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EVALUATION OF ANTIBACTERIAL ACTIVITY OF *PUNICA GRANATUM* PEEL EXTRACT

Mr.S.Subin[1*], Dr.Anusha Bhaskar[2]

[1*]Research Scholar, Sarvapalli radhakrishan university, Bhopal and Sri venkateshwara Research Centre, Thanjavur.
[2]Professor, Sarvapalli radhakrishan university, Bhopal, India

Abstract

The present study was carried out to identify the phytochemicals present in the *Punica granatum* a medicinally important plant of the Punicaceae family. In the present study the ethanolic rind extract of *Punica granatum* fruit has been subjected to GC-MS analysis and antibacterial assay. This analysis revealed that ethanolic extract of *P. granatum* Linn contains 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, Nitroisobutylglycerol, Ethyl. alpha. -d-glucopyranoside, 3H-indole-3-carbaldehyde (4-amino-5-methyl-4H-1,2,4-triazol-3-yl) hydrazone, Maltol etc., justifying the use of this plant to treat many aliments in folk and herbal medicine. The antibacterial investigation was performed against *Staphylococcus aureus, Bacillus subtilis, S. epidermidis, Escherichia coli and Proteus vulgaris* and the fungal strain *Candida albicans* as test organisms. This screening was determined both by disk diffusion and agar-well diffusion methods. The most susceptible bacteria in agar well diffusion method were, *S.epidermidis, B. subtilis P.vulgaris, S.aureus* and *Candida albicans* while the most resistant bacteria were *E.coli* and *Pseudomonas aeroginosa*.

Introduction

In the Indian subcontinent's ancient Ayurveda system of medicine, the pomegranate has extensively been used as a source of traditional remedies for thousands of years. The rind of the fruit and the bark of the pomegranate tree are used as a traditional remedy against diarrhea, dysentery and intestinal parasites. The seeds and juice are considered a tonic for the heart and throat, and classified as having bitter astringent taste plus a range of taste from sweet to sour, depending on ripeneness [1]. The astringent qualities of the flower juice, rind and tree bark are considered valuable for a variety of purposes, such as stopping nose bleeds and gum bleeds, toning skin, firming-up sagging breasts and treating hemorrhoids. Ayurveda differentiates between pomegranate varieties and employs them for different remedies. Pomegranate has been used as a contraceptive and abortifacient by means of consuming the seeds, or rind, as well as by using the rind as a vaginal suppository. This practice is recorded in ancient Indian literature, in medieval sources, and in modern folk medicine [2]. In the present day the antibacterial study of the different parts of the plant extract of *P. granatum* is a subject of research by many. We have attempted to study the antibacterial activity of the peel extract and have also resolved the phytochemicals present in it by GC MS analysis.

Materials and methods

Preparation of plant extract

The *Punica granatum* L fruits were obtained at a public market in Triuvananathapuram. The peel of the fruit was removed and shade dried and powdered with the help of a hand grinding mill. About 200 g of the powder was exhaustively extracted with ethanol. The extract was concentrated to a residue. The crude extract was used for further investigation for its phytochemical compounds and potential antioxidant properties.

Column chromatography 10 g of the crude extract was subjected to column chromatography over silica gel (100-200 mesh) and eluted with nhexane, chloroform, ethanol and methanol respectively.

n-Hexane and Chloroform did not elute much of the compounds. The ethanol fraction of the *Punica granatum* fruit rind was taken for GC-MS analysis.

Gas Chromatography- Mass Spectrum Analysis (GC-MS)

GC-MS technique was used in this study to identify the phytocomponents present in the extract.

GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (Length : 30.0 m, Diameter : 0.25 mm, Film thickness : 0.25 µm Composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51ml/min and an injection volume of 1µl was employed (split ratio: 10). Injector temperature 240°C; Ion-source temperature 200°C. The oven temperature was programmed from 70°C (isothermal for 3 min.), with an increase of 300°C for 10 min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC MS solution ver .2.53.

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having more patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The name, molecular weight, molecular formula and structure of the component of the test material were ascertained.

Antimicrobial Screening of *P. granatum*

Test Microorganisms Used

All microbial strains were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. Bacterial strains used were *Staphylococcus aureus* (MTCC – 7443), *Bacillus subtilis* (MTCC – 2274), *S. epidermidis* (MTCC 435), *Escherichia coli* (MTCC – 40) *and Proteus vulgaris* (MTCC – 1771) and the fungal strain *Candida albicans* (MTCC – 7253) were used for the in vitro studies. All bacterial strains were maintained on Nutrient Agar medium (NAM), Typticase Soy Agar (TSA), except fungal strain, which was maintained on Malt Yeast Agar (MYA), Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and stored at 4ºC. All these strains were subcultured every month on the same media. Paraffin stocks were prepared by overlaying agar slants with it.

Preparation of bacterial and fungal Inoculum

A loop full of grown culture from the slants was transferred into 5-6 ml of fresh Muller Hinton or Sabouraud dextrose broth for for bacteria and fungi respectively. This was incubated at appropriate temperature of 35-37 ° C for 24 h for bacteria and 25–28 °C for 72- 96 h for fungi respectively. The broth was standardized by using sterile normal saline (0.85% NaCl) to obtain a population of 1.5 × 108 cfu/ml by 0.5 McFarland standard methods for bacteria and by using flexi loop for fix amount of fungal culture i.e.1.5 × 108 cfu/ml. From this 0.1 ml was served as working inoculums for screening of antibacterial and antifungal activities

Determination of Antimicrobial activity

Antibacterial activity assay using disk diffusion method

The antibacterial effects were tested by the disk diffusion method , briefly, Muller Hinton Agar plates were culture with a standardized inoculums (1.5 ×108 CFU/ml equal to 0.5 McFarland) of each bacterial strains. Then the disks contain specific amount of extracts were carefully placed at

the labeled seeded plate. The plates were incubated aerobically at 37º C for 24 hours. The diameter of inhibition zones were measured in mm and the results were recorded. Inhibition zone ≥ 12mm were considered as good inhibitory effect of extract [3,4]. Each experiment was done 3 times.

Antibacterial activity assay using Well method

Muller Hinton Agar plates were culture with a standardized inoculums (1.5 ×108 CFU/ml equal to 0.5 McFarland) of each bacterial strains using a sterile cotton swab. Agar wells were prepared with the help of sterilized cork borer with 7mm diameter.Using a micropipette100 microliters of different concentration of spices extracts were added to different wells in the plate. The plates were incubated in an upright position at 37º C for 24 hours. The diameter of inhibition zones were measured in mm and the results were recorded. The inhibition zones with diameter ≥ 12mm were considered as having Antibacterial activity [8,9].

Results and discussion

The GC-MS study of *Punica granatum* fruit rind (Figure 1) shows many phytochemicals which contributes to the medicinal activity of the plant. Table 1 shows the major components which present in the fruit rind *of Punica granatum* and in Table 2 is presented the important biological activities of these constituents.

The GC-MS study of *Punica granatum* peels has shown many phytochemicals which contributes to the medicinal activity of the plant (Tables 1). The major components present in the peels of the plant *Punica granatum* peel was Maltol (RT: 5.200), Isopropylmethylnitrosamine (RT: 6.136) 3,5-Dihydroxy-6-methyl-2,3 - dihydro-4H-pyran-4-one (RT:6.183), 3H-Indole-3-carbaldehyde (4-amino-5-methyl4H-1,2,4-triazol 3 yl) hydrazone ,(RT: 9.795), Pyrogallol (RT: 10.078), Nitroisobutylglycerol (RT: 10.547), 4-deuterio-trans-3,4-dihydroxy-cyclopentene (RT: 12.457), Ethyl .alpha.-d-glucopyranoside (RT: 12.743), and 4-O-Methylmannose (RT: 13.203) present in the peels of the plant *Punica granatum* and their activities were shown in Table 3.

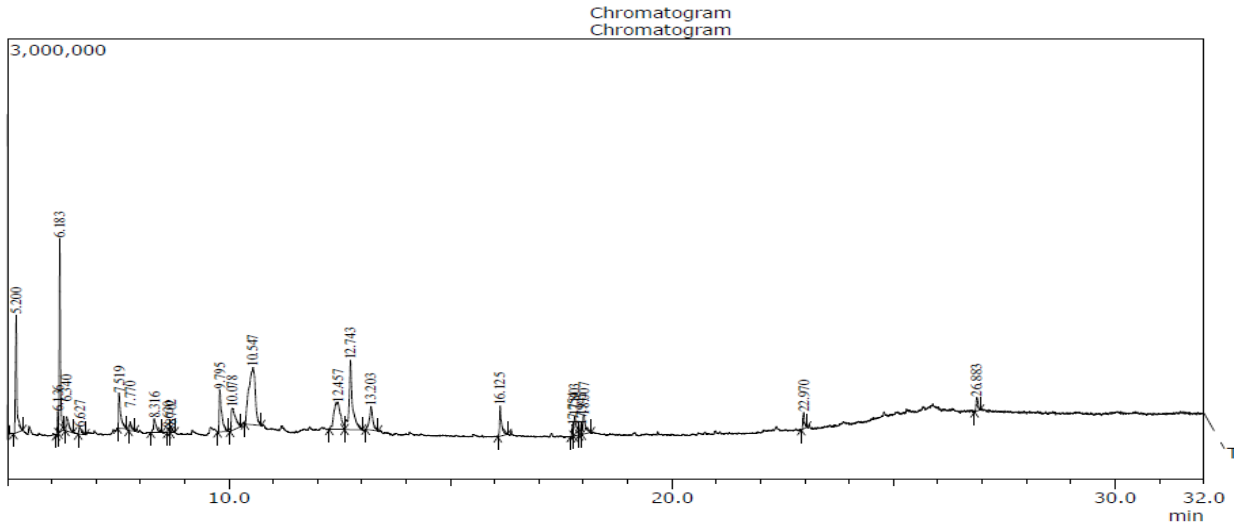

Figure 1. GC MS chromatogram of *Punica granatum*

Table 1: GC-MS Report of *Punica granatum*

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Peak Report TIC

PEAK#	R.TIME	AREA	AREA%	NAME
1	5.200	2149643	9.46	Maltol
2	6.136	269643	1.19	Isopropylmethylnitrosamine
3	6.183	2688485	11.83	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one
4	6.340	455719	2.01	Glycerol
5	6.627	199340	0.88	4-Hydroxydihydro-2(3H)-furanone
6	7.519	885004	3.90	5-Hydroxymethylfurfural
7	7.770	180689	0.80	Glycerin monoacetate
8	8.316	438651	1.93	4-Hydroxy-3-methyl-2-butanone
9	8.630	86902	0.38	3-Methyldecanoic acid
10	8.702	101184	0.45	2-Ethyl-3-nitroso-1,3-oxazinane
11	9.795	1186444	5.22	3H-INDOLE-3-CARBALDEHYDE (4-AMINO-5-METHYL-4H-1,2,4-TRIAZOL-3-YL)HYDRAZONE
12	10.078	1054084	4.64	Pyrogallol
13	10.547	4320919	19.02	Nitroisobutylglycerol
14	12.457	2138298	9.41	4-DEUTERIO-TRANS-3,4-DIHYDROXY-CYCLOPENTENE
15	12.743	2873674	12.65	Ethyl .alpha.-d-glucopyranoside
16	13.203	936991	4.12	4-O-Methylmannose
17	16.125	681696	3.00	Palmitic acid
18	17.754	171967	0.76	10,12-HEXADECADIEN-1-OL
19	17.803	575356	2.53	Oleic Acid
20	17.945	180101	0.79	14-METHYL-8-HEXADECYN-1-OL
21	18.007	540315	2.38	Stearic acid
22	22.970	287073	1.26	9-OCTADECENAMIDE
23	26.883	316345	1.39	gamma.-Sitosterol
		22718523	100.00	

Table 2. Activity of Phytochemicals Present in GC-MS of *Punica granatum*

RT	Name	Peak area %	Activity
5.200	Maltol	9.46	Anticonvulsant, Antifatigue, Antioxidant, antitumour
6.136	Isopropylmethylnitrosamine	1.19	Antioxidant, antihyperglycemic
6.183	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	11.83	Antimicrobial, anti-inflammatory, Antiproliferative.
9.795	3H-Indole-3-carbaldehyde (4-amino-5-methyl-4H-1,2,4-triazol-3-yl) hydrazone	5.22	Antioxidant, anticonvulsant, analgesic, Antibacterial, Antifungal
10.078	Pyrogallol	4.64	Antibacterial, Antidermatitic, Antimutagenic, Antioxidant,
10.547	Nitroisobutylglycerol	19.02	Oxytocin-induced activity, Antioxidant, Anti-staphylococcal Activity.
12.457	4-deuterio-trans-3,4-dihydroxy-cyclopentene	9.41	antiviral activity, Anticancer Activitiy
12.743	Ethyl .alpha.-d-glucopyranoside	12.65	Antituberculous Activity, Antioxidant, alpha amylase inhibitory activity, Hypolipemic activity, Anticonvulsant
13.203	4-O-Methylmannose	4.12	Antibacterial activity

Table 3. Antibacterial activity of ethanolic extract of *Punica granatum* using disk diffusion and agar well diffusion method

S. No	Organism	Well method				Disk method			
		1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml
1	*E.coli*	8	0	0	0	0	0	0	0
2	*S.aureus*	17	16	14	13	11	8	0	0
3	*P.aeroginosa*	0	10	8	7	0	8	0	0
4	*S. epidermidis*	23	20	18	17	15	12	10	8
5	*B.subtilis*	20	18	14	12	16	14	10	8
6	*P.vulgaris*	18	17	15	12	10	8	7	0
5	*Candida albicans*	18	15	13	11	10	8	0	0

The antibacterial activity of ethanolic extract *Punica granatum* rind extract was assayed against six gram positive and negative bacteria and *C. albicans* by disk diffusion and agar well diffusion methods and the results of inhibition zones have shown in Table 3.

The most susceptible bacteria in agar well diffusion method were, *S.epidermidis*, *B. subtilis P.vulgaris*, *S.aureus* and *Candida albicans* with diameter of inhibition zones 23,20,18,17 and 18 mm respectively, while the most resistant bacteria were *E.coli* and *Pseudomonas aeroginosa*. The largest zone of inhibition obtained against *S.epidermidis (23mm).*

The phytochemicals listed in table 2 from GC MS results show that some compounds viz., 3H-Indole-3-carbaldehyde (4-amino-5-methyl-4H-1,2,4-triazol-3-yl) hydrazone, Pyrogallol, 4-O-Methylmannose etc., having antibacterial activity.

Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study. Further investigations into the pharmacological importance of *Punica granatum* peel and their diversity and detailed photochemistry may add new knowledge to the information in the traditional medical systems.

References

[1]. Jafri, M.A., Aslam, M., Javed, K., & Singh, S. (2000). Journal of Ethnopharmacology, 70: 309-314.

[2]. Ventura, J., Alarcon-Aguilar, F., Roman-Ramos, R., Campos-Sepulveda, E., Reyes-Vega, M.L., Daniel Boone-Villa, V., Jasso-Villagomez, E.I., & Aguilar, C.N. (2013). Food Chemistry. 136: 109-115.

[3] J.M .Androw. *J. Antimicrobial Chemotherapy* **2001**; 7 (5): 48 - 57.

[4] A.Nostro,M.P.Ger,V.D.Angelo,M.A.C.Cannatelli. *Applied Microbiol*.**2001**, 15: 379 - 85.

[5] C.Walter, Z.K.Shinvari, I.Afzal, R.N. Malik. *Pak. J. Bot*. **2011**,43: 155-162.

[6] B.Kivack,T.Mert ,H. Tansel.*Turkish Journal of Biology*.**2001**, 26: 197-200.