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An Investigation of the Total Antioxidant Activity and 2,2-diphenyl-1-picrylhydrazyl of Hydro Alcoholic Pomegranate and Lemon Fruit Peel Extract

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Abstract

Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases. The total antioxidant capacity and 2,2-diphenyl-1-picrylhydrazyl were studied. The literature on value addition to fruit derived waste is diverse. This paper presents the Hydro alcoholic extracts of *Pomegranate* and *Lemon* fruit peel which are traditionally used in medicine were screened for antioxidant activity, in which *Punica granatum* L. extract had a high total antioxidant scavenging activity of 38.9% at 100 g/ml concentration by which Lemon extract has a strong scavenging activity of 118.3 percent at a concentration of 40 g/ml. and Pomegranate peel high DPPH radicals found (46.235) at 60 g/ml of extract concentration. and Lemon 75.54 percent at a concentration of 60 g/ml. it is critical given the growing popularity through, fruit therapies around the world.

Plants or their fruits with therapeutic properties, as well as their secondary metabolites, have been recognized and used in recipes since human history began. Globally, the fruit processing industry generates more than 0.5 billion tonnes of trash. The worldwide availability of this feedstock, as well as its untapped potential, has prompted academics to do extensive research on the value-added potential of fruit processing waste.

Fruit processing waste is observed to be selective and concentrated in nature when compared to general food or other biomass-derived trash. Previous many researches have revealed that the availability of phenolic chemicals is often responsible for the medicinal effects of the herbs studied, which is always a part of antioxidant. Antioxidants are also characterized as substances capable of blocking certain oxidising enzymes, reacting with oxidising agents prior to causing harm to other molecules, sequestering metal ions, or even mending systems such as iron transporting proteins[8]. Agricultural resources, particularly fruit waste, can be used to make a variety of vital medications. Solid–liquid and liquid–liquid extraction procedures can be used to isolate polyphenolic chemicals from waste biomass such as CP [1]. Fruits of the pomegranate (*Punica granatum*) are high in phytochemicals that have antioxidative, anti-inflammatory, anti-

cancer, and antibacterial properties[7]. Lemon, on the other hand, is one of the most frequently used citrus fruits in the world, used for nutritional, medicinal, and cosmetic purposes. As an antioxidant and oily extract, the large level of vitamin C in this food is useful in enhancing skin health and avoiding certain diseases. However, most people are unaware that lemon peel contains more vitamins than lemon juice and is high in fiber and minerals, despite the fact that it is frequently discarded. As a result, this work discusses the antioxidative the antioxidant properties of the two plant extracts were investigated. Pomegranate and Lemon fruit peels have been used in Traditional medicinal plants' fruits for a long time throughout the world, particularly in Asian nations (India, Pakistan, Iran, Saudi Arabia, and others)[3]. The antioxidant activity of both plant fruit peel extracts was assessed using the Total Antioxidant and DPPH in the current study. Extensively used in different communities and elucidates which will employ in helping entire system functions against illnesses attacking human bodies.

Reagents and chemicals: 5mM Sodium Nitroprusside, ethanol, D.W Griess reagent (1% sulphonil amide, 0.1% N 1-naphthylethylenediamine, 2% orthophosphoric acid), Phosphate buffer (pH- 7.4),0.1 Mm solution of DPPH in methanol, Ascorbic acid (1%).

Sample Collection & Soxhlet Extraction :

Pomegranate fruit was picked from the Ashta regional market in Madhya Pradesh in January 2020, the peel was removed, and was sliced into small pieces and sun-dried for 15 to 20 days at an ambient temperature of 20-25 ^{o}C. The second (*Citrus*) lemon fruit sample was obtained in March 2020 from a local market lemon shop in Bhopal, Madhya Pradesh. The peels were thoroughly washed with ordinary water, then distilled water, and the surplus pulp was removed completely. The peels were then dried for 12-15 days in the shade at room temperature. The dry sample was finely pulverized with a mortar and pestle before being processed into powder with a grinder. The type of solvent employed in the extraction process has a big impact on the success of determining physiologically active chemicals from plant material. We used Soxhlet extraction for plant powder extraction. The sample 60 gm. dried friut peel bark powder) was extracted with 500 ml organic solution of ethanol and D.W. in Soxhlet apparatus. Extraction was performed at 65°C over 72 h. and the extract was evaporated at 45 °C to form a paste. if the target component has a low solubility in the solvent and the impurity is insoluble in that solvent the Soxhlet extraction is necessary. If the desired component has a high solubility in the solvent, it can be separated from the insoluble substance using simple filtration. Instead of sending many portions of the heated solvent through the sample, only one batch of solvent is recycled in this technique[5].

Total antioxidant capacity assay :

The assay for total antioxidant capacity was performed as described by[6] The extract was collected at various concentrations (0.5–2.5 g/ml) and 1.0 ml of the reagent solution was added. The tubes were capped and incubated for 90 minutes at 95°C in a

thermal block. The absorbance of each aqueous solution was measured at 695 nm against a blank after cooling to room temperature. The overall antioxidant capacity was represented as equivalents of ascorbic acid or gallic acid, with ascorbic acid as the benchmark[6].

2,2-diphenyl-1-picrylhydrazyl :

This was accomplished using the Molyneux approach[4]. As a blank, methanol was utilized, DPPH 50 g/mL was used as a control, and ascorbic acid was used as a standard, 2 ml of standard or sample was produced in various concentrations, then added to 2 ml DPPH 50 g/ml solution and incubated for 30 minutes before being analyzed by UV-visible spectrophotometry at 517 nm. Using a DPPH scavenging activity calibration curve, the IC_{50} of a sample or standard was computed[2].

Analysis of Data :

Hydro alcoholic extract was determined. The ability to scavenge Antioxidant was calculated using the following equation: %= [(OD sample-OD blank) / (OD ascorbic acid-OD blank)] X100.

All of the studies were repeated three times, and the percentage of antioxidant obtained from each experiment was computed using Microsoft Excel as mean standard deviation.

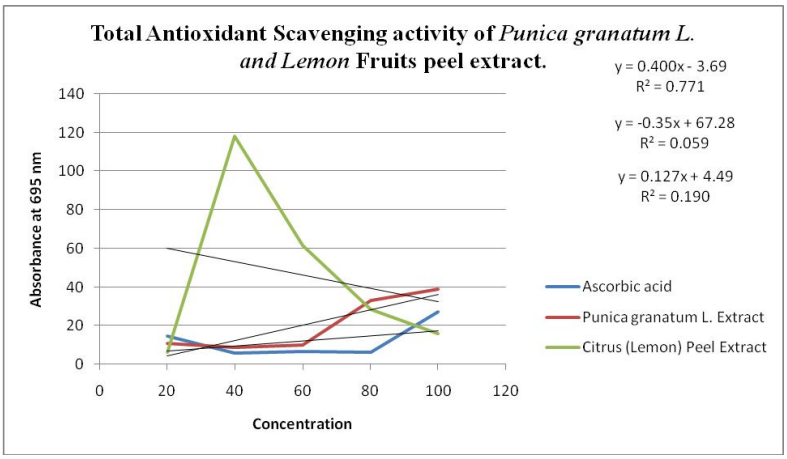
At 100 g/ml concentration, total antioxidant scavenging activity corresponding to ascorbic acid was found to be 27.3 percent. *Punica granatum* L. extract had a high total antioxidant scavenging activity of 38.9% at 100 g/ml concentration. *Lemon* extract has a strong scavenging activity of 118.3 percent at a concentration of 40 g/ml. The free radical scavenged by the extracts are shown in table 1,2 and graph 1 at concentrations ranging from 20 to 100 g/ml of the hydro alcoholic extract of *Punica granatum* and *Citrus* (*Lemon*). While IC_{50} *Citrus* (*Lemon*) (62.18) and *Punica grantum* had the greatest ability to scavenge DPPH radicals (46.235). At 60 g/ml of extract concentration, the DPPH scavenging activity equivalent of ascorbic acid was 91.18 percent. *Punica granatum* L. 91.9 percent at a concentration of 20 g/ml, and *Citrus* 75.54 percent at a concentration of 60 g/ml Hydroalcoholic extracts of both plants with concentrations ranging from 20 to 100 g/ml. Table number 3,4 and, graph number 2 show the results.

Table-1. Total Antioxidant Activity of *Pomegranate & Citrus* Hydroalcoholic Extracts

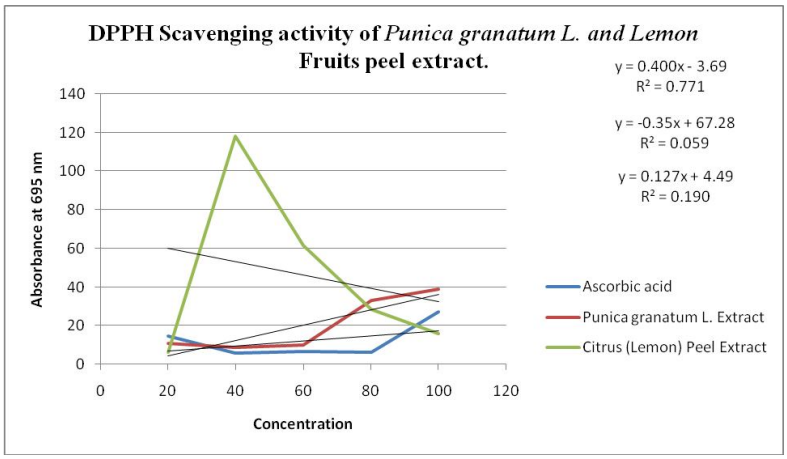
Concentration (µg/ml)	Ascorbic acid	*Punica granatum* L. extract	*Lemon* peel Extract
20	14.7 %	10.9 %	6.4 %
40	5.9 %	8.9 %	118.3 %
60	6.6 %	10.00 %	61.8 %
80	6.2 %	33.00 %	28.7 %
100	27.3 %	38.9 %	16.2 %

Table-2. DPPH scavenging Activity of *Pomegranate & Citrus* Hydroalcoholic Extract

Concentration (µg/ml)	Ascorbic acid	*Punica granatum* L. extract	*Lemon* peel Extract
20	89.1 %	91.9 %	57 %
40	90.09 %	71.18 %	61 %
60	91.18 %	82.09 %	75.54 %
80	91.9 %	84.63 %	66.09 %
100	87.9 %	89.36 %	55.9 %



Graph 1: Total Antioxidant Scavenging activity of *Punica granatum L.* and *Lemon* Fruits peel extract.



Graph 2: DPPH Scavenging activity of *Punica granatum L.* and *Lemon* Fruits peel extract.

Table-3. Total Antioxidant and DPPH of *Pomegranate* & Lemon Fruits Peel extract with Standard ascorbic acid

Sample	IC_{50} value of Total Antioxidant
Ascorbic acid	45.383
Punica granatum L. extract	45.91
Lemon extract	17.63

Table-4. DPPH of *Pomegranate* & Lemon Fruits Peel extract with Standard ascorbic acid

Sample	IC_{50} Value of DPPH
Ascorbic Acid	21.03
Punica granatum L.	46.235
Lemon extract	62.18

A fruit's medicinal property is determined by the existence of several natural compounds with bio-active potential, and the balanced proportion of these components provides curative or therapeutic capabilities. In this study, we found that the antioxidant chemicals found in the extracts of utilized plant fruits are involved in scavenging free radicals. Although the findings of this study are preliminary, they are critical given the growing popularity of plant fruit therapies around the world. Pomegranate and lemon, in particular, were found to have good antioxidant potential. Though more research into the actual performance of these plants in physiological systems is needed, their antioxidant capacities have been demonstrated by this study's preliminary qualitative and quantitative screening. As a result, more *in vivo* investigations of these plant fruit peels are needed, as well as a systematic examination of these antioxidant-rich species, therefrom this type of fruit peel will place to be used as preventative medicine. Therefore, further *in vivo* studies of these species are required, and a systematic investigation of these antioxidant rich species is needed before they can be used in the food processing industry and as preventive medicine.

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Declaration of competing interest:

The authors declare that there is no conflict of interest regarding the publication of this article.

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