

**Research Article****EXTRACTION OF BIOACTIVE CHEMICAL COMPOUNDS AND ANTIBACTERIAL ACTIVITY OF BIOWASTE FRUIT PEELS EXTRACT OF *PUNICA GRANATUM***Richa Sharma¹, Richa Saxena¹ and Bankim Chandra Nandy*²¹Department of food and Biotechnology, Jayoti Vidyapeeth Women's University, Jaipur- 303122, Rajasthan, India²Department of Pharmaceutical Sciences, Jayoti Vidyapeeth Women's University, Jaipur- 303122, Rajasthan, India

Received 17 August 2015; Accepted 14 October 2015

ABSTRACT

Recently, research has gained a renewed focus on herbal products and their biowaste that play an important role in the developing world due to limited modern health service. Various parts of *Punica granatum* was shown to exhibit antibacterial effects against a wide variety of bacteria. This study conferred the screening of qualitative and quantitative analysis of the major bioactive constituents of biowaste peel extract of *Punica granatum* with their comparative antibacterial efficacy of ethanolic and methanolic extracts against pathogenic bacteria (*Staphylococcus aureus* and *Proteus vulgaris*). The percentage value of yield extraction in ethanolic extract was 55.82 % and methanolic extract was 52.48 %. The total phenolic content was 337 ± 0.012 and 329 ± 0.02 mg/g of dry weight of ethanolic and methanolic extracts, respectively, expressed as gallic acid equivalents. The total flavonoid content was 197 ± 0.032 and 193 ± 0.061 mg/g for ethanolic and methanolic extracts, respectively, expressed as quercetin equivalents. Hence, it could be pursued further for obtaining phytomedicine. The results showed that the crude ethanolic extract has highest activity then methanolic extract. These observations could be the basis for the usefulness of ethanolic extract of the biowaste fruit peels of *Punica granatum* in the treatment remedies for microbial infections.

Keywords: Herbal medicines, bioactive compounds, extract *Punica granatum***INTRODUCTION**

Medicinal plants are the most productive source of natural bioactive chemical compounds and drugs of natural origin, for thousands of years. The World Health Organization predicts that up to 80 percent of world population still relies mostly on traditional remedies for their preliminary health care (Shanley and Luz, 2003; Jasuja et al., 2014). The potential of medicinal plants as a source for treatment of diseases are still largely unexplored. Eventually, 250'000- 500,000 plant species, only very little percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening. Most of the bioactive compounds isolated from plants are the secondary metabolites, which include alkaloids (Navarro and Delgado, 1999), tannins, flavonoids (Mandalari et al., 2007), steroids, terpenoids (Funatogawa et al., 2004; Harvey, 2008), saponins (Avato et al., 2006) and anthroquinone (Ayo, 2010; Prasad and Bisht, 2011; Wang et al., 2009; Mandalari et al., 2007) have great antimicrobial

activity (Ateş et al., 2003; Şengül et al., 2005; Nair, 2005; Dülger et al., 2005; Kumar et al., 2006; Mathabe et al., 2006). Screening techniques of pharmacologically active beneficial compounds have been conducted on well-known species of plants used in traditional medicines. *Punica granatum* commonly known as pomegranate belongs to family Punicaceae. Pomegranate has been known to be a reservoir of secondary metabolites (Li et al., 2006). This is a resourceful area of research as many parts of *Punica granatum* are used in traditional medicine as well as exhibits various pharmacological properties while their chemistry indicates varied chemical structures (Jasuja et al., 2012). Therefore it is necessary that a systematic and significant assessment of the future directions of research in this field and its application be undertaken. The presence of antibacterial substances in the medicinal plants are well established as they have provided a source of inspiration for novel drug compounds as herbal medicine have made significant contribution towards human health (Srivastava et

al., 1996; Farombi, 2003). This study has been focused on utilization of biowaste fruit peel of *P. granatum* as compare to other parts of plant. However, up to now, very few studies have been conducted on the antimicrobial activity of *P. granatum* peels. Therefore, the basic aim of this research was to determine the total phenolic and flavonoids content with their concentrations in ethanolic and methanolic extracts of biowaste peel of *Punica granatum* using spectrophotometric methods, as well as to examine qualitative phytochemical analysis of plant extracts and also determined the comparative antibacterial activity of ethanolic and methanolic extracts. It also highlights the scientific basis for future research on plants in this genus, including their potential for development as herbal drugs.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used in this study were of analytical grade and obtained from Merck Company, Germany.

Collection and identification of plant

Biowaste fresh fruit peel of *Punica granatum* were collected from juice shop (National Handloom Juice centre, Vaishali nagar), Jaipur during the month of May 2012. Further plant material was identified and registered (Rrg. No. RUBL – 21111) by 'Herbarium' Department of Botany, University of Rajasthan, Jaipur. Fruit peel were then cut into smaller pieces and then first washed with tap water followed by washing with distilled water. It was than dried under hot air oven at 40 °C temperature for 4 to 5 days. The dried plant samples were powdered by mechanical grinder and sieved to give particle size 50- 150 mm. The powder was stored in polythene bags at room temperature before extraction.

Preparation of Extracts

P. granatum fruit peel powdered (35 g) was also filled in the thimble and extracted successively with 95 % ethanol (ethanol: distilled water; 9.5: 0.5) and 95 % methanol (methanol: distilled water; 9.5: 0.5) solvents in soxhlet extraction unit for 48 hours (Lin et al., 1999). Ethanolic and methanolic extracts were filtered and then concentrated using rotary evaporator at 40 °C and each extract were transferred to glass vials and kept at 4° C before

use. Yield of the extract obtained was calculated by formula as mentioned below:

$$\text{Extractive yield value} = \frac{\text{Weight of concentrated extract}}{\text{Weight of plant dried powder}} \times 100$$

Qualitative Analysis

Different qualitative phytochemical tests were performed for determination of presence or absence of bioactive constituents. The methanolic and ethanolic extracts were reported the many bioactive chemical constituents such as carbohydrate, alkaloids, glycosides, saponins, phenolic compound, tannins and flavonoids (Practical Pharmacognosy by C. K. Kokate, 2004).

Quantitative phytochemical Analysis

Total Phenols Determination

The total phenolic contents in the peel extracts of *P. granatum* were determined by the Folin-Ciocalteu assay with slight modification (Kim et al., 2003; Katasani, 2011). An aliquot (1 ml) of extracts or standard solution of gallic acid was added to a volumetric flask, containing deionised distilled water (9 ml). A reagent blank using deionised distilled water was also prepared. Folin-Ciocalteu's phenol reagent (1 ml) was added to the mixture and shaken. After 5 min 10 ml of 7 % sodium carbonate solution was added. The final volume of test sample were made upto 25 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a UV spectrophotometer. A calibration curve was constructed using gallic acid solution as standard (Figure 1) and total phenolic content of the extract was expressed in terms of milligrams of gallic acid equivalents (GAE) per gram of dry weight (Table 2). The estimation of the phenolic compounds was carried out in triplicate.

Total flavonoids determination

Aluminum chloride colorimetric method was used for flavonoids determination according to Katasani (2011) with slight modification. Each plant extracts (0.5 ml) in methanol were separately mixed and added into a volumetric flask, containing 9 ml of distilled water with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1ml of 1 M potassium acetate and diluted with 2.8 ml of distilled water. The solution was mixed well and after incubation

of 30 min at room temperature; the absorbance of the reaction mixture was measured at 510 nm with an UV/Visible spectrophotometer. The calibration curve was prepared with quercetin as standard (Figure 2). The total flavonoids content was expressed as milligrams of quercetin equivalents (QE) per gram of dried sample (Table 3).

Micro-organisms

The pathogenic bacterial strains were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. Bacterial strains used were *Staphylococcus aureus* (MTCC – 7443) and *Proteus vulgaris* (MTCC – 1771) for antibacterial studies.

Preparation of Inoculum

A loopful of each selected bacteria was taken and sub-cultured in test tube containing 10 ml of nutrient broth. The test-tubes were incubated at 37°C for 24 hours. The broth was standardized using sterile normal saline (0.85% NaCl) to obtain a population of 1.5×10^8 cfu/ml by 0.5 McFarland standard methods.

Determination of Antibacterial activity

The Disc diffusion method was used for evaluation of antibacterial activity (Baur et al., 1966; Rios et al., 1988; Kim et al., 2012). Standard size Whatman No. 1 filter paper discs, 5.0 mm in diameter, sterilized by moist heat at 121 lb in an autoclave for 15 min were used to determine antibacterial activity. Muller hinton agar (MHA) medium were prepared for disc diffusion test. After sterilization, it was poured into sterilized petriplates and allowed to solidify. Then, one day old culture of bacteria will be used for inoculums preparation. A suspension that was just turbid (~0.5 McFarland standard) by visual inspection was prepared by suspending bacteria in 0.85% NaCl solution and the homogeneous suspension was used for inoculation. Using a sterile cotton swab, bacterial cultures were swabbed on the surface of sterile agar plates. Sterile 5 mm discs were impregnated with extract (10 mg/ disc) and placed on the surface of agar plates inoculated with a microbial culture. Each extract was tested in triplicate. Gentamycin sulphate and streptomycin sulphate (0.1mg/disc) served as a control. The plates were incubated at 37°C for 24 h. The diameter of the

inhibition zones was measured in millimeter. Three replicates were kept in each case and average values were calculated.

RESULTS

In the present study, ethanolic and methanolic extracts of *P. granatum* biowaste fruit peels were subjected to usual qualitative and quantitative chemical investigation to identify the nature of phytochemical constituents present in them. Biowaste fruit peels of *P. granatum* were extracted using solvent 80% ethanol and 80% methanol by the earlier mentioned method. In the extractive values were found 55.82 % of ethanolic extract and 52.48 % of methanolic extract (Table 1). In this study, qualitative analysis of both extracts of *P. granatum* revealed the presence of phytochemical constituents such as alkaloids, phytosterols, glycosides, tannins and phenolic compounds, flavonoids, carbohydrates, saponins. Protein, amino acids, fixed oils and fat were absent in the ethanolic and methanolic extracts of *P. granatum* (Table 2). The amount of total phenol was determined with Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: $y = 0.098x + 0.021$, $R^2 = 0.995$ (Figure 1); where y is absorbance at 750 nm and x is total phenolic content in the extracts of *P. granatum* expressed in mg/gm. The total phenolic content was found in the ethanolic and methanolic extract of *P. granatum* (337 ± 0.012 and 329 ± 0.02 mg/gm) (Table 3). The amount of total flavonoids was determined with aluminum chloride reagent. Quercetin was used as a standard compound and the total flavonoid was expressed as mg/g quercetin equivalent using the standard curve equation: $y = 0.027x + 0.024$, $R^2 = 0.993$ (Figure 2); where y is absorbance at 510 nm and x is total flavonoid content in the extracts of *P. granatum* expressed in mg/gm. The total flavonoid was 197 ± 0.032 and 193 ± 0.061 mg/gm in the ethanolic and methanolic extracts, respectively (Table 3). The results obtained from the present study showed that the ethanolic extract of *P. granatum* contain highest amount of phenolic and flavonoid compounds. Further work is going on this plant in order to isolate, identify, characterize and elucidate the structure of the bioactive principles with the help of advanced technologies to develop

plant derived drugs. This study also revealed that the *P. granatum* biowaste peel extract may be useful as a broad spectrum antibacterial agent following extensive investigation. The results of the disk diffusion test for antibacterial efficacy of extracts and standard antibacterial drugs against *Staphylococcus aureus* and *Proteus vulgaris* at same concentrations (10 µg/disk) were depicted Table 4. The DMSO used for dissolution of the extract did not show any activity. It is used as a negative control. The highest zone inhibition activity of ethanolic extract was 18 ± 0.57 mm and 19 ± 0.57 mm against *S. aureus* and *P. vulgaris* respectively. So, ethanolic extract was most active against *S. aureus* and *P. vulgaris* at low concentration (10 µg/ disc) than methanolic extract.

DISCUSSION

Researchers have comprehensively studied the medicinal properties of *Punica granatum* and their results showed that this plant is ethno medically valuable (Shibumon and Benny, 2010). Nowadays, antibacterial resistance is a burning issue. Synthetic drugs are effective against various pathogenic microorganism but these are causing number of clinical infections in different areas (Mycek et al., 2000). Most important reasons of this issue are frequently use of antibiotics against a minor problem of healthcare, due to which microbes became resistance to the antibiotics. So, it is necessary to find out the understanding of this emerging issue to minimize this problem in healthcare (Anderson and Keye, 2009). This research work provides a scientific validation for utilization of ecofriendly biowaste fruit peel of medicinal plant *P. granatum*, in having potential to be a good drug. It is a base for the further investigation of qualitative and quantitative bioactive chemical compounds with their comparative antibacterial activity of ethanolic and methanolic extract against *S. aureus* and *P. vulgaris*. In the qualitative estimation of phytoconstituents of ethanolic and methanolic extracts of *P. granatum* peels were showing encouraging results for the presence of various bioactive compounds like alkaloids, phytosterols, glycosides, tannins and phenolic compounds, flavonoids, carbohydrates, saponins but protein, amino acids, fixed oils and fat were absent in the ethanolic and methanolic extracts. Ethanolic

extracts were contains highest amount of phenolic (337 ± 0.012 mg/gm) and flavonoids (197 ± 0.032 mg/gm) content then methanolic extract. Chandrasekharnath et al., (2013) were also studies on phytochemical analysis of bioactive compounds of *P. granatum* peels extract and antimicrobial activity of selected microorganism (*Klebsiella*, *Enterococci*, *Pseudomonas*, *E.coli*, *S.aureus*). *Punica granatum* fruit peel extracts are currently used for treatment of respiratory diseases and in the preparation of therapeutic formulae. The tannin rich ellagitannins and phenolic acids of *Punica granatum* have antibacterial, antifungal and antiprotozoal activity (Prashanth et al., 2001; Vasconcelos et al., 2003; Voravuthikunchai et al., 2004; Supayang et al., 2005; Voravuthikunchai et al., 2005). In the present results ethanolic and methanolic extract of *P. granatum* peels was found inhibitory against *S. aureus* and *P. vulgaris* and diameter of zone of inhibition was to be 18 ± 0.57 and 15 ± 0.66 mm against *S. aureus* and was to be 19 ± 0.57 and 17 ± 0.33 mm against *P. vulgaris* respectively. The current finding coincided with Chandrasekharnath et al., (2013) who reported that aqueous, methanolic and ethanolic extract show an inhibitory zone diameter of 12, 14, 16 mm respectively and ethanolic extract shows best result as compare to standard antibiotic ampicillin (14 mm). Our results also coincide with Khan and Hane, (2011) who revealed that antibacterial activity of *P. granatum* extracts (hot aqueous, methanolic and ethanolic) were evaluated against *E. coli*, *P. aeruginosa* and *S. aureus*. Hot aqueous, methanolic and ethanolic extracts of *Punica granatum* pericarp show an average zone of inhibition diameter was 23.3, 22.3 and 24.5 mm respectively which indicates that ethanolic extract shows best result than that of the standard antibiotic Tetracycline (20.1mm). Similarly, Altuner, (2011) reported that ethanol and a solvent cocktail (dH₂O: ethanol: methanol: acetone: CH₂Cl₂) extracts of *P. granatum* fruit peel and peel ashes were tested against *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* Serotype Typhimurium SL 1344 and *Shigella flexneri*. The results indicated that *P. granatum* fruit peel ashes showed promising degrees of antimicrobial activity. Egharevba, (2010) reported that *P. granatum* fruit bark and leaves extract exhibited varying activity against different microbes with

zones of inhibition ranging from 14 to 34mm. Further, research work is going on this plant in order to isolate, identify, characterize and

elucidate the structure of the bioactive principles with the help of advanced technologies to develop plant derived drugs.

Table 1: The percentage yield by soxhlet extraction method of crude extract of *Punica granatum*

Solvent	Weight of Powdered Plant Material	Volume of Solvent	Weight of Extract	% of yield of Extraction
95 % Ethanol	35 gm	200 ml	19.54 gm	55.82 %
95 % Methanol	35 gm	200 ml	18.37 gm	52.48 %

Table 2: Preliminary phytochemical test of extract of *P. granatum*

S. No.	Phytochemical tests		Observation of plant extract	
			Methanol extract	Ethanol extract
	Chemical Constituents	Test		
1.	Alkaloids	Mayer's Test	Positive	Positive
		Wagner's test		
		Dragendroff's reagent		
		Hager's test		
2.	Phytosterols	Salkowski's test	Positive	Positive
		Liebermann Burchard's test		
3.	Glycoside	Borntrager's test	Positive	Positive
		Legal's test		
4.	Tannins & Phenolic compounds	Ferric Chloride test	Positive	Positive
		Gelatin – salt Test		
		Iodine Test		
		Nitric acid Test		
5.	Flavonoids	Alkaline reagent test	Positive	Positive
		Lead acetate test		
		With H ₂ SO ₄ ,		
		Zinc test		
6.	Saponins	Froth Test	Positive	Positive
		Foam Test		
7.	Carbohydrate test	Fehling's test	Positive	Positive
		Benedict's test		
		Molisch's test		
8.	Protein and amino acids	Biuret test	Negative	Negative
		Ninhydrin test		
		Xanthoproteic test		
9.	fixed oils and fat test	Spot test	Negative	Negative

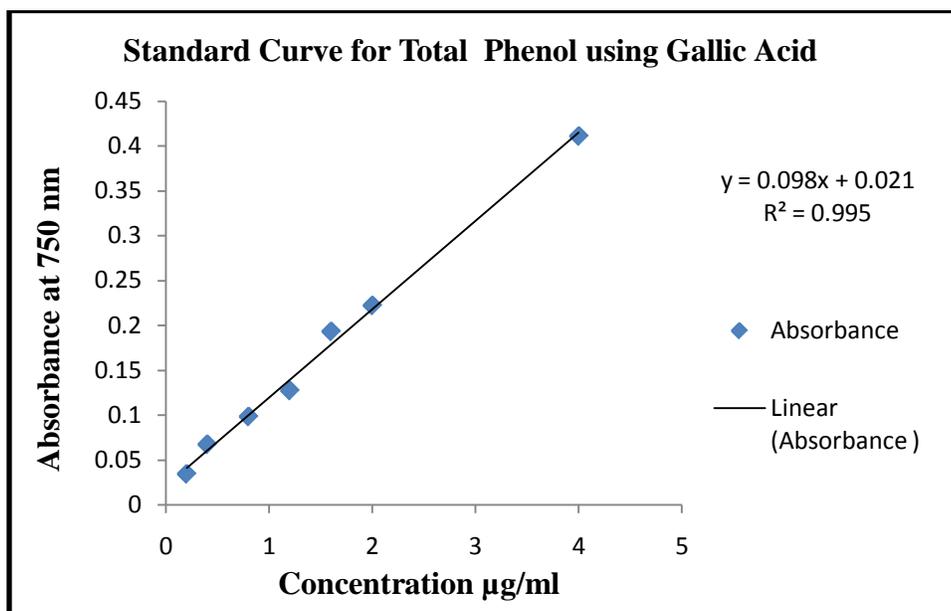


Figure 1: Standard Curve for total phenol using gallic acid

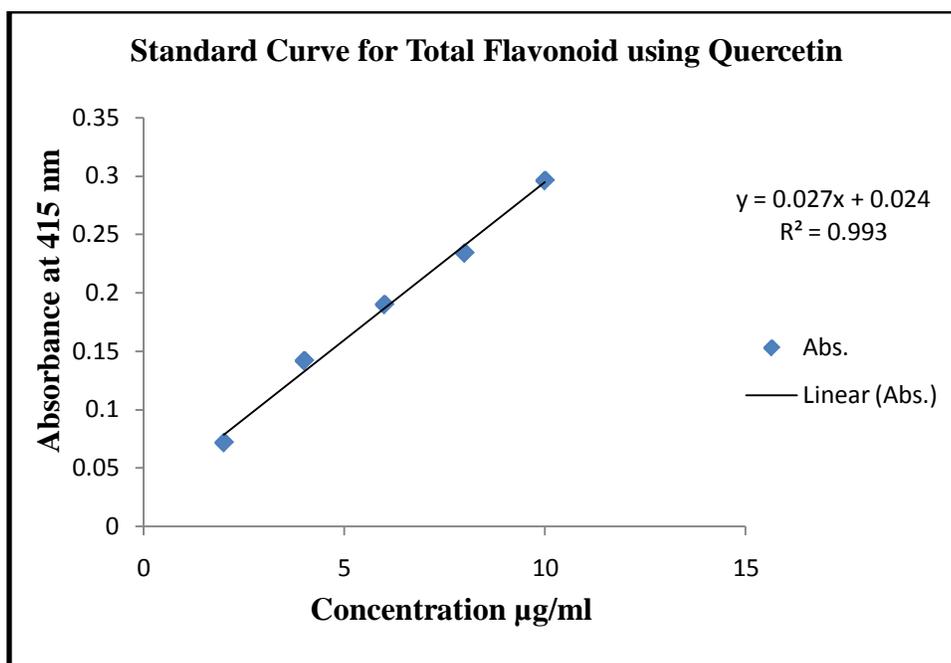


Figure 2: Standard Curve for total flavonoids using Quercetin

Table 3: Quantitative Estimation of Total phenolic and flavonoids content of different extracts of *Punica granatum*

<i>Punica granatum</i>	Total phenolic (mg /gm)*	Total flavonoids (mg /gm)*
Ethanollic extract	337 ± 0.012	197 ± 0.032
Methanollic extract	329 ± 0.02	193 ± 0.061

*Results expressed as Mean ± SEM from three observations.

Table 4: *In vitro* antibacterial activity of ethanolic and methanolic extract of biowaste peel of *Punica granatum*

Microorganism	Zone of Inhibition (mm)			
	Ethanolic Extract (10 mg/ disc)	Methanolic Extract (10 mg/ disc)	Streptomycin Sulphate (0.1 mg/ disc)	Gentamycin Sulphate (0.1 mg/ disc)
<i>Staphylococcus aureus</i>	18 ± 0.57	15 ± 0.66	24 ± 0.28	24 ± 0.16
<i>Proteus vulgaris</i>	19 ± 0.57	17 ± 0.33	26 ± 0.33	27 ± 0.33

*Results expressed as Mean ± SEM from three observations

CONCLUSION

Convincingly, the results show that methanolic extracts of waste pomegranate peels have great potential as antibacterial compounds against *Staphylococcus aureus* and *Proteus vulgaris*. Ethanolic extracts were also having a vast range for total phenolic content and total flavonoids content than methanolic extract. Thus, they can be remarkably effective in the treatment of infectious diseases caused by the *S. aureus* and *P. vulgaris*. Further isolation, purification and characterization of bioactive compounds are in progress to determine the active components responsible for the inhibitory effect of *S. aureus* and *P. vulgaris*; also, clinical trials will be required to confirm its antibacterial action *in vivo*. In order to study the structure of bioactive compounds of this plant will be done by various techniques such as high performance liquid chromatography (HPLC), fourier transform infrared (FTIR) spectroscopy and Nuclear magnetic resonance (NMR) etc.

ACKNOWLEDGEMENT

The authors are grateful to Department of Science & Technology, Government of Rajasthan, Jaipur for financial support and Department of Biotechnology and Allied Sciences, Jayoti Vidyapeeth Women's University, Jaipur (Rajasthan), for providing the laboratory facilities used in this study.

REFERENCES

- Altuner EM: Investigation of antimicrobial activity of *Punica granatum* L. fruit peel ash used for protection against skin infections as folk remedies especially after male circumcision. African Journal of Microbiology and Research 2011; 5: 3339- 3342.
- Anderson DJ and Kaye KS: Controlling antimicrobial resistance in the hospital. Infect Dis Clin North Am 2009; 23: 847-864.
- Ateş A and Erdoğan ÖT: Antimicrobial activities of various medicinal and commercial plant extracts. Türk J Biol 2003; 27: 157-162.
- Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z and Jurzysta M: Antimicrobial activity of saponins from *Medicago* sp. structure-activity relationship. Phytother Res 2006; 20: 454-457.
- Ayo RG: Phytochemical constituents and bioactivities of the extracts of *Cassia nigricans* Vahl: A review. Journal of Medicinal Plants Research 2006; 4: 1339-1348.
- Baur AW, Kirby WM, Sherris JC, Turck M: Antibiotic susceptibility testing by a standard single disk method. Am J Clin Path 2006; 45: 493-496.
- Chandrasekharnath N, Venkanna B, Mahlakshmi YV and Uma A: Phytochemical analysis and Antimicrobial activity of various solvent extracts of *Punica granatum* and *Phyllanthus niruri*. Pharnanest: An Int J Advances Pharm Sci 2013; 4: 191- 196.
- Dülger B, Gönüz A, Bican T: Antimicrobial studies on three endemic species of *Sideritis* from Turkey. Acta Biologica Cracoviensia Series Botanica 2005; 47:153-156.
- Egharevba, Henry Omoregie; Kunle, Oluyemisi, Folashade; Iliya, Ibrahim; Orji Peace Nkiruka; Abdullahi, Makailu Sabo; Okwute, Simon Koma; 1Okogun, Joseph Ibumeh: Phytochemical Analysis and Antimicrobial Activity of *Punica granatum* L. (fruit bark and leaves). New York Sci J 2010; 3: 91-98.
- Farombi EO: African indigenous plants with chemotherapeutic properties and biotechnological approach to the production of

- bioactive prophylactic agents. African Journal Biotechnology 2003; 2: 662-671.
11. Funatogawa K, Hayashi S, Shimomura H, Yoshida T, Hatano T, Ito H, Hirai Y: Antibacterial activity of hydrolyzable tannins derived from medicinal plants against *Helicobacter pylori*. Microb Immunol 2004; 48: 251-261.
 12. Harvey AL: Natural products in drug discovery. Drug Discovery Today 2008; 13: 894 -901.
 13. Jasuja ND, Saxena R, Chandra S and Sharma R: Pharmacological Characterization and Beneficial uses of *Punica granatum*. Asian J Plant Sci 2012; 11: 251-267.
 14. Jasuja ND, Saxena R, Chandra S, Bhargava S and Joshi SC Pharmacological evaluation of an ethnomedicinal and endangered desert plant: *Mimosa hamata*. J Biol Sci 2014; 14: 52-59
 15. Khan JA and Hanee S: Antibacterial Properties of *Punica granatum* Peels. Int J Applied Biol Pharm Technol 2011; 2: 23- 27.
 16. Katasani D: Phytochemical screening, quantitative estimation of total phenolic, flavanoids and antimicrobial evaluation of *Trachyspermum ammi*. J Atoms Mole 2011; 1: 1-8.
 17. Kim DJ, Chung SG, Lee SH, Choi JW: Relation of microbial biomass to counting units for *Pseudomonas aeruginosa*. Afr J Microbiol Res 2012; 21: 4620-4622.
 18. Kim DO, Jeong SW, Lee CY: Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem 2003; 81:321- 326.
 19. Kokate CK Practical Pharmacognosy. Published by Nirali Prakashan New Delhi, 2004; pp. 29-107.
 20. Kumar PV, Chauhan SN, Padh H, Rajani M: Search for antibacterial and antifungal agents from selected Indian medicinal plants. J Ethnopharmacol 2006; 107: 182-188.
 21. Li Y, Guo C, Yang J, Wei J, Xu J, Cheng S : Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem 2006; 96: 254-260.
 22. Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK and Staden JV : Preliminary screening of some traditional zulu medicinal plants for anti- inflammatory and anti-microbial activities. J Ethnopharmacol 1999; 68: 267-274.
 23. Mandalari G, Bennett RN, Bisignano G, Trombetta D, Saija A, Faulds CB, Gasson MJ, Narbad A: Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry. J Appl Microb 2007; 103: 2056-2064.
 24. Mathabe MC, Nikolova RV, Laly N, Nyazema NZ : Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa J Ethnopharmacol 2006; 107: 286-293.
 25. Mycek MJ, Harvey RA and Champe PC: Lippincotts Illustrated Reviews; Pharmacology, 2nd Edn., Lippincotts Williams and Wilkins, 2000; pp.311-320.
 26. Nair R, Kalariye T, Chanda S: Antimicrobial Activity of some selected Indian medicinal Flora. Turk. J Biol 2005; 29: 41-47.
 27. Navarro V, Delgado G: Two antimicrobial alkaloids from *Bocconia arborea*. J Ethnopharmacol 1999; 66: 223-226.
 28. Prasad K, Bisht G : Evaluation of nutritive, antioxidant and mineral composition of *Pavetta indica* Linn. leaves. Res J Phytochem 2011; 5: 54-59.
 29. Prashanth D, Asha M, Amit A: Antibacterial activity of *Punica granatum*. Fitoterapia. 2001; 72: 171-173.
 30. Rios JL, Recio MC, Vilar A: Screening methods for natural products with antimicrobial activity: A review of the literature. J Ethnopharmacol 1988; 23: 127-149.
 31. Russel AD, Fur JR: Antibacteria activity of a new chloroxylenol prepration containing ethylenediamine tetra acetic acid. J Appl Bacteriol 1977; 43: 253-260.
 32. Şengül M, Öğütçü H, Adıgüzel A, Şahin F, Kara AA, Karaman I and Güllüce M Antimicrobial effects of *Verbascum georgicum* Bentham extract. Turk J Biol 2005; 29: 105-110.
 33. Shanley P and Luz L: The impacts of forest degradation on medicinal plant use and implication for health care in Eastern Amazonia. BioSci 2003; 53: 573 – 584.
 34. Shibumon G and Benny P J: A review on the medicinal significance of common fruits. Int J Biomed Res Analysis 2010; 1: 60-64.
 35. Srivastava J, Lambert J, and Vietmeyer N: Medicinal plants: an expanding role in

- development. World Bank technical, 1996; pp. 320.
36. Supayang PV, Treechada S, Surasak L, Thanomjit S, Tetsuya I, Takeshi H: Inhibitory effect of active compounds from *Punica granatum* pericarp on verocytotoxin production by enterohaemorrhagic *Escherichia coli* O 157: H 7. J Health Sci 2005; 51: 590-596.
 37. Vasconcelos LCD, Sampaio MCC, Sampaio FC, Higino JS: Use of *Punica granatum* as an antifungal agent against candidosis associated with denture stomatitis. Mycoses. 2003; 46: 192-196.
 38. Voravuthikunchai S, Lortheeranuwat A, Jeeju W, Sririrak T, Phongpaichit S, Supawita T: Effective medicinal plants against enterohaemorrhagic *Escherichia coli* O157:H7. J Ethnopharmacol 2004; 94: 49–54.
 39. Voravuthikunchai S, Sririrak T, Limsuwan S, Supawita T, Iida T, Honda T: Inhibitory effects of active compounds from *Punica granatum* pericarp on verocytotoxin production by enterohaemorrhagic *Escherichia coli* O157:H7. J Health Sci 2005; 51: 590–596.
 40. Wang J, Peng Q, Li G: New compounds of natural resources in 2008. Afr J Biotechnol 2009; 8: 4299-4307.