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Study On Vitro Anti-Microbial Activity and Phytochemical Analysis of Selected Fruit Wastes

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ABSTRACT
Antibiotics and synthetic medications that have major side effects are not without their drawbacks, but medicinal plants are a rich source of potentially beneficial phytochemical substances. The waste materials from the fruits, Punica granatum peel and Vitis vinifera seeds, were extracted in the current study using ethanol as the solvent. The antibacterial potential and phytochemical content of the ethanolic extract of both fruit components were examined. A qualitative phytochemical examination of the chosen extracts revealed the presence of powerful bioactive components as polyphenols, terpenoids, flavonoids, tannins, alkaloids, cardiac glycosides, carbohydrates, and phytosterols. The in vitro antibacterial activity of the two chosen extracts was assessed against the bacterial strains Staphylococcus aureus, Bacillus circulans, Klebsiella pneumonia, Vibrio vulnificus, and Salmonella typhi. Candida tropicalis, Candida albicans, and Cryptococcus neoformans were the fungi strains chosen for the study. The lowest inhibitory concentration was also assessed, and the antibacterial activity was tested using the agar plate well diffusion method. The studied organisms were effectively inhibited by both extracts' antibacterial activities. The antibacterial potential of P. granatum peel extracts was higher than that of V. vinifera seed extract, nevertheless. Staphylococcus aureus growth was entirely suppressed at lower concentrations by both of the extracts used for this study in the lowest inhibitory concentration study, which was conducted using the micro dilution technique.

Key Words-: *Punica granatum; Vitis vinifera; Antibacterial activity; phytochemicalanalysis; Fruit waste; disc diffusionmethod.*

I. Introduction

The use of medicinal plants in human health care is significant, and it is among the earliest types of medical treatment that have ever been used by humans. Over 2600 plant species have been used in India's ancient medical practices, including Ayurveda, Unani, and Siddha (Khandelwal, 1999). India is ideally suited for the development of pharmaceuticals with plant origins. Numerous naturally occurring chemicals have been identified to have antibacterial properties in nutritional and medicinal herbs, fruits, and vegetables (Kouassi and Shelef, 1998; Larson et al., 1996). Plant-based antimicrobials have a huge therapeutic potential and have been utilized for centuries. Due to their substantial adverse effects, they have been demonstrated to be effective substitutes for synthetic chemical antibacterial agents and antibiotics (Pawar and Nabar, 2010; Olila et al., 2001). The rise of multidrug-resistant pathogenic strains poses a danger to the clinical efficacy of many currently available antibiotics (Bandow et al., 2003). Secondary metabolites such as essential oil, xanthones, benzophenones, coumarins, and flavonoids were among the antibacterial compounds that were identified from plants (Belguith and Kthiri, 2010).

Punica granatum L., a native plant to Asia and the Mediterranean region, has a long history of usage in traditional medicine. This plant's bark, leaves, flowers, fruits, and seeds have all been utilized for a long time to treat illnesses (Jayaprakasha et al., 2006). According to numerous reports (Faria et al., 2007; Adhami and Mukhtar, 2006), pomegranates have anti-virus, antioxidant, anti-cancer, and anti-proliferative properties. Because of its potent astringency, pomegranate peels are used in traditional medicine and are a well-liked treatment all over the world. It was used for dysentery and diarrhea as an aqueous decoction (i.e., boiling the hulls in water for 10–40 minutes), and it can also be used for stomatitis as a mouthwash, douche, or enema.

Today, grapes (Vitis Vinifera) are grown around the world, regardless of climate (Gruenwald et al., 2004). According to reports, grape seed extract offers a wide range of therapeutic and pharmacological actions, including anti-inflammatory, anti-oxidative, and antibacterial properties, as well as cardioprotective, hepatoprotective, and neuroprotective effects. The grape seed is used as a nutritional supplement and in herbal

therapy (Shenoy et al., 2007). According to Chedea et al. (2010), GSE is regarded as a potent antioxidant nutritional supplement that guards against illnesses and premature aging.

II. Materials And Methods

Collection of plant material

The plant specimens for the proposed study were collected from Local market, Saharanpur, India of same cultivar. The fruits *Punica granatum* belonging to Lythraceae family and *Vitis vinifera* belonging to *Vitaceae* family were selected for this study. Care was taken to select healthy fruits. The *Punica granatum* peel and *Vitis vinifera* seeds were removed, washed and used for the studies.

Preparation of the extracts

P.granatum peel and *V.vinifera* seeds were removed from the fruits and washed thoroughly using sterilized water. The selected plant materials were shade dried and pulverized to fine powder in a mechanical grinder individually. 100g of Course powder were weighed and extracted with 1 Litre of Ethanol as solvent. These extract were collected after filtration using Whatman No.1 filter paper. The solvent were concentrated under reduced pressure in a rotary evaporator until solvents are completely evaporated from the extract. This obtained alcoholic extracts were used for further studies.

Determination of antimicrobial activity by disc diffusion assay

Bacterial and fungal strain

Bacterial strains *Staphylococcus aureus* (MTCC 1144), *Bacillus circulans* (MTCC 7635), *Klebsiella pneumonia* (MTCC 3040) and *Vibrio vulnificus* (ATCC33817) were selected along withfungal strain *Candida albicans* (MTCC 227) and *Candida trophicalis* (MTCC 184).All the above selected strains were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India and AmericanType Culture Collection (ATCC), USA. Fungal strains *Cryptococcus neoformans*, and bacterial strain *IMTECH Chandigarh*, India. Strains were maintained at 4ºC on nutrient agar slants. Each of the microorganisms was freshly cultured prior to susceptibility testing by transferring them into a separate sterile test tube containing nutrient broth and incubated overnight at 37°C. A microbial loop was used to remove a colony of each bacterium and fungus from pure culture and transferit into nutrient broth.

Preparation of inoculums

The organisms selected for studies were recovered and sub cultured on fresh media. A loopful inoculum of each bacterium and fungus was suspended in 5ml of nutrient broth and incubated overnight at 37ºC. These overnight cultures were used for the study. Composition of nutrient broth were Beef extract -3.0 g, Peptone -5.0 g, Distilled water-1000 ml. The medium was adjusted to pH 7.4 and sterilized byautoclaving at 15 lbs pressure (121ºC) for 15 min.

The disc diffusion method (Bauer AW *et al*, 1966) was used to screen the anti- microbial activity. *In-vitro* antibacterial assay was done using Muller Hinton Agar (MHA, pH 7.3 ± 0.1) for anti-bacterial and Potato dextrose agar for anti-fungal activity. The Muller Hinton Agar and Potato dextrose agar were prepared and autoclaved at 121 C for 20min.The plates were prepared by pouring 15ml of molten media into sterile petri plates.

The plates were allowed to solidify for 10 minutes.0.1% standardized inoculums suspension was swabbed uniformly, and the inoculums were allowed to dry for 5 minutes. Sterile HiMedia paper disc (6mm) were soaked in 20µl of the extract with different concentration diluted in 25% DMSO and dried at 37 C overnight. The loaded disc was placed on the surface of medium, the compound was allowed to diffuse for 5 minutes, and the plates were kept for incubation at 37 C for 24hrs.

Antibiotic disc containing Chloramphenicol (30µg) was used as controls. A disc loaded with 25% DMSO alone served as the negative control. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone formed around the discs. In antifungal activity determination the loaded disks were placed in the surface of medium and the plates were kept for incubation at 28^0c for 72 hours. Antibiotic disc containing Amphotericin B was used as controls. The antifungal activity was evaluated by measuring the diameter ofthe inhibition zone formed around the discs. These studies were performed in triplicate.

Minimum inhibitory concentration(MIC) determination

The antimicrobial activity of natural products was studied by employing a micro-dilution method, using two different culture media: Muller-Hinton broth and Luria Bertania (LB). The inoculums were prepared as described in above. Extracts were dissolved in DMSO (10% of the final volume) and diluted with culture

broth to aconcentration of 2 mg/ml. Further 1:2 serial dilutions were performed by addition of culture broth to reach concentrations ranging from 2 to 0.01575 mg/ml; 100 l of each dilution were distributed in 96-well plates, as well as a sterility control and a growth control (containing culture broth plus DMSO, without antimicrobial substance).Each test and growth control well was inoculated with 5 l of a bacterial suspension (108 CFU/ml or 105 CFU/well). All experiments were performed in triplicate and the micro dilution trays were incubated at 36ºC for 18 hours. MIC values were defined as the lowest concentration of each natural product, which completely inhibited microbial growth. The results were expressed in milligrams per milliliters (Souza *et al.*, 2005).

III. Results And Discussion

Qualitative phytochemical analysis

The result of qualitative phytochemical analysis of the selected fruit extracts revealed the presence of various potent phytochemical such as flavonoids, tannins, alkaloids, cardiac glycosides, carbohydrates, terpenoids, chalcones, phlobatannins and phytosterols were present in both the extract whereas saponins was present only in *V.vinifera* seed extract and steroids in *P.granatumpeel* extract (Table 1). Flavonoids and tannins were abundant in both the selected extract.

The antimicrobial efficacy of *Punica granatum* peel and *Vitis vinifera* seeds against the bacterial and fungal strains was evaluated by determination of the zones of inhibition. The results of the antibacterial activity of both the extracts were presented in Table 2,3. Both the extracts showed bactericidal activity against the selected Gram negative and Gram positive bacteria.Chloramphenicol was used as a positive control. The zone of inhibition showed by *P.granatum* peel extract against test bacteria ranged from 7-16 mm and seed extract produced zone of inhibition at a range of 7-15 mm. Both the selected extracts showed higher antibacterial activity against *Staphylococcus aureus*(Tables 2, 3). The two extracts selected for this study showed antifungal activity against all the three fungal strains. Both the extract showed maximum zone of inhibition against *Candida tropicalis.*(Table 4)

Table.1 Qualitative analysis *Punica granatum* Peel and *Vitis vinifera* seed extractAntimicrobial activity by Agar well diffusion method

Phytochemicals	***Punica granatum* Peel**	***Vitis vinifera* seeds**
Flavonoids	++	++
Anthraquines	-	-
Saponins	-	+
Tannins	++	++
Aminoacids	+	+
Carbohydrates	++	+
Phlobatannins	+	+
Chalcones	+	+
Alkaloids	+	++
Steroids	+	-
Terpenoids	+	+
Phytosterol	++	+
Cardiac Glycosides	+	+

Table.2 Antibacterial activity of *Punica granatum* peel extract against selected bacteria

S.No	**Microorganism**	**Zone of Inhibition (mm)**					
		1000 µg	**500 µg**	**250 µg**	**100 µg**	**DMSO**	**Chloramphenicol30µg**
1.	*Staphylococcus aureus*	16	14	13	12	-	26
2.	*Bacillus circulans*	13	12	10	8	-	21
3.	*Salmonella typhi*	11	8	7	-	-	15
4.	*Klebsiella pneumonia*	13	12	11	9	-	20
5.	*V.vulnificus*	12	10	8	7	-	20

Table. 3 Antibacterial activities of *Vitis vinifera* seed extract against selected bacteria

S.No	Microorganism	Zone of Inhibition (mm)					
		1000 µg	500 µg	250 µg	100 µg	DMSO	Chloramphenicol30µg
1.	*Staphylococcus aureus*	15	13	12	11	-	27
2.	*Bacillus circulans*	12	10	8	8	-	20
3.	*Salmonella typhi*	8	7	7	-	-	15
4.	*Klebsiella pneumonia*	13	11	11	10	-	21
5.	*V.vulnificus*	11	10	8	8	-	17

Table. 4 Antifungal activity of fruit extracts against selected strains

S.No	Microorganism	*Candida albicans*			*Cryptococcusneoformans*			*Candida tropicalis*		
		Zone of Inhibition (mm)								
		Day 1	Day2	Day3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
1.	*Punica granatum* Peel	7	8	11	7	8	10	10	12	15
2.	*Vitis vinifera seeds*	6	8	9	7	7	8	-	7	10
3.	Amphotericin B	14	15	16	13	15	17	15	18	20

The alcoholic extract of *Punica granatum* peel and *Vitis vinifera* seeds showed antimicrobial activity against each of the tested strains at different concentration. The values of *Punica granatum* peel extract ranged from 62.50 to 1000 g/ mL and *Vitis vinifera* seed extract showed activity varying from 125 to >1000 g/mL against the selected strains. The MICs determined by micro dilution method confirmed the results obtained using the disc diffusion method. The MIC results showed both *Punica granatum* peel and *Vitis vinifera* extracts showed growth inhibition of *Staphylococcus aureus* at lowest concentration (Tables 5, 6). Phytonutrients and secondary metabolites naturally occurring from plants possess antimicrobial activity (Srikumar *et al*., 2007).The antibacterial activity of the extracts may be due to the presence of several metabolic toxins. Several metabolites from plant materials, including alkaloids, tannins and sterols possess antimicrobial activity (Leven *et al*., 1979)

.The site and the number of hydroxyl groups on the phenol components may increase the toxicity against the microorganisms. Tannins are water soluble polyphenols known as tannic acid acts as antimicrobial agents. Presence of tannins is to prevent the development of microorganism by precipitating microbial proteins (Pranay Jain and GulhinaNafis, 2011)**.** The antimicrobial properties of tannins might be related to their ability to inactivate microbial adhesions, enzymes, and cell envelope transport proteins, their complexity with polysaccharides, and their ability to modify the morphology of microorganisms **(**Cowan, 1999). Sterol molecules Gamma.-Sitosterol is present in both the selected extracts whereas Stigmasterol is present only in *V.vinifera* seed extract (Ashok kumar and Vijayalakshmi, 2011). Sterol molecules shows antibacterial activity and their mode of action may be due to surface interaction of it with the bacterial cell wall and membrane ultimately leading to pore formation and degradation of the bacterial components (Devjani Chakraborty and Barkha Shah, 2011).*P.granatum* peel *and V.vinifera* seed extracts contains 4H- Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- a flavonoid fraction (Ashok kumar and Vijayalakshmi, 2011) which possess anti-microbial, anti-oxidant and anti-inflammatory activities (Teoh *et al*., 2011: Kumar and Maneemegalai 2008; Kumar *et al*., 2010) which may play a role in anti- microbial activity of the extract against the selected microbial strains.

Most of the antibiotics used nowadays have lost their effectiveness due to the development of resistant strains of microbes, which is primarily due to the expression of resistance genes (Davis,1994; Service, 1995).The antibiotics are sometimes associated with serious side effects such as hypersensitivity, immune suppression and allergic reactions (Ahmad *et al.*, 1998). Therefore, more interest is shown to develop alternative antimicrobial drugs for the treatment of infectious diseases without side effects. (Berahou *et al*., 2007; Salomao *et al*., 2008). The results of present study support the traditional usage of plant materials *P.granatum* and *V.vinifera* extracts which possess compounds with antibacterial and antifungal potential that can be used as anti-microbial agents as new drugs for the therapy of infectious diseases caused by pathogens.

Table.5 Minimum Inhibitory Concentration of *Punica granatum* peel extractagainst selected microorganisms

S.No	Microorganism	Minimum Inhibitory Concentration (g/ml)							
		>1000	1000	500	250	125	61.50	31.25	15.175
1.	*Staphylococcus aureus*	-	-	-	-	-	-	+	+
2.	*Bacillus circulans*	-	-	-	+	+	+	+	+
3.	*Salmonella typhi*	-	-	+	+	+	+	+	+
4.	*Klebsiella pneumonia*	-	-	-	-	+	+	+	+
5.	*V.vulnificus*	-	-	+	+	+	+	+	+
6.	*Candida albicans*	-	-	-	+	+	+	+	+
7.	*C.neoformans*	-	-	+	+	+	+	+	+
8.	*Candida tropicalis*	-	-	-	-	+	+	+	+

Table.6 Minimum Inhibitory Concentration of *Vitis vinifera* seedextract against selected microorganisms

S.No	Microorganism	Minimum Inhibitory Concentration (g/ml)							
		>1000	1000	500	250	125	61.50	31.25	15.175
1.	*Staphylococcus aureus*	-	-	-	-	-	+	+	+
2.	*Bacillus circulans*	-	-	-	+	+	+	+	+
3.	*Salmonella typhi*	-	+	+	+	+	+	+	+
4.	*Klebsiella pneumonia*	-	-	-	+	+	+	+	+
5.	*V.vulnificus*	-	-	+	+	+	+	+	+
6.	*Candida albicans*	-	-	+	+	+	+	+	+
7.	*C. neoformans*	-	-	+	+	+	+	+	+
8.	*Candida tropicalis*	-	-	-	+	+	+	+	+

+ = growth of organism, - = inhibition of growth

References

[1]. Adhami, V.M., and Mukhtar, H. 2006. Polyphenols from green tea andpomegranate for prevention of prostatecancer. Free. Rad. Res. 40(10): 1095- 104.

[2]. Ahmad, I., Z. Mehmood and Mohammad,F. 1998. Screening of some Indian medicinal plants for their antimicrobialproperties. J. Ethnopharmacol. 62: 183 93.

[3]. Ashok Kumar, K., and Vilayalakshmi K. 2011. GC-MS Analysis of phytochemical constituent in Ethnolic extract of *Punica granatum* peel and *Vitis vinife*ra seeds. Int.J.Pharma. Bio sci. 2 (4): 461 468.

[4]. Bandow, J. E., H. BrotzLeichert, L.I.O.Leichert, H. Labischinski and Hecker, M. 2003. Agent. Chemother. 47: 948-955.

[5]. Bauer, A.W., M.M. Kriby, J.C. Sherris andTruck, M .1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin.Pathol.45: 493-6.

[6]. Belguith, H., and Kthiri, F. 2010.Inhibitory effect of aqueous extract of Allium Sativum. Afri. J. Microbiol. Res. 4(5): 328-338

[7]. Berahou, A., A. Auhmani, N. Fdil, A. Benharref, M. Jana and Gadhi, C. A. 2007. Antibacterial activity of *Quercus ilex* bark s extracts.J. Ethnopharmacol. 112: 426 9.

[8]. Chedea,V.S., C. Braicu and Socaciu, C. 2010. Antioxidant/prooxidant activityof polyphenolic grape seed extract. Food. Chem.121: 132-139,

[9]. Cowan, M, M., 1999. Plants products as antimicrobial agents. Clin.Microbio.Rev. 12: 564 82(3): 192-199.

[10]. Davis, J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. Science. 264: 375 82.

[11]. Faria, A., R. Monteiro, N. Mateus, I. Azevedo and Calhau, C. 2007. Effect of pomegranate (*Punica granatum*) juice intake on hepatic oxidative stress.Eur. J. Nutr. 46(5): 271-8.

[12]. Gruenwald, J., B.A. Brendler and Jaenicke, C. 2004. PDR for herbal medicines, 3rd ed., Thomson PDR: Montvale, NJ. Harborne, J.B., 1998. Phytochemicalmethods guide to modern Technique ofPlant Analysis,3rd Edition, Chapmanand Hall, London.1 pp. 135.