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Comparative evaluation of *in vitro* antioxidant potential of *Punica granatum L.* leaves extracts

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Abstract

A comparative evaluation of tannins, flavonoid, phenol quantification and antioxidant potential of aqueous, methanol, *n*-hexane, acetone and chloroform extracts of *Punica granatum* leaves were determined. Quantification of phenolic was carried out by the technique of Folin-Ciocalteau, using rutin as standard flavonoids were evaluated through the technique of colorimetry, tannin was measured by the difference of total phenolics and free phenolics assay procedure. Antioxidant activities were evaluated by four standards antioxidant techniques including Superoxide dismutase (SOD)-like activity, Hydrogen Peroxide (H_2O_2) scavenging activity,1, 1'-diphynyl- 2-picrylhydrazyl (DPPH) activity and Nitric oxide (NO) scavenging activity method using ascorbic acid as standard. The antioxidant activities showed that methanol extract at 500µg/mL calculated maximum DPPH inhibition activity was 78±1%, H_2O_2 scavenging activity was 90±0%, SOD-like activity was 88±0% and NO scavenging activity was 90±0%. The outcomes indicated the major antioxidant actions were carried out by the extract of methanol; the entire potential was increased in the directive of methanol> chloroform> acetone>aqueous>n-hexane extracts. The results indicate that *Punica granatum* leaves extracts to have potent antioxidant activities that would have beneficial effects on human health and methanol extracts are superior with better antioxidant potential. The leaves extracts of *Punica granatum* could be of enormous attention in the enhancement of top value-added secondary products and the appliance of functional and green substitutes in the cosmetics, food and pharma industries.

Keywords: Free Radicals, Natural antioxidant**,** Leaves extracts, Phenols, Flavonoids, Reactive oxygen species, Diseases.

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1. Introduction

Antioxidants are micronutrients that have picked up significance recently because of their capability to destroy free radicals or their activities. Reactive oxygen species (ROS) such as H_2O_2, $^{\bullet}OH$, dioxygen (singlet) and superoxide radicals are frequently synthesized as a result of organic response or from exogenous elements (Nalini and Anuradha 2015). Antioxidant substances secure man from the destructive outcome of ROS and DNA/protein damages (Javid *et al*., 2018).

Reactive oxygen species are the end products of ordinary cell digestion, having both harmful and valuable impacts on the body. The harmony between the synthesis of free radicals and the antioxidant defense of the body has vital safety plans. In the event, that there are excesses of free radicals delivered and else few antioxidants, a condition of "oxidative stress" produces which may reason unending injury to the body (Hemayet*et al*., 2012).

All single human cells protected themselves against free extreme harm by catalysts, for example, glutathione, ascorbic corrosive, catalase or mixes, superoxide dismutase (SOD) and tocopherol. Now and again these protein systems are upset by different obsessive procedures and cell reinforcement additions are vigorous to fight against oxidative injury. Nowadays, abundant reflection has been synchronized to the progression of ethno solutions with solid antiradical potential with low cytotoxic effect (Rajan*et al*., 2011).

Botanically pomegranate is known *Punica granatum* and belongs to the family Punicaceae. It is a small tree or shrub of approximately five to eight meter in height. The stem is short with evergreen leaves and fruits are packed with fleshy, juicy pulp; the fifty-two percent whole fruit is comprised of seeds. Pomegranate is both crosses and self-pollinated (Seday *et al*., 2013). Pomegranate has been planted in prehistoric times throughout the region of Asia, Europe and Africa (Vinodhini *et al.*, 2016). Pomegranates have enormous dietary values and numerous health advantages and they are utilized for diverse treatments like digestive disorders, skin disorders, throats, and coughs (Bhowmik *et al*., 2013). *Punica granatum* has many phyto compounds be active, in reality, as anti-inflammatory and antioxidant compounds, and stand for an effective protective means against radiation-induced tissue damage, microbial infections, male infertility, arthritis, diabetes, Alzheimer's disease, obesity, childhood cerebral ischemia, cardiovascular diseases and cancer (Montefusco *et al*., 2021). Leaves of Pomegranate have been used by experience based in traditional medicine. Its ointment or paste is applied as a treatment for skin injuries and arthritis. The infusion or decoction has been utilized to care for stomach disorders, urinary tract infection, and to treat sore throat and renal colic (Yu *et al*., 2021). Current research on products of *Punica granatum* has considerably augmented and profitably drained customers' concentration to medicinal and nutritional values, growing the pomegranate industry's expansion globally. *Punica granatum* is one of the main significant marketable produce crops in industrial food processing and fresh consumption. Furthermore, *Punica granatum* leaves, seeds and peel extracts are utilized for therapeutic purposes (Chen *et al*., 2022).

Considering the above scientific and research-based facts, the present design study is reasonably defensible. In actuality, the pomegranates world production, the limited efforts prepared to time to value-added the secondary products (leaves), the research still needed regarding the utilization of pomegranate leaves powders for photochemical extraction (aqueous and organic solvents) and its antioxidant potential to validate additional study to investigate the theme. The results investigated to date in the technical text to a great extent endorse research in this part, to give additional comprehensive information about the real advantages to be derived in terms of best solvents extraction system and antioxidants model potential approach is adopted. This aspect represents the novelty of the current paper.

Consequently, the novelty of the current study work is based on the sustainable and mass disposal of the leaves of the pomegranate industries and the production of high added value bioactive compounds having a high potential for antioxidants. A further novelty that our present work provides is feature extraction task is done using which solvent is best for photochemical extraction and antioxidant potential.

Thus, this study aims to carry out the quantification of tannins, flavonoids, phenol and antioxidant potential of n-hexane, aqueous, methanol, chloroform and acetone extract of pomegranate leaves. The data and research would provide useful information and reference for researchers, herbal medicines practitioners, and commercial utilization of pomegranate leaves in the food, pharmacy, cosmetics, agriculture and poultry industries.

2. Materials and Methods

2.1 Punica granatum L. Leaves Collection and Processing

The *Punica granatum* L. leaves were collected from the Medicinal Botanical Garden of Pakistan Council of Scientific and Industrial Research (PCSIR) Peshawar-Pakistan. The procured leaves were dehydrated in an Air Cabinet Dryer (England) at 35 °C for three days. The dried leaves were ground to make a powder in a Waring® Commercial Laboratory Blender USA.

2.2 Solvent Extraction

Hundred-gram (100g) *Punica granatum* L. leaves powder was soaked in one liter (01L) of methanol, *n*-hexane, acetone and chloroform independently at room temperature for 07 days. The blended mixer was filtered through filter paper Whatman® No. 1 (England). After every extraction process, the remaining extracted cake was again soaked in each solvent for 07 days at room temperature. Through filter paper Whatman® No. 1 (England) the extracts were filtered. These processes were repeated one more time. For water extraction, a 100-gram dried sample was extracted using one litter (01 L) of boiling H_2O for fifteen minutes. After cooling the extract was filtered through filter paper Whatman® No. 1 (England). The extracted mixer cake was again extracted using one litter (01L) of boiling H_2O for fifteen minutes. After cooling the extract was filtered through Whatman® No. 1 (England) filter paper. This process was repeated one time more. Each solvent was evaporated through a rotating evaporator (Buchi Rotavapor R-200, Switzerland, Buchi Heating Bath B-490) to obtain paste-type concentrate. To acquire a dry extract, each paste concentrate was kept in a large crucible on hot pale at 40°C. The dry extract is kept in an amber glass bottle and stored at - 20 °C till being utilized.

2.3 Phenol Determination

The *Punica granatum L.* leaves extract total phenolics content was calculated using Folin- Ciocalteau reagent by the technique. Take separately about 20 µg of extract and it was diluted with distilled water up to 1 mL. Then diluted Folins-phenol reagent 500 µL (1: 1 ratio with H_2O) and twenty percent $Na_2 CO_3$ 2. 5 mL were added. The blend was well shaken for forty minutes and incubated in a dark cabinet for colour development. Afterwards, incubation, at 725 nm absorbance was recorded. A gallic acid calibration curve was created and linearity was got in the 10 - 50 µg/mL range. The total phenolic content in the leaves was calculated as mg of gallic acid (Jamuna *et al*., 2014).

2.4 Flavonoid Determination

The flavonoid quantification was carried out the defined technique. A total of one m L of *Punica granatum L.* leaves extracts were mixed with distilled H_2O 200 µL distinctly tracked by the addition of 150 µL $NaNO_2$ solution (5%). This blend was kept for five minutes and then added $AlCl_3$ solution (10%)150 µL and allowed for 06 minutes to stand. Formerly added 4% of NaOH solution 2 mL and marked up to five mL with condensed H_2O. The blend was well shaken and leftward at room temperature for fifteen minutes. At 510 nm the absorbance was calculated. The occurrence of flavonoid content was displayed with the arrival of pink colour. Based on using a standard curve the flavonoids measurement was specified as equivalent to rutin mg RE/g on a dry weight extract (Jamuna *et al*., 2014).

2.5 Tannins Determination

Tannin's content of *Punica granatum L.* leaves extracts was assessed by the technique of Jamuna *et al*., 2014. A total extract of 500 µL was booked separately in a test tube and 500 µL distilled H_2O was added and polyvinyl polypyrrolidone (100 mg). This blend was incubated for four hours at 4 °C. Then centrifuged the sample for five minutes at 5000 r/min and the supernatant 20 µL was picked. With the polyvinyl polypyrrolidone, the tannins would have been sideways precipitated, so this upper layer has demonstrated phenolics free tannins. At 725 nm the upper layer phenol was calculated and on the dry substance basis, the outcome was known as free phenolics. So based on the following formula, the tannin was measured:

Tannins (mg GAE/g matter) = Total phenol (mg GAE/g matter) - Phenol Free (mg GAE/g matter)

2.6 In Vitro Antioxidant Capacities

2.6.1 DPPH Scavenging Procedure

The individual amount of each standard/fixation (500, 400, 300, 200 and 100 µg/mL) were arranged in 95% methanol and mixed this blended preparation of one mL with one mL methanol (95%) preparation of 0.004% DPPH and additionally with standard Vitamin C preparation autonomously. Methanol (95%) and DPPH with 1:1 mL were applied as clear. These preparations were reserved in a dark room for twenty minutes and absorbance was calculated at 517 nm by U-2900 Hitachi Tokyo Japan UV/VIS Spectrophotometer (Goyal *et al*., 2013). Using the flowing equation 1 the scavenging inhibition (I %) was determined.

$$\text{Scavenging Percent Inhibition} = (A - B)/A \text{ X}100 \quad (1)$$

where A is the Absorbance of Control and B is the Absorbance of Tests.

2.6.2 Hydrogen Peroxide Scavenging Activity

A 2 mM aliquot of Hydrogen peroxide and each concentrate/standard at diverse complexes (500, 400, 300, 200 and 100 µg/mL) were blended (1:0.6 v/v) and at room temperature brooded for ten minutes. Later appearing darkly menacing, optical density was taken at 230 nm on Hitachi Tokyo Japan (U-2900) UV/VIS Spectrophotometer in contradiction of a pure preparation containing buffer phosphate absent H_2O_2. The amount of hunting act of $H_2O_2$was determined by applying the No. 1 equation (Goyal *et al*., 2013).

2.6.3 Superoxide Dismutase (SOD)-like Activity

The mixture reaction comprising 0.2 mL of every standard/concentrate (100, 200, 300, 400 and 500 μ g/mL), tris support (EDTA 10 mM, pH 8.5, 50 mMtris) 2.6 mL and M pyrogallol 0.2 mL of 7.2 m, and for ten minutes at 25°C hatched. Later addition to 1 N HCl (0.1 mL), the reacted pyrogallol was calculated on Hitachi Tokyo Japan (U-2900) UV/VIS Spectrophotometer at 420 nm. The SOD-like activities were connected to the reduction degree of optical density as designated by the associated state (Jong *et al*., 2010).

SOD-like activity %= [1− (Absorbance of Test/ Absorbance of Control)] ×100.

2.6.4 Nitric Oxide Scavenging Activity

One milliliter (01 mL) of each concentrate/standard (500, 400, 300, 200 and 100 µg/mL) was fused with 1 of the rejoinder preparations comprising 10 mmol/L of sodium nitroprusside in20 mmol/L, pH 7.4 phosphate cradle. At 37 °C brooding for one hour was pursued, and 0.5 milliliter of the sample taken was then blended with 0.5 milliliter Griess reagent. U-2900 Hitachi Tokyo Japan Spectrophotometer was used to estimate the absorbance at 540 nm (Rop *et al*., 2014). The scavenging activity of Nitric oxide was determined by equation 1.

3. Statistics

Every trial was run three times and the results were expressed as average ± standard deviation. An assessment of $p<0.05$ was calculated as significant.

4. Results

4.1 Phenols, Flavonoids and Tannins

Entire phenol, flavonoids and tannins content are shown in Table 1. The highest concentrations of phytochemicals were found in methanol extract as compared to the rest of the extract.

Table 1. Bioactive Components in *Punica granatum* L. Leaves Extracts.

Extracts	Total phenolic content	Total flavonoids	Tannins
n-Hexane	05±1	10±1	44±1
Methanol	14±1	26±1	100±2
Aqueous	07±1	12±1	60±1
Acetone	09±1	16±1	80±1
Chloroform	12±1	18±1	90±1

Results value expressed average ± SD (n = 3). Tannin quantity (as catechin equivalents in mg CE/g). Total phenolic quantity (as mg gallic acid equivalents (GAE)/g of extract). Total flavonoids (as mg rutin equivalents (RE)/g of extract).

4.2 DPPH Antioxidant Activity

Punica granatum L. scavenging activity of leaves extracts in terms of DPPH is shown in Figure 1. Altered concentrations (500, 400, 300, 200 and 100 µg/mL) of leaves were utilized to govern the antioxidant potential. From the above-mentioned concentrations, the concentration of 500 µg/ml showed the highest scavenging action 78±1% in methanol extract showing good antioxidant activity as linked to the ascorbic acid standard showed 79±2%. The results showed that the DPPH activities were in a dose-dependent manner.

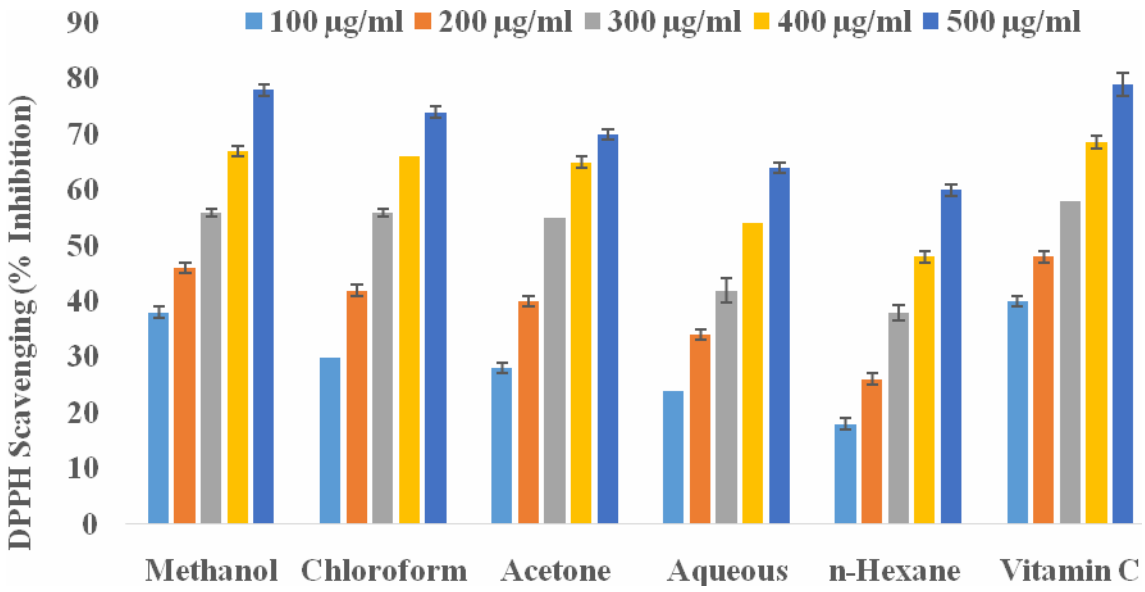

Fig. 1. DPPH Scavenging (% Inhibition) Activity of *Punica granatum* L. Leaves Extracts

4.3 H_2O_2 Scavenging Assay

The *Punica granatum L.* leaves antioxidant was evaluated by the well-known procedure by way of a Hydrogen peroxide hunting action. Since the results, it displays that the Hydrogen peroxide hunting activity of the extracts is noteworthy corresponding to that of the Vitamin C (Figure 2). The antioxidant properties of n-hexane, aqueous, methanol, acetone and chloroform extracts of *Punica granatum L.* leaves at 05 (five) different concentrations (500, 400, 300, 200 and 100 µg/mL) on H_2O_2 were stated alike % inhibition and likened to the Vitamin C standard. Leaves extracts were talented to counteract Hydrogen peroxide in diverse

quantities. The outcomes exposed for methanol extract at a maximum dosage of 500 µg/mL were 90±0%. The results showed that the greater the concentration increased the % inhibition.

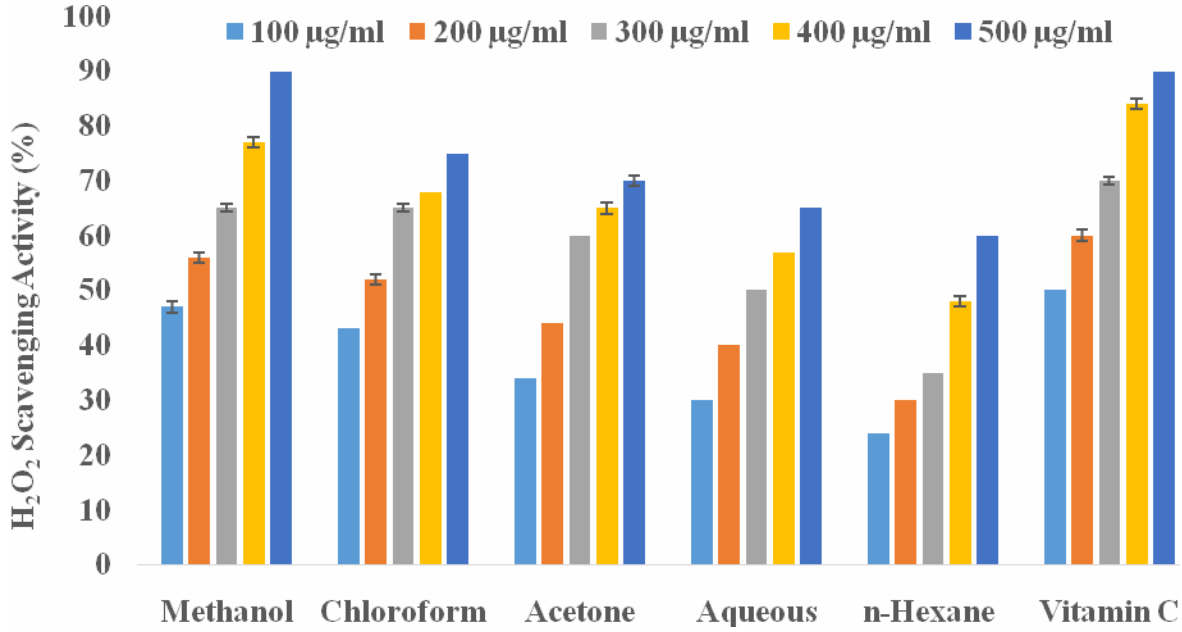

Fig.2. H_2O_2 Scavenging Activity (%) of *Punica granatum* L. Leaves Extracts

4.4 Superoxide Radical Scavenger Potential

The *Punica granatum L.* leaves extract antioxidant potential in terms of superoxide radical hunting and were compared to the known amount of Vitamin C in a range from 100 to 500 μ g/mL as accessible in Figure 3. The outcomes had proposed that the maximum rate (88±0%) at 500 μ g/ml goes to the methanol extract (Figure 3). In an amount contingent style, overall, the extracts had hunting potential on the superoxide radicals. However, once connected to Vitamin C, the superoxide hunting abilities of the extracts were found to be significantly lesser. The graph showed that increasing the concentration, increased the % rate of SOD scavenging potential.

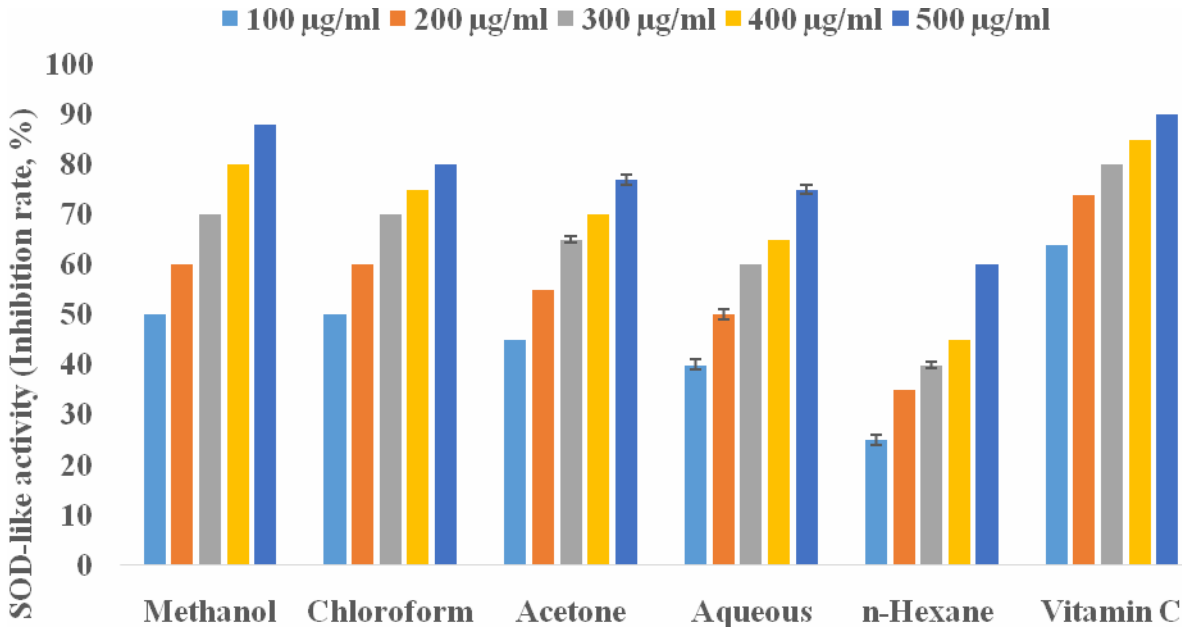

Fig. 3. SOD-like activity (Inhibition rate %) of *Punica granatum* L. Leaves Extracts

4.5 NO Radical Scavenging Activity

The scavenging capacities of NO by *Punica granatum L.* crude extract leaves were augmented in quantity-reliant means. However, a higher inhibition (90±0%) was reached at a 500 µg/mL maximum concentration. Figure 4 displays a considerable reduction in the NO radical due to the hunting capability of extract. The results depicted that NO scavenging potential is dose-dependent manner.

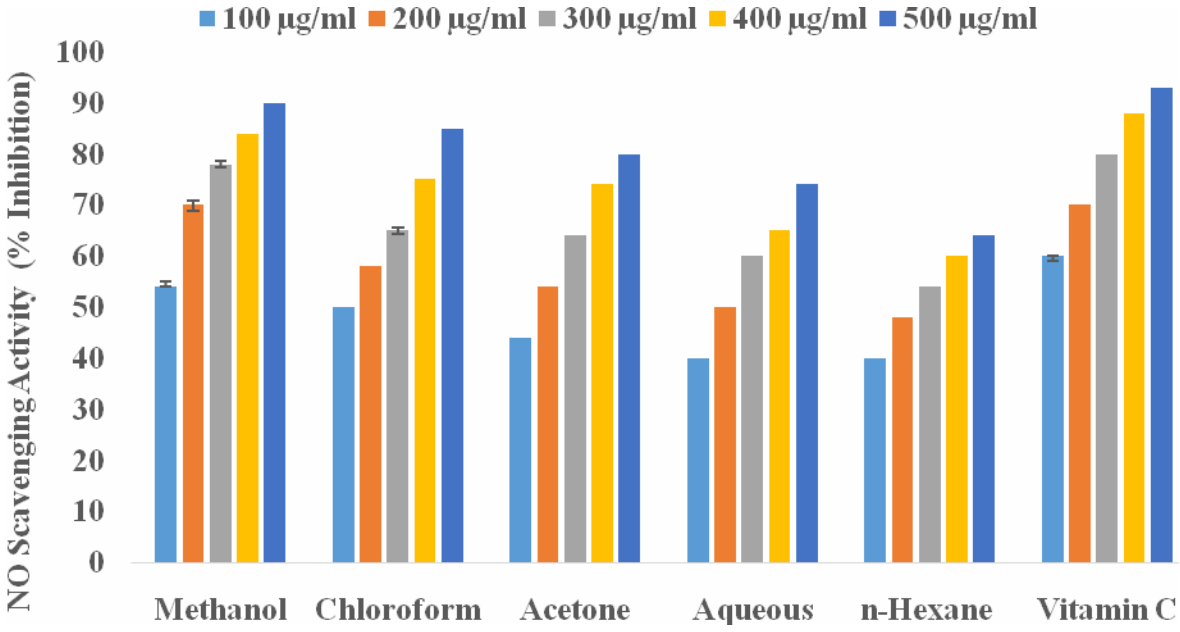

Fig. 4. Nitric Oxide Scavenging Activity of *Punica granatum* L. Leaves Extracts

5. Discussion

5.1 Phenols, Flavonoids and Tannins

The alcoholic and aqueous concentrate of *Punica granatum* natural product skin yielded 176±5.29 and 122.33±6.42 mg/g gallic corrosive proportional phenolic content, 81.33±6.1 and 135.33±8.08 mg/g quercetin proportionate flavonoid and 114.23±12.16 and 81.66±3.51 mg/g tannic corrosive equal tannins separately (Rajan*et al*., 2011). In our study, this phytochemical concentration was in low quantity, because of the parts differences and geographical location. Pomegranate leaves total polyphenol (GAE mg/g dry weight) in aqueous and methanol extract were 9.85 and 14.78 respectively, while total flavonoids (RE mg/g dry weight) in aqueous and methanol 12.77 and 26.08 respectively and similarly hydrolysable tannins (TAE mg/g dry weight) values in aqueous and methanol 64.40 and 128.02 respectively (Elfalleh *et al*., 2012). Our study showed that flavonoids in methanol and water extract have similar concentrations, tannins were in low concentration and phenols have a slightly high concentration in methanol and low concentration in the aqueous extract as compared to Elfalleh *et al*., 2012 study. The study by Lamiae *et al*., 2021 reported that pomegranate peel extract was 283.86 mg GAE/g dw and 185.37 mg QE/g dw polyphenols and flavonoids respectively, while aril extract of pomegranate was polyphenols and flavonoids 166.90 mg GAE/g dw and 57.43 mg QE/g dw respectively. The studies showed that leaves infusion of pomegranate reported that tannins, flavonoids and total phenols contents were 24.20 mg EC/g DW, 55.84 mg RU/g DW and 133.47 mg/g Gallic acid equivalent respectively (Yu *et al*., 2021). Peels and seeds of pomegranate extracts were higher in total phenolics (0.16–0.73 mg GAE/mg extract) from conventional extraction, while higher contents of total flavonoids (0.019–0.068 mg CATE/mg extract) obtained from sonication-assisted extraction (Campos *et al*., 2022). Total phenolic contents and total flavonoids contents of Punica granatum fruits from Jhelum valley Pakistan were 1.94 mg/g of Gallic acid equivalent and 4.30 mg/g of Rutin hydrate equivalent respectively (Ismat *et al*., 2022). But, the variation in bioactive contents could be depended on the environmental conditions, experimental procedures, extraction protocols, cultivars studies and phytochemical composition (Lamiae *et al*., 2021).

5.2 DPPH Scavenging Assay

Scavenging assay in terms of DPPH of pomegranate leaves extracts variance in amongst the extracts was typically attributed to the occurrence of numerous plant compounds. DPPH radical scavenging activity was determined with rising concentrations of pomegranate peel phenolic extract (PPPE) and pomegranate aril phenolic extract (PAPE. The results observed that both PPPE and

PAPE display noteworthy antioxidant potential in a dose-dependent way. Though, PPPE reported 1.73-fold elevated potential as compared to PAPE, seeing that by the values of IC_{50} which were IC_{50} = 12.49 µg/mL for PPPE and IC_{50} = 21.58 µg/mL for PAPE (Lamiae et al., 2021). Extracts of peels and seeds of pomegranate antioxidant activity showed that DPPH radical scavenging activity was IC_{50}, 0.01–0.20 mg/mL (Campos et al., 2022). Pomegranate Peel hydro alcoholic extract DPPH assay testing samples at 3 mg/ml was 92% (Francesca et al., 2022). DPPH antioxidant activity of pomegranate fruit at 6.42 mg/g was 71% (Ismat et al., 2022). These dissimilarities when compared to our studies could be justified by the pomegranate varieties, country origin, climate, growing conditions, extraction methods and the analytical methods used.

5.3 Hydrogen Peroxide Scavenging Activity

The compounds which were present in the extracts; show worthy electron givers and may reduction of H_2O_2 to H_2O. The cell inside, when H_2O_2 enter into membrane cell and it can react with iron and copper ions to practice OH, this might be the foundation of numerous deadly outcomes. Rare enzymes have a weak oxidizing substance that straight deactivates the H_2O_2, regularly by oxidation of vital thiol ($^-$SH) clusters. Consequently, eliminating hydrogen peroxide and O^{2-} is tremendously vital for food system safety (Nakayama *et al*., 1993). However herbal plants can reduce hydrogen peroxide into H_2O the substances could give an electron. The donation of electron ability and antiradical activity is proportional directly (Vinodhini *et al*., 2016). The study observed that pomegranate peel phenolic extract (PPPE) has a superior potential to hunt hydrogen peroxide free radicals (IC_{50} = 19.96 µg/mL) as compared to PAPE (IC_{50} = 37.06 µg/mL. In expressions of antioxidant activity (AA%) at the same concentration, ascorbate displayed the maximum AA% followed by PPPE and then PAPE. At 200 ug/mL, the AA% was 98.21%, 87.8%, and 64.12%, respectively, for ascorbic acid, PPPE, and PAPE. Hydrogen peroxide antioxidant activity was probable influenced by the quantification of phenolic molecules. Because phenol molecules are strong chain-breaking antioxidants, they could accelerate the decomposition of Hydrogen peroxide to water and oxygen (Lamiae *et al*., 2021). It is so naturally useful for tissues to administer the amount of hydrogen peroxide that is allowable to gather. The ability of leaves pomegranate to feed hydrogen peroxide might be known to the existence of tannins and phenols, which could share electrons, thus neutralizing it into H_2O.

5.4 Superoxide Radical Scavenging Activity

Superoxide is a sensitive O (oxygen) agent, superoxide participates sure damaging abilities that could be forced to the DNA and tissues and afterwards calls many ailments. Consequently, a suggestion has to endure recognized to quantify the comparative stoppage potential of the antiradical abstracts to hunt the superoxide compound (Esmaeili *et al*., 2015). *Punica granatum* is a wealthy resource of numerous tannins and phenolic substances which give an extremely high antioxidant potential. These bioactive compounds can decrease oxidative stress in unhealthy cells giving anti-invasive, pro-apoptotic effects and antiproliferative as revealed in numerous in vivo and in vitro findings. As a result, the antioxidant potential qualities of *Punica granatum* extract have a huge potential as medicinal abilities are helpful for nutraceutical industries (Francesca *et al*., 2022).

The study of bioactive compounds in the varieties (Ganesh and Kesar) exposed that, the phenolics quantification ranged from 49.3 to 602 mg TAE/g and the flavonoids content ranged from 0.27 to 18.8 mg RE/g. The high amount of flavonoids and phenolics was calculated in the extracts of the rind as compared to the arils and leaves in both cultivars. From a contrasting point of view, the free radical scavenging activity determined by diverse antioxidant protocols displayed different levels of antioxidant activity in diverse herbs parts of the varieties and was observed to be maximum in aril and rind extracts as compared to leaves. Along with the solvents ethanol and methanol possessed maximum yield extraction than that of H_2O and acetone. Total flavonoids and phenolics were considerably dissimilar with ethanol and methanol being the mainly competent solvent, while acetone being the slightest capable one (Kolar et al., 2021).

5.5 NO Radical Hunting Activity

Nitric oxide is a powerful pleiotropic inhibitor of a biological system such as regulation of tissue refereed poisonousness, platelet clump inhibition, smooth muscle relaxation and signaling of neuronal. Nitric oxide is a free radical and diffusible, which holds various properties like as an effector piece in organization together with anti-tumor activities, antimicrobial potential, vasodilatation and neuronal messenger (Hagerman *et al*., 1998). Yet, nitric oxide excess production is correlated with different kinds of diseases (Lalenti *et al*, 1992).

Aqueous extract showed 65.44±4.2% inhibition of *Punica granatum* fruit peel showing at 100µg/mL NO scavenging potential and the alcoholic extract was 73.03±0.87% (Rajan *et al*., 2011). Nitric Oxide displays an active part in numerous inflammatory developments. Progressive stages of these radicals are fatal to organs and promote vascular letdown. Overexcited concentration appearance of NO radical is connected with much ulcerative colitis and carcinoma. Many diseases like inflammation, cancer and other pathological circumstances are concerned with free radical NO (Rajan *et al*., 2011). The inhibition quantity of the NO free radicals was initiated to be increased by augmenting the quantity of the *Punica granatum L.* extracts, and this shows that the *Punica granatum L.* might contain substances talented at preventing the synthesis of NO and offers technical proof for the practice of *Punica granatum L.* infusion to treat many diseases. Free radical scavenging action alterations are because of the ingredient quantity and fineness of the phenols in the various fragment of the extract. The phenolics' antioxidant action is generally because of their redox properties which spot them turn as metallic chelating potential, singlet oxygen quenchers, hydrogen contributors and reducing substance (Rice-Evans *et al*., 1996). Collaboration among the antioxidants in the blend made the antioxidant potential not

only reliant on the quantity but then similarly on the assemblage and the interface among the antioxidants (Djeridane*et al*., 2006). Plants produced numerous kinds of antiradical substances like phenolic agents; in herbs, they stop oxidative stress. After these herbs are utilized as nutrition by man, they protect persons from sicknesses (Javid *et al*., 2018). In addition, the comparative potential of methanol extracts was considered best as compared to the rest of the extracts. Really, because of various antiradical abilities of diverse substances, the antiradical potential of infusion powerfully dependents on solvent extraction (Elfalleh *et al*., 2012).

6. Conclusion

The current study concluded that it could be well-known that *Punica granatum* leaves extract marked polyphenol concentration and confirm high anti-radical abilities. The curative potential against a diversity of illnesses might be exploited, especially those connected with oxidative stress. The investigational results depict that the extracts of leaves are credible to have the ability of free radicals hunting and therefore can be combined into medicine, cosmetics and food for healthy skin, healthiness and anti-ageing products. Pomegranate leaves crude extracts could carry benefits to the health of humans and might be added to the pharmaceutical and food industries. Generally, the current research offers better information on numerous properties contributing to the healthiness profit and advertising of pomegranate leaves that can be utilized to help growers and breeders to react to industrial and consumer preferences, as well as give confidence in the expansion of agriculture wastes strategies for the utilization of pomegranate leaves as value-added ingredients or nutraceutical for custom-tailored supplemented foods.

Though, knowing the broad variability originates, additional studies on the diverse regions, varieties and leaves maturity stages from each genotype pomegranate is necessary to finest utilize their food, agro-industrial abilities and boost sustainability by falling biomass wastes from the fruits and vegetables food-manufacture systems. Future research may perhaps judge its appliance in clinical and preclinical experiments to enhance the use of its precise curative profile measures concerning the human beings' health benefits. More prospective studies could be designed to separate the therapeutic phytochemical free radical scavenging molecules on an industrial and pilot scale, produced it on a commercial scale, and utilize it in the food, pharmaceutical, cosmetics, poultry and agriculture industries to help the third world countries of the regions, especially the poor countries. Limitations of this current research study comprised lack different extraction methods, small sample size and short duration.

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