

Phytochemical Screening of Pomegranate Peel using crude Hydro-alcoholic Extract and pharmacological activities

Nitin Kumar^{*}, Pooja upadhyay^{**}, Gaurav saxena^{***}

^{*} Department, Institute Name

^{**} Department, Institute Name, if any

Abstract- Pomegranate is a curative plant from ancient times which belongs to punicaceae and Lythraceae family. This plant is developed as small trees or shrubs in several countries like Iran, Iraq, Afghanistan, Pakistan, India, Russia and Mediterranean area. From the peel extract the tests were carried out to find the presence of the following chemical compounds such as carbohydrates, reducing sugars, glycosides proteins, amino acids, phenolic compounds, tannins, alkaloids, flavonoids, saponins, sterols, etc. This paper shows a comparative report of the screening test of pomegranate peel using crude, hydro alcoholic extract and In vitro antimicrobial activity.

Index Terms- pomegranate peel extract, hydro alcoholic extract, antimicrobial activity,

I. INTRODUCTION

Pomegranate (*Punica granatum* L.); the common name is derived from the Latin words *ponus* and *granatus*, a granular apple which is a delectable fruit consumed worldwide. The total production of pomegranate around the world is 1,500,000 tons, in that 60% is the weight of the peel itself. *Punica granatum* Linn. (Pomegranate) is a plant belongs to Punicaceae family locally East for more than 5,000 years ago and it is found in Iran, Egypt, India, Bangladesh, Sri Lanka, North Africa. Pomegranate peels are used as a popular remedy throughout the world and exploited in traditional medicine because of their strong mordancy properties (Nitave and Patel, 2014). Bark, leaves, immature fruits and fruit rind of different parts of pomegranate have valuable therapeutic potential. Several studies focused on prevention and treatment of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction and skin allergy investigations were carried out to determine antioxidant, anticarcinogenic and anti-inflammatory properties of pomegranate constituents. Pomegranate peels are distinguished by an internal network of membranes encompassing almost 26–30% of total fruit weight and are characterized by considerable amounts of phenolic compounds, including flavonoids such as anthocyanins, catechins and other complex flavonoids and hydrolysable tannins (punicalagin, punicalin, pedunculagin, ellagic and gallic acid). Preliminary phytochemical screening of the aqueous extract of *P. granatum* peels gave positive tests for tannins, flavonoids, and alkaloids and showed that the hydroalcoholic extract of *P. granatum* peels may contain some biologically active principles that may be the basis for its traditional use (Qnais et al., 2007).

The ethno pharmacological Profile of pomegranate Peel makes it a prized traditional asset due to its antimicrobial, antimutagenic and antioxidant, anti-inflammatory properties.

II. MATERIALS AND METHODS

Collection of plant materials: fresh peel of *punica granatum* were collected from local market of dehradun and transported in to laboratory. The peel removed from fruit carefully by sharp knife and allowed to dry. The peel washed with distilled water and dry in oven at room temperature.

Preparation of extract: the dried peel converted into powder with the help of grinder and pass through the sieve no 120. The peel *Punica granatum* (5g) was extract with 100ml hydro alcoholic solvent. The hydro alcoholic solution was prepared water: ethanol (40:60). The extraction process were done hot maceration method and crude extract was stored at 20°C in refrigerator.

Phytochemical screening: phytochemical screening of *punica granatum* peel extract were assessed by the standard method as describe by khandelwal et al 2009

✓ **Alkaloids** Dried extract were treated with dilute hydrochloric acid, shake well and then filtered. The filtrate is further used for different test.

Mayer's test: Filtrates were treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow cream precipitate indicates the presence of alkaloids.

Wagner's test: Filtrates were treated with Wagner's reagent (potassium bismuth iodide). The formation of brown and reddish brown precipitate indicates the presence of alkaloids.

Dragendorff's test: Filtrates were treated with Dragendorff's reagent (iodine in potassium iodide). The formation of red precipitate indicates the presence of alkaloids.

Hager's test: Filtrates were treated with Hager's reagent (saturated solution of picric acid). The formation of yellow precipitate indicates the presence of alkaloids.

✓ Carbohydrates

Molisch's test: Extracts were treated with alcoholic α -naphthol solution in a test tube and concentrated sulphuric acid is added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of the carbohydrates.

Fehling's test: Extracts are hydrolyzed with dilute hydrochloric acid, neutralize with alkali and heated for 5-10 minute on water bath after adding Fehling A and B solution. First yellow, then brick red precipitate indicates the presence of reducing sugars.

✓ Glycosides

Determine free sugar content of the extract, Hydrolysed the extract with mineral acid. Again determine the total sugar content of the hydrolysed extract. Increase in sugar content indicates of glycosides.

Legal's test: The extract was treated with sodium nitroprusside in pyridine and methanolic alkali. The formation of pink to red colour indicated presence of cardiac glycosides.

Killer Killani test: A small amount of dried extract was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. These were kept for some time after addition of 1ml of concentrated H_2SO_4 . A brown ring obtain at the presence cardenolides.

✓ Saponin glycosides

Foam test: The extract was diluted with 20ml of distilled water separately and shaken for 15min. in graduated cylinder. A layer of foam, measuring about 1cm, indicates the presence of saponins.

✓ Coumarins glycosides

With Ammonia: A drop of Ammonia is taken on a filter paper and a drop of aqueous extract was added to it. Fluorescence was observed if coumarins are present and fluorescence was not there if coumarin absent.

With dilute HNO_3 : Small quantity of extract was added to dilute HNO_3 presence of coumarin was confirmed by the formation of yellow precipitated

✓ Flavonoids

Shinoda test: A small amount of dried extracts are extracted with 10ml of ethanol for 15 min on a boiling water bath and filtered. To the filtrates, added a small piece of magnesium ribbon and concentrate HCl. Formation of pink colour indicates the presence of flavonoids.

$FeCl_3$ test: To the sample solution, added $FeCl_3$ solution. A change of colour from green to black indicates presence of flavonoids.

✓ Proteins and Amino acids

Million's test: The extracts were treated with Million's reagent. The formation of white precipitate, which turned to red upon heating, indicates the presence of proteins and amino acids.

Ninhydrin test: To the extracts, 0.25% Ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates presence of amino acids.

✓ Tannins

Ferric chloride test: The extract was treated with few drops of neutral ferric chloride. Solution (5%). The formation of bluish black colour indicates the presence of tannins.

Lead acetate test: The extract was treat with few drops of neutral Lead acetate solution (10%). The formation of yellow precipitate indicates the presence of tanins.

✓ Steroids and Triterpenoids

Liebermann-Burchard reaction: To the dried extracts ethanol, chloroform and acetic acid were added. To the above mixture, 1 to 2 drops of concentrated sulphuric acid is added. Dark green coloration of the

Solution indicates the presence of steroids and dark pink coloration of the solution indicates the presence of triterpenoids.

Salkowaski reaction: Chloroform was added to the dried extracts followed by addition of a few drops of concentrated sulphuric acid, shaken well and allowed to stand for some time. Red colour appearance in the lower layer indicates the presence of steroids and formation of yellow colure lower layer indicated the presence of triterpenoids.

✓ Fixed oil and fats

Greasy spot test: Small quantity of extract was pressed between two filter papers. An oil stain on filter paper indicates the presence of fixed oil.

✓ Volatile oil

Sudan red: Small amount of extract was added to Sudan red solution to produce red colour precipitate .It shows presence of volatile oil.

$CuSO_4$ solution: Small amount of extract was added to Copper sulphate solution to produce clear solution It show presence of volatile oil.

| Phyto constituents test | Reagent name | Hydro alcoholic extract |
|-------------------------|-------------------------|-------------------------|
| Tannin | 1. Ferric chloride test | + |
| | 2. Lead acetate test | + |
| Alkaloids | 1. Mayer reagent | + |
| | 2. Dragendroff reagent | + |
| | 3. Wagner reagent | + |
| | 4. Hager reagent | + |

| | | |
|------------------------|---|-------------|
| Carbohydrate | 1. Molisch test 2. Fehling solution 3. Osazone test | - - - |
| Glycoside | 1. Legal's test 2. Killer Killani test | - - |
| Saponins | 1. Foam test | - |
| Flavonoids | 1. Shinoda test 2. Ferric chloride test | + + |
| Steroids | 1. Liebermann-Burchard reaction 2. Salkowaski reaction | + + |
| Protein and amino acid | 1. Million's test 2. Ninhydrin test | - - |
| Coumarins | 1. With Ammonia 2. With dilute HNO ₃ | + + |
| Fixed oil | 1. Gray spot test 2. Sudan red test | - - |

Pharmacological activity

Antimicrobial activity

Agar well diffusion method

Preparation of agar media

- Suspended 9.5gm MHA agar in a 500ml conical flask and 250ml distilled water will be added.
- Then, it will be heat on hot plate with frequent agitation until it completely dissolves.
- Then, media will be sterilizing in autoclave at 121°C for 1 hour.

Procedure

Approximately 25ml of Mueller- Hinton agar (MHA) will be pouring into sterile Petri- dish and allow to solidify. 50 micro liter of bacterial inoculums will be spread on the solidify MHA media by using sterile spreader. In each of these plates four wells (5mm diameter) will be punching into the agar by using sterile cork borer. Then, working concentration of 25mg, 50mg, 75mg and 100mg dilution will be prepare from 500mg/ml of stock solution of each extracts and 100micro liter of each extract will be separately adding into wells and allowed to diffuse at room temperature. Equal volume of alcohol will be use as negative control and standard antibiotics (Gentamicin, amikacin, tetracycline and ofloxacin) using as positive control. The plates will be incubate for 24 hours at 37°C & the diameter (in mm) of clear zone of growth inhibition will be recording and measure with the help of radius scale ((Natarajan *et al.*, 2010).

Test organism used in antimicrobial activity

- *E. coli*- gram negative bacteria(rod)
- *Pseudomonas*- gram negative bacteria(rod)
- *S. aureus*- gram positive bacteria(coccus)
- *Klebsiella*- gram negative bacteria(rod)

Determination of total phenolic and flavonoids content hydro alcoholic extract

Determination of total phenolic content: The total phenolic content of all extracts of drug estimate according to the method described in Ayurvedic Pharmacopoeia of India. The total phenolic content expressed in milligrams of Gallic acid equivalents per gram of extract. Stock solution (1 mg/ml) of the extract in methanol is prepared. From the stock solution, suitable quantity of the extract is taken into 25ml volumetric flask and added 10 ml of water and 1.5ml of folin ciocalteau reagent. Kept the mixture for 5 min and then added 4 ml of 20% Na₂CO₃ solution and made up to 25 ml with double distilled water. Kept the mixture for 30 min and recorded absorbance at 765 nm. Calculated percentage of total phenolics from calibration curve of gallic acid prepare by using the above procedure and expressed total phenolics as percentage of gallic acid according to Ayurvedic Pharmacopoeia of India (2008).

• Reagents

1. Sodium carbonate solution (20%): 20 g Sodium carbonate was dissolved in 100 ml water, and then it was filtered after allowing it to stand overnight.
2. Gallic acid stock solution (1 mg/ml): 10 mg Gallic acid was dissolved in 10ml distilled water

5.9.2 Determination of Flavonoids Content: The total flavonoids content of all extracts estimated according to the aluminum chloride method as follows. The aluminum chloride method is used for the determination of the total flavonoids content of the sample extracts. Aliquots of extract solutions are taken and the volume is made up to 3 ml with methanol. Then 0.1 ml aluminum chloride (10%), 0.1 ml potassium acetate (1 M) and 2.8 ml distilled water added sequentially. The test solution is vigorously shaken. Absorbance at 415 nm is recorded after 30 minutes of incubation. A standard calibration plot is generated at 415 nm using known concentrations of rutin. The concentrations of flavonoids in the test samples calculated from the calibration plot and express as mg rutin equivalent/g of sample. (Mervat *et al.*, 2009).

Reagents

1. Aluminum chloride solution 10 %: 10g Aluminum chloride was dissolved in 100 ml methanol.
2. 1 M Potassium acetate solution: 9.815g Potassium acetate was dissolve in 100 ml of distilled water.
3. Standard rutin solution (1mg/ml): 10 mg rutin was dissolved in 10 ml methanol.

Result and discussions

Pomegranate peel is rich in tannins, high-molecular weight plant polyphenols, which can be categorized into two chemically and biologically separate groups: condensed hydrolysable tannin and tannin, the latter composed of glycosyl esters and phenolic acids. Hydrolyzable tannins are parted into gallotannins containing gallic acid and ellagitannins, containing ellagic acid. The cytoprotective and inhibitory effects of peel demonstrates the potential to prevent some human carcinomas. Table 1 lists the phytochemical constituents of Punica granatum peel extracts. All the phytochemical constituents tested were present in aqueous

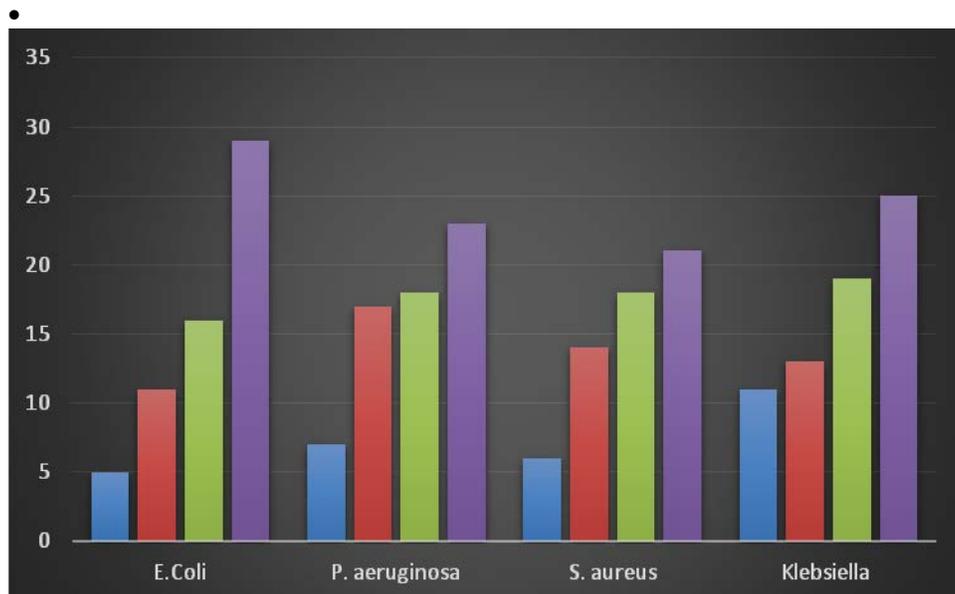
extract of *P. granatum* peel except glycosides and anthocyanin. It was noted that ethanolic peel extract of *Punica granatum* showed the presence of all phytochemical constituents except tannins, glycosides. The prophylactic potential of the peel against viral epidemics and pandemics, specifically influenza, may open up new avenues for research in the nutritional and medical science domains. In the present study, anti-bacterial activity was studied against *E.Coli*, *Pseudomonas aeruginosa*, *S.aureus* and *S.typhii*. It can be seen that both the tested strains were susceptible to the aqueous peel extract as dose dependent manner (Figure 1). A steady increase in inhibitory zone was recorded in high concentration. In the case of *P.aeruginosa*, maximum zone of inhibition was recorded at 100µL with 20mm followed by 50µL with 19mm; 10µL revealed 11mm of zone of inhibition whereas in the case of *S.aureus* and *S.typhii*, the zone of inhibition was around 21mm at the highest dosage level of 100µL. *E.coli* showed high sensitivity to high concentration of aqueous peel extract (100µL, 50µL, 10µL); 22, 21 and 7mm of zone of

inhibition has been observed at the respective concentrations (Table 1) (Graph 1).

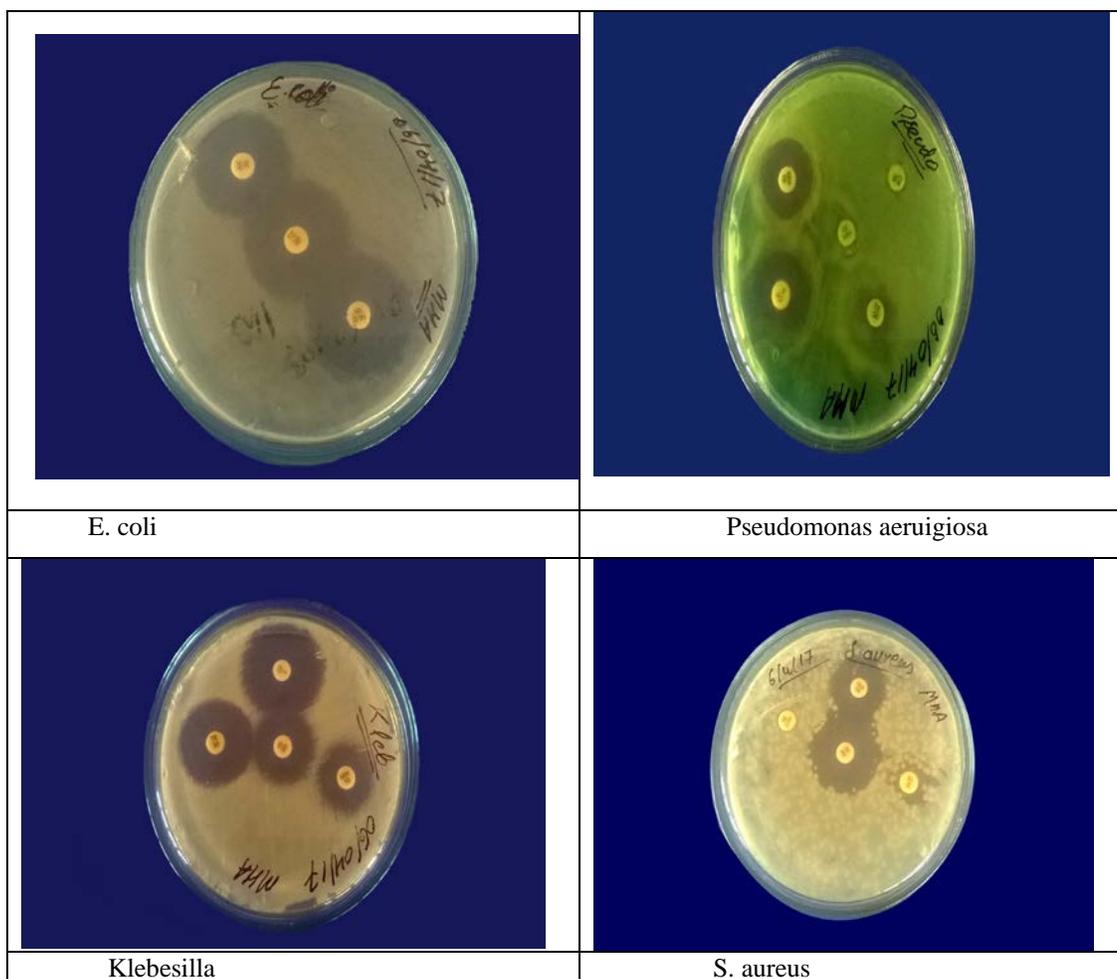
Table 2 Antimicrobial activity of hydro alcoholic extract by well diffusion method

| S.no | Organism | Zone of inhibition | 10µL | 50 µL | 100µL |
|------|----------------------|-------------------------|------|-------|-------|
| | | Positive control | | | |
| 1 | <i>E.Coli</i> | 29mm | 5 | 11 | 16 |
| 2 | <i>P. aeruginosa</i> | 23mm | 7 | 17 | 18 |
| 3 | <i>S. aureus</i> | 21mm | 6 | 14 | 21 |
| 4 | <i>Klebsiella</i> | 25mm | 11 | 13 | 19 |

Graph 1 zone inhibiton (mm) of hydro alcoholic extract at different concentration against pathogenic micro organism



- Positive control
- 10µL
- 50µL
- 100µL

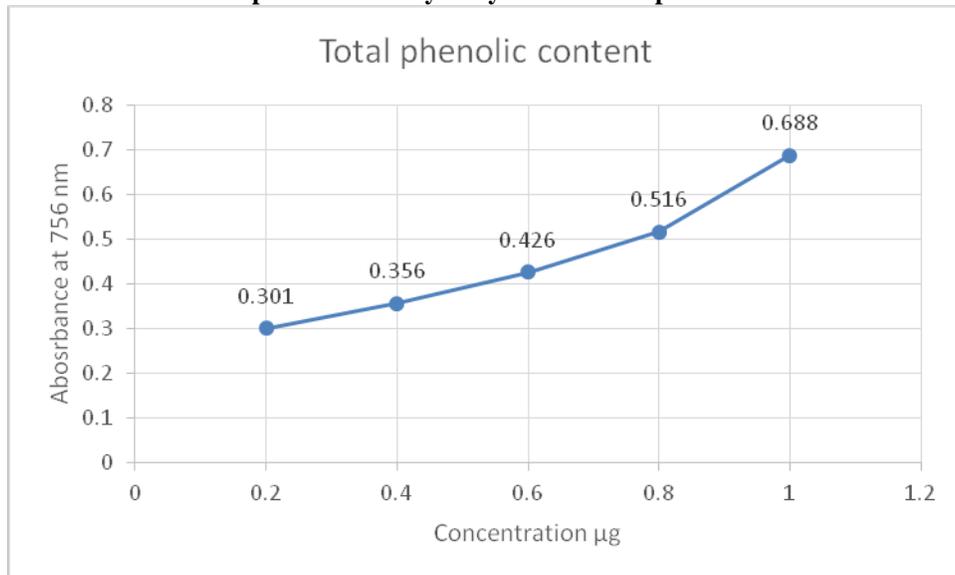


In the present study, antimicrobial activity was studied against the *E. coli*, *pseudomonas aeruginosa*, *klebesilla*, and *S. aureus*. It can be seen both tested both strains were susceptible to hydro alcoholic peel extract as dose depend manner (Figure 1). A steady increase zone inhibitory was recorded in high concentration. In case of *S. aures* maximum zone of inhibition was recorded at 100µL with 21mm followed by 50µL with 14mm; 10µL with 6mm zone of inhibition. Whereas case of *S aureus* and *klebesilla* was around 21 and 19 at highest level of 100µL. *E.coli* has showed highly sensitivity of high

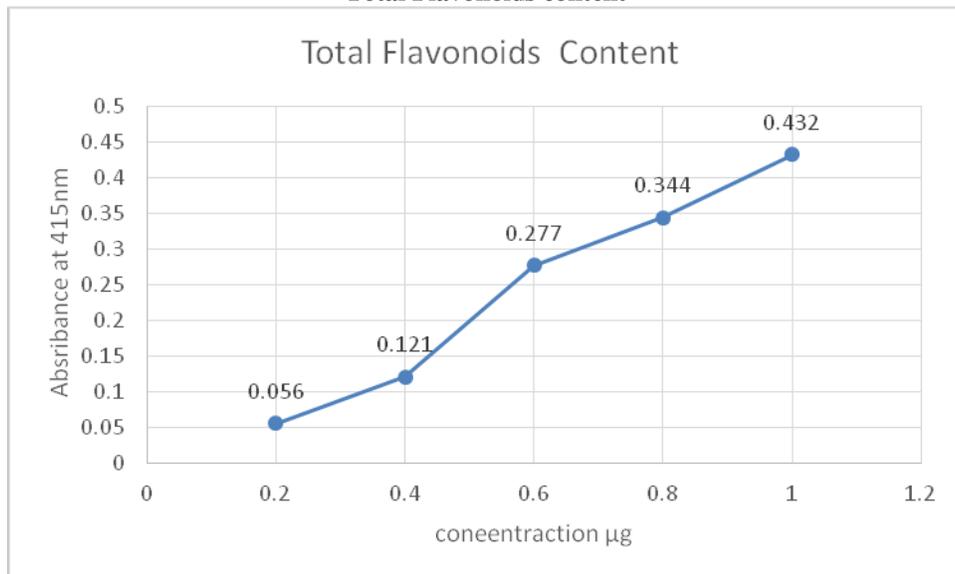
concentration of hydro alcoholic extract (100, 50, 10µL); 19, 13 and 11 zone of inhibition has been observed at respective concentration(table 2) and (graph 1).

Plants are opulent in secondary metabolites, including phenolics, carotenoids and flavonoids due to their chemical structures and redox properties. It gives us a complete estimation of reducing capacity of antioxidant. This particular study represented the higher percentage of phenolics, flavonoids.

Total phenolic activity of hydro alcoholic peel extract



Total Flavonoids content



III. CONCLUSION

Our result suggest that the hydro alcoholic extract of pomegranate is potential source of antimicrobial activity. The specific isolation of the active component in the hydro alcoholic extract and characterization should be further examined. Further phytochemical analysis is initiated to isolate the elements of peel that show a wide spectrum of pharmacological activity.

CONFLICT OF INTEREST

Conflict of interest as none

REFERENCES

[1] Aviram M, Volkova N, et al. Pomegranate phenolics from the peels, arils, and byproduct administration to apolipoprotein flowers are antiatherogenic:

Studies in vivo in atherosclerotic apolipoprotein E-deficient (E 0) mice and in vitro in cultured macrophages and lipoproteins. *J Agric Food Chem.* 2008 Feb 13; 56(3):1148-57

[2] Pai V, Chanu TR, et al. Evaluation of the antimicrobial activity of Punica granatum peel against the enteric pathogens: an in vitro study. *Asian Journal of Plant Science and Research.* 2011; 1:57-62

[3] Braga LC, Shupp JW, et al. Pomegranate extract inhibits Staphylococcus aureus growth and subsequent enterotoxin production. *J Ethnopharmacol.* 2005 Jan 4; 96(1-2):335-9

[4] Reddy MK, Gupta SK, et al. Antioxidant, antimalarial and antimicrobial activities of tanninrich fractions, ellagitannins and phenolic acids from Punica granatum L. *Planta Med.* 2007 May; 73(5):461-7.

[5] Vasconcelos LC, Sampaio FC, et al. Minimum inhibitory concentration of adherence of Punica granatum Linn (pomegranate) gel against S. mutans, S. mitis and C. albicans. *Braz Dent J.* 2006; 17(3):223-7

[6] M. Dikmen, N. Ozturk, and Y. Ozturk, "The antioxidant potency of Punica granatum L. fruit peel reduces cell proliferation and induces apoptosis on breast cancer," *Journal of Medicinal Food*, vol.14, no.12, pp.1638-1646, 2011

[7] E. P. Lansky and R. A. Newman, "Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer," *Journal of Ethnopharmacology*, vol.109, no.2, pp.177-206, 2007

- [8] Akter, S., Sarker, A. and Hossain, M.D. 2013. Antidiarrhoeal activity of rind of *Punica granatum*. *Int. Curr. Pharmaceut. J.* 2(5): 101-104.
- [9] Ashok Kumar, K. and Vijayalakshmi, K. 2013. In vitro anti-microbial activity and phytochemical analysis of selected fruit wastes. *Int. J. Curr. Microbiol. Appl. Sci.* 2(5): 196-204.
- [10] Raskin, I., Ribnicky, D.M., Komarnytsky, S., Ilic, N., Poulev, A., Borisjuk, N., Brinker, A., Moreno, D.A., Ripoll, C. and Yakoby, N. 2002. Plants and human health in the twenty first century. *Trend. Biotechnol.* 20: 522-531. 8
- [11] Prakash, C.V.S. and Prakash, I. 2011. Bioactive chemical constituents from pomegranate (*Punica granatum*) juice, seed and peel-a review. *Int. J. Res. Chem. Environ.* 1(1): 1-18
- [12] Nitave, S.A. and Patil, V.A. 2014. Study of antibacterial and antifungal activity of *Punica granatum* peel and its phytochemical screening. *World J. Pharmaceut. Res.* 3(10): 505-512.
- [13] C. C. Lin, Y. F. Hsu, T. C. Lin, and H. Y. Hsu, "Antioxidant and hepatoprotective effects of punicalagin and punicalin on acetaminophen-induced liver damage in rats," *Phytotherapy Research*, vol.15, no.3, pp.206–212, 2001.
- [14] N.P.Seeram, Y.Zhang, R.McKeever et al., "Pomegranate juice and extracts provide similar levels of plasma and urinary ellagitannin metabolites in human subjects," *Journal of Medicinal Food*, vol.11, no.2, pp.390–394, 2008
- [15] V. M. Adhami, I. A. Siddiqui, D. N. Syed, R. K. Lall, and H. Mukhtar, "Oral infusion of pomegranate fruit extract inhibits prostate carcinogenesis in the TRAMP model," *Carcinogenesis*, vol.33, no.3, pp.644–651, 2012
- [16] Y. Wang, S. Zhang, S. Iqbal et al., "Pomegranate extract inhibits the bone metastatic growth of human prostate cancer cells and enhances the in vivo efficacy of docetaxel chemotherapy," *The Prostate*, vol.74, no.5, pp.497–508, 2014.
- [17] M. I. Gil, F.A. Tomas-Barberan, B. Hess-Pierce, D.M. Holcroft, and A. A. Kader, "Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 10, pp. 4581–4589, 2000.

AUTHORS

First Author – Nitin Kumar

Second Author – Pooja upadhyay

Third Author – Gaurav saxena