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PHARMACOGNOSTIC INVESTIGATION, ANTIOXIDANT ACTIVITY, ANTICANCER ACTIVITY AND HPLC ANALYSIS OF *PUNICA GRANATUM* EXTRACT

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Abstract

This article offers an extensive examination of Punica Granatum, a small tree cultivated in various regions such as Iran, India, China, Japan, Russia, and the USA. It has been widely utilized in traditional medicine for treating diverse health issues. The study aimed to assess Pharmacognostic parameters, phytochemical constituents, antioxidant activity, and anticancer potential across different parts of the Punica Granatum plant. Initial observations involved macroscopic characterization of fresh leaves and stems, noting characteristics like color, odor, taste, shape, and size. The extractive values were determined using various solvents, and ash values were measured to gauge plant material quality. Phytochemical analysis revealed significant levels of bioactive constituents like flavonoids, phenolic compounds, tannins, and alkaloids, emphasizing the therapeutic potential of Punica Granatum. Antioxidant activity was assessed through multiple assays, demonstrating the extracts' ability to combat oxidative stress and prevent degenerative diseases. Furthermore, the anticancer potential against various cancer cell lines was examined, indicating promising inhibitory effects on pancreatic, breast, colon, larynx, and liver cancer cells. High-Performance Liquid Chromatography (HPLC) analysis of methanolic extracts from leaves, stem, and fruit peel revealed the presence of potent compounds, further supporting the plant's medicinal value and providing crucial insights for future research and drug development. These findings underscore Punica Granatum's significance as a rich source of bioactive compounds with notable therapeutic effects, calling for further investigation to harness its medicinal properties for novel drug and treatment development.

Keywords: Antioxidant Activity, HPLC Analysis, Pharmacognostic Investigation, Phytochemical Screening, Punica Granatum (L.).

1. INTRODUCTION

Plants have played a fundamental role in traditional medicine across the globe for centuries, offering mankind a continuous stream of new remedies. India, known for its rich culture and extensive natural biodiversity, offers a once-in-a-lifetime chance for drug discovery researchers.[1] Before undertaking any testing, the World Health Organisation (WHO) emphasizes the necessity of verifying the authenticity and purity of medicinal plants by macroscopic and microscopic description.[2] Pharmacognosy encompasses various disciplines, including drug identification, origin, morphology, microscopic studies, assessment of drug quality, chemical compositions, therapeutic effects, and more.[3]

Punica Granatum (L.), a tiny tree that may provide health advantages, thrives in countries such as India, Iran, China, Japan, Russia, and the USA, as well as other regions in the East. Traditional medicine has utilized different parts of the tree, including the pericarp, roots, bark, and juice, for treating various conditions such as colitis, diarrhea, dysentery, leucorrhea, menorrhagia, oxyariasis, paralysis, rectocele, and headaches.[4] The fruit is known for its antioxidant and cancer-preventive

properties, while the stem exhibits antibacterial activity. Notably, Punica Granatum leaf has shown promising anticandidal and antibacterial effects, and the flowers have demonstrated hepatoprotective properties.[5]

Degenerative diseases, including cancer, can be triggered by free radicals. Consequently, there is a growing interest in identifying medicinal plants with potent antioxidant activity to mitigate oxidative stress and tissue damage. One such plant is Punica Granatum. This study investigates the antioxidant activity of the plant's methanolic extract using various assays.[6]

Cancer is a major global health concern, demanding several health concerns throughout the world. The limits of conventional cancer therapies, such as chemotherapy, radiation, surgery, and immunosuppression, have resulted in high rates of death and morbidity. As a result, there is an urgent need for effective and safe therapeutic techniques.[7] Herbal therapies in dietary supplements and botanical preparations have resurfaced as promising possibilities. Medicinal plants include a diverse range of distinct chemical compounds with medicinal potential, and many have been utilized as folk medicines for centuries.[8]

HPLC, a flexible and extensively used technology, is critical in the isolation of natural compounds. In phytochemical and analytical chemistry, this chromatographic approach is used to detect, measure, and purify specific components within a mixture.[9] Given its resolving strength and capacity to swiftly analyze multi-component samples, HPLC is becoming more popular for fingerprinting research in the quality control of herbal plants.[10] HPLC has been used by many researchers to characterize and quantify secondary metabolites in plant extracts, including phenol compounds, steroids, flavonoids, and alkaloids.[11]

The current study intends to analyze numerous pharmacognostic characteristics of the Punica Granatum plant, including macroscopy, physicochemical properties, phytochemical contents, antioxidant activity, anticancer potential, and HPLC analysis.

2. METHOD AND MATERIAL

2.1 Plant Material

In October 2021, Punica Granatum was introduced to the herbal garden located at the Noida Institute of Engineering and Technology (Pharmacy Institute) in Greater Noida, India. The leaves, stems, and fruit peels were carefully cleansed with water and left to air-dry in the shade for 4-5 days. Following this, the dried components of the plant were finely ground using a grinder and subsequently stored in a well-ventilated container under cold and dry conditions to prevent any potential contamination. A voucher plant specimen (NIET/Pharmacy Institute/R&D/16) was maintained and authenticated by the Botanical Garden of the Indian Republic in Noida, India.

2.2 Extraction

Using the Soxhlet apparatus, 100 gm of dried Punica Granatum components powder was extracted for each thimble. The solvent used for extraction was methanol. 800 cc of solvent was used for the extraction, and each solvent was used for 15 hours. Before being used in the tests, each crude extract was filtered and dried at 60°C in a rotatory evaporator. It was then stored in a dry, cool place.[12]

2.3 Macroscopy

The leaf and stem components were separated from the rest and underwent a thorough cleaning process, followed by rinsing, drying, and subsequent storage for future utilization. Observations of the fresh leaves and stems included a detailed examination of their macroscopic characteristics, encompassing aspects such as colour, scent, texture, size, shape, surface, veins, presence or absence of petiole, apex, border, base, lamina, and overall texture, among other features.[13]

2.4 Determination of Extractive Values

Various solvents, including distilled water, petroleum ether, chloroform, methanol, ethyl acetate, and benzene, were employed to determine extract values due to the diverse chemical composition of the drug. Five grams of powdered material underwent continuous Soxhlet extraction using 100 milliliters of each solvent. It is essential to analyze the chemical composition whenever assessing the preventive potential of a medicinal substance. Following extraction, the resulting extracts were dried in vacuum desiccators and concentrated using a rotary evaporator. Subsequently, the anticipated values were computed by dividing the weight of the air-dried drug by the percentage weight of the solvent-soluble extract.[14]

2.7 Determination of Ash Values

Approximately 5 grams of powder were dispersed within a silicon crucible, which was previously filled and pre-heated to determine the drug's ash content. Subsequently, the crucible underwent gentle agitation to eliminate any remaining traces of carbon. Upon cooling, the crucible was weighed to determine the overall ash quantity, and the levels of sulphated, water-soluble, and acid-insoluble ash was analyzed. The reference point for calculating the percentage of total ash was based on an air-dried sample.[15]

2.8 Extract Analysis

2.8.1 Determination of total flavonoid content

The test solution consists of one milliliter of plant extract and three milliliters of sodium nitrite solution (5%), which is then allowed to incubate at room temperature for five minutes. After the incubation period, 2 mL of 1 M NaOH and 0.3 mL of 10% AlCl3 are introduced. Finally, distilled water is added to make up the remaining 10 mL, and the mixture is thoroughly blended. The absorbance is then measured at 510 nm. The total flavonoid concentration of the extract is calculated using the quercetin standard curve as a reference.[16]

2.8.2 Determination of Total Phenolic Content

A combination of 2.5 mL of 20% Na2CO3, 0.5 mL of 2 M Folin-Ciocalteu reagent, and 1 mL of plant extract was allowed to sit at room temperature for 20 minutes. The absorbance was then recorded at 725 nm. The phenol concentration was determined by referencing the pyrogallol standard curve. Subsequently, the total phenol content of the extract was calculated based on the pyrogallol standard curve.[17]

2.8.3 Determination of Tannin Content

The tannin content was determined using the Folin-Sialocteau method. Plant extracts (2 mL) were treated with a solution comprising 0.3 mL of 0.1 N FeCl3 in 0.1 N HCl (3 mL). Furthermore, 0.3 milliliters of 0.0008 M potassium ferricyanide were included as

a zero-point concentration. The absorbance of the solution was measured at 720 nm. The tannin concentration was evaluated using the standard curve.[18]

2.8.4 Determination of Alkaloid Content

To 1 mL of plant extract, 5 mL of 60% H2SO4 was added. The tubes were then combined and left at room temperature for three hours. The absorbance of the resultant solution was measured at 565 nm. The concentration of the alkaloids was determined using the atropine standard curve. [19]

2.9 Determination of Antioxidant Activity

2.9.1 DPPH Radical Scavenging Assay:

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, following Bliss' methodology, was employed to evaluate antioxidant activity. The reaction mixture consisted of various chemical concentrations (ranging from 0.5 to 2.5 µg/ml) and 100 µM DPPH in methanol. After incubating for 30 minutes at room temperature, the absorbance at 517 nm was measured, and the scavenging activity was determined as the percentage of radical reduction. Each experiment was repeated three times, and BHT was used as a chemical control.[20]

2.9.2 Nitric Oxide Scavenging Assay:

The impact of Punica Granatum methanolic extracts on nitric oxide (NO) was assessed through the nitrite detection method. Sodium nitroprusside (10 mM) dissolved in 0.5 M phosphate buffer at pH 7.4, which inherently generates NO in an aqueous environment, served as the NO source. Nitrites were formed as a result of NO reacting with oxygen to form stable molecules. Following a 60-minute incubation at 37°C, the Griess reagent (consisting of 0.1% naphthyl-ethylenediamine in water and 1% sulphanilic acid in 5% H3PO4) was applied. The same reaction mixture was utilized as a control, where the sample extracts were omitted and an equal volume of distilled water was substituted. Ascorbic acid was employed as a positive control.[21]

2.9.3 Hydrogen Peroxide Radical Scavenging Assay:

The efficacy of extracts in eliminating hydrogen peroxide (H2O2) was assessed following Navi's protocol. A 40 mM solution of hydrogen peroxide was prepared in phosphate buffer with a pH of 7.4. After a ten-minute interval, the absorbance at 230 nm was gauged to determine the quantity of hydrogen peroxide relative to an empty solution comprising phosphate buffer without hydrogen peroxide. Ascorbic acid was employed as the benchmark.[22]

2.9.4 Ferric Reducing Antioxidant Power (FRAP) Assay:

The FRAP method was strictly followed for the strain evaluation. In summary, the FRAP reagent was freshly prepared and warmed to 37°C, comprising 5 mmol of FeCl3 (20 mmol/L) and 50 ml of TPTZ (2, 4, 6-tripyridyl-S-triazine) (10 mmol/L) in 40 mmol/L HCL with 5 ml of acetate buffer (0.3 mmol/L, pH = 3.6). After a 10-minute incubation at 37°C, 3 mL of FRAP reagent was combined with the sample extract (0.5-2.5 g/mL), and the absorbance of the reaction mixture was measured at 593 nm using spectrophotometry.[23]

2.9.5 Phosphomolybdenum Method

Fruit peel, leaves, and stems were dissolved in methanol at concentrations ranging from 50 to 500 mg/mL. A phosphomolybdenum reagent was combined with 0.5 ml of trilobata methanolic extract in a solution containing 2 mM sodium phosphate and 4 mM ammonium molybdate. Sealed test tubes containing 0.6 M sulfuric acid were then subjected to incubation in a water bath at 95°C for 100 minutes. After cooling the test tubes to room temperature, dilution absorbance at 695 nm was measured using a UV spectrophotometer. In the blank, the drug was substituted with 0.3 mL of methanol and phosphomolybdenum reagent.[24]

2.10 Determination of Anticancer Activity

The pancreatic cancer cell line (AsPC-1), breast cancer cell line (MCF-7), colon cancer cell line (HCT116), larynx cancer cell line (HEP2), and liver cancer cell line (HEPG2) were provided by the tissue culture unit of the Institute of Microbial Technology in Chandigarh, India. These cells were cultured according to established protocols in a 37°C incubator with 5% CO2. The cell viability assay, utilizing 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), was previously described by Mosmann.[25] For each cancer cell type, 7000 cells per well were seeded onto a 96-well plate and allowed to grow overnight before being treated in triplicate. The extracts, serially diluted, were tested at six different concentrations (5, 10, 50, 100, 150, and 200 µg/mL) in 100 µL wells of the medium. After 24 hours, the medium was aspirated and replaced with 150 µL of MTT reagent (5 mg/mL). Following a 4-hour incubation period, the medium was aspirated again, and then 100 µL of dimethyl sulfoxide (DMSO) was added to each well.[26] After an additional hour, the absorbance of the solution in each well was measured at a wavelength of 570 nm using a 96-well plate reader. The percentage of inhibition was calculated using the following formula:

The IC50, or the concentration that inhibited 50% of the cancer cell lines, was determined using the dose-response curve.

2.11 HPLC Analysis

An examination of the plant's literature revealed that the methanolic extract of Punica Granatum's leaves, stems, and fruit peel exhibited exceptional effectiveness compared to other extracts. For the HPLC analysis conducted in this study, 50 mg of methanolic extract from the fruit peel, stem, and leaf was utilized. A C18 2504 column measuring six meters in length was employed for the analysis.[27] The sample was introduced after adjusting the wavelength to 254 nm. Acetonitrile (solvent system A) and a buffer consisting of 0.1% fa[5:95:ACN: H2O] (solvent system B) were utilized for chromatographic separation. These solvent systems were filtered using a vacuum pump equipped with a filter. The mobile phase was pumped at a rate of 1.5 ml/min at room temperature.[28]

3. RESULTS

3.1 Macroscopy

Macroscopic examinations of leaves show that they were decussate, opposite, and simple in composition. The leaf measured between 4 and 2 cm in length and 2 cm in breadth on average. It was noted that the leaf was green in color. The leaf is glossy, shaped differently, and has a distinct smell.

The dried peels used in this experiment have a dark reddish-brown hue, vary in size, and are brittle. The peel pieces have a concavo-convex appearance. The exterior is smooth and woodsy. The pith within is septate, yellowish brown, and has a membrane wall. It also has the seeds' imprints on it. The peel powder tastes astringent and has a subtle fragrant scent.

3.2 Estimation of Extractive Values

The estimation of different extractive values is shown in Table 1.

Table 1: Extractive values of leaves, stem, and fruit peel of *Punica granatum*.

S.No.	Type of solvents	% Extractive value Mean ±SD		
		Leaves	Stem	Fruit peel
1.	Distilled water	7.128±0.68	5.235±0.25	6.328±0.59
2.	Petroleum ether	2.632±0.63	0.965±0.45	2.963±0.45
3.	Chloroform	1.235±0.56	2.056±0.58	3.956±0.23
4	Methanol	9.973±0.59	8.188±0.85	10.976±0.99
5	Ethyl acetate	5.234±0.23	1.523±0.56	3.328±0.89
6	Benzene	3.629±0.52	1.235±0.85	2.568±0.58

3.4 Estimation of Ash Values

The estimation of different ash values is shown in Table 2.

Table 2: Ash values of leaves, stem, and fruit peel of *Punica granatum*.

S. No.	Ash Values	% w/w (mean ± SD)		
		Leaves	Stem	Fruit Peel
1.	**Total ash**	6.42±0.52	5.23±0.46	6.95±0.25
2.	**Acid insoluble ash**	2.21±0.78	2.04±0.80	2.33±0.66
3.	**Water insoluble ash**	2.92±0.55	2.13±0.59	3.46±0.28
4.	**Sulfated ash**	0.97±0.58	0.76±0.24	1.12±0.36

3.5 Physiological composition of the plant extracts

3.5.1 Estimation of Total Flavonoid Content

Using aluminum chloride as a spectrophotometric tool, the flavonoid content of methanolic extracts of leaves stems, and fruit peels was determined. The quercetin equivalents of the flavonoid contents were calculated using the standard curve equation (Y= 0.0005X + 0.0034, R2= 0.9981).

The concentration was found to be mg of quercetin equivalent/g; Shown in Table 3.

Table 3: Total Flavonoid content of leaves, stem, and fruit peel of *Punica granatum*

Part of plant	Total Flavonoid Content
Leaves	4.82 mg
Stem	2.83 mg
Fruit Peel	5.97 mg

3.5.2 Estimation of Total Phenolic Content

Using the Folin-Ciocaltue's reagent, the total phenolic contents of methanolic extracts of leaves stems, and fruit peel are expressed in terms of gallic acid equivalent (Y=0.0004X + 0.0116, R2= 0.9921). The acquired total phenol levels are given as milligrams of GA per gram of extract.

The total phenolic content in the examined extract was found against the gallic acid equivalent/g; Shown in Table 4.

Table 4: Total Phenolic content of leaves, stem, and aerial fruit peel of *Punica granatum*

Part of plant	Total Phenolic Content
Leaves	8.78 mg
Stem	6.23 mg
Fruit peel	10.65 mg

3.5.3 Estimation of Total Tannin Content

Using folin-ciocalteu's reagent, the tannin content of methanolic extracts of leaves, stems, and fruit peels was investigated. The results are given in gallic acid equivalents (Y= 0.0006X + 0.0019, R2= 0.9958, standard curve equation).

The concentration of tannin was found in mg of gallic acid equivalent/g; Shown in Table 5.

Table 5: Total Tannin content of leaves, stem, and fruit peel of *Punica granatum*.

Part of Plant	Total Tannin Content
Leaves	6.81 mg
Stem	9.67 mg
fruit peel	8.24 mg

3.5.4 Estimation of Total Alkaloid Content

Examined and measured in terms of atropine equivalent/g of extract, the alkaloid levels were expressed using the standard curve equation (Y=0.0007X + 0.0083, R2=0.9985).

The concentration of alkaloid was found to be in mg of atropine equivalent/g; Shown in Table 6.

Table 6: Total Alkaloid content of leaves, stem, and fruit peel of *Punica granatum*.

Part of Plant	Total Alkaloid Content
Leaves	2.22 mg
Stem	1.95 mg
fruit peel	3.02 mg

3.6 Antioxidant Activity of *Punica Garnatum*

Natural antioxidants might be useful therapeutic targets since eating fruits and vegetables high in antioxidants lowers the incidence of metabolic and cancer disorders (reference).

The present investigation demonstrated that plant extracts had a high capacity for antioxidants, which increased as the extract concentration increased. Plants with higher levels of phytochemical components have a better antioxidant ability concerning those components. It is widely established that the capacity for antioxidants increases as the amount of phytochemicals increases.[29]

Through the detoxification of reactive oxygen species, antioxidants have a therapeutic impact (ROS). By lessening and repairing ROS-induced cell damage, enhancing immunological function, and lowering the risk of cancer and degenerative illnesses, these oxidants serve as natural scavengers.[30]

Using the DPPH, H2O2, Nitric oxide, phosphomolybdinum, and ferric antioxidant methods, the antioxidant activity of methanolic extracts of *Punica garnatum* leaves stems, and fruit peel was assessed. These techniques were chosen because they are low-cost and straightforward.[31]

3.6.1 Estimation of Ferric Reducing Antioxidant Assay

The methanolic extract of leaves stem, and fruit peel had IC50 values of 17.05 µg/mL, 18.68 µg/mL, and 19.77 µg/mL, respectively, according to the results of the ferric-reducing antioxidant technique.

These findings exceeded the ascorbic acid standard values, which are 12.63 µg/mL (IC50 values). This demonstrates that, in comparison to the plant's stem and fruit peel, Penuca garnatum leaves have a higher level of antioxidant activity.

3.6.2 Estimation of DPPH Assay

The methanolic extracts of leaves stem, and fruit peels had IC50 values of 18.64 µg/mL, 39.07 µg/mL, and 11.6 µg/mL, respectively, according to the results of the DPPH free radical scavenging technique.

The IC50 values of BHT, which are 7.11 µg/mL, were greater than these standard values. This demonstrates that, in comparison to the plant's stem and foliage, *Punica garnatum* fruit peels have higher levels of antioxidant activity.

3.6.3 Estimation of Hydrogen peroxide (H_2O_2) Assay

The methanolic extract of leaves stems, and fruit peel had IC50 values of 31.04 µg/mL, 47.89 µg/mL, and 29.43 µg/mL, respectively, in the H2O2 free radical scavenging technique.

These readings were more than the ascorbic acid standard values, which are 18.61 µg/mL (IC50 values). This demonstrates that, in comparison to the plant's stem and foliage, *Punica garnatum* fruit peels have higher levels of antioxidant activity.

3.6.4 Estimation of Nitric Oxide Assay

The methanolic extract of leaves, stem, and fruit peel had IC50 values of 41.57 µg/mL, 31.23 µg/mL, and 22.60 µg/mL in the Nitric oxide free radical scavenging technique, according to the data.

These findings exceeded the ascorbic acid standard values, which are 15.38 µg/mL (IC50 values). This demonstrates that, in comparison to the plant's stem and foliage, Penuca garnatum fruit peels have higher levels of antioxidant activity.

3.6.5 Estimation of Phosphatmolybdinum Assay

The methanolic extract of leaves stem, and fruit peel had IC50 values of 33.95 µg/mL, 32.05 µg/mL, and 24.31 µg/mL in the Phosphomolybdinum free radical scavenging assay, according to the data.

These findings exceeded the ascorbic acid standard values, which are 16.23 µg/mL (IC50 values). This demonstrates that, in comparison to the plant's stem and foliage, Penuca garnatum fruit peels have higher levels of antioxidant activity.

3.7 Anticancer activity of plant extract

This study highlights the crucial role of phytochemical constituents in plant extracts and their antioxidant capacity in combating cancer cell damage. As shown in Table 7, the results indicate that extracts with elevated levels of antioxidant activity and phytochemical constituents exert a potent anticancer effect.[32]

For instance, the fruit peel extract from Punica granatum, possessing the highest concentration of phytochemical components and antioxidant potential, demonstrated an IC50 against the studied cancer cell lines of less than 20 µg/mL.

According to the standards set by the American National Cancer Institute (NCI), a crude extract's threshold for action is defined by an IC50 of less than 30 µg/mL, signifying the extract's promising potential as an anticancer agent. Notably, most alternative extracts displayed IC50 values below 30 µg/mL across various cancer cell lines.

Specifically, the Punica Granatum leaf extract exhibited an IC50 exceeding 30 µg/mL against AsPC1 and HEP2, but values below 30 µg/mL against other cancer cell lines under investigation. Similarly, the Punica Granatum stem extract demonstrated an IC50 exceeding 30 µg/mL against HCT116 and HepG2, yet values below 30 µg/mL against other cancer cell lines.[33]

These extracts, showing limited anticancer activity, also exhibited reduced levels of antioxidants and phytochemical content, in line with the observation that the anticancer effect correlates with higher levels of phytochemical constituents and antioxidant capacity.[34]

Table 7: IC50 (µg/Ml) of the plant extracts on the different cell lines (Pancreatic cancer cell line (AsPC1), Breast cancer cell line (MCF5), Colon cancer cell line (HCT116), Larynx cancer cell line (HEP2) and Liver cancer cell line (HepG2))

Plant extract	Cell lines				
	AsPC1	MCF5	HCT116	HEP2	HepG2
Leaves extract	38.02±0.56	25.58±0.26	21.89±0.72	41.25±0.56	17.65±0.51
Stem extract	29.56±0.28	23.56±0.84	40.10±0.22	25.62±0.48	31.25±0.89
Fruit peel extract	8.50±0.40	6.40±0.32	9.91±0.58	6.04±0.25	11.67±0.096

Values are given as means of replicates ± standard error

3.8 Estimation of HPLC Analysis

Using the optimized chromatographic condition baseline was recorded. The chromatogram of f5ruit peel, leaves, and stem extracts at 254 nm in acetonitrile solvent system (A) and solvent system (B) are found in figure 1,2,3 respectively. The extract solution was injected and chromatograms were recorded.

Figure 1 shows multiple peaks in the chromatogram of fruit peel extract at the retention time of 1.30 min, 1.75 min, 1.89 min, 2.46 min, 3.28 min, 4.86 min, 5.66 min, 7.57 min, 8.49 min, 9.70 min, 10.76 min, 17.99 min, and 18.93 min.

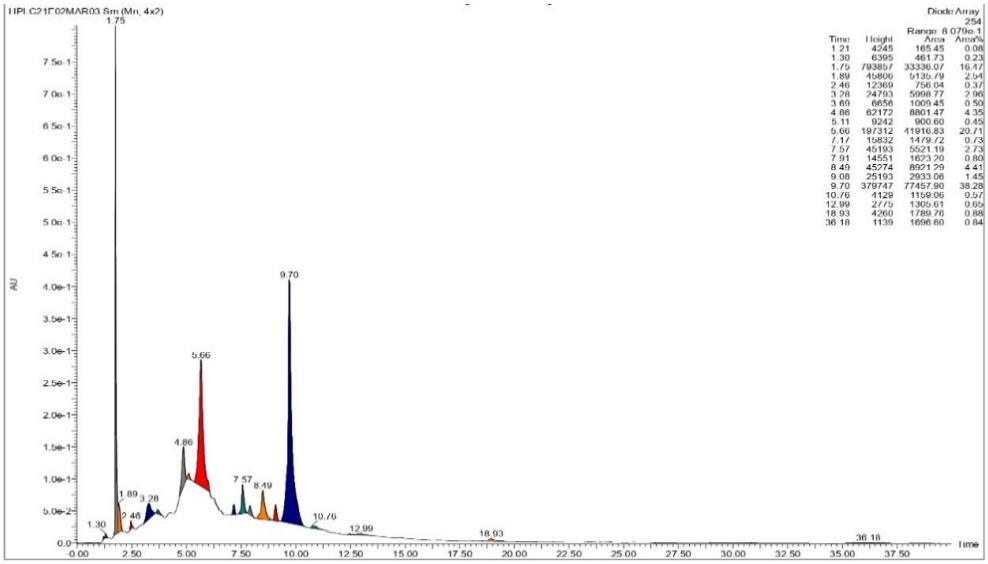

Figure 1: HPLC profile of extracts from the fruit peel of *Punica Granatum* at 254 nm on SUNFIRE C18 250 X 4.6, 5uM column

Figure 2 shows multiple peaks in the chromatogram of leaf extract at the retention time of 1.74 min, 1.89 min, 3.47 min, 4.85 min, 5.66 min, 7.15 min, 7.90 min, 8.48 min, 9.54 min, 10.90 min, 12.99 min, 14.81 min, 19.12 min, and 23.44 min.

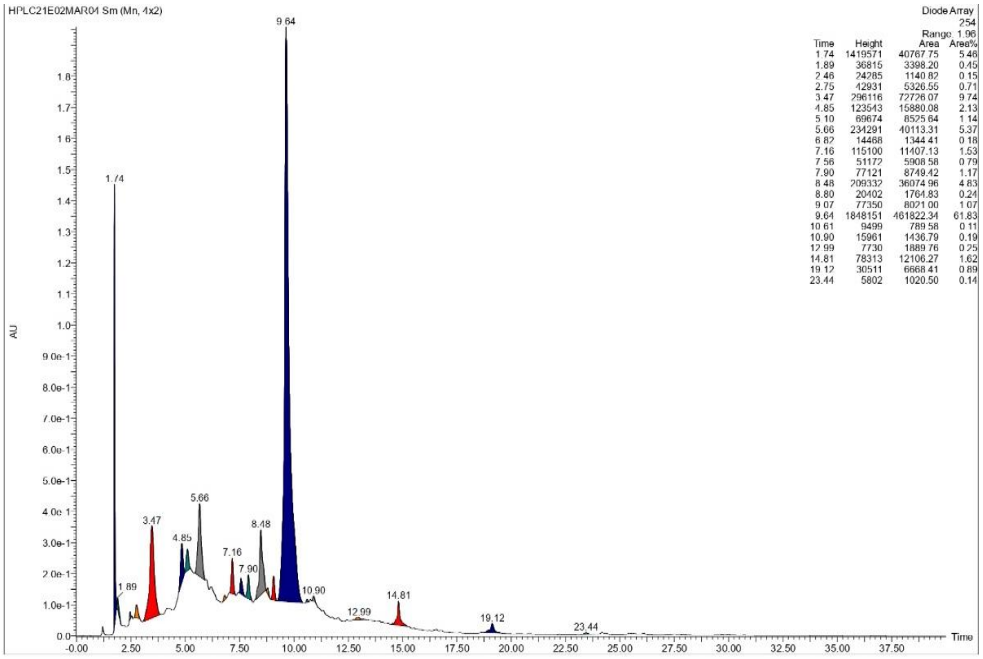

Figure 2: HPLC profile of extracts from leaves of *Punica Granatum* at 254 nm on SUNFIRE C18 250 X 4.6, 5uM column

Figure 3 shows multiple peaks in the chromatogram of stem extract at the retention time of 1.74 min, 2.47 min, 9.58 min, 10.24 min, 12.94 min, 14.37 min, 14.95 min, 15.33 min, 16.55 min, 17.42 min, 17.62 min, 18.62 min 20.14 min, 24.13 min, 24.77 min, 26.10 min, 26.49 min, 26.84 min, 28.21 min 29.01 min and 30.28 min.

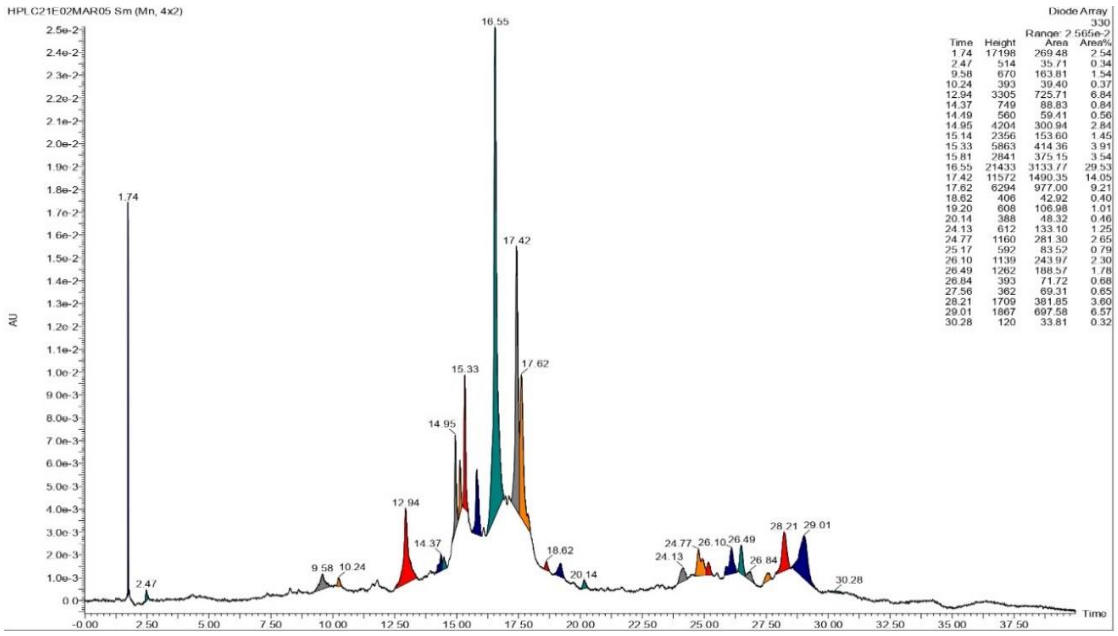

Figure 3: HPLC profile of extracts from stems of *Punica Granatum* at 254 nm on SUNFIRE C18 250 X 4.6, 5uM column

4. CONCLUSION

Several standardized measures, including macroscopic, Pharmacognostic, and phytochemical screening, were used in the current work and may be useful in the authentication of Punica Granatum (L.). It may be inferred from the aforementioned studies of the antioxidant test conducted in various models that Punica Granatum in methanol has strong antioxidant activity. The anticancer effect of many Punica Granatum extracts was demonstrated in this study, demonstrating their ability to suppress cancer cell lines. The current study's findings will also be used as a source while the monograph is being prepared.

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Conflict Of Interests

The authors declared no conflicts of interest.

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