ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

ANTIOXIDANT PROPERTY ANALYSIS OF POMEGRANATE PEELS IN AYURVEDIC FORMULATIONS

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Manuscript Info

Manuscript History:

Received: 26 July 2014
Final Accepted: 29 August 2014
Published Online: September 2014

Key words:

Punicagranatum, Pomegranate, Ayurveda, Antioxidant, H2O2, Rutin

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Abstract

The aim of this research is to determine total antioxidant activity of water and ethanolic extracts of an Ayurvedic preparation of *Punicagranatum (pomegranate peels).* In the Indian subcontinent's ancient Ayurveda system of medicine, the pomegranate has extensively been used as a source of traditional remedies. Pomegranate is considered a healthful counterbalance to a diet high in sweet-fatty component. It is also effective in reducing heart disease risk factors, including LDL oxidation, macrophage oxidative status, and foam cell formation. In this study, antioxidant activities of *P.granatum* were measured by Rutin method, H_2O_2 radical scavenging assays. In conclusion, the Ayurvedic formulations of *P.granatum*had total antioxidant activity.

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Introduction

An attractive shrub or small tree, to 20 or 30 ft (6 or 10 m) high, the pomegranate is much-branched, more or less spiny, and extremely long-lived[2]. The pomegranate tree is native from Iran to the Himalayas in northern India and has been cultivated since ancient times.Pomegranate contains polyphenols, such as ellagitannins and flavonoid, punicic acid (65.3%), palmitic acid (4.8%), stearic acid (2.3%), oleic acid (6.3%) and linoleic acid (6.6%). The juice of wild pomegranates yields citric acid and sodium citrate for pharmaceutical purposes. It enters into preparations for treating dyspepsia and is considered beneficial in leprosy.The bark of the stem and root contains several alkaloids including *iso*pelletierine which is active against tapeworms. The tannin rich extracts have been investigated for various properties[6, 10]. Because of their tannin content, extracts have been given as astringents to halt diarrhea, dysentery and hemorrhages. Pomegranates have displayed hypotensive, antispasmodic and anthelmintic activity in bioassay[7].

Some of the reactiveoxygen species, including hydrogen peroxide, singlet oxygen, hydroxyl and superoxide radicals, have positive roles in energy production *in vivo* systems, phagocytosis, intercellular signal transfer, regulation of cell growth and the synthesis of important biological compounds[8]. Additionally, reactive oxygen species modify DNA and membranes by attacking the lipids, proteins, and carbohydrates in cell membranes and tissues[9].

In the organism, the rates of production and removal of free radicals are in balance, known as oxidative balance. An increase in the rate of production or a decrease in the rate of removal disrupts this balance and increases the levels of reactive oxygen species. This condition, which is called oxidative stress, indicates a serious imbalance between the production of free radicals and the antioxidant defense systems, resulting in tissue damage[11].

The aim of this research is to determine the total antioxidant activity of the Ayurvedic alternative formulations of *P.granatum*, with the help of rutin.

MATERIAL AND METHODS

1. PROCUREMENT OF RAW MATERIALS AND FORMULATION

P.granatum fruits were locally procured in Pune, India. The peels of the fruits were collected and dried in a hot air oven at 100^0 Celsius for two hours. The dried peels were then grinded and a fine powder was made. For extraction (ethanol or water), 5g powder of the pomegranate samples (ayurvedic preparations) were mixed in a solvent system of ethanol(15mL per sample). Extraction continued until the extraction solvents became dark in colour but clear. The obtained extracts were filtered over Whatman No. 1 paper and the filtrate was collected, then solvent was removed by a rotary evaporator at 50°C[5].

2. ESTIMATION OF PHYSICOCHEMICAL PROPERTIES

It was observed that both the formulations possessed a dark colour and lower density as compared to the crude pomegranate. . Also, it was observed that the characteristic odour of crude drug was lost after the formation, increasing its pharmaceutical palatability. On vigorous shaking with an organic solvent, both the formulations showed foaming equivalent to the crude drug, indicating the presence of saponins. When compared to the crude drug, formulation1 showed presence of tannins than the formulation2. Anthraquinones were present in the crude drug as well as in the formulation2. The crude drug as well as both the formulations showed absence of alkaloids. The test for carbohydrates using Molisch reagent and heating the extract showing a violet-blue colouration was absent for both the formulations, along with the crude drug[1].

3. HYDROGEN PEROXIDE SCAVENGING CAPACITY

The ability of the *P. granatum*extracts to scavenge hydrogen peroxide was determined. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4)[3]. Both extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide.

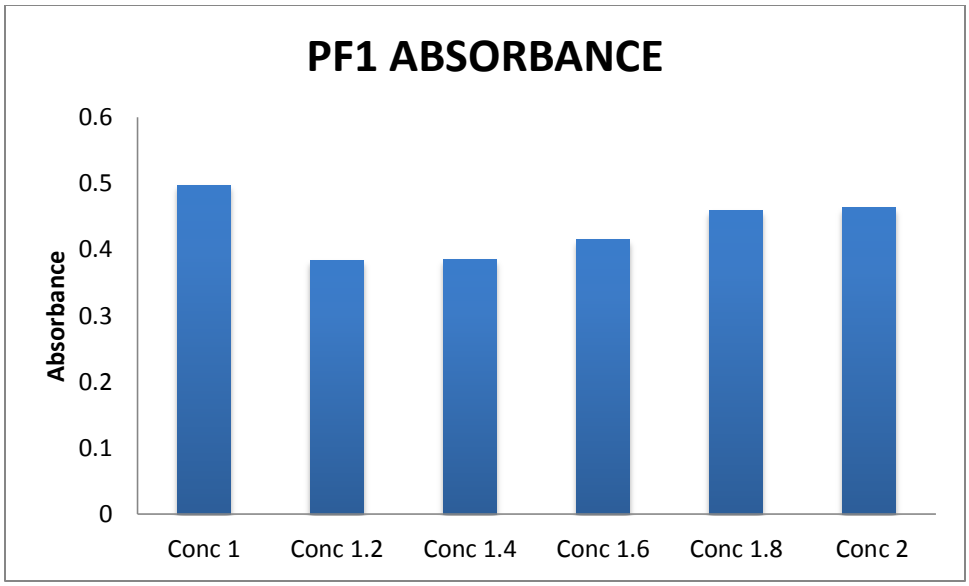
4. ANTIOXIDANT ANALYSIS WITH RUTIN

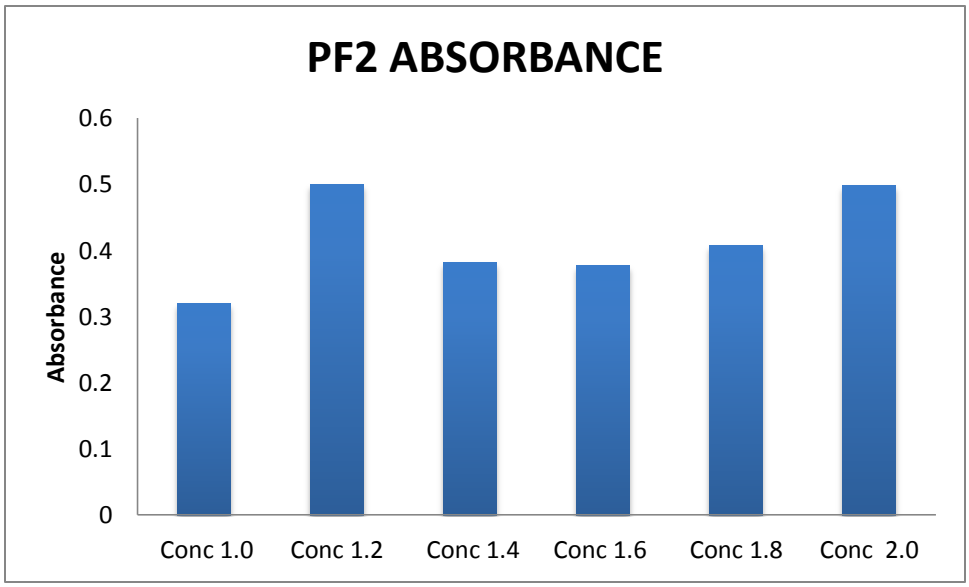
The ability of the formulation to show antioxidant activity was determined using the Rutin method. The drug powder was macerated with methanol and filtered through Whatmann filter paper. 1ml of this filtrate was taken and mixed with 1ml 2% solution of $AlCl_3$ and then diluted with methanol to make up the volume to 10ml. The solution was incubated for an hour and absorbance measured at 415nm using rutin as the standard. Methanolic solution of Rutin was prepared by diluting 0.01mg in 1ml[4].

RESULT AND DISCUSSIONS:

1. HYDROGEN PEROXIDE SCAVENGING PROPERTY:

The antioxidant properties of Formulation 1 (PF1) and Formulation 2 (PF2) are seen in the following graphs:





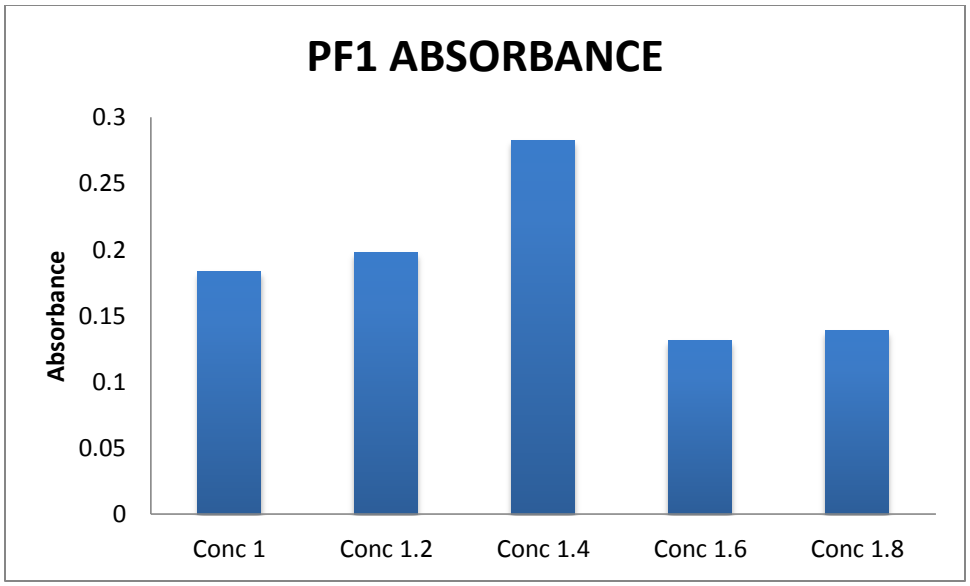
In both the formulations, a linear graph is seen, indicating that there is linear relationship between the concentration and the scavenging property showing that as one increases the concentration, a potential increase in scavenging abilities is seen.

In PF1, a linear increase is seen in the graph as the concentration increases. This indicates that an increase in antioxidant properties is seen. In PF2, a more uniform graph is seen with only a slight drop in antioxidant activity at around 1.4 and again picking up on higher concentration. From these graphs, it can be inferred that as we progress with dilutions, the AVC of H_2O_2 scavenging activity till the concentration of 1.8 is ellipsoidal which indicates rhythmic successive decline in activity. Above 1.8, the graphs become arrhythmic. If peak plasma levels are studied, it can show similar outputs.

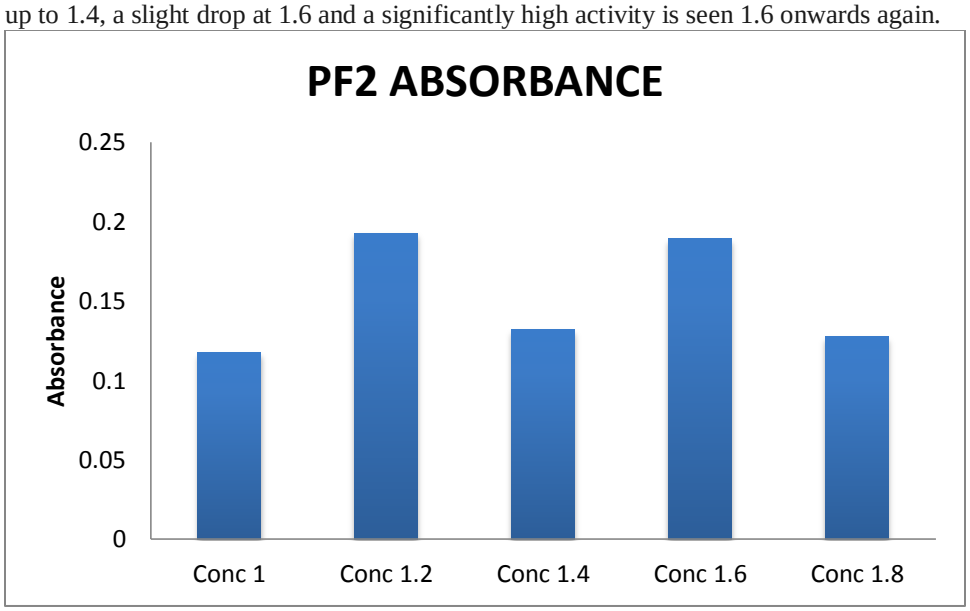
The slight change in the antioxidant properties of the two formulations can be explained by the difference in the way the pyrolysis of the pomegranate peels was carried out. Heat was provided in different ways to both, which caused different changes in its chemical moiety, explaining the difference in their scavenging properties.

2. RUTIN ANALYSIS:

The antioxidant properties of Formulation 1 (PF1) and Formulation 2 (PF2) are seen in the following graph:



In PF1, a linear increase is seen up to concentration of 1.4, after which a gradual drop is seen till 1.6 and again a linear increase is seen for the upper concentrations. This indicates that an increase in antioxidant properties is seen up to 1.4, a slight drop at 1.6 and a significantly high activity is seen 1.6 onwards again.



In PF2, an alternate pattern of increasing and decreasing antioxidant property is seen.
This pattern of association and dissociation of the solute in the solvent is changing with every alternate dilution which might be because of the time lag of the molecular rearrangement which takes place during the reaction incubation. Because of the fact that direct formulation was taken. The cellular structures in the formulation have been observed to interfere in the methodology of release of the drug constituents in the solvent system which has not been seen with other methods of formulation. This indicates that, the extract must be prepared and evaluated for the accuracy of its antioxidant property.

ACKNOWLEDGEMENTS

The authors are grateful to the Principal and laboratory facilities at Maharashtra Institute of Pharmacy, Pune

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