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ORIGINAL ARTICLE

Bioaccessibility of bioactive compounds of biowaste fruit peel of *P. granatum* with antifungal potential against plant pathogenic fungi

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ABSTRACT
The worldwide attention in scientific examination is to discover the herbal and natural medicine, as a result provided that them for pharmacological production and food security without any harmful property on human healthiness. Punica granatum plant is rich in medicinal properties and has high-bioavailability. Pomegranate fruit has a biowaste fruit peel with potential therapeutic applications. Pomegranate biowaste fruit peel extract and fractions are most potential due to their high efficiency. The earlier study confirmed the significant antioxidant and antibacterial properties of pomegranate coating as herbal food additives. This paper highlights the relevant and recent antifungal applications against plant pathogenic fungi established in the prime alteration of biowaste fruit peel of P. granatum. Antifungal activity of the ethanolic extract and fractions of selected plant part were carried out by disk diffusion method against selected plant pathogenic fungi. The results showed that selected fractions of P. granatum biowaste fruit peel (PG II) have great potential as antifungal compounds against Colletotrichum gloeosporiodes (22 mm) than Rhizoctonia solani. The MIC value of both selected fungi was evaluated by agar dilution method varied as of 0.1 mg/ml to 2 mg/ml. Therefore, the current study aim to account the PG II fraction of biowaste fruit peel of P. granatum is a major source of polyphenolic complexes. Although, the present study recommended the pomegranate biowaste fruit peel that can place as a relatively more precious plant source of herbal secondary metabolites for rising novel efficient food-pharma constituents with encouraging human being fitness.
Keywords: *P. granatum, Green technology, plant pathogens, secondary metabolites.*

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INTRODUCTION

Punica granatum L. (Punicaceae) is a nutritional fruit and have various therapeutic phytochemical compounds [1, 2]. *Punica granatum* generally well-known as pomegranate is a small tree belonging to the family *Punicaceae.* Pomegranate is developed in India, USA, Iran and most of the East Countries with worldwide application in folk medicine [3, 4]. Amid fruits, pomegranate is one of the commonly passionate fruit in the earth for good health. Formerly, the fruit is consumed and the peel is discarded as waste. But pomegranate peels are as valuable as the pomegranate. *P. granatum* are acknowledged to have antibiotic, antifungal and enzymatic activity. It is rich in vitamins and fiber content. Pomegranate and their fruit peels are have vital significance as an antioxidant for health concern against potential hazard of synthetic antioxidants in the food industry. Fungi are most responsible for plants; fruits and vegetables infection and they are extremely mordant and extensive to plant life. Plant fungal pathogen enters in to the vegetables and harvested fruits during wounds, bruises and cracks for the period of harvesting method. Indeed, plant fungi can build food crops unusable for utilization during storage, through varying the nutritional impact of the seeds, fruits or creating mycotoxins that are hazardous for human fitness [5,

6]. Globe tendency are stirring to reduce pesticide exploit in vegetables and fresh fruits. Beside with this tendency, numerous physical and biological resources have been estimated as broad-spectrum control in favor of the exploit of chemically synthesized fungicides. *Punica granatum* biowaste fruit peels attributes of the natural biologically active compounds contain antioxidant, antitumor, antibacterial, antiviral, antimutagenic, antibiotic, cardioprotective activities and enzymatic properties [7, 8]. Secondary metabolic compounds of fruits and their biowaste peels e.g. polyphenolic compounds are mostly recognized as health-improving compounds. While extracts generated from pomegranate fruit peels inhibit the growth of microorganisms, the distribution of these properties to the diverse subgroups of substances, most potent components, is still unidentified. So, in the previous study of Saxena et al. [9], polyphenols from pomegranate biowaste fruit peels were extracted and separated into polyphenolic fraction using TLC and column chromatography. Phenolic compounds were determined by UV-spectrophotometry, HPLC (high performance liquid chromatography) and LC- MS (liquid chromatography - mass spectrometry) techniques in the previous research paper of Saxena et al. [9]. All fractions of fruit peel of *P. granatum* were named as PG I, PG II, PG III, PG IV and PG V respectively. The study of Saxena et al. [9] were also reported that the most potent total phenolic and flavonoids content were examined in the PG II fraction of fruit peel extract (617 ± 0.017 mg/g and 546.33 ± 0.032 mg/g correspondingly) in contrast to further fractions. Although, the aim of this present study was to investigate the antifungal effect of the pomegranate fruit peel extract and its fractions against plant pathogenic fungi (e.g. *Colletotrichum gloeosporiodes* (MTCC – 9663) and *Rhizoctonia solani* (MTCC – 4633) respectively) by the disc diffusion technique and MIC (Minimum Inhibitory Concentration) was evaluated by agar well diffusion technique.

MATERIALS AND METHODS

Collection and Identification of biowaste fruit peel

Fresh waste fruit peels of pomegranate were assembled from the juice shop (National Handloom Juice Centre, Vaishali Nagar, Jaipur, Rajasthan). The sample specimen was recognized by 'Herbarium' Department of Botany, University of Rajasthan, Jaipur derived from the taxonomical characteristics and then registered. Registration number was RUBL – 21111 i.e. allotted to fruit peel of *P. granatum*.

Preparation of Crude Extract of biowaste fruit peel

The extraction process was carried out as per the process reported earlier by Perumal Samy and Gopalakrishnakone [10]. Pomegranate fruit peel powder was packed in the thimble and extracted consecutively with 95 % ethanol (ethanol: distilled water; 95: 5) solvents in soxhlet extraction machine for 48 hours. The fruit peel extracts were filtered and then concentrated via rotary evaporator at 40 ºC.

Qualitative and Quantitative Screening of phenolic compounds

Several quantitative and qualitative screening of bioactive compounds was tested in the earlier study of Sharma et al. [8] and Saxena et al. [9] by the methods of Practical Pharmacognosy by C.K. Kokate [11].

Fractionation of biowaste fruit peel by chromatographic techniques

Therefore, in the previous study of Saxena et al. [9], polyphenols from pomegranate biowaste fruit peels were extracted, separated and identified into polyphenolic fraction using TLC, column chromatography, UV- spectrophotometry, HPLC and LC- MS techniques. All fractions of fruit peel were named from PG I to PG V correspondingly.

Antifungal screening via disc diffusion technique

The Disc diffusion technique was employed for assessment of antifungal effect with slight modification [12, 13]**.** Sterilized 5 mm discs were soaked by extracts (10 mg/ disc) and fractions (1 mg/ disc) of biowaste fruit peel and put on the SDA agar plates which have the microbial culture on the surface. The petri-dishes were incubated at 28 ºC for 5 to 7 days for the growth of fungal cultureand observed the diameters of the inhibition zones. All the experiments were completed in triplicate and the zone of inhibition (in mm) were expressed as their mean values.

Screening of Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) value of extracts and fractions of *P. granatum* was evaluated by agar dilution technique for fungal cultures [14, 15]. The extracts and selected fractions were incorporated into specific nutrient medium at various concentrations and uniform gently in sanitary petri-plates and after that permitted to set. The plates were then incubated at 28 ^{o}C up to 5 to 7 for fungi after inoculation. The lowest concentration preventing the visible expansion in each determination and that was taken as the MIC (Minimum Inhibitory Concentration). The growth of microorganisms was indicated through visual examination. All the experiments were performed in triplicate. ketoconazole served as a control for fungi. Standard gallic acid, caffeic acid was also employed as a control for comparison of fruit peel extract and fractions with different concentrations.

Data Examination

Statistical investigation was processed by SPSS version 16.0 software for antifungal property of fruit peel extract and fractions. The outcome express as arithmetic mean ± SD (standard deviation) and analysis was carry out by student T-test and analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Exposure of drug resistance in plant pathogenic fungi as well as harmful side effects of antibiotics has created huge attention in the exploration for new antifungal drugs of plant origin [16]. The antifungal property of the fruit peel crude ethanolic extract and selected fractions (PG II fraction) was assessed by the presence or absence of inhibition zone against selected fungal cultures i.e. *C. gloeosporiodes* (MTCC No. 9663) and *R. solani* (MTCC No. 4633) respectively. The outcome of the disc diffusion method for antifungal values of extract and fractions of selected plant part were depicted in Figure 1. The current findings were also demonstrated the noteworthy antifungal activity of PG II fraction of *P. granatum* displaying the greatest antifungal effect against *Colletotrichum gloeosporiodes* (22 mm) in comparison to standard drug (ketoconazole) at the same concentration 50 µg /ml respectively. However, no antifungal activity was found by *P. granatum* (ethanolic extract and PG II fraction) against *R. solani* at the specific dose (50 µg /ml). Moreover, no significant antifungal activity was also observed with standard compound i.e. caffeic acid and gallic acid against *R. solani* at the same dose level 50 µg /ml as shown in Table 1.1. The total phenolic and flavonoids content and chromatographic identification of extract and fractions of *P. granatum* fruit peel were summarized in the earlier study of Saxena et al. [9]. Most significant motives of this issue are generally reduce the utilization of synthetic pesticides against a minor problem of phytopathogenic fungi in plants due to which microbes produce toxins in plant seeds and fruits. Therefore, it is essential to discover the perceptive of this potential concern to reduce this issue in healthcare system [16, 17]. This investigation affords a scientific evidence for exploitation of biodegradable biowaste fruit peel of pomegranate which have potential to become a good drug source against plant pathogenic fungi. The data were expressed as mean ± SEM (n =3). Statistical significance for antifungal activity of biowaste fruit peel against fungal cultures was determined by two way ANOVA test. A 95 % confidence interval, P values less than 0.05 were measured as significant. It was clear from the present results that PG II fraction of fruit peel of pomegranate had a broad spectrum of antifungal activity against the *Colletotrichum gloeosporiodes*. The MIC was performed to find out the minimum concentration of the tested extract and PG II fraction fruit peel of pomegranate that inhibited the growth of fungal strains with MIC values ranging from 100 to 2000 µg/ml [Table 1.2]. Table 1.2 represented the results of MIC of PG II fraction of *P. granatum* against *C. gloeosporiodes* was found to be 800 µg/ml and 1000 µg/ml correspondingly and further no visual growth was observed at this concentration up to 10 days for fungal cultures. Furthermore, the antifungal activity of *P. granatum* against mycelial fungi was also reported by various scientists [18, 19, 20]. Current results were also coincides with Duman et al. [21], who demonstrated the antimicrobial effect of diverse six varieties of *P. granatum*, grown in the Mediterranean region of Turkey, associated with the responses of phytonutrient belongings, e.g. anthocyanin and phenolic compounds and three varieties i.e. Kan, Serife and Eksi of *P. granatum* accounted the affirmative results regarding the inhibition of *Candida albicans*. Although, other three varieties i.e. dikenli incekabuk, katirbasi and tatli had no significant effect on *C. albicans.* Similarly, Endo et al. [22] examined the prominent antifungal property of punicalagin against *C. parapsilosis* and *C. albicans*, signifying this essence as an effective antifungal agent. Moreover, the current pronouncement were also approved by earlier scientist Glazer et al. [23], who demonstrated that pomegranate peel extracts were analyzed by *in vitro* for their antifungal effect against *Stemphylium botryosum*, *Alternaria alternata,* and *Fusarium* species. These are responsible for causing fruit and vegetable decay during storage and also suggested that punicalagins and ellagitannins both were the principal constituents in pomegranate fruit peels. They can be utilized as a control agent of storage diseases and to decrease the utilization of synthetic fungicides. The recent finding were matched with the investigation of Rongai et al. [24] and Rongai et al. [25] that reported the utilization of pomegranate fruit peel extract to control phytopathogenic fungal diseases. In contrast, the potent efficiency of antifungal action confirmed with diverse pomegranate fruit peel extract in laboratory research motivated scientists to further examine this field of application. Infact, existing field information suggests the potential use of pomegranate fruit peel extracts to control a wide variety of diseases caused with hemibiotrophic, biotrophic and necrotrophic fungal pathogens. For example, the incorporation of a pomegranate fruit peel extract in soils artificially inoculated with *F. oxysporum* extensively remove the population of the pathogen and improved the amount of healthy tomato plants.

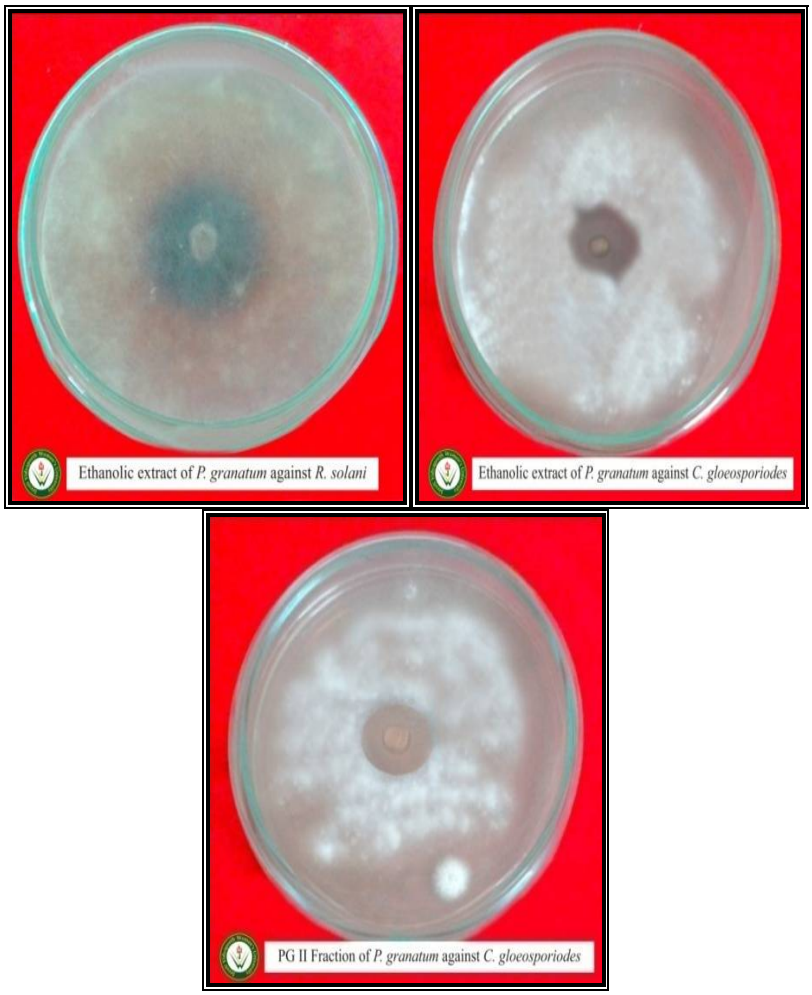

Figure 1: Antifungal Activity of Ethanolic Extract and PG II Fraction of peels of *Punica granatum* against *R. solani and C. gloeosporiodes*

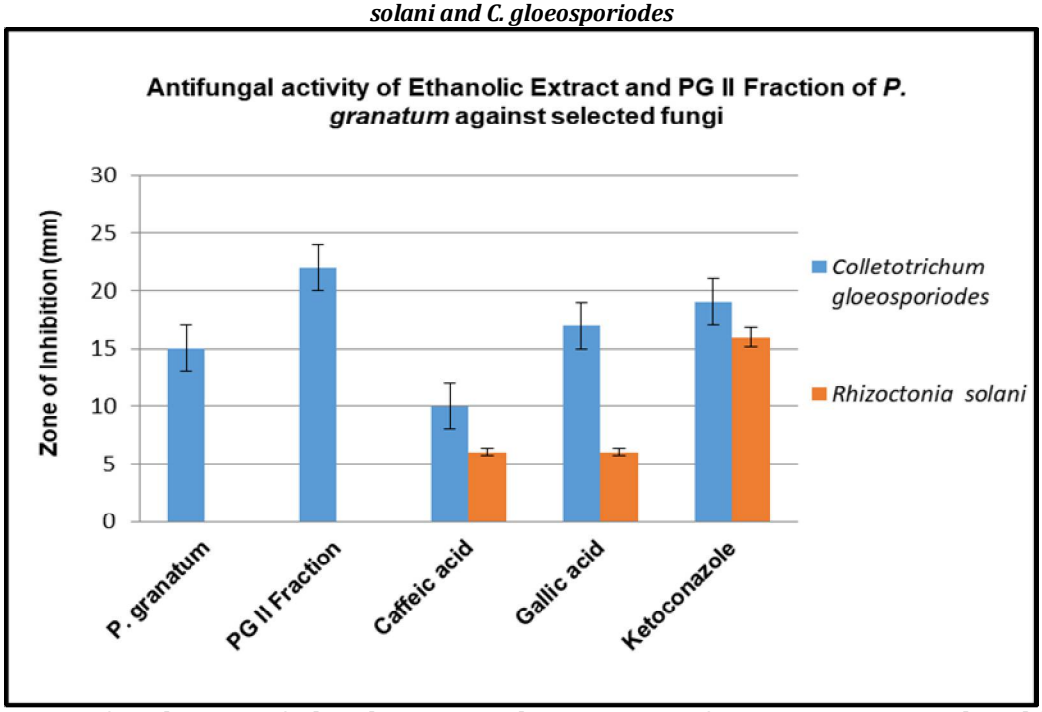

Figure 2: Antifungal activity of Ethanolic Extract and PG II Fraction of *P. granatum* against selected fungi (*Results expressed as Mean ± SEM from three observations)

Table 1: Minimum Inhibitory Concentration (MIC) results of Ethanolic Extract and PG II Fraction of *Punica granatum* against *C. gloeosporiodes* fungal culture

Conc. (µg/ml)	Visible growth of Microorganism *C. gloeosporiodes*	
	P.g.E.	PG II Fraction
100	+4	+4
200	+ 4	+3
400	+ 3	+2
600	+3	+2
800	+3	+1
1000	+2	No growth
1200	+2	0
1400	+1	0
1600	+1	0
1800	+1	0
2000	No growth	0
Control without Extract/Fraction	100 % growth	100 % growth

*Growth was scored in the following manner: +3 good growths comparable to that of the Extract free control; +2, light growths approximately that of the control + 1, very light growths approximately that of the control; 0, No visible growths.

CONCLUSION

The findings of the present study concluded that biowaste fruit peel of pomegranate can effectively control phytopathogens. The biowaste fruit peel extract and fractions of *P. granatum* showed antifungal activity and was used here for inhibition of phytopathogenic fungi. This study covers the mode for the enhancement of bioactive natural products with phytohygienic appliances, with the additional wage of an ecologically secure and cost-effectively feasible product.

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