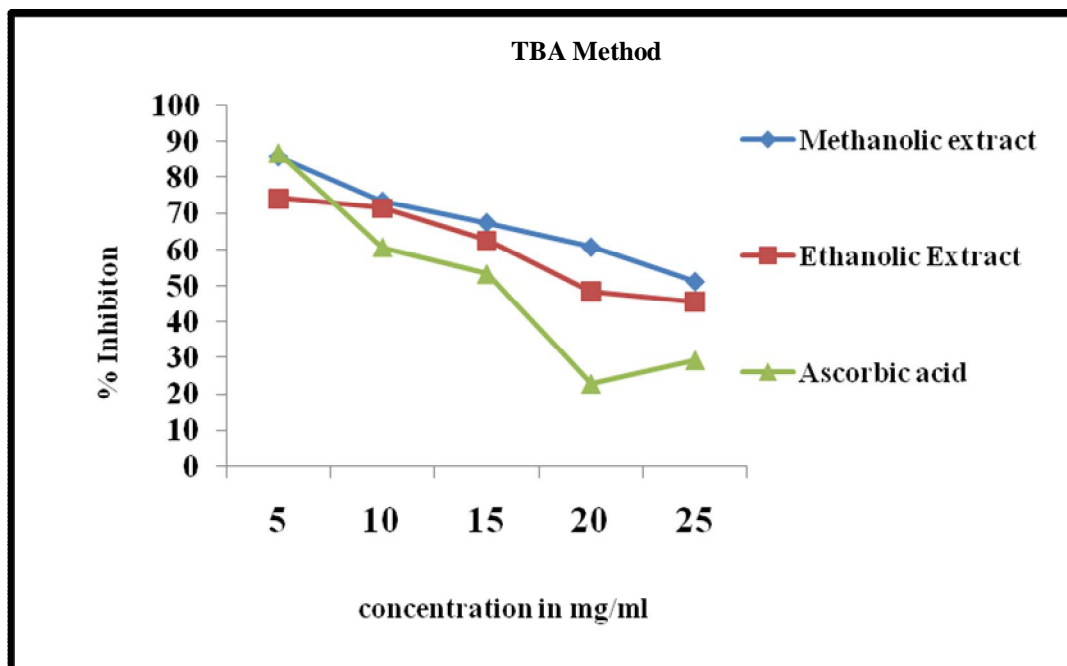
Determination of antioxidant activity by TBA assay gave different % inhibition for different concentration (5mg/ml, 10mg/ml, 15 mg/ml, 20 mg/ml, and 25 mg/ml) of peel extract and Reference. Methanolic extract showed percent inhibition from 91.27% to 57.38% with respect to concentrations. Obtained data of ethanolic extract clearly indicates percent free radical scavenging activities from 89.9% to 48.99%. Here reference (Ascorbic acid) gave % inhibition from 84.46% to 39.59% by TBA assay.

The linear Regression plot (Graph no.-2) gave detail account on percent inhibition of Methanolic, Ethanolic extract and reference Ascorbic acid.

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Graph no.-2: The Linear Regression plot of TBA assay

Point to be noted that from Linear Regression plot (Graph no.2) Ascorbic acid reference exhibit lowest scavenging activity than both Methanolic and Ethanolic extract. It also clears from graph no.2 methanolic extract showed maximum percent inhibition than ethanolic extract.

IC₂₀ Value

IC₂₀ Value of was calculated by plotting slope on graph no.2. Slope showed 30mg/ml IC₂₀ Value for methanolic peel extract. Calculations indicate that Ethanolic peel extract have IC₂₀ Value at 23mg/ml. Here Ascorbic acid which is taken as a reference gave about 19mg/ml IC₂₀ Value. Thus Methanolic peel extract showed maximum IC₂₀ Value than Ethanolic peel extract and reference by both DPPH and TBA Assay.

V. CONCLUSION

The results expressed in this study are the first information on the antioxidant activity of *Punica granatum*. Antioxidants are the substances that when present in low concentration significantly delay or reduce the oxidation of the substrate [27]. Pomegranate fruit peels are by-product of the food industry. Eighty percent methanol was a better solvent for extracting active constituents of peels. Phenolics from peel extracted exhibited a potent antioxidant activity [23]. From result it can be conclude that methanolic extract of peel showed maximum free radical scavenging activities than the ethanolic extract and reference Ascorbic acid with respect to both DPPH and TBA method.

Linear Regression Plot of different concentration, IC₂₀ Value 20mg/ml and 30mg/ml by DPPH and TBA assay indicates methanolic extract is more efficient and potent than Ethanolic extract and reference Ascorbic acid. So pomegranate peels can be employed as a potent natural antioxidant agent. Thus all over present work clearly showed the by-product of pomegranate which is generally considered as a waste and thrown away can be utilized as natural antioxidant agent in regular routine.

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