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Fruit of the Pomegranate (*Punica granatum*) Plant: Nutrients, Phytochemical Composition and Antioxidant Activity of Fresh and Dried Fruits

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Abstract:- Since the beginning of time, medicinal plants have been considered nature's undiscovered but precious resources, or nature's pharmacy. Almost 35,000 different species have been used as a safe and effective source of medicine in nearly every human culture on the planet (representing over 75% of the global population). The body uses plant-based materials known as nutrients to aid in tissue development and repair. A natural defense mechanism against disease, phytochemicals are bioactive compounds that are present in fruits, vegetables and aromatic plants. Phytochemicals are a broad class of chemical substances present in natural goods. These include polyphenols, flavonoids, steroidal saponins, organo-sulfur compounds etc. Numerous biological advantages of natural antioxidants include their ability to reduce inflammation, prevent atherosclerosis and fight cancer. Pomegranate have also been investigated in recent years to determine the presence of certain phytoconstituents. Pomegranate has a wide range of pharmacological properties, including anti-inflammatory, wound-healing, antidiabetic, antioxidant, anti-cancer, anti-mutagenic, anti-microbial properties. Hence the objectives of this present study is to analyse the nutrient composition, to identify the phytochemicals, to estimate the antioxidant activity of the fresh and dried pomegranate fruits. Nutrients such as carbohydrate, protein, fibre, iron, vitamin-c, calcium were done by AOAC method. Preliminary qualitative phytochemical analysis was carried out by the standard methodology with extraction through maceration process to identify the secondary metabolites like alkaloids, flavonoids, quinones and etc in various solvents like aqueous, ethanol, methanol, acetone, petroleumether and chloroform. Antioxidant activity were done by DPPH method. Hence dried fruit have better profile of phytochemicals and antioxidants so it can be used for therapeutic purposes mainly digestive problems and pharmaceutical industries.

Keywords:- *Medicinal Plants, Defense System, Bioactive Compounds, Antioxidants, Pharmaceutical*

I. INTRODUCTION

Humans have struggled with a variety of illnesses, discomforts, and ways to provoke them over the years. The use of medicinal plants to cure a variety of diseases is one of the many strategies used to combat illnesses. The trend toward herbal medicine is gaining traction despite the introduction of several key medicines because of growing worries about the growing toxicities associated with main line therapies. Using medicinal plants in conjunction with other forms of treatment is now regarded as a complementary and alternative therapy (Khalid *et al.*, 2022).

The rapid therapeutic benefits of synthetic drugs propelled allopathic medical systems to popularity at the start of the 20th century, but regrettably, they also came with a host of unfavorable side effects. This is causing a trend in the existing healthcare system to change from synthetic to herbal. The "Return to Nature" movement is the name given to it. Plant-based natural medicines have a wide range of phytochemicals and essential oils, which suggests that they have therapeutic potential for humans (Sharma *et al.*, 2014).

According to data released by the United Nations Food and Agriculture Organization, fruit output has been increasing consistently over the past few decades. However, with the growing global population and current consumer preferences for organic, high-quality food products and a healthy lifestyle, the amount of fruits produced cannot meet the world's needs for the greatest food. Furthermore, a significant amount of by-products will inevitably be produced as fruit output rises. Precup *et al.*, (2022) suggest that incorporating these by-products into functional foods is a viable way to decrease waste production and improve the nutritional value of various diets.

A significant amount of scientific evidence indicates that eating fruits and vegetables and other plant foods can help prevent chronic diseases, which supports the idea that these foods should be an essential part of a balanced diet. A wide variety of chemical compounds, particularly secondary metabolites with a polyphenolic character, are found in fruits and vegetables. These chemical compounds, which include ascorbic acid, carotenoids, and polyphenols like flavonoids and phenolic acids, have advantageous biological and antioxidant action that is significant to humans. Furthermore,

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these compounds create easily excretable weak radicals and halt fast-moving oxidative processes (Torralba *et al.*, 2018).

These days, pomegranates are reported to have therapeutic benefits for a wide range of illnesses and conditions, such as obesity, diabetes, aging, and inflammation, in international literature. In particular, it has been demonstrated that treating obesity using pomegranate-derived natural chemicals is effective (Viladomiu *et al.*, 2013).

Pomegranates contain a variety of bioactive components, including secondary metabolites like tannins, organic acids, phenolic acids (including gallic acid, ellagic acid (EA), and caffeic acid), anthocyanins, flavonoids, vitamins, and terpenes, in addition to primary metabolites like sugars, lipides, and fatty acids. However, tannins, such as gallotannins and ellagitannins, appear to be the primary secondary metabolites (Vucic *et al.*, 2019).

Pomegranates have a unique biochemical profile with over 124 phytochemicals that provide them a wide spectrum of anti-inflammatory, anti-oxidant, and anti-mutagenic effects. High molecular weight hydrolysable tannins, such as ellagitannins, a variety of anthocyanins that protect against degenerative diseases, hydroxybenzoic acids, hydroxycinnamic acids, minerals, vital lipids, and complex polysaccharides, are thought to be stored in pomegranate fruit and its fractions, such as the flower, peel, juicy sacs, and seeds. It typically has a pH of less than 4.0 and contains 70–180 g/L of sugar, mostly glucose and fructose. According to Orgil *et al.*, (2014), the fruit's edible portion is composed of 85% water, sugars, pectin, organic acids, phenolics, and flavonoids, mostly anthocyanins.

Thus, the goal of this research is to determine the amount of free radical scavenging activity in both fresh and dried pomegranate fruits, as well as to analyze nutrients and screen for phytochemicals. Hence the present study is carried out by following objectives are to analyse the nutrient composition of fresh and dried pomegranate fruits, to analyse the phytochemical composition of fresh and dried pomegranate fruits, to estimate the antioxidant activity of fresh and dried pomegranate fruits.

II. MATERIALS AND METHODS

A. Selection and Collection of Leaves:

The pomegranate fruit is berry-like with a leathery rind (or husk) enclosing many seeds surrounded by the juicy arils, which comprise the edible portion of the fruit. The aril juice sack is composed of many epidermal cells. According to cultivar, arils range from deep red to virtually colorless, whereas the enclosed seed varies in content of sclerenchyma tissue, which affects seed softness. Pomegranate is full of powerful antioxidants like anthocyanins, tannins and punicalagin. These elements are known to be powerful antioxidants to reduce inflammation and prevent chronic health issues. Pomegranates can have up to three times more antioxidants than green tea or red wine. They provide plenty of macro- and micronutrients, as well as bioactive compounds that promote health. They also have anti-diabetic, anti-carcinogenic activity and improve urinary health. Hence it is selected for the present study for due to the presence of bioactive compounds and therapeutic purposes.

B. Processing of Fresh and Dried Fruits:

The sample was first visually examined for any kind of infection, spores, damage, discoloration, and distortion. Undamaged samples of fruits were collected, grinded and filter to get extract. Natural shade drying is the most accepted storage method for aromatic medicinal herbs because of its low cost and minimum loss of volatile constituents. So the fruits are shadow dried for 3-4 days, collected & then grinded into a fine powder and stored in a air tight container.

Fig 1: Cut it into Two Halves

Fig 2: Fresh Pomegranate

Fig 3: Shadow Drying

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Fig 4: Fine Pomegranate Powder

C. Extraction of Fresh and Dried Pomegranate Fruits:

Extraction is the first step in isolating the desired natural compounds from the basic materials. The extraction principle states that pressing, sublimation, distillation, and solvent extraction are examples of extraction procedures. The solvent extraction method is the most widely used strategy.

➢ *Extraction Process – Maceration:*

Maceration is the process of placing coarsely crushed drug material—such as leaves, stem bark, or root bark—into a container and pouring menstrual fluid over it until the drug material is completely covered. Following that, the container is sealed and left for at least three days. If the material is stored in a bottle, it is shaken and mixed on a regular basis to ensure complete extraction. After extraction, the micelle and mark are separated via decantation or filtration. The micelle is next evaporated in an oven or over a water bath to separate it from the menstruum. This method is practical and suitable for materials that are thermostable in plants (Abubakar *et al.*, 2020).

The extraction was done with different solvents such as aqueous, methanol, ethanol, chloroform, petroleum ether, acetone for phytochemical analysis. For antioxidant and nutrient analysis the extract was done with aqueous solvent.

D. Determination of Nutrient Analysis of Fresh and Dried Pomegranate Fruits:

The nutritional analysis is the description of the method used to determine the amounts of the nutrients in a particular food. The extraction was carried out by aqueous solvent.

Table 1: Nutrient Analysis in Aqueous Extract

Nutrients	Method	Author & Year
Carbohydrate	Anthrone reagent method	E.E.Layne, (1975), David T. Plummer (1990)
Protein	Lowry's method	Chang-Hui Shen (2023)
Crude Fibre	Weende method	D. O. Holst (1982)
Vitamin-c	Titration method	Earle Willard Mchenry and Murray Graham (1935)
Iron	Colorimetric method	Braunschweig (2012)
Calcium	Titration method	Kahandal S (2017)

E. Qualitative Phytochemical Analysis:

The presence of various phytochemicals in fresh and dried Pomegranate fruits were analyzed. Phytochemical such as alkaloids, saponin, tannin, terpenoids, quinones, flavonoids, phenol and steroids were analysed by standard procedures with different solvents as mentioned above. Qualitative Phytochemical screening procedure is given below:

Table 2: Qualitative Phytochemical Analysis in Various Solvent Extract

Phytochemicals	Procedure	Observation
Alkaloids (Wagner test)	2ml extract, 2 to 3drops of Fecl	Greenish to black color indicates the presence of alkaloids
Flavonoids (Alkaline reagent test)	2ml extract +Few drops of NaOHsolution	Intense yellow color which become colorless onaddition of dil Hcl
Phenol (Ferric chloride test)	2ml extract +5%Fecl	Deep blue or green color indicates presence of phenol
Saponins (Foaming test)	2ml extract +6ml distilled H_2O and shakevigorously	Staple foam indicates thepresence of saponins
Tannins (Braymers test)	2ml extract + Alcoholic $Fecl_3$	Blue or green colour indicates the presence of tannins
Terpenoids	1ml chloroform+2mlextract+few drops C.H_2SO_4	Reddish brown precipitateindicates presence of terpenoids
Quinone	2ml extract +con HCL	Yellow precipitate indicates the presence of quinone
Steroids (Salkowski test)	2ml extract +chloroform +H_2SO_4	Development of reddish brown colour indicates thepresence of steroids

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F. Estimation of Antioxidant Activity of Fresh and Dried Pomegranate Fruits:

Antioxidants are compounds that minimize oxidative damage to biological processes by giving free radicals an electron and making them appear harmless (Shantabi *et al.*, 2014). Antioxidant compounds can scavenge free radicals and prolong shelf life by postponing the lipid peroxidation process, which is one of the primary causes of food and pharmaceutical product deterioration during processing and storage (Halliwell 1997). The antioxidant activity of fresh and dried pomegranate fruits was assessed using the DPPH radical scavenging test (DPPH)

- *2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay:*

DPPH is a stable deep purple chromogen radical. It is not required to be generated prior to the test and can be purchased commercially. In order to eliminate DPPH radicals, the DPPH scavenging assay depends on antioxidants' capacity to contribute electrons. The reaction causes a change in the color of DPPH, which is visible at 517 nm. This discoloration can be used to determine how efficient the antioxidants are.

In a test tube, 100 µL of leaf extract and 3 mL of DPPH working solutions were combined. Three milliliters of DPPH-containing solution in 100 µL of methanol is a typical standard. After then, the tubes were kept completely dark for thirty minutes. Therefore, the absorbance was calculated at 517 nm.

$$\% \text{ Scavenging Activity} = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample} \times 100}{\text{Absorbance of the control}}$$

III. RESULTS AND DISCUSSION

Table 3: Nutrient Analysis of Fresh and Dried Pomegranate Fruits

Nutrients	Fresh Pomegranate fruits	Dried Pomegranate fruits	
Carbohydrate	0.60g	0.42g	
Protein	23g	16.5g	
Crude Fibre	5.55%	7.4%	
Vitamin-C	22mcg	17.6mcg	
Iron	50mg	100mg	
Calcium	3.6mg	5.6mg	

Table - 3 depicts the nutrient analysis of the fresh and dried pomegranate with aqueous extract. Carbohydrate, protein & vitamin-c present in fresh fruits are higher in amount compared to dried fruits while in fibre, iron & calcium are present in dried fruits are higher in amount when compared to fresh fruits.

Table 4: Qualitative Phytochemical Analysis of Fresh and Dried Pomegranate fruits in Various Solvents

	Aqueous		Ethanol		Methanol		Acetone		Petroleum Ether		Chloroform	
Phytochemicals	**Fresh**	**Dried**	**Fresh**	**Dried**	**Fresh**	**Dried**	**Fresh**	**Dried**	**Fresh**	**Dried**	**Fresh**	**Dried**
Alkaloids	-	-	+	+	+	+	+	-	-	+	-	-
Flavonoids	+	+	+	+	+	+	+	+	-	+	-	+
Phenols	+	+	+	+	+	+	+	+	-	-	+	+
Saponins	-	+	+	-	+	+	+	+	+	+	+	-
Tannins	+	+	+	+	+	+	+	+	-	+	-	+
Terpenoids	-	-	-	+	-	+	-	-	-	-	+	+
Quinones	-	-	-	+	-	-	-	+	+	+	-	-
Steroids	+	+	+	+	+	+	+	-	+	+	+	+

Table – 4 depicts the qualitative phytochemical analysis of fresh and dried pomegranate fruits with various solvent extract. Aqueous extract of fresh fruits reveals that it contains flavonoids, phenols, tannins and steroids while in dried fruits contains phytochemicals such as flavonoids, phenols, saponins, tannins and steroids except alkaloids, terpenoids and quinones.

Ethanol extract of fresh fruits contains all phytochemicals except terpenoids & quinones while in dried fruits contains all phytochemicals except saponins.

Methanol extract of fresh fruits contain all phytochemicals except terpenoids & quinones while in dried fruits contains all phytochemicals except quinones

Acetone extract of fresh fruits contains all phytochemicals except terpenoids and quinones while in dried fruits contains phytochemicals such as flavonoids, phenols, saponins, tannins & quinones.

Petroleum ether of fresh fruits contain saponins, quinones and steroids while in dried fruits contains most of the phytochemicals except phenols & terpenoids.

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Chloroform extract of fresh fruits contains phenol, saponins, terpenoids and steroids while in dried fruits contains all phytochemicals except saponin and quinones.

Majority of the tested phytochemical were present in all solvent extract of the dried fruits.

Fig 5: Aqueous Extract

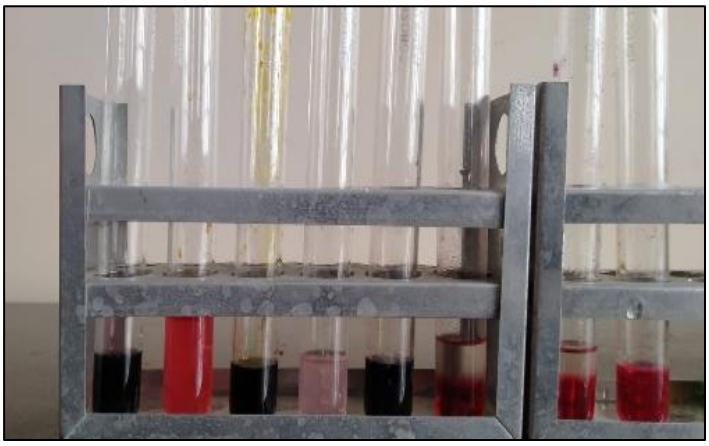

Fig 6: Ethanol Extract

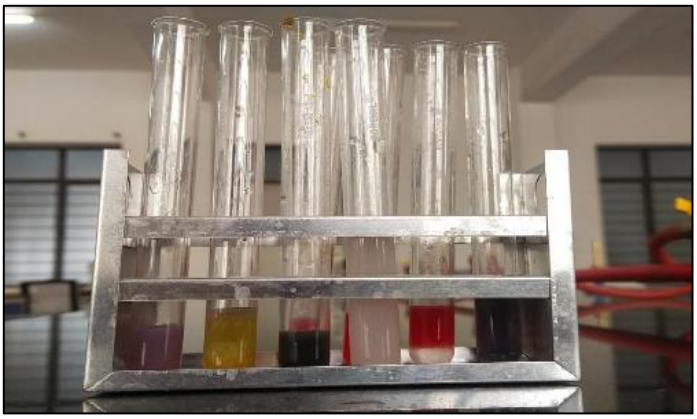

Fig 7: Methanol Extract

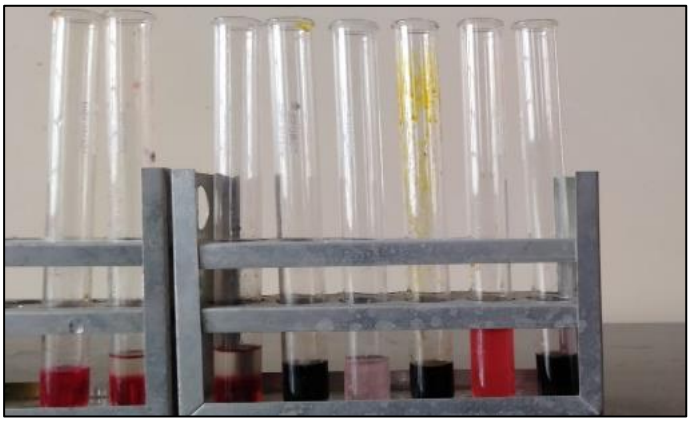

Fig 8: Acetone Extract

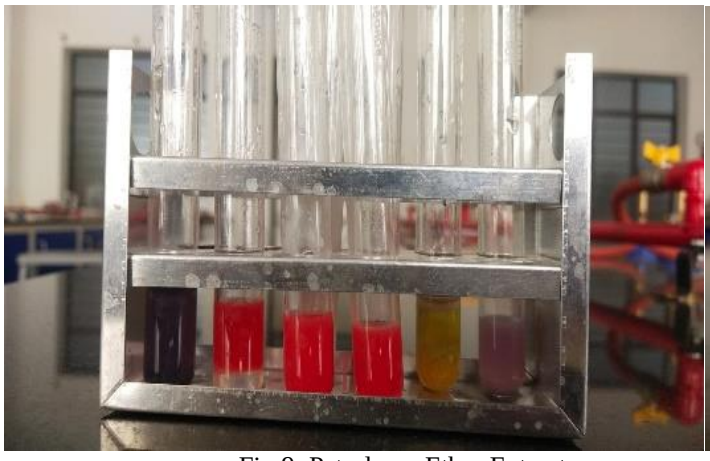

Fig 9: Petroleum Ether Extract

Fig 10: Chloroform Extract

Table 5: Antioxidant Activity of Fresh and Dried Pomegranate Fruits

Concentration(µg/ml)	Absorption		% of inhibition	
100	**Fresh**	**Dried**	**Fresh**	**Dried**
200	4.131	3.101	9.00	31.69
300	4.068	2.123	10.03	52.85
400	3.154	1.839	30.26	59.34
500	2.123	0.356	53.47	92.19

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Table – 5 depicts the antioxidant activity of fresh and dried pomegranate fruits. DPPH radical scavenging assay in dried fruits (31.69%, 52.85%, 59.34%, 92.19%, 93.24%) contain more antioxidant than the fresh leaves (9.00%, 10.03%, 30.26%, 53.47%, 55.17%).

Fig. 11: Fresh Fruit

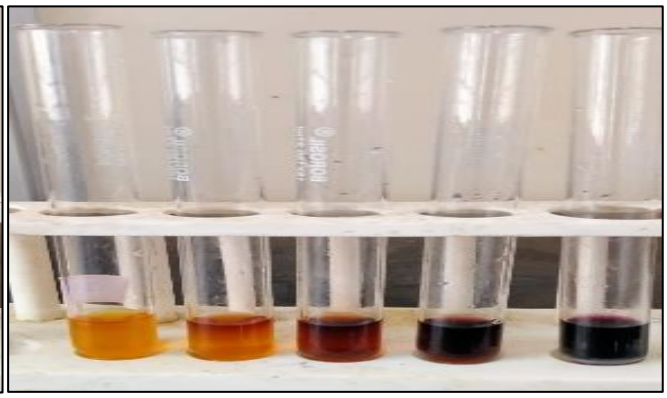

Fig 12: Dried Fruit

IV. CONCLUSION

The pomegranate (*Punica granatum*) is the most valued and well-known plant in the Ayurvedic medical system. It is one of the most versatile plants and has several medicinal applications. Fruits have unique medicinal qualities because of their vitamins, minerals, antioxidants, and phytochemicals. The fruits of pomegranate are potent of antioxidants and rich in vitamins & minerals. Pomegranates can help to lowering the blood pressure as well as the blood glucose levels, anti-cancer, wound healing properties. The present study revealed that the nutrients like carbohydrate, protein & vitamin-c are rich in fresh fruits while fibre, calcium and iron are rich in dried fruits. Dried pomegranate fruits have major phytochemical constituent in most of all solvent extraction than fresh pomegranate fruits. Antioxidant activity are high in dried fruits compared to fresh fruits. Using pomegranate in food industry & pharmacology can address the growing demand for nutraceuticals and functional foods. Products made from dried samples would have a higher nutritional profile than those made from fresh samples.

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