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# Evaluation Radical Scavenging Activity of *Punica grantum* and *Citrus* plant Waste Peel Hydro Alcoholic Extracts from Improved Processing

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## ABSTRACT

Free radicals are atoms or molecules that have one or more unpaired electrons on its outer orbital, highly reactive, and could damage cell inside human body. Antioxidants are the substances which inhibit oxidation, which have the ability to remove the potentially damaging oxidizing agents in a living organism. Many phytochemicals present in the plants are able to reduce or prevent the oxidative damage to the human cells which can cause even cancer in humans. It is highly vital to know about the antioxidant activities of each plant and the phytocompounds responsible for that. In this study, the DPPH free radical scavenging activity of the extracts of Pomegranate and Citrus (Lemon) is analyzed. And we found Citrus peel extract 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH) assay activity better on comparing Pomegranate peel extract.

**Keywords:** Herbal Medicinal Plant; Pomegranate; Citrus; DPPH scavenging activity.

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## INTRODUCTION

Antioxidant capacity is a broadly used term as a parameter to characterize different substances and food samples with the ability of scavenging or neutralizing free radicals. This capacity is associated to the presence of compounds capable of protecting a biological system against harmful oxidation. Due to increased health awareness, consumers are increasingly demanding functional health products. This has led to an expansion of the health food market and research; and food industry is actively searching for new functional materials from natural resources.[1] Plant – based foods are not only importance source of nutriments such as carbohydrates, proteins, sugar etc. but also of secondary metabolites. The latter are becoming increasingly popular for their beneficial effects on user health, for instance, by providing antioxidants. In the recent years the focus on natural antioxidants, especially from plant materials[2]. These antioxidant compounds can be derived from natural and chemical sources. Natural sources are much safer to use due to less toxicity and side effects. Antioxidant are also defined as a substance which are capable of inhibiting a specific oxidizing enzymes or a substance that reacts with oxidizing agents prior to causing damage to other molecules or a substance that sequesters metal ions or even a substance capable of repairing system such as iron trans- porting protein [3]. As such, production of free radicals and other reactive oxygen species in the human body by numerous physiological and bio-chemical processes is reported[4]; however, overproduction of these could lead toward development of diseases. These antioxidants may help to relive oxidative stress, i.e. preventing free radicals from damaging biomolecules such as proteins, DNA and lipids [5]

DPPH assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration. DPPH is a common abbreviation for the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl. It is a dark- colored crystalline powder composed of stable free-radical molecules. [6] When DPPH radicals encounter a proton donating substance such as an antioxidant, it would be scavenged and the absorbance is reduced. Thus, the DPPH radicals were widely used to investigate the scavenging activity of some natural compounds[7]. In this concept plant based chain, DPPH study On *Pomegranate* (*punica granatum*) and *Citrus* (*Lemon*) fruit peel because both are using in Traditional medicinal plant fruits long time in world specially Asian countries (India, chain, Pakistan, Iran, Saudi Arabia etc). [8] in different types disses. Therefore the present study was conduct to determine the DPPH (Antioxidant) activity of Hydro alcoholic extract of *pomegranate and Lemon (Citrus)* waste fruits peel and obtain $IC_{50}$ values.

## MATERIALS AND METHOD

Plant Material- The *punica granatum L* plant fruits were collected from regional market Ashta Madhya Pradesh INDIA in the month of January 2020 and second *lemon* (*Citrus*) plant fruits collected in summer season from the lemon shop of local market Bhopal, M.P., India. The botanical identification of the collected plant was done by Dr Suman Mishra; Vindhya Herbal Bhopal. Powdered peels material was extracted in water or ethanol (2.5l) using soxhlet apparatus for 48hours. The resultant crude Hydro alcoholic extract was evaporated to dryness using rota vapour whilst the aqueous extract was concentrated using a freeze drier. The dried crude extracts were then stored in the fridge until ready for use.

**Chemicals**

1-0.1 Mm solution of DPPH in methanol was prepared and used in the study.

2-Ascorbic acid 1%.

**2, 2-Diphenyl-1-picryl-hydrazyl (DPPH) assay**

Antioxidant activity was done by Molyneux method [9] Methanol was used as blank, DPPH 50 µg/mL as control and ascorbic acid as standard. 2 ml of standard or sample was prepared in various concentration then added into 2 ml DPPH 50 µg/ml solution and incubated for 30 minutes before measured by UV-visible spectrophotometry at λ 517 nm and performed triplicate. IC50 of sample or standard was calculated by DPPH scavenging activity calibration curve [10]

The scavenging potency of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical of pomegranate and Citrus (Lemon) peel Hydro alcoholic was determined . The ability to scavenge DPPH radicals was calculated using the following equation

Inhibition (%) = (A control – A test) / A control 100. Where;

A control = The absorbance of the control reaction.

A test = The absorbance of the Extract. The results were expressed as the half maximal inhibitory concentration (IC50) and compared with standard. All measurements were fulfilled in triplicate and mean values were calculated.

## RESULTS AND DISCUSSION

DPPH is stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, then losing colour stoichometrically with the number of electrons consumed, which is measured spectrophotometricallty at 517 nm. Radical scavenging activity using DPPH radical are shown in and expressed, radical µg/ml extract. The Hydro alcoholic extract of Punica grantum and citrus

fruit peel studied showed maximum radical scavenging property. Maximum DPPH radical scavenging ability was observed *Citrus (Lemon)* (62.18) and *Punica grantum* (46.235).

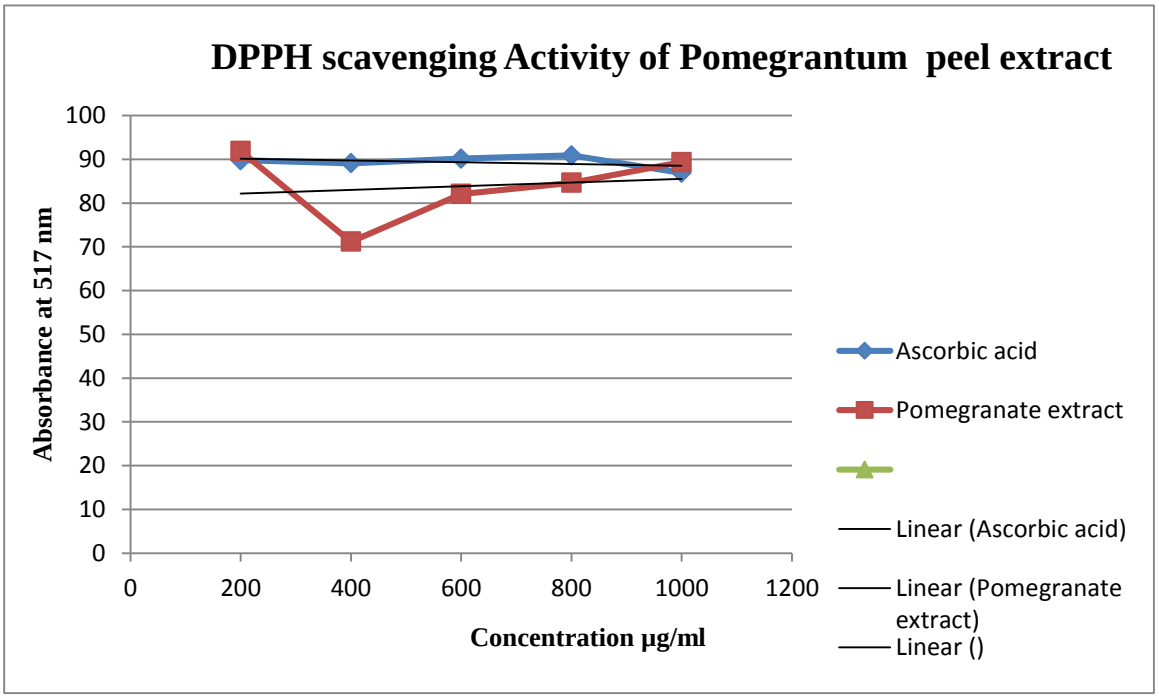
Results as show in Table 1, Graph 1, 2 and table 3.

**Table 1: Percent DPPH scavenging of Ascorbic acid and Plant peel extracts in various concentrations**

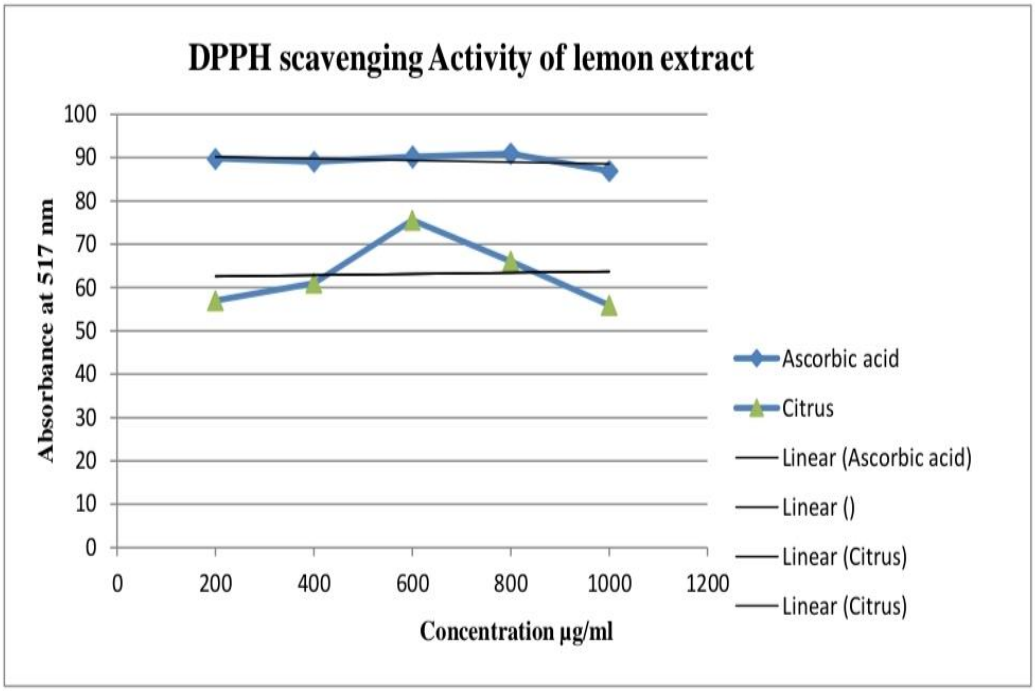
| Concentration (µg/ml) | Ascorbic Acid | Pomegranate extract | Citrus |
|---|---|---|---|
| 200 | 89.1 % | 91.9 % | 57 % |
| 400 | 90.09 % | 71.18 % | 61 % |
| 600 | 91.18 % | 82.09 % | 75.54 % |
| 800 | 91.9 % | 84.63 % | 66.09 % |
| 1000 | 87.9 % | 89.36 % | 55.9 % |

**Table 2: IC $_{50}$ Values of Ascorbic Acid and Both plants.**

| Sample | IC $_{50}$ Value |
|---|---|
| Ascorbic Acid | 21.03 |
| Pomegranate | 46.235 |
| Citrus | 62.18 |




**Graph 1: DPPH Scavenging activity of Pomegranate peel extract.**




**Graph 2: DPPH Scavenging activity of *Citrus* peel extract.**

## CONCLUSION

In the present research, an effective and antioxidant preserving method was developed to obtain highly active antioxidant extract. The present study was conducted to evaluate the antioxidant activities of the Hydro alcoholic extracts of whole plant on *Punica grantum* and *Citrus (Lemon*) In present study, both the plant extract demonstrated scavenging of stable 2,2- diphenyl-2-picrylhydrazyl (DPPH) radical. Among these plants maximum antioxidant activity was shown by Citrus (62.18) and than pomegranate (46.235). It is best antioxidant agent. already proven benefits, the extracts are promising candidates as commercial food supplements.

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