



The aqueous extract of *Punica granatum* peel has antibacterial, antifungal, antioxidant, and phytochemical activity

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Abstract

Pomegranate (*Punica granatum* L.) is a natural medication and a supply of bioactive compounds in India and different elements of the world. It is used to stimulate urge for food and deal with digestive sicknesses. The contemporary take a look at is asking into the antibacterial, antifungal, antioxidant, and phytochemical interest of pomegranate peel. The peel extracts have been examined for synergistic antimicrobial interest the use of the Agar Well diffusion approach with 4 consultant human pathogenic micro-organismf and fungal organisms. On a triplicate plate with nicely dimensions of 6 mm, the agar nicely diffusion approach turned into used to decide the synergistic motion of those extracts in a couple of combinations. The aqueous extract verified more antibacterial interest, with inhibition zones starting from three.1 to 18 nm and antifungal interest starting from three. 2 to 15 nm. Phytochemical investigations of phenol, carbohydrates, protein, flavonoid alkaloids, and saponin The capacity of peel extracts to scavenge the solid 2,2, diphenyl-1, picryl hydroxyl (DPPH) loose radical turned into used to decide their antioxidant ability, and the extracts verified 81.4 percentage antioxidant interest. Overall, the pomegranate peel turned into determined to be a rather treasured supply of bioactive compounds that would be used to enhance the practical homes of food.

Keywords: pomegranate peel, antimicrobial, antioxidant and phytochemical research

Introduction

The predominance of life-threatening sicknesses resulting from pathogenic microorganisms like micro organism, fungi, viruses, and protozoa has multiplied globally, and it's far now a first-rate purpose of unwholesomeness and demise in immunocompromised sufferers in advanced countries ^[1]. Hospital go contamination have to be managed urgently through enhancing antibiotic use and lowering sanatorium go contamination ^[2]. However, the improvement of recent antibiotics must be endured due to the fact it's far vital to retaining the efficacy of antimicrobial remedy ^[3]. The contemporary situation of a couple of drug resistance to human pathogenic microorganisms has caused a look for new antimicrobial materials derived from flora. Medicinal flora and arrangements crafted from them have lengthy been used to deal with infections in a big part of the community. Antibiotics have plenty of facet results at the human body, which has rekindled hobby withinside the look for better flora of herbal foundation with antimicrobial interest. A medicinal plant has homes just like pharmaceutical drugs ^[4-6]. Oxidative strain contributes substantially to the pathophysiology of a extensive variety of pathological conditions, which include cardiovascular dysfunction, atherosclerosis, irritation, carcinogenesis, drug toxicity, reperfusion injury, and neurodegenerative sickness ^[7]. Plants (fruits, vegetables, medicinal herbs) incorporate a various variety of loose radical scavenging molecules, which include phenolic compounds, nitrogen compounds, vitamins, terpenoids, and different endogenous metabolites with excessive antioxidant interest. Pomegranate (*Punica granatum* L.) is hired as a flavouring drug in India and opportunity countries. Pomegranates are a essential supply of bioactive homes. Pomegranate fruit is set 5 inches extensive and has a deep red, coriaceous pores and skin, a bomb shape, and a pointed coil ^[8-10]. The pomegranate belongs to the Punicaceae own circle of relatives and is sent anywhere withinside the world; it has a excessive natural manner price. It has numerous healing uses. They're hired in growing pomegranate leaf tea, healthful juices and salads, and meditative supplements. A healthful leaf is flat and shiny, mild green. Pomegranates have effective antioxidant, antimicrobial, and antifungal interest. There is a range of phytochemical compounds in pomegranates equal to phenols, carotenoids, tannins, flavonoids, tocopherols, and opportunity secondary metabolites that display organic interest ^[11-12]. It is used to stimulate urge for food and deal with organic manner troubles in Ayurveda. Pomegranate leaves useful resource in weight reduction and deal with plenty of issues and illnesses along with insomnia, stomach pain, dysentery, cough, jaundice, mouth ulcers, pores and skin aging and irritation of the pores and skin like eczema. Pomegranate leaves are hired in supplementary and one of a kind clinical aids. Supplementary remedy is using sustenance and minerals. Human research have proven that every day intake of pomegranate juice lowers strain stages in hypertensive subjects, delays the coronary-artery sickness approach and will increase the overall inhibitor status of the blood. Several research suggest that pomegranate

extracts also are used as herbal options for the remedy of a extensive sort of microorganisms and infectious retailers due to their antimicrobial interest [13-16]. This take a look at is centred at assessing the antibacterial, antifungal, antioxidant, and phytochemical evaluation of pomegranate peel extracts in opposition to six consultant human pathogenic microorganisms.

Materials and Methods

Plant Powder Preparation

A fruit cover of pomegranate peel turned into amassed from a juice shop (Padma Juice Corner, Tiruchirappalli, Tamil Nadu).

Peels have been then dug into smaller items, so that they have been first washed with water accompanied through laundry with distilled water. It have been dried beneathneath daytime until the water droplets had absolutely evaporated. The cowl turned into then unbroken in a warm air kitchen equipment for three–four days, so it would get dried. The dried pericarp turned into then taken for grinding with the help of a mixer grinder. Then a pulverised form of plant pattern turned into then used at some point of the take a look at.

Extraction Procedure

The powdered pomegranate peel pericarp turned into dissolved in a binary compound solvent. The 5 grams of floor cowl have been accessorial to 50ml of warm boiling water, which turned into left withinside the quandary tub for an hour at 70 °C, so secondary metabolites were given absolutely extracted. The extract turned into then filtered with the help of whatman no-1 paper and unbroken in a warm air kitchen equipment for drying. Then the dried extract turned into dissolved in a double quantity of DMSO (dimethyl sulphoxide) in order that the attention of the pattern reached 5 hundred mg/ml. The filtrate turned into dried to result in the antimicrobial that turned into in addition dissolved in double the quantity of the extract, for this reason growing the closing quantity of the extract to 500 mg/ml.

Microorganisms and Culture

Normal lines of being have been hired on this take a look at and have been acquired from Cauvery Medical Hospital, Tiruchirappalli, Tamil Nadu. The microorganism species used have been the gram negative microorganism genus *Bacteroides fragilis*, *Pseudomonas vulgaris*, and consequently the gram positive microorganism are *Propionibacterium acnes* and *Staphylococcus aureus*. Fungus *Candida albicans* and *Aspergillus niger* have been vegetation species. Microorganisms have been considerable in Mueller Hinton Agar and fungi have been grown in Sabouraud Agar. The attention of bacterial suspensions turned into adjusted to 10⁸ cells mL⁻¹ and that of fungal suspensions to 10⁷ cells mL⁻¹.

Culture media preparation

Preparation of Muller-Hinton agar medium Thirty-8 grams of the powder of Mueller Hinton agar have been weighed, dissolved in distilled H₂O, and allowed to soak for ten mints. The medium turned into positioned in a water tub to dissolve, swirled to mix and sterilised through autoclaving for fifteen mins at 121°C, cooled to forty-seven °C and combined nicely, then poured into sterile Petri dishes. Preparation of Sabouraud agar Sixty-grams of pulverised Sabouraud dextrose agar have been weighed, unfold in 1.zero L of water, swirled to mix, then sterilised through autoclaving for a 15 minutes at 121 °C, cooled to 37 °C, mixed nicely, and poured into sterile Petri dishes.

The Agar nicely diffusion approach is used to check for medication interest. *In vitro* antibacterial interest of numerous crude extracts acquired through successive extraction turned into evaluated the use of the victimisation Agar nicely diffusion approach. In 5% dimethyl sulfoxide (DMSO), crude extract answers at a attention of a hundred mg mL⁻¹ have been prepared.

The test microorganisms have been seeded into numerous media through the spread plate approach. Once solidified, a cork borer with a diameter of 6. zero millimetres turned into inseeded with 50µl, 100 µl, 250 µl, and 500 µl of crude extracts accompanied through drying off. Well have been disbursed onto the floor of the inoculated agar plates, and Petri plates have been incubated for twenty-4 hours at thirty-seven degrees. The diameters of the clean zones of inhibition made spherical the discs have been measured and recorded. To this end, an equal method advanced for antibacterial interest assays has been used. Check antifungal interest, Sabouraud agar urned into used. The inoculated medium turned into incubated at twenty-seven C for two days for the *Candida albicans* and three days for the fungus genus *Aspergillus niger* [15].

Determination of inhibitor Activity DPPH radical scavenging assay

To a hundred µl of plant extracts accessorial zero. Four millilitre of DPPH (zero.1mM) solution and zero. four ml of 50mM Tris HCl. The answers are combined nicely and incubated in darkish situation at temperature for thirty min. as soon as 30 min, the absorbance of the combination turned into test at 517 nm. 3ml of DPPH turned into taken as control. The water-soluble nutrition is taken as trendy. The percent radical scavenging interest of the plant extracts turned into calculated victimization the following formula, (Where, RSA is the Radical Scavenging Activity). DPPH (%) = control OD – test OD / control OD x one hundred carried out math evaluation [17].

Statistical evaluation

Antimicrobial interest test turned into executed in triplicates and facts have been given as shows that \pm variance (SD). Carried out math evaluation for all of the assays consequences have been executed victimization Microsoft stand out program (2010).

Phytochemical evaluation

The initial phytochemical screening of numerous extracts turned into executed to decide the presence of bioactive components. The presence of alkaloids (dragendroff, Mayer, Wagner), flavonoids, tannins, phlobatannins, triterpenes, steroids, saponins and inner organ glycosides was firm through J. B. Horborne [30].

Results and Discussion

The antibiotic interest of the binary compound extracts from quiescent pomegranates turned into resolved in opposition to the gram positive *Propionibacterium acnes* and *Staphylococcus aureus* and consequently the gram-negative micro-organism genus *Bacteroides fragilis* and *Pseudomonas vulgaris*. As proven in Table 1 and 2; Figure 1, absolutely one of a kind concentrations confirmed variable interest in opposition to the examined microorganism, as proven in Table One. For *S.aureus*, the inhibition area values have been among four.2 nm and 18.1 nm. Repressive results in *P. vulgaris* rages from four.15–11.seventy five nm have been determined to be at an all-time low. By growing attention, the most important diameter of the increase inhibition area turned into achieved. The antifungal interest of peel extract was firm in opposition to *Candida albicans* and fungi of the genus *Aspergillus niger*. The inhibition area values have been among three.1 and 15nm at *A. niger*, proven in Table 1 & 2 and Figure1. Antibiotics are powerful treatments for the inhibition of microorganism increase. Antibiotic resistance in opposition to being, on the alternative hand, is one of the maximum extreme troubles withinside the use of antibiotics in opposition to microorganisms [16-20]. Medicinal flora and their extracts were appreciably studied through researchers with the intention to deliver ability drug treatments with decreased toxicity. Therefore, this take a look at investigated the ability clinical interest of pomegranate peel extracts. This method is based on the power of the inhibitor to cut back the purple-coloured solid radical of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) right into a yellow-colored 2, 2-diphenyl-1-picrylhydrazine molecule. The DPPH loose radical is decolorized through an well-known companion diploma lepton this is given through an antioxidant compound. The scavenging ability of the pattern extract is proportional to the diploma of DPPH discoloration. The trendy of the antioxidants within side the extracts turned into decided through the IC50 price, because of this that that the powerful attention of the pattern had to inhibit 50% of the preliminary DPPH loose radical. The IC50 price is reciprocally proportional to the antioxidant interest. Within the take a look at, the extracts of pomegranate peel have been geared up to decolorize DPPH. The values of the DPPH radical scavenging sports of pomegranate peel extract are calculated as percent and IC50 values as given in Table 4-6. The percentage of radical scavenging interest and IC50 of binary compound extracts various from 70.sixty two percent to 81.40% and 10 g mL⁻¹ to 5 hundred g mL⁻¹, respectively. These consequences are relatively clever as compared with the proportion DPPH radical scavenging interest of water-soluble vitamins (92.230%) as an inhibitor trendy. It has been found that the very first-class DPPH scavenging interest is in acetone extract and consequently the bottom in chloroform extract [21-21]. The dissolver extract of pomegranate peel suggests the maximum DPPH scavenging interest in contrast to binary compounds, fermentation alcohol, and dissolving agent extracts. In the contemporary take a look at, the aqueous extracts acquired through display better attention scavenging interest than the ones acquired the use of much less decrease attention. This might be attributed to the excessive content material of artificial resin compounds in pomegranate fruit peel which might be chargeable for the inhibitor interest. These compounds are from time to time extracted with polar solvents equal to aqueous acetone and methanol [25-29]. It can be express that pomegranate peel extracts have been clever DPPH radical scavengers. The separation of phytochemicals through solvents turned into derived through carrying out equal phytochemical tests to peer their presence withinside the extracts. Per the take a look at for tannins of pomegranate rind extract, the rind includes hydrolysable tannins and condensed tannins.

Table 1: Antibaceterial activity of *Punica granatum* peel

S.NO	Name of the test organism	Zone of inhibition (mm) SD \pm Mean				
		500 μ g/ml	250 μ g/ml	100 μ g/ml	50 μ g/ml	Pc
1.	<i>Propionibacterium acnes</i>	14.5 \pm 0.7	13.25 \pm 0.35	7.2 \pm 0.28	3.15 \pm 0.21	9.5 \pm 0.7
2.	<i>Bacteroides fragilis</i>	12.75 \pm 1.06	10.5 \pm 0.7	7.25 \pm 0.35	3.1 \pm 0.14	18.5 \pm 0.7
3.	<i>Staphylococcus aureus</i>	18 \pm 1.41	14.35 \pm 0.49	9.25 \pm 0.35	4.2 \pm 0.28	10.5 \pm 0.7
4.	<i>Proteus vulgaris</i>	11.75 \pm 1.06	9.35 \pm 0.49	7.25 \pm 0.35	4.15 \pm 0.21	7.5 \pm 0.7

SD – Standard Deviation, *Significance - $p < 0.05$.

Table 2: Antifungal activity of *Punica granatum* peel

S. No	Name of the test organism	Zone of inhibition (mm) SD \pm Mean				
		500 μ g/ml	250 μ g/ml	100 μ g/ml	50 μ g/ml	PC
1.	<i>Candida albicans</i>	13.5 \pm 0.7	11.25 \pm 0.35	4.15 \pm 0.21	0	5.5 \pm 0.7
2.	<i>Aspergillus niger</i>	15 \pm 1.41	13.35 \pm 0.49	10.25 \pm 0.35	3.2 \pm 0.28	17.5 \pm 0.7

Table 3: Phytochemical analysis of *Punica granatum* peel

S. No	Phytochemical Test	Present / Absent
1	Phenol	Present
2	Tannin	Present
3	Flavonoids	Present
4	Steriods	Absent
5	Triterpenoids	Absent
6	Alkaloids	Present
7	Carbohydrates	Present
8	Protein	Present

Table 4: Antioxidant activity of *Punica granatum* Peel

S.No	Tested sample concentration (µg/ml)	OD Value at 517 nm (in triplicates)		
1.	Control	1.312	1.316	1.363
2.	500 µg/ml	0.217	0.254	0.271
3.	250 µg/ml	0.285	0.283	0.275
4.	100 µg/ml	0.293	0.296	0.287
5.	50 µg/ml	0.302	0.320	0.338
6.	10 µg/ml	0.373	0.391	0.408
7.	Ascorbic acid	0.08	0.11	0.12

Table 5: *Punica granatum* peel IC50 value

S. No	Tested sample concentration (µg/ml)	OD Value at 517 nm (in triplicates)		
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6.	10 µg/ml	0.373	0.391	0.408
7.	Ascorbic acid	0.08	0.11	0.12

Table 6: *Punica granatum* peel IC50 value

Log (inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50		1.770
HillSlope		-1.346
IC50		58.95
Std. Error		
LogIC50		0.06927
HillSlope		0.2915
95% Confidence Intervals		
LogIC50		1.621 to 1.920
HillSlope		-1.975 to -0.7162
IC50		41.77 to 83.19
Goodness of Fit		
Degrees of Freedom		13
R square		0.8835
Absolute Sum of Squares		2141
Sy.x		12.83
Number of points		
Analyzed	3	15

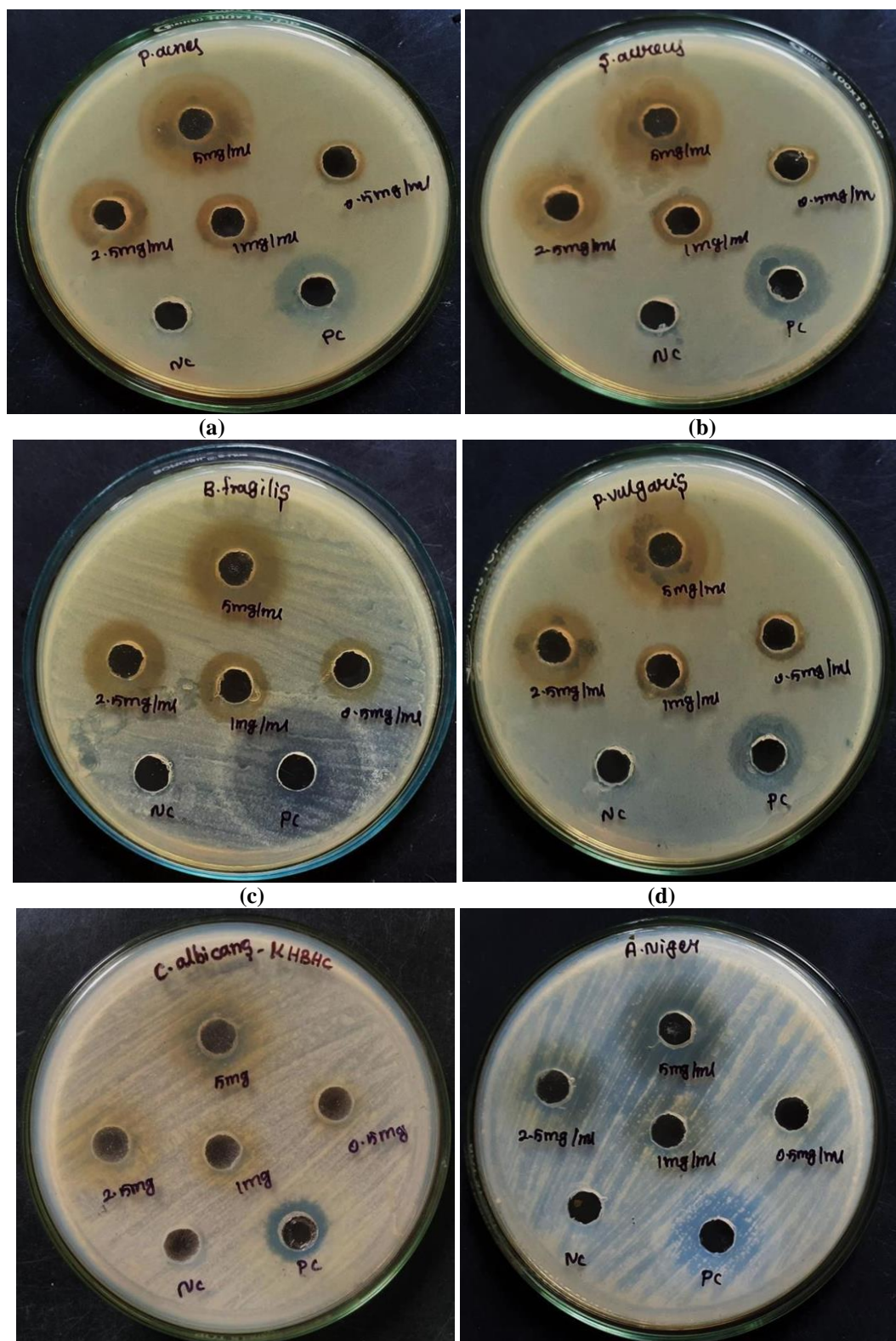


Fig 1: Effect of sample testing against Bacteria *P. acnes* b. *S. aureus* c. *B. fragilis* d. *P. vulgaris* e. *C. albicans* f. *A. Niger* Inhibition zone

Conclusion

The current study found that *P. granatum* peel aqueous extracts have antibacterial activity against a variety of human infections, suggesting that it could be useful in the treatment of infectious disorders caused by microbes. Furthermore, total phenolic and total flavonoids were shown to be abundant in *P. granatum* extracts. Antioxidant activity against DPPH was also discovered in the extracts. In general, the findings support the plant's usefulness as an antioxidant and antibacterial agent. As a result, the plant's good attributes in terms of its use in traditional medicine have been proven. The current findings are intended to be useful to anyone dealing with medicinal plant advantages everywhere in the world. To confirm their antibacterial and antioxidant effects, identify and

isolate the active compounds, and characterise their pharmacological properties, more research is needed, including *In vitro* and *In vivo* examinations.

References

1. Alexandre EMC, Silva S, Santos SAO *et al.* "Antimicrobial activity of pomegranate peel extracts performed by high pressure and enzymatic assisted extraction," *Food Research International*,2019;115:167-176.
2. Amri Z, Zaouay F, Lazreg-Aref H *et al.* "Phytochemical content, fatty acids composition and antioxidant potential of different pomegranate parts: comparison between edible and non-edible varieties grown in Tunisia," *International Journal of Biological Macromolecules*,2017;104:274-280.
3. Arun KB, Jayamurthy P, Anusha CV, Mahesh SK, Nisha P, "Studies on activity guided fractionation of pomegranate peel extracts and its effect on antidiabetic and cardiovascular protection properties," *Journal of Food Processing and Preservation*,2017;41(1):e13108.
4. Elfalleh W, Tlili N, Nasri N *et al.* "Antioxidant capacities of phenolic compounds and tocopherols from Tunisian pomegranate (*Punica granatum*) fruits," *Journal of Food Science*,2011;76(5):C707-C713.
5. Elsherbiny EA, Amin BH, Baka ZA. "Efficiency of pomegranate (*Punica granatum* L.) peels extract as a high potential natural tool towards *Fusarium* dry rot on potato tubers," *Postharvest Biology and Technology*,2016;111:256-263.
6. Fawole OA, Makunga NP, Opara UL. "Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract,"
7. Gözlekçi S, Saraçoğlu O, Onursal E, Özgen M. "Total phenolic distribution of juice, peel, and seed extracts of four pomegranate cultivars," *Pharmacognosy Magazine*,2011;7(26):161-164.
8. Kahramanoglu I, Usanmaz S. *Pomegranate Production and Marketing*, CRC Press Taylor & Francis Group, Boca Raton, FL, USA, 2016.
9. Kahramanoglu I. "Trends in pomegranate sector: production, postharvest handling and marketing," *International Journal of Agriculture Forestry and Life Sciences*,2019;3(2):239-246.
10. Licciardello, F., S. Kharchoufi, G. Muratore, and C. Restuccia, "Effect of edible coating combined with pomegranate peel extract on the quality maintenance of white shrimps (*Parapenaeus longirostris*) during refrigerated storage," *Food Packaging and Shelf Life*,2018;17:114-119.
11. Singh B, Singh JP, Kaur A, Singh N. "Phenolic compounds as beneficial phytochemicals in pomegranate (*Punica granatum* L.) peel: a review," *Food Chemistry*,2018;261:75-86.
12. Sorrenti V, Randazzo CL, Caggia C *et al.* "Beneficial effects of pomegranate peel extract and probiotics on pre-adipocyte differentiation," *Frontiers in Microbiology*,2019;10:660.
13. Stojanovic K, Šavikin N, Djedovic *et al.* "Pomegranate peel extract ameliorates autoimmunity in animal models of multiple sclerosis and type 1 diabetes," *Journal of Functional Foods*,2017;35:522-530.
14. Surendhiran D, Li C, Cui H, Lin L. "Fabrication of high stability active nanofibers encapsulated with pomegranate peel extract using chitosan/PEO for meat preservation," *Food Packaging and Shelf Life*, vol. 23, Article ID 100439, 2020.
15. Wafa BA, Makni M, Ammar S *et al.* "Antimicrobial effect of the Tunisian Nana variety *Punica granatum* L. extracts against *Salmonella enterica* (serovars Kentucky and Enteritidis) isolated from chicken meat and phenolic composition of its peel extract," *International Journal of Food Microbiology*,2017;241:123-131.
16. Wu X, Cao G, Prior RL. "Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry," *The Journal of Nutrition*,2002;132(7);1865-1871.
17. Barros ZMP, Salgado JM, Melo PS, de Oliveira Biazotto F. Enrichment of commercially-prepared juice with pomegranate (*Punica granatum* L.) peel extract as a source of antioxidants. *Journal of Food Research*,2014;3(6):179.
18. Dadwal V, Bhatt Sh, Sonkhla K, Joshi R, Gupta M. Quantification of free and bound phenolics in Bio-Waste pomegranate peel and formulation of punicalagin rich riceextruded snacks. *International Journal of Food and Nutritional Science*,2017;(2):98-104.
19. Farag R, Abdelatif M, Emam S, Tawfeek L. Phytochemical screening and polyphenol constituents of pomegranate peels and leave juices. *Agr Soil Sci*,2014;1(6):86:93.
20. Hmid I, Elothmani D, Hanine H, Oukabli A, Mehinagic E. Comparative study of phenolic compounds and their antioxidant attributes of eighteen pomegranate (*Punica granatum* L.) cultivars grown in Morocco. *Arabian Journal of Chemistry*,2017;10:S2675-S84.
21. Wu S, Tian L. Diverse phytochemicals and bioactivities in the ancient fruit and modern functional food pomegranate (*Punica granatum*). *Molecules*,2017;22(10):1606.
22. Alexandre EM, Silva S, Santos SA, Silvestre AJ, Duarte MF, Saraiva JA *et al.* Antimicrobial activity of pomegranate peel extracts performed by high pressure and enzymatic assisted extraction. *Food research international*,2019;115:167-76.
23. Rowayshed G, Salama A, Abul-Fadl M, Akila-Hamza S, Mohamed EA. Nutritional and chemical evaluation for pomegranate (*Punica granatum* L.) fruit peel and seeds powders by products. *Middle East Journal of Applied Sciences*,2013;3(4):169-79.
24. Entessar H, Al-Mosawe A-SI, Al-Saadi I. The Extraction and purification of gallic acid from the pomegranate rind. *Al-Mustanisinyah J Sci*,2012;23:184-92.

25. Bhandari PR. Pomegranate (*Punica granatum* L). Ancient seeds for modern cure? Review of potential therapeutic applications. International Journal of Nutrition, Pharmacology, Neurological Diseases,2012;2(3):171.
26. Singh R, Chidambara Murthy K, Jayaprakasha G. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *In vitro* models. Journal of agricultural and food chemistry,2002;50(1):81-6.
27. Kaneria MJ, Bapodara MB, Chanda SV. Effect of extraction techniques and solvents on antioxidant activity of pomegranate (*Punica granatum* L.) leaf and stem. Food Analytical Methods,2012;5(3):396-404.
28. Panichayupakarananta P, Issuriya A, Sirikatitham A, Wang W. Antioxidant assay-guided purification and LC determination of ellagic acid in pomegranate peel. Journal of chromatographic science,2010;48(6):456-9.
29. Middha SK, Usha T, Pande V. A review on antihyperglycemic and antihepatoprotective activity of eco-friendly *Punica granatum* peel waste. Evidence-Based Complementary and Alternative Medicine, 2013.
30. Harborne JB, Phytochemical analysis: A guide to modern techniques of plant analysis. Chapman A and Hall, London, 1973.