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Full Length Research Paper

Antimicrobial activity and qualitative phytochemical analysis of *Punica granatum* Linn. (PERICARP)

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Pomegranate, *Punica granatum* L. is frequently used for the treatment of gastero-intestinal resistant organisms, oral bacteria, highly pathogenic and antibiotic-resistant organisms (*Salmonellae* and *Pseudomonas aeruginosa*) and a few skin infecting pathogens, including fungus and methicillin-resistant *Staphylococcus aureus* (MRSA). In this study, an *in vitro* antimicrobial activity and phytochemical analysis of ethanolic pericarp extract of *P. granatum* was carried out. A total of 21 microorganisms (19 bacteria and 2 fungi) were used for antimicrobial activity by disc diffusion, broth dilution methods and a standard procedure was employed to identify the phytoconstituents. Ethanolic extract of pericarp showed significant bactericidal activity against *Yersinia enterocolitica* (25.8 mm), *Staphylococcus epidermidis* (24.6 mm), *Salmonella enterica* (21.2 mm), *Burkholderia cepacia* (19.4 mm), *S. aureus* (19.2 mm), *Salmonella paratyphi* A (18.8 mm), *Salmonella typhimurium* (18.6 mm), *Escherichia coli* (18.4 mm) and *P. aeruginosa* (18.2 mm) were documented. Based on broth dilution technique, the lowest concentration of ethanolic pericarp extract (512 µg/ml) showed significant inhibitory activity against *S. epidermidis* and *Y. enterocolitica* and 1,024 µg/ml was observed as a minimum inhibitory concentration (MIC) value against *S. aureus*, *Streptococcus mutans*, *S. paratyphi* A, *S. typhimurium*, *Salmonella brunei*, *P. aeruginosa* and *B. cepacia*. The results of ethanolic extract of *P. granatum* pericarp showed significant inhibitory activity when compared with other organisms. The highest minimum inhibitory activity was observed against bacteria when compared with fungi. The inhibitory effect could be due to the presence of some of the secondary metabolites like phenolic compounds, flavonoids, terpenoids, phytosterols, glycosides and tannins detected in the ethanolic pericarp extract of *P. granatum*.

Key words: *Punica granatum*, phytoconstituents, antimicrobial activity, disc diffusion and broth dilution methods, minimum inhibitory concentration (MIC).

INTRODUCTION

Medicinal plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years.

Plants are the gifts of nature used to cure number of human diseases (Deepa et al., 2012). Recently, there has been a rekindling interest in "rediscovering natural products" (Cragg and Newman, 2005) due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and also due to side effects of synthetic antibiotics (Renu, 2010).

Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases (Deepa et al., 2012). The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of phytomedicines to act against microbes (Kumaraswamy et al., 2008).

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However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security.

Punica granatum belongs to the family Punicaceae, commonly known as pomegranate, is a shrub or small tree with several upright, thorny stems, the leaves are elliptic, roughly 2×1 inches, the flowers are white or red, double-flowered races, native of Asia and Mediterranean Europe (Egharevba et al., 2010). The traditional knowledge reveals that the bark and rind fruits have been used in tanning and as vermifuge, especially in cough and cold (Vijayanand and Hemapriya, 2011). For centuries, the barks, leaves, flowers, fruits and seeds of this plant have been used to ameliorate diseases (Jayaprakasha et al., 2006). The potential therapeutic properties of pomegranate are wide-ranging and include treatment and prevention of cancers, cardiovascular disease, diabetes, dental conditions, erectile dysfunction and prevention from ultra violet (UV) radiation. The pericarp of *P. granatum* is used to treat infections found in human sexual organs as well as mastitis, acne, folliculitis, pile, allergic dermatitis, tympanitis, scalds, diarrhoea and dysentery (Singh et al., 2002). The constituents of *P. granatum* include gallocatechins, delphinidin, cyanidin, gallic acid, ellagic acid, pelargonidin and sitosterol, which are well known for their therapeutic properties (Lansky and Newman, 2007). In addition, *P. granatum* is reported to have antioxidant (Heber et al., 2007; Parmar and Kar, 2008), anti-atherosclerotic (Aviram et al., 2004; Parmar and Kar, 2007), antibacterial (Braga et al., 2005; Naz et al., 2007) and antiviral (Zhang et al., 1995) properties. The present study is aimed at providing information on the type of phytoconstituents present in the plant and exploring the antimicrobial properties.

MATERIALS AND METHODS

Collection of plant materials

Fresh fruit pericarp of *P. granatum* was collected from the natural habitat of Tiruchengode, Namakkal, Tamil Nadu. The plant species was preliminarily identified in the Department of Botany, Vivekanandha College of Arts and Sciences for Women, Elayampalayam, Tiruchengode. Further, it was authentified by the Scientists of the Botanical Survey of India (BSI) in Coimbatore.

Extraction of plant

The pericarp of *P. granatum* fruit was shade dried and pulverized. 250 g of the powdered material was packed in Soxhlet apparatus and subjected to continuous hot percolation for 8 h using 450 ml of ethanol (75% V/V) as solvent. The ethanol extract was concentrated under vacuum and dried in a dessicator.

Phytochemical screening

The preliminary qualitative phytochemical tests of ethanolic pericarp extract of *P. granatum* were analysed and the methods were described by Harbourne (1984), Hebert et al. (1984), Basset et al. (1985) and Kokate (1990). The following phytoconstituents, namely, carbohydrates, glycosides, fixed oils and fats, protein and amino acids, saponins, tannins, phenolic compounds, phytosterols, terpenoids, alkaloids and flavonoids were screened.

Antimicrobial screening

Source of microbial strains

Strains of human pathogenic microorganisms used in this study are as follows: three Gram-positive bacteria, *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435) and *Streptococcus mutans* (MTCC 890); sixteen Gram-negative bacteria, *Escherichia coli* (MTCC 739), *Klebsiella pneumoniae* (MTCC 432), *Enterobacter aerogenes* (MTCC 111), *Proteus vulgaris* (MTCC 742), *Proteus mirabilis* (MTCC 425), *Salmonella typhi* (MTCC 733), *Salmonella paratyphi* A (MTCC 735), *Salmonella typhimurium* (MTCC 98), *Salmonella infantis* (MTCC 1167), *Salmonella enterica* (MTCC 660), *Salmonella brunei* (MTCC 1168), *Pseudomonas aeruginosa* (MTCC 424), *Burkholderia cepacia* (MTCC 1617), *Vibrio parahaemolyticus* (MTCC 451), *Haemophilus parahaemolyticus* (MTCC 1776) and *Yersinia enterocolitica* (MTCC 80); two fungus, *Candida albicans* (MTCC 183) and *Cryptococcus neoformans* (clinical isolate). The microorganisms were originally obtained from Microbial Type Culture Collection Centre (MTCC), Institute of Microbial Technology, Chandigarh, India. Cultures were maintained as respective agar slants in screw-capped bottles and were stored at 4°C. All cultures were checked for viability and purity by regular plating.

MIC: Disc diffusion method

The antimicrobial activities of the successive ethanolic extracts of pericarp were tested by disc diffusion method (Bauer-Kirby et al., 1966). The culture plates were prepared by pouring 20 ml of sterile Hi-sensitivity (Himedia-M 486) agar medium. The depth of the medium was approximately 4 mm. Three to four similar colonies of pure cultures were inoculated with tryptone soy broth (Himedia-M 323), further, it was incubated at 37°C for 2 to 8 h and inoculum size was adjusted to yield uniform suspension containing 10^5 to 10^6 cells/ml (McFarland's standard). The agar surface of the plates was swabbed in three directions, turning the plates at 60° between each swabbing. Confluent growth is desirable for accurate results. The sterile discs (6 mm-Himedia) were used for loading ethanolic pericarp crude extract. Five different concentrations were prepared (250, 500, 750, 1,000 and 1,250 µg) and loaded in appropriate discs. The impregnated discs were incubated at 37°C for an hour. The dried discs were placed over the surface of the swabbed medium with equal distance to avoid the overlapping of the zones of inhibition. Then discs were pressed gently on the surface of the medium. The plates were allowed to stand at refrigerator for 30 min (Pre-diffusion time). The plates were incubated at 37°C for 16 to 18 h during which the activity was evidenced by the presence of zones of inhibition surrounding the discs. Each experiment was done in triplicate. A panel of antibiotics was used against each microbial strain and antibiotic that is sensitive with a particular organism is used as control.

MIC: Broth dilution method

Tube dilution method was used to determine the MIC of the extracts in Muller Hinton broth (Himedia-M 391) and Sabouraud Dextrose broth (Himedia-M 033) as specified by National Committee for Clinical Laboratory Standard (NCCLS, 1998). A total of 10 ml of

Table 1. Phytochemical analysis (qualitative) of ethanolic pericarp extract of *Punica granatum*.

S/N	Name of the phytoconstituents	Ethanolic extract
1	Carbohydrates	+
2	Glycosides	+
3	Fixed oils and fats	-
4	Protein and amino acid	+
5	Saponins	-
6	Tannins	+
7	Phenolic compounds	+
8	Phytosterols	+
9	Terpenoids	+
10	Alkaloids	-
11	Flavonoids	+

+: **P**resent; -: Absent.

each broth was dispensed into separate test tube and was sterilized at 121°C for 15 min and then allowed to cool. Two-fold serial dilutions of the extracts in the broth were made from the stock concentration of the extracts to obtain 8 to 4,096 µg/ml for ethanolic pericarp extracts. About 0.1 ml of the standardized inoculums of the microbes was inoculated into the different concentration of the extracts in the broth. The test tubes of the broth were incubated at 37°C for 24 h and 30°C for 1 to 2 days for bacteria and fungi, respectively and were observed for turbidity. The lowest concentration (highest dilution) that showed no turbidity (growth) in the test tube was recorded as the MIC.

Determination of activity index

The activity index of the crude plant extract was calculated as;

$$\text{Activity Index (AI)} = \frac{\text{Zone of inhibition of the extract}}{\text{Zone of inhibition obtained for standard antibiotic (drug)}}$$

RESULTS

The preliminary qualitative phytochemical tests of ethanolic pericarp extract of *P. granatum* are as shown in Table 1. The ethanolic extract showed the presence of carbohydrates, glycosides, protein and amino acids; tannins, phenolic compounds, phytosterols, terpenoids and flavonoids were identified, and saponins, alkaloids, fixed oils and fats were absent.

The antimicrobial activity of the ethanolic pericarp extract at different concentrations was screened by the disc diffusion method and zones of inhibition were expressed in mean value. The data pertaining to the antimicrobial potential of ethanolic pericarp extract of *P. granatum* were presented in Table 2. The MIC was determined by the broth dilution method and the results were given in Table 3.

DISCUSSION

The use of medicinal plants still plays a vital role to establish the basic health requirements in developing countries. Nearly 80% of the world population relay on traditional medicine for primary health care, most of which involves the use of natural products (Sandhya et al., 2006). Based on this concept, plants continue to be a rich source of therapeutic agents. Many researchers made an extensive study on the biologically active principles of *P. granatum* and their potential results showed that this plant is ethno medically valuable (Shibumon and Beny, 2010). *P. granatum* pericarp extracts are currently used for treatment of respiratory diseases, gastro-intestinal disorders, and in the preparation of tinctures, cosmetics and other therapeutic formulae. Natural products have been evaluated as source of antimicrobial agents with efficacies against a variety of microorganisms. *P. granatum* has been used extensively as a traditional medicine in many countries for the treatment of dysentery, diarrhoea, helminthiasis, acidosis, haemorrhage and respiratory disorders (Ricci et al., 2006; Sánchez-Lamar et al., 2007). Recently, a number of antibiotics have lost their effectiveness due to the development of resistant strains of bacteria, which has primarily occurred through the expression of resistance (Davies, 1995; Service, 1995), antibiotics are sometimes associated with opposing effects such as hypersensitivity, immune-suppression and allergic reactions (Ahmed et al., 1998). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases (Berahou et al., 2007; Salomoa et al., 2008).In this study, crude ethanolic extract of the pericarp of *P. granatum* was evaluated for antimicrobial activity against 21 microorganisms. The result showed that the pericarp possesses some broad-spectrum antimicrobial properties; contents of ethanolic extract are sufficient to inhibit the growth of more than 95% of the tested microorganisms. The ethanolic extract of pericarp exhibits significant antibacterial activity when compared with antifungal activity. Another important result of this study was that the microorganisms which is responsible for diarrhoea and typhoid diseases (*S. aureus*, *E. coli*, *S. typhi*, *S. paratyphi* A, *S. typhimurium*, *S. infantis*, *S. enterica*, *S. brunei*, *V. parahaemolyticus* and *Y. enterocolitica*), wound infection (*P. aeruginosa*, *V. parahaemolyticus*, *P. mirabilis*, *E. coli* and *S. aureus*), respiratory disorders (*K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *H. parahaemolyticus*) and skin infection (*S. aureus*, *S. epidermidis* and *C. albicans*) were inhibited considerably. The antimicrobial activity of pomegranate was previously studied. Indeed, it is reported that the bark, leaves, flowers and pericarp of pomegranate are widely used as phytotherapeutic agents in Brazil (Mathabe et al., 2005). The present investigation of antimicrobial activity of *P. granatum* pericarp is utmost comparably correlated with many research outcomes. Ahmed and Beg (2001) reported that alcohol extracts of pomegranate fruits showed antimicrobial activity where tested against *S. aureus*, *E. coli*, and *Shigella dysenteriae*. Prashanth et al. (2001) also reported metha-

Table 2. Antimicrobial activity of ethanolic pericarp extract of *Punica granatum*

S/N	Name of the organism	Zone of inhibition in mm								
		µg/disc					Mean ± SEM	Standard antibiotic µg/disc	Zone (mm)	Activity index (AI)
		250	500	750	1000	1250				
1	*S. aureus*	16	18	19	20	23	19.2 ± 1.15	Amoxycillin (30)	40	0.48
2	*S. epidermidis*	23	24	24	24	28	24.6 ± 0.87	Cloxacillin (5)	27	0.91
3	*S. mutans*	13	15	16	16	21	16.2 ± 1.31	Ciprofloxacin (5)	32	0.50
4	*E. coli*	15	18	18	19	22	18.4 ± 1.12	Ciprofloxacin (5)	30	0.61
5	*K. pneumoniae*	14	16	17	17	19	16.6 ± 0.81	Nalidixic acid (30)	29	0.57
6	*E. aerogenes*	13	14	15	17	21	16.0 ± 1.41	Ciprofloxacin (5)	30	0.53
7	*Proteus mirabilis*	15	15	17	19	21	17.4 ± 1.16	Lomefloxacin (10)	25	0.69
8	*Proteus vulgaris*	15	16	17	18	21	17.4 ± 1.02	Co-trimoxazole (1.25/23.75)	26	0.66
9	*S. typhi*	13	15	16	19	21	16.8 ± 1.42	Chloramphenicol (30)	29	0.57
10	*S. paratyphi A*	15	17	19	20	23	18.8 ± 1.35	Chloramphenicol (30)	27	0.69
11	*S. typhimurium*	15	16	18	21	23	18.6 ± 1.50	Chloramphenicol (30)	29	0.64
12	*S. infantis*	13	13	15	18	18	15.4 ± 1.12	Chloramphenicol (30)	29	0.53
13	*S. enterica*	15	18	21	24	28	21.2 ± 2.26	Chloramphenicol (30)	29	0.73
14	*S. brunei*	13	15	17	19	23	17.4 ± 1.72	Chloramphenicol (30)	29	0.60
15	*P. aeruginosa*	13	17	19	20	22	18.2 ± 1.52	Amikacin (10)	32	0.56
16	*B. cepacia*	15	19	20	20	23	19.4 ± 1.28	Amikacin (10)	29	0.66
17	*V. parahaemolyticus*	11	12	12	13	14	12.4 ± 0.50	Ciprofloxacin (5)	29	0.42
18	*H. parahaemolyticus*	13	14	15	17	21	16.0 ± 1.41	Nalidixic acid (30)	26	0.61
19	*Y. enterocolitica*	25	25	25	26	28	25.8 ± 0.58	Ciprofloxacin (5)	29	0.88
20	*C. albicans*	12	14	15	18	21	16.0 ± 1.58	Nystatin (100)	24	0.66
21	*C. neoformans*	-	-	-	-	-	-	Ketoconazole (10)	35	0.00

SEM: Standard error of mean.

nolic extracts of *P. granatum* fruit rind to be active against all microorganisms tested in their study. These results are in accordance with the results obtained in the present study for bacteria wherein antibacterial activity was observed for all the bacterial cultures (19 bacteria) and *C. albicans* culture tested. Pomegranate fruit pericarp compound punicalagin is reported to have antimicrobial activity against *S. aureus* and *P. aeruginosa* (Burapadaja and Bunchoo, 1995). Jayaprakasha et al. (2006) extracted pomegranate pericarp with different polar solvents at room temperature, and water extracts were evaluated against both Gram-positive and Gram-negative bacteria. The acetone extract showed the highest antibacterial activity. Results obtained in our study, contrarily, ethanolic extract of *P. granatum* pericarp showed significant antibacterial activity against all bacteria.

In vitro studies have revealed that extract of pomegranate inhibited the growth of oral bacteria and *Candida* species (Pereira, 1998; Vasconcelos et al., 2003). Punicalagin isolated from the fruit peel of pomegranate was reported to have antimicrobial activity against *C. albicans* (Burapadaja and Buncho, 1995). Similar results also observed in this present study, the ethanolic extract of *P. granatum* pericarp inhibited *S. mutants* and *C. albicans* and these organisms are responsible for dental disorder and oral thrush. Brazilian researchers evaluated the synergistic effect of a *P. granatum* methanolic extract with five antibiotics on 30 clinical isolates of methicillin resistant *S. aureus* (MRSA) and methicillin sensitive *S. aureus* (MSSA) (Machado et al., 2002). Their results pointed out the evidence for the presence of antimicrobial compounds in the crude ethanolic extracts of these plants. These findings and the results obtained in our study clearly confirm the inhibitory activity of *P. granatum* pericarp ethanolic extract on *S. aureus.* In the overall assessment, the antimicrobial activity of ethanolic extract of *P. granatum* pericarp was associated with the presence of principal constituents (tannins, phenolic compounds, flavonoids, terpenoids, phytosterols and glycosides) in it. Current research seems to indicate that the most therapeutically beneficial pomegranate constituents are ellagic acid ellagitannins (including punicalagins), punicic acid, flavonoids, anthocyanins, anthocyanins and estrogenic flavonols and flavones. In other hand, pericarp of pomegranate consist of phenolic punicalagins, gallic acid and other fatty acids, catechin, quercetin, rutin and other flavonols, flavones, flavonones and anthocyanins (Amakura et al., 2000; De Pascual-Teresa et al., 2000; Artik, 1998; Nawwar et al., 1994;

Table 3. MIC of ethanolic pericarp extract of *P. granatum*.

S/N	Name of the organism	Concentration of extracts (µg/ml)											
		4096	2048	1024	512	256	128	64	32	16	8	Control	MIC (µg/ml)
	Ethanolic pericarp extract												
2	*S. epidermidis*	-	-	-	-	+	+	+	+	+	+	+	512
3	*S. mutans*	-	-	-	+	+	+	+	+	+	+	+	1024
4	*E. coli*	-	-	+	+	+	+	+	+	+	+	+	2048
5	*K. pneumoniae*	-	-	+	+	+	+	+	+	+	+	+	2048
6	*E. aerogenes*	-	-	+	+	+	+	+	+	+	+	+	2048
7	*P. mirabilis*	-	-	+	+	+	+	+	+	+	+	+	2048
8	*P. vulgaris*	-	-	+	+	+	+	+	+	+	+	+	2048
9	*S. typhi*	-	-	+	+	+	+	+	+	+	+	+	2048
10	*S. paratyphi A*	-	-	-	+	+	+	+	+	+	+	+	1024
11	*S. typhimurium*	-	-	-	+	+	+	+	+	+	+	+	1024
12	*S. infantis*	-	-	+	+	+	+	+	+	+	+	+	2048
13	*S. enterica*	-	-	-	+	+	+	+	+	+	+	+	1024
14	*S. brunei*	-	-	-	+	+	+	+	+	+	+	+	1024
15	*P. aeruginosa*	-	-	-	+	+	+	+	+	+	+	+	1024
16	*B. cepacia*	-	-	-	+	+	+	+	+	+	+	+	1024
17	*V. parahaemolyticus*	-	-	+	+	+	+	+	+	+	+	+	2048
18	*H. parahaemolyticus*	-	-	+	+	+	+	+	+	+	+	+	2048
19	*Y. enterocolitica*	-	-	-	-	+	+	+	+	+	+	+	512
20	*C. albicans*	-	-	+	+	+	+	+	+	+	+	+	2048
21	*C. neoformans*	+	+	+	+	+	+	+	+	+	+	+	-

Noda et al., 2002). An explosion of interest in the various therapeutic values of *P. granatum* over the last decade has led to numerous *in vitro*, animal and clinical trials. The results obtained in the study clearly stated broad-spectrum antibacterial activity of *P. granatum* against Gram-positive, Gram-negative and fungal (*C. albicans*) strains. The antimicrobial activity of *P. granatum* pericarp extract might be associated with its antibiotic compounds or to the presence of metabolic products. Among the various microorganism tested, *Salmonellae* have been inhibited significantly by the ethanolic extract of *P. granatum* pericarp. On the other hand, the unknown minor components present have not been elucidated in terms of their broad-spectrum activity. The ethanolic extract of *P. granatum* pericarp effect on 19 bacterial and two fungal pathogens only have been tested *in vitro*; however, further studies are needed to ascertain the antimicrobial activity based on clinical trials.

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REFERENCES

Ahmad I, Beg AZ (2001). Antimicrobial and photochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. Ethnopharmacol. 74:113-123.

Ahmad I, Mehmood Z, Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 62(2):183-193.

Amakura Y, Okada M, Tsuji S, Tonogai Y (2000). Determination of phenolic acids in fruit juices by isocratic column liquid chromatography. J. Chromatogr. A. 891:183-188.

Artik N (1998). Determination of phenolic compounds in pomegranate juice by using HPLC. Fruit Process. 8:492-499.

Aviram A, Rosenblat M, Gaitini et al., (2004). Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. Clin. Nutr. 23:423-433.

Basset J, Denny J, Jeffery JH,Mendham J (1985). Volgel's Textbook of Practical Pharmacognosy, Baillere, London.

Bauer AW, Kirby MDK, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by standardized single disc diffusion method. Am. J. Clin. Pathol. 45:493-496.

Berahou A, Auhmani A, Fdil N, Benharref A, Jana M, and Gadhi CA (2007). Antibacterial activity of *Quercus ilex* bark's extracts. J. Ethnopharmacol. 112(3):426-429.

Braga LC, Shupp JW, Cummings C et al., (2005). Pomegranate extract inhibits *Staphylococcus aureus* growth and subsequent enterotoxin production. J. Ethnopharmacol. 96(1-2):335-339.

Burapadaja S, Bunchoo A (1995). Antimicrobial activity of tannins from *Terminalia citrine*. Planta Med. 61:365.

Cragg GM, Newman DJ (2005). Biodiversity: A continuing source of novel drug leads. Pure Appl. Chem. 77(1):7-24.

Davies J (1994). Inactivation of antibiotics and the dissemination of resistance genes. Science 264(51-57):375-382.

De Pascual-Teresa S, Santos-Buelga C, Rivas-Gonzalo JC (2000). Quantitative analysis of flavan-3-ols in Spanish foodstuffs and beverages. J. Agric. Food Chem. 48:5331-5337.

Deepa T, Elamathi R, Kavitha R, Kamalakannan, Sridhar S, Suresh Kumar J (2012). Screening for physical, phytochemical and antimicrobial activities of leaf extracts of *Sapindus emarginatus* Vahl. Int. J. Pharma. Tech. Res. 4(1):392-397.

Egharevba HO, Kunle OF (2010). Preliminary phytochemical and proximate analysis of the leaves of *Piliostigma thionningii* (Schumach.) Milne-Redhead. Ethnobot. Leaflets 14:570-77.

Harbourne JB (1984). Phytochemical methods- Guide to modern techniques of plant analysis, 2nd Edition, Chapman and Hall, London pp.4-120.

Heber D, Seeram NP, Wyatt H, Henning SM, Zhang Y, Ogden LG, Dreher M, Hill JO (2007). Safety and antioxidant activity of a pomegranate ellagitannin-enriched polyphenol dietary supplement in overweight individuals with increased waist size. J. Agric. Food Chem. 55:10050-10054.

Hebert E, Brain, Ellergy W, Kenneth (1984). Textbook of Practical Pharmacognosy, Baillere, London p.363.

Jayaprakasha GK, Negi PS, Jena BS (2006). Antimicrobial activities of Pomegranates. In pomegranates: Ancient roots to modern medicines, Eds., CRC press. Boca Raton, FL, USA, pp 167-168.

Kokate CK, Purohit AD, Gokhale (1990). Pharmacognosy, 1st Edition, Nirali Prakasan, Pune-123.

Kumaraswamy MV, Kavitha HU and Sathish S (2008). Antibacterial evaluation and phytochemical analysis of *Betula utilis* against some human pathogenic bacteria. Word J. Agri Sci. 4(5):661-664.

Lansky EP, Newman RA (2007). *Punica granatum* (Pomegranate) and its potential for prevention of treatment of inflammation and cancer. J. Ethnopharmacol. 109:177-206.

Machado TB, Leal CR, Amerl ACF, Santos KRN, Silva MG, Kuster RM (2002). Anti *Punica granatum* fruits. J. Brazil. Chem. Soc.13:606-610.

Mathabe MC, Nikolova RV, Lall N, Nyazema NZ (2005). Antibacterial activities of medicinal plants used for the treatment of diarrhea in Limpopo Province. South Afr. J. Ethnopharmacol. 105:286-293.

Nawwar MA, Hussein SA, Merfort I (1994). NMR spectral analysis of polyphenols from *Punica granatum*. Phytochemi. 36:793-798.

Naz S, Siddiqi R, Ahmad S, Rasool SA, Sayeed SA (2007). Antibacterial activity directed isolation of compounds from *Punica granatum*. J. Food Sci. 72(9):M341-M345.

NCCLS (National Committee for Clinical Laboratory Standard), (1999). Performance standards for antimicrobial susceptibility testing, 9th Int. Suppl. M100-S9, Wayne Pa.

Noda Y, Kaneyuki T, Mori A, Packer L (2002). Antioxidant activities of pomegranate fruit extract and its anthocyanidins: dephinidin, cyanidin, and pelargonidin. J. Agric. Food Chem. 50:166-171.

Parmar HS, Kar A (2007). Protective role of *Citrus sinensis, Musa paradisiaca and Punica granatum* pericarp against diet-induced atherosclerosis and thyroid dysfunctions in rats. Nutr. Res. 27(11):710-718.

Parmar HS, Kar A (2008). Medicinal values of fruit pericarp from *Citrus sinensis, Punica granatum and Musa paradisiaca* with respect to alterations in tissue lipid peroxidation and serum concentration of glucose, insulin and thyroid hormones. J. Med. Food 11(2):376-381.

Pereira JV (1998). Atividade antimicrobianodoestrato hidroalcoolico da P. granatum Linn, sobre microorganisms formadores da placa bacterium, Joao Pessoa, Post-graduation in Dentistry, Thesis, Federal University of Paraiba p.91.

Prashanth DJ, Asha MK, Amit A (2001). Antibacterial activity of *Punica granatum*. Fitoterapia 72:171-173.

Renu S (2010). Some medicinal plants with antibacterial activity. Pharmacie. Globale (IJCP). 4(10).

Ricci D, Giamperi L, Bucchini A, Fraternale D (2006). Antioxidant activity of *Punica granatum* fruits. Fitoterapia. 77(4):310–312.

Salomao K, Pereira PRS, Campos LC et al., (2008). Brazilian propolis: correlation between chemical composition and antimicrobial activity. Evidence-Based Complemen. Alter. Med. 5(3):317–324.

Sánchez-Lamar A, Fonseca G, Fuentes JL, Cozzi R, Cundari E, Fiore M, Ricordy R, Perticone P, Degrassi F, De Salvia R (2007). Assessment of the genotoxic risk of *Punica granatum* L. (Punicaceae) whole fruit extracts. J. Ethnopharmacol. 115(3):416-422.

Sandhya B, Thomas S, Isabel W, Shenbagarathai R (2006). Ethnomeicinal plants used by the Valiyan community of Piranmallai Hills (Reserved forest), Tamil Nadu, India. A pilot study. Afri. J. Tradi. Complemen. Alter. Med. 3:101-114.

Service RF (1995). Antibiotics that resist resistance. Science 270(5237):724-727.

Shibumon G, Benny PJ (2010). A review on the medicinal significance of common fruits. Int. J. Biomed. Res. Anal. 1(2):60-64.

Singh RP, Chidambara MKN, Jayaprakasha GK (2002). Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. J. Agric. Food Chem. 50(1):81-86.

Vasconcelos LC, Sampaio MC, Sampaio FC, Higino JS (2003). Use of *Punica granatum* as an antifungal agent against candidosis associated with denure stomatits. Mycos. 46:192.

Vijayanand S, Hemapriya J (2011). In vitro antibacterial efficacy of peel and seed extracts of *Punica granatum* L. against selected bacterial strains. Int. J. Microbiolog. Res. 1(4):231-234.

Zhang J, Zhan B, Yao X, Gao Y, Shong J (1995). Antiviral activity of tannin from the pericarp of *Punica granatum* L. against genital Herpes virus *in vitro*. Zhongguo Zhong yao za zhi 20(9):556-576.