

**ASPERGILLUS NIGER MEDIATED SOLID STATE FERMENTATION
(SSF) OF POMEGRANATE PEELS, YIELDS BIOACTIVE PRODUCT
HAVING ANTIBACTERIAL, ANTIOXIDANT, RADICAL
SCAVENGING AND ANTI-UV PROPERTIES**

Harshada Satish Dipnaik¹ Priyanka Shrivastava² Salgo Merin Jacob^{3*}

^{1,2}Department of Biotechnology, Viva college of Arts, Commerce and Science, University of
Mumbai, Maharashtra, India.

³Department of Biotechnology, University of Mumbai, Kalina, Santacruz East, Mumbai,
Maharashtra, India.

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***Correspondence for
Author**

Dr. Salgo Merin Jacob
Department of
Biotechnology,
University of Mumbai,
Kalina, Santacruz East,
Mumbai, Maharashtra,
India.

ABSTRACT

Pomegranates contain several bioactives, majorly phenolic compounds, which have been reported to have numerous health benefits. Several industries require anti-oxidant, anti-UV and anti-microbial activities in their products which are obtained by incorporating various chemicals. With a view to assess these applications *Punica granatum L.* (pomegranate) peels were evaluated. **Methods:** The pomegranate peels were subjected to Solid State Fermentation (SSF) using *Aspergillus niger*. The bioactives were extracted using methanol as a solvent. Extracts were analysed for phytochemicals, antimicrobial activity (agar-cup method and AATCC 100-2004 method for fabric), Total Phenolic Content (Folin-ciocalteau method), antioxidant potential activity (Ferric-ion-Reducing-Antioxidant-Potential), superoxide-scavenging (alkaline DMSO method) and anti-UV activity. Bioactive

Compounds were characterized using UV spectrophotometer and FTIR. **Result:** Phytochemical analysis and characterization revealed that the compounds of interest are aromatic and phenolic in nature. The methanolic extract showed antibacterial activity against *S.aureus*, *P.aeruginosa* and *B.subtilis*. An 86.10% and 92.60% reduction of *S.aureus* and *K.pneumoniae* count was observed on treated fabric respectively. On exposure to UV the extract protected *E.coli* upto 40 seconds of exposure to UV. The total phenolics were 59.73 ± 0.46 mg gallic acid equivalence gm^{-1} pomegranate peels, whereas an average antioxidant

potential was 0.18 ± 0.018 mg ascorbic acid equivalence gm^{-1} of pomegranate peel. A 65% superoxide scavenging activity was observed at 0.5 mg/ml of peel extract. The correlation coefficient for anti-oxidant potential and phenolic content was $R^2 = 0.928$. **Conclusion:** Results indicated significant contribution of isolated phenolic compounds to antibacterial, anti-UV, antioxidant and radical scavenging activity of the *Punica granatum L.*

KEYWORDS: *Punica granatum*, SSF, TPC, FRAP, antimicrobial, anti-UV.

INTRODUCTION

Interest in the development of Solid State Fermentation (SSF) bioprocess for the production or extraction of bioactive compounds from natural sources has increased, in recent years, due to the potential applications of these compounds in food, chemical, and pharmaceutical industries. Filamentous fungi have a significant potential to produce bioactive compounds by SSF, and therefore, they are the most commonly used microorganisms for this purpose.^[12]

Punica granatum L. (Pomegranate) are cultivated around the world in subtropical and tropical regions with different microclimatic zones such as in Iran, California, Turkey, Egypt, Italy, India, Chile and Spain.^[3] Pomegranate fruits have been the focus of recent interest among researchers for their role in human health and particularly in prevention of chronic diseases. Pomegranate fruit is a rich source of bioactive compounds such as flavonoids, phenolic acids, tannins and vitamin C and are attributed with diverse medicinal properties and health benefits that are highly desirable.^[6]

The non-edible fruit sections also have high Total Phenolic Content (TPC) and high levels of antioxidant capacity^[41]. Pomegranate fruit peel constitutes about 50% of the total fruit weight and it is often discarded as waste by food processing industries^[4]. Studies have also demonstrated that pomegranate peel contain substantial amount of phenolic compounds as compared to the juice.^[6]

These phenolic compounds, derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants are well known for anti-oxidant activity.^[8] The biological activities exhibited are scavenging free radicals, inhibiting microbial growth, and decreasing the risk of cardio and cerebrovascular diseases, some cancers and other conditions related with viruses such as HIV-1, influenza.^[9, 7, 43]

The antioxidant activity of phenolics may be attributed to various mechanisms, such as prevention of chain initiation, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging action ^[28]. The reducing capacity of the compound serves as a significant indicator of its potential antioxidant activity. Many *in vitro* and *in vivo* studies pointed out to high nutritional and potential tissue specific action of Pomegranate. ^[22] Pomegranate peel powder has exhibited potential anti-oxidant activity *in-vivo* and was able to protect liver against the oxidative stress of CCl₄ in rats. ^[24]

The anti-microbial activity of phenols is exhibited by bactericidal actions due to cross linking, coagulating, clumping the bacterial cells. At higher concentration these compounds penetrate and disrupt the cell wall and make the cell proteins fall out of suspension. Studies have recorded that most of the isolated bacteria from waste water were sensitive to pomegranate peels extract. A number of extracts of pomegranates has been tested against a range of bacteria (*S. aureus*, *E.coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Bacillus subtilis* and *Salmonella typhi*), and activity against all isolates is reported. ^[21]

Antibacterial activity of the extracted compound also has its application in textile industry for production of fabric used under aseptic conditions like surgical cloth, bandages etc. In this context the compounds extracted from Pomegranate peels are also tested for their antibacterial activity on textile cloth by AATCC method in the present study. This test method provides a quantitative procedure for the evaluation of the degree of antibacterial activity on textile cloth.

Polyphenols such as various kinds of flavonoids, which are photo protective in nature, can be found in many plant extracts. They are known to contribute to an important function in plants, providing protection from UV radiation. This is attributed to absorbance or reflection of UV radiations by the compounds. These compounds also prevent negative effects of UV radiations including mutagenesis, cellular death by dimerisation of pyrimidines or thymine units in DNA, genomic as well as mitochondrial DNA fragmentation, apoptosis of keratinocytes and generation of reactive oxygen species (ROS). ^[32] Pomegranate peel extract has been reported to be effective at protecting human skin fibroblasts from cell death following UV exposure. ^[33] In this study, (SSF) with *Aspergillus niger* is used as the bioprocess that successfully converted pomegranate peel waste to bioactive phenolic compounds which were studied for antioxidant, anti-UV, anti-bacterial properties.

MATERIALS AND METHODS

Sample collection: The Pomegranate fruit peels, a waste product of food processing units such as juice centres, were obtained from the several juice centres in the city Mumbai, Maharashtra. The peels were cleaned with running water to make them free from any contaminants. They were finely chopped and powdered using a mixer grinder. The powder was autoclaved at 121 °C at 15psi for 20mins.

Solid state fermentation

Solid state fermentation was carried out using finely chopped, ground and autoclaved pomegranate peels. Spore suspension of *Aspergillus niger* was inoculated into Sabaraud's broth. A 2.5 ml portion of the inoculated broth was added to the pomegranate peel substrate to initiate fermentation for 0, 2, 4, 6 and 8 days at room temperature $32 \pm 2^\circ\text{C}$ (RT).

Extraction of bioactive compounds

25 gm of SSF product of designated day of fermentation was extracted separately using 200 ml methanol (85%) in Soxhlet apparatus. Whatman filter paper No.1 was used to prepare a thimble and extraction cycle was carried out for 9 hours at 65°C until the solvent was found to be colourless and the thimble was removed. Methanol was recovered and concentrated extract was collected and refrigerated till further studies.

Qualitative analysis of Phytochemicals

The methanolic extract of sample of defined period of fermentation was evaluated for its phytochemical composition. The extracts were screened for bioactive compounds using standard methods. [23, 24, 28]

Table 1: Phytochemical analysis of peel extract.

Test	Method	Observation	Inference
Proteins	Millon's test	No formation of white precipitate	Proteins Absent
Amino acids	Ninhydrin test	No appearance of violet coloration	Amino acids Absent
Reducing sugars	Fehling's test	Brick red precipitation	Reducing sugars present
Carbohydrates	Benedict's test	Reddish brown precipitation	Carbohydrates Present
	Molish's test	Appearance of a violet ring at the inter phase	
Starch	Iodine test	Dark blue coloration	Starch Present
Phenols & tannins	Ferric chloride test	Blue-green or black coloration	Phenols & tannins Present
	Sodium Bicarbonate test	Effervescence indicate aromatic nature	
	Libermann's test	Deep greenish blue coloration	Phenols Confirmed
	Lead acetate test	Yellow precipitate	Tannins Confirmed

	Gelatin test	White precipitate	
Flavanoids	Shinoda test	Pink scarlet coloration	Flavanoids Present
	Alkaline reagent test	An intense yellow coloration which turns colourless on addition of few drops of diluted acid	
Saponins	Foam test	Formation of a stable foam	Saponins Present
Glycosides	Salkowskis test	Reddish brown coloration	Cardiac glycosides Present
	Keller-Killiani test	Brown ring formation at the inter phase	
Steroids	Crude extract + 2ml Chloroform + conc. H ₂ SO ₄	No development of a greenish coloration	Steroids Absent
Terpenoids	Crude extract dissolved in 2ml Chloroform + conc. H ₂ SO ₄ + gentle heating	No development of a greyish coloration	Terpenoids Absent
Alkaloids	Mayer's test	Turbidity of the solution	Alkaloids Present

Antibacterial and antifungal activity

Antibacterial and antifungal activity of the extracted compounds was tested through agar cup method. Erythromycin (100mcg/ml), Ampicillin (50 mcg/ml), Chloramphenicol (100mcg/ml) were the standard antibiotics used for testing antibacterial activity against the test Gram positive organisms *B.subtilus* NCIM NO.2724 and *S.aureus* NCIM NO.2121, Gram negative organisms *P.aeruginosa* NCIM NO.2659 and *E.coli* NCIM NO.2830 on the other hand Fluconazole (100 mcg/ml) was used as a standard against fungal cultures namely (*C.albicans* NCIM NO.3471 and *S.cerevisiae*). Culture suspension of density 1×10^8 of all microorganisms was prepared. A range of 10-100% concentrations of the SSF processed peel extract was prepared using DMSO as a diluent and 40µl of each concentration was added into each agar cup of 10mm diameter. Zone of Inhibition were observed and recorded after incubation of the plates for 24 hours by referring to Kirby Bauer's chart.

Antibacterial activity on textile

Since Day 6 methanolic extract (sample) showed highest antibacterial activity, it was sent to SASMIRA (Synthetic and Art Silk MILLS Research Association) Worli, Mumbai-400030, for assessment of antibacterial activity on textile cloth using AATCC 100-2004 protocol. The test organisms used were *Staphylococcus aureus* ATCC6538 and *Klebsiella pneumonia* ATCC4352 after standardisation. Briefly in this method fabric swatches of Test (with sample extract) and Control (without sample extract) were inoculated with the test organisms and incubated for 24 hours. Bacterial loads on both Control and Test fabrics were determined at "time zero" and reduction in the load was determined after incubation for 24 hours by elution

of bacteria in a large volume of neutralizing broth, followed by dilution and plating. A control was run to verify that the neutralization/elution method effectively neutralizes the antimicrobial agent in the fabric. The reduction in bacterial load is calculated by formula, % Reduction (R) = 100 (B-A/B); where B = Bacterial load (CFU) at 0 hour and A = Bacterial load (CFU) at 24 hours; CFU: Colony forming units = No. of micro-organisms.

Quantitative analysis of Phenolics (TPC)

Total phenolic content of methanolic extract was determined by Folin - Ciocalteu method and expressed as Gallic Acid Equivalents (GAE). Gallic acid (0.8mg/ml) was used as a standard. 1 ml of the extract was mixed with 5mL of Folin-Ciocalteu's reagent and allowed to stand for 2 minutes at room temperature after which 15ml of 20% sodium bicarbonate was added to the reaction mixture and incubated in dark for 2 hrs. Distilled water was used as blank and absorbance was noted using visible spectrophotometer [Equip-tronics/NO EQ 820]. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained at λ 760nm. A standard curve was prepared using Gallic acid with different concentrations (25-800 mcg/ml) and the total phenolic content was expressed in GAE.

***In vitro* antioxidant activity (FRAP)**

The Ferric Reducing Antioxidant Power (FRAP) of the Day 6 methanolic extract was determined by using the potassium ferricyanide-ferric chloride method. A 200 μ l portion of the extract at different concentrations [100, 200, 300, 400, 500 mcg/ml] was added to 2ml of 0.2M, Phosphate buffer (pH 6.6) and 2.5ml of 1% Potassium ferricyanide solution and incubated at 50°C for 20 min. 2.5ml of 10% TCA was added to reaction mixture after cooling. 2.5 ml of reaction mixture was transferred to equal amount of distilled water and 1ml of 0.1% FeCl₃. Standard solution of Ascorbic acid (1 mg/ml) and Control (85% methanol) were also kept. Ferric reducing antioxidant power to ferrous complex was measured using spectrophotometer [Equip-tronics/NO EQ 820]. The absorbance was recorded at λ 700nm and expressed as ascorbic acid equivalents.

Superoxide scavenging activity by DMSO

The superoxide scavenging activity of Day 6 methanolic extract with different concentrations (100-500 μ g/ml) and Ascorbic acid (standard 10-50 μ g/ml) made in hydrogen peroxide solution was analysed by alkaline DMSO method. To the reaction mixture containing 0.3 ml of the samples, 0.1 mL of NBT was added, 1 ml of alkaline DMSO (1 ml DMSO containing, 0.1ml, 5 mM NaOH in water) reagent was added making the final volume to 1.4ml. A blank

containing plain DMSO instead of the test sample or ascorbic acid was also included under the same condition. Superoxide scavenger inhibits the formation of a red dye, formazan. Absorbance was recorded at λ 560nm using spectrophotometer [Equip-tronics/NO EQ 820]. The superoxide scavenging activity was calculated using formula % Superoxide radical scavenging activity = [(Test absorbance - Control absorbance)/Test absorbance] x 100.

Anti UV radiation test

Anti ultraviolet radiation effect of compound was evaluated on *E.coli* (1×10^8 cells/ml). Culture (5 ml) was added to 6 sterile empty petriplates containing 100 μ l of test (extracted compound) or 100 μ l control (85% methanol without test compound) plates respectively and each plate of set was exposed to ultra violet light at different intervals (20, 40, 60, 80, 100 seconds). Serially diluted test and control samples were then spread plated on sterile nutrient agar plates (0.1 ml of test culture on each plate). Results were observed after 24 hrs of incubation at 37°C.

Determination of structural and functional properties

UV spectroscopy (UV/Visible spectrophotometer LAB INDIA) was used to confirm the aromatic character of the extracted compound recording absorbance at λ 440nm using 25 ml of methanolic extract as a sample. The functional group were assessed by FTIR analysis using (JASCO FT/IR-4100) using 1:5 diluted methanolic extract as a sample and FTIR spectrum were recorded at absorbance range of λ 190nm to 1100nm at a speed of 100nm/min.

RESULTS

Qualitative analysis of the phytochemicals in peel extract of *Punica granatum* L.

The methanolic extract showed presence of carbohydrates, reducing sugars, phenols, tannins, flavanoids, saponins, glycosides and alkaloids where as proteins, steroids, terpenoids were not present.

Antimicrobial test

Methanolic extract of Day 0, Day 2, Day 4, Day 6 and Day 8 solid state fermentation product showed antimicrobial activity against *S. aureus*, *B. subtilis*, and *P. aeruginosa*. The extract of Day 6 fermentation product showed the largest zone of inhibition.

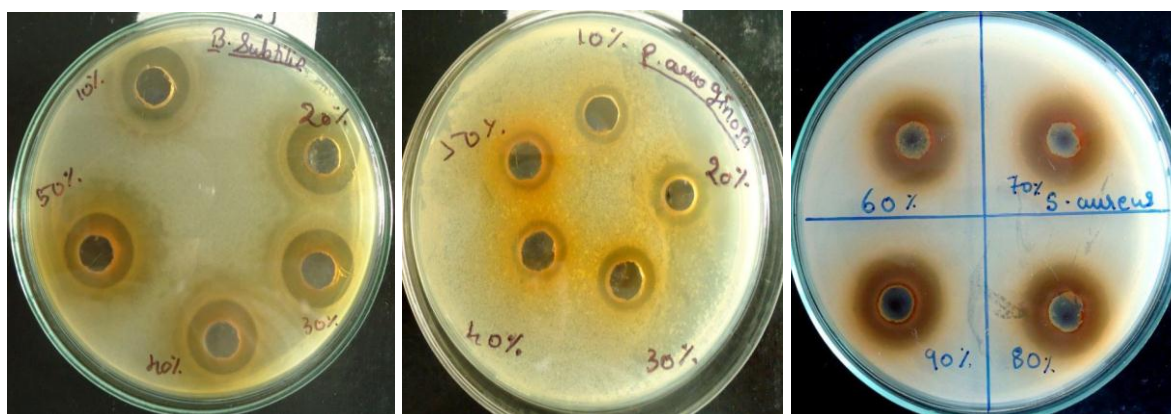


Fig 1: Antibacterial activity of day6 methanolic extract against *B.subtilis*, *P. aeruginosa* and *S.aureus*.

Table 2: Observation table of antimicrobial activity against *B. subtilis*, *P.aeruginosa* and *S.aureus*.

ZONE OF INHIBITION in (mm)																	
<i>B.subtilis</i>						<i>P.aeruginosa</i>						<i>S.aureus</i>					
Dilution of extract	D0	D2	D4	D6	D8		D0	D2	D4	D6	D8		D0	D2	D4	D6	D8
10 %	-	-	15	16	15		-	-	-	14	-		-	-	15	13	11
20%	-	-	-	18	15		-	-	-	14	-		-	-	14	16	12
30%	-	-	12	19	15		-	-	13	15	-		-	-	12	15.5	12
40%	-	14	11	17	16		-	-	12	14	-		-	-	11	16	13
50%	-	15	15	19	18		-	-	16	14	-		-	-	11	16	16
60%	-	-	-	13	12		-	-	14	14	11		-	-	11.5	16	-
70%	-	-	-	12	13		-	-	12	15	12		-	-	12	16.5	-
80%	-	-	-	13	13		-	-	12	16	13		-	11	13	17	-
90%	-	15	12	15	12		-	-	12	16	15		-	11	13	17.5	-
100%	-	14	14	13	11		-	-	13	17	14		-	-	15	18	-
Cont	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
DMSO	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Cam	16	17	17	17	17	Ery	20	20	12	22	19	Ery	30	25	30	24	37

D0: day0, D2: Day2, D4: day4, D8: day8; Cont: Control, ABX: Antibiotic, Cam: Chloramphenicol, Ery: Erythromycin, (-): no zone of inhibition obtained.

Antibacterial activity on textile

Since Day 6 samples showed highest zone of inhibition compared to other day samples, these were sent for evaluation of textile related antibacterial activity to SASMIRA (Synthetic and Art Silk MILLS Research Association) lab, Worli, Mumbai-400030. The reduction in bacterial load on fabric swatches recorded before and after incubation with samples was as follows.

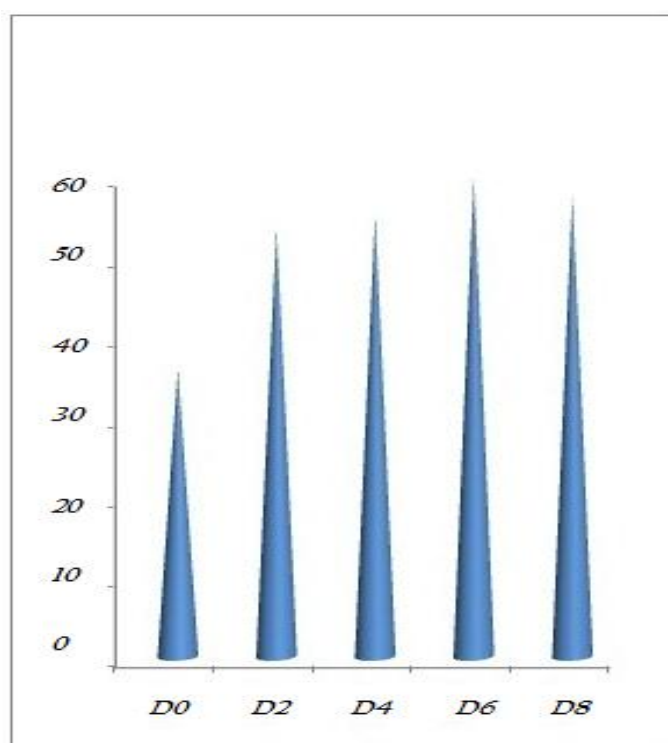
Table 3: Reduction of bacterial load on textile cloth.

Sample Code	Test organisms	No. of Bacteria per sample at CFU		Percentage Reduction (R)
		'0' hours (B)	'24' hours (A)	
Fabric	<i>S.aureus</i>	1.12×10^5	0.29×10^5	86.10%
	<i>K.pneumoniae</i>	2.84×10^5	0.21×10^5	92.60%

$$\% \text{ Reduction (R)} = 100 (B-A/B)$$

Quantitative analysis of Phenolics

TPC: Total Phenolic content, GAE: Gallic Acid Equivalence

**Fig 2: Graph of TPC of each day methanolic extract.**

D0: Day 0, D2: Day 2, D4: Day 4, D6: Day 6, D8: Day 8

Day 6 methanolic extract recorded the highest TPC value of 59.73 ± 0.46 mg Gallic acid equivalence in 1 gm of the sample.

In vitro antioxidant activity (FRAP)

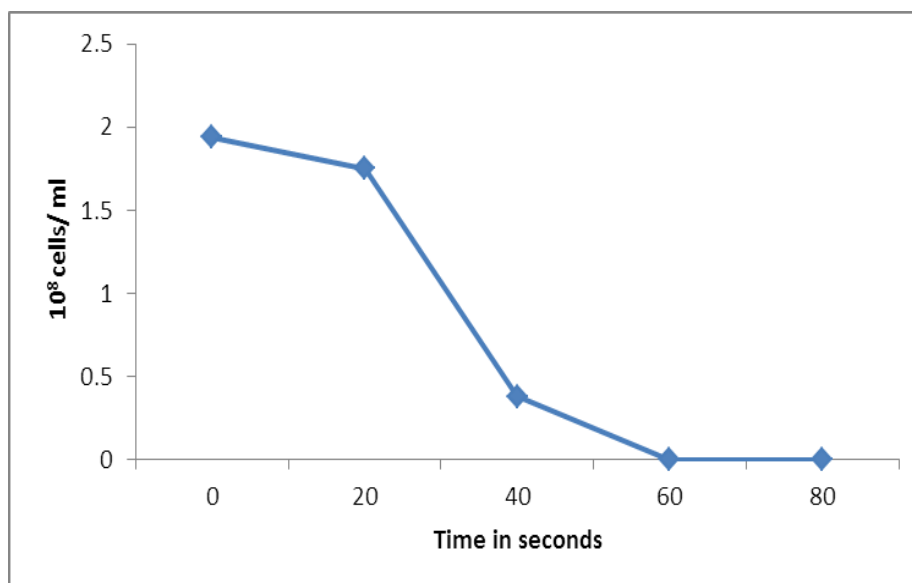
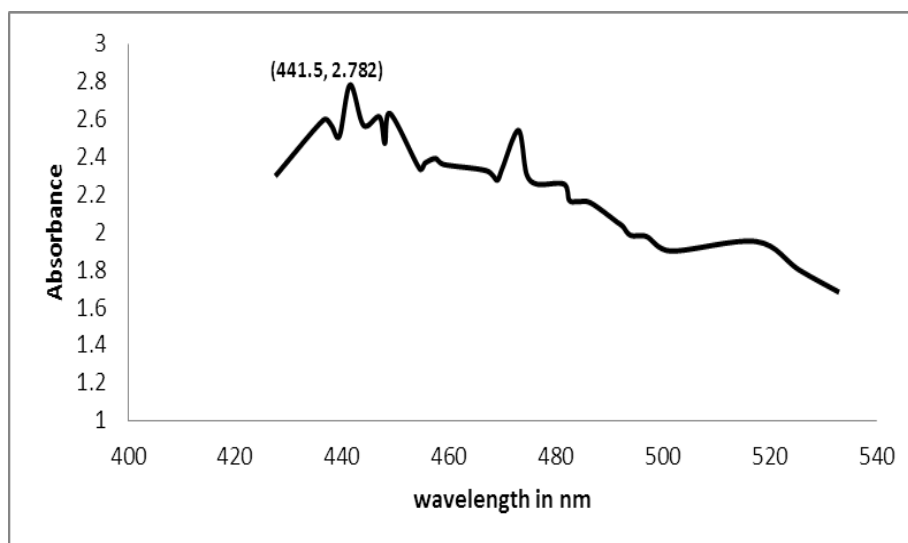
Average antioxidant potential is 0.18 ± 0.018 mg Ascorbic acid equivalence in 1 gm of peel of *Punica granatum L.*

Table 4: Correlation of Phenols with FRAP.

Phenols	FRAP
59.73 ± 0.46	0.18 ± 0.018
Correlation R2	0.928

Determination of superoxide scavenging activity by DMSO**Table 5: Result of % Scavenging activity of Ascorbic acid and Sample**

S. No	Ascorbic acid (Standard)		Day6 Methanolic extract	
	Concentration (mg/ml)	% scavenging activity	Concentration (mg/ml)	% scavenging activity
1	0.01	61.2	0.1	52.9
2	0.02	60.0	0.2	57.9
3	0.03	63.6	0.3	61.9
4	0.04	65.2	0.4	60.0
5	0.05	68	0.5	65.21

Evaluation of Anti-UV effect**Fig 3: Survival of average no. *E. coli* /ml Vs exposure to UV in seconds.****Determination of the functional group and organic nature of the sample****Fig 4: UV- spectroscopy of the sample.**

Absorbance of the sample at wavelength range 400-600nm (interval: 0.50nm), Spectral bandwidth: 2.00nm. Absorbance peak maxima (2.782) obtained at 441.5nm.

Table 6: Analysis of readings obtained from FTIR by comparing with standard chart.

Wavenumber obtained	Range	Probable functional group
3448	3550-3200	Alcohol/Phenol O-H group

DISCUSSION

Being a symbol of super-fruit and famous for its numerous biological effects, the interest in pomegranate (*Punica granatum* L.) continue, in response to an increasing number demand, especially food processing industry. These have increased the number of developed co-products with significant quantities of waste being generated. Solid state fermentation being an eco-friendly and economically important technology, can reduce the toxicity of accumulating agro wastes in natural environments by converting them into commercially valuable products. SSF is reported as a technique to produce higher yields of secondary metabolites in shorter times with lower capital investments as compared to submerged fermentation (SmF) ^[44]. Though the choice of the micro-organism used relies on the desired end product, filamentous fungi are the most commonly used organisms since they have a great potential to produce bioactive compounds by SSF and characteristically grow in low or near to no moisture requirements. *Aspergillus niger* has a suitable choice for SSF of many substrates like wheat and soya brans to produce polygalactarunose, apple pomace and cotton seed powder to produce xylanase, grapes to produce glycosidase, etc ^[45]. In this study we try to explore the possibility of the commercial use of pomegranate peel, a waste product of the industry. The Pomegranate peel substrate were subjected to SSF by *Aspergillus niger* to effectively extract bioactive phenolic compounds, similar to a recent study in which pomegranate husks were successfully used as support and nutrient sources for production ellagic acid by SSF with *Aspergillus niger* GH1 ^[34]. Such process is also highly economical for large scale production, as essential valuable bioactive products are obtained from low cost substrate.

Antimicrobial activity results depicted that *B. subtilis*, *P.aeruginosa*, *S.aureus*, were inhibited by phyto-constituents present in the sample extract where as *E. coli*, *S. cerevisiae* and *C. albicans* were resistant to the compounds in the peel extract. Also the peel extract showed significant anti-bacterial activity in comparison to standard antibiotics used (Chloramphenicol and Erythromycin). However previous research has shown that

pomegranate peel extracts have strong anti-bacterial activity against *B.subtilis*, *S.aureus*, *P.aeruginosa* and *E.coli*.^[37] The peel extract also showed significant reduction of both Gram positive (*S.aureus* : 86.10%) and Gram negative (*K. Pneumonia* : 92.60%) organisms on the textile cloth which is better in comparison to previous study on pomegranate peel extract where maximum reduction observed was 36% for Gram positive (*S.aureus*) and 56% for gram negative (*E.coli*) organisms on the textile cloth^[38], suggesting that the pomegranate peel fermentation product has potential application in the textile industry as a bacterostatic component for reduction of bacterial load on fabric, especially for fabrics used in hospital environments to reduce infections.

Quantitative analysis of phenolics in Day 6 peel extract of pomegranate exhibited highest content of total phenolics i.e 59.73 ± 0.46 mg GAE/gm pomegranate peel which was higher than a similar study done on hydro-alcoholic (methanol, water, ethanol) peel extracts of Egyptian pomegranate in which the sample extract had TPC of 6.2 mg GAE/g dry solids^[36]. On the contrary, in the cases of pomegranate varieties from India (Kashmir district), higher TPC of 124.3 - 249.4 mg GAE/g dry solids have also been reported.^[35] In order to tap the extract's antioxidant capacity, FRAP (Ferric reducing Antioxidant potential) assay was carried out. FRAP value is based on reduction of the ferric ion by the antioxidants, as they are capable of donating a single electron or hydrogen atom for reduction. Higher FRAP values thus, relate to higher antioxidant capacity. In the present study antioxidant capacity was found to be increasing on increase in concentration of the sample. A similar trend was observed in the previous study on antioxidant activity recorded for methanolic extract of seven pomegranate cultivars cultivated in South Africa that showed increasing antioxidant activity with increase in concentration of the sample extracts.^[1] Antioxidants play an important role in scavenging free radicals. Superoxide anion is produced from molecular oxygen due to oxidative enzymes.^[40] These (ROS) are also generated by UV radiations which are very effectively scavenged by polyphenols.^[32] The superoxide radical scavenging activity of the pomegranate peel extract was assessed by Alkaline DMSO method in which Super oxide free radicals formed by alkaline DMSO react with Nitro Blue Tetrazolium salt to produce coloured formazon. The scavenging activity of the peel extract was measured by its ability to inhibit formazon formation. At the maximum concentration of 0.5 mg/ml of the peel extract, 65.21% of superoxide radicals were scavenged, which is significant in comparison standard used, ascorbic acid which scavenged 68% superoxide radicals at the concentration of 0.05 mg/ml. Thus pomegranate peelextract showed significant anti-microbial and

antioxidant activity as exhibited previously by Hayrapetyan et al, 2012 ^[53]. Also the correlation coefficients for antioxidant potential and phenolic content of methanolic extract of pomegranate peel was found to be 0.928 suggesting significant contribution of phenolic compounds to anti-oxidant activity which is higher than the correlation coefficient that was noted ($R^2 = 0.509 - 0.885$) for TPC to antioxidant activity (FRAP) in pomegranate juice. ^[25]

The extracted compound also showed significant anti-UV characteristic thereby allowing the survival of *E.coli* on an exposure to UV light. It was observed that *E. coli* was able to survive till 40 seconds of exposure to UV radiation when treated with peel extract where as in control the growth of *E. coli* was inhibited within 10 seconds. This suggests that the compound extracted is protective in nature against UV radiation either due to absorbance of UV radiation or reflection of UV radiation. Similar observations have been recorded by Yip, Cheng-Wai for fruit peel extracts which protected *E.coli* and allowed their survival when exposed to UV radiations, which were otherwise lethal to *E.coli* in the absence of the peel extract. ^[31]

Analysis of UV and FTIR spectrum, support the view that the methanolic extract primarily contains compounds of aromatic in nature with OH functional group.

CONCLUSION

From the present study it can be concluded that SSF is an effective method to improve producing bioactives from agro wastes such as pomegranate peels. Further, as the extracts showed anti microbial, anti-oxidant and anti-UV activities it can be used for various applications in different industries from food, textile, pharmaceutical to cosmetics. Better optimization of reacting parameters like temperature and pH during SSF could definitely increase the yield of bioactive compounds.

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