



Available online through

www.jbsoweb.com



Research Article

ANTIMICROBIAL STUDY OF METHANOLIC EXTRACT OF *PUNICA GRANATUM* LINN AND *CYMBOPOGON*

R.Y.Patil¹, P. N. Jagtap¹, Lad Meenal², A.C. Sankh¹, A.V.Patil^{1*}, O. R. Walwadkar¹¹Shankarrao Ursal College of Pharmaceutical sciences and Research center, Kharadi, Pune, Maharashtra, India²Department of Dravyaguna and Research Methodology, P. D. E. A College of Ayurveda and Research Centre, Akurdi, Pune, India

*Correspondence	Abstract
<p>A.V. Patil Shankarrao Ursal College of Pharmaceutical sciences and Research center, Kharadi, Pune, Maharashtra, India</p> <p>DOI: 10.7897/2321-6328.01311</p> <p>Article Received on: 12/08/13 Accepted on: 22/09/13</p>	<p>A wide range of medicinal plants are used as drugs. The different parts used as drugs include root, stem, flower, fruit, twigs exudates and modified plant organs. The <i>Punica granatum</i> Linn and <i>Cymbopogon</i> are native shrubs of occidental Asia and Mediterranean Europe that has a rich history of traditional uses in medicine. For centuries, the peels, fruits and seeds, leaves of these plants show antimicrobial activity. In this research project combination of pomegranate peels and lemon grass has antimicrobial activity, they fight against bacteria. The present study has evaluated antimicrobial activity of methanolic extract of <i>Punica granatum</i> Linn and <i>cymbopogon</i>. In this research Phytochemical, Physicochemical, Antimicrobial test of Pomegranate and Lemon grass was done. Extraction of pomegranate peel's and lemon grass leaves was carried out by maceration method. The crude extract of Pomegranate and Lemon grass has antimicrobial activity against Gram positive and Gram negative bacteria. The results obtained in this study have confirmed its Antimicrobial activity.</p> <p>Keywords: Anti-microbial activity, <i>Punic granatum</i> Linn, <i>Cymbopogon</i></p>

INTRODUCTION

Punica granatum Linn (Pomegranate) belonging to family Punicaceae, had been long esteemed as food and medicine, and is diet in convalescence after diarrhea¹. Peels of some plants as *Punica granatum* Linn (having antimicrobial properties) which are generally treated as wastes are true antibiotics as they are available for no cost, has no side effects and the most important benefit in that antibiotic resistant pathogens would be easily killed by these new and natural antimicrobials because they would take at least a few decades to get mutated and resistant to them². It is used in Siddha, Ayurvedha and Unani medicine especially for the treatment of Gastro-Intestinal (GI) diseases³. Pomegranate is a fruit of great antiquity and is known to have been cultivated in the Middle East more than 5,000 years ago. The plant is found all over India. Pomegranate has been considered important since prehistoric times as an agency of longevity. The fruit is good for dysentery, diarrhea and gastralgia. Hindu physicians use the rind of the fruit and flowers, combined with aromatics, such as cloves, cinnamon, coriander, pepper etc as bowel astringent in diarrhea⁴. In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. In Ayurvedic medicine the pomegranate is used as an anti parasitic agent, a "blood tonic," and to heal aphthae, diarrhea, and ulcers^{5,6}. Ingestion of pathogens can cause many different infections. These may be confined to the GIT or initiated in the gut before spreading to other parts of the body. A syndrome characterized by GI symptoms including nausea, vomiting, diarrhea and abdominal discomfort. Worldwide, diarrhea diseases are the second leading cause of death; about 25 million enteric infections occur each year. These

infections cause significant morbidity and death, particularly in elderly people and children younger than age 5⁷. It has been estimated that 4 to 6 million children die each year from diarrhea particularly in developing countries in Asia and Africa. Even in developed countries, significant morbidity occurs as a result of diarrhea illness, although acute diarrheal syndromes are usually self-limited, some persons with infectious diarrhea will require diagnostic studies and treatment. The last decade has seen a resurgence of global interest in medicinal plants as therapeutic agents⁸. Lemon grass belongs to the section of *Andropogon* called *Cymbopogon* of the family Gramineae. Medicinal use of lemon grass is known to mankind since antiquity. Its leaves has been used to cure various ailments like cough, cold, spitting of blood, rheumatism, lumbago, digestive problems, bladder problems, leprosy and as mouth wash for the toothache and swollen gums. It is also been claimed to be stimulating, diuretic, anti purgative and sudorific to reduce fever. This traditional treatment approach is much significance in the world especially in India due to the endemic presence of infective diseases, which are the major causes of infant and adult mortality. Knowing the activity of *Punica granatum* Linn and *cymbopogon* a study has been carried out to evaluate its antimicrobial activity, which has been reported in this paper. This research reveals investigated phytochemical, physicochemical and antimicrobial properties of Pomegranate and Lemon grass. Extraction of pomegranate peel's and lemon grass leaves was carried out by maceration method. The crude extract of Pomegranate and Lemon grass was found to antimicrobial against Gram positive and Gram negative bacteria.

MATERIAL AND METHODS

Plant Material

The peels of *Punica granatum* Linn and leaves of *Cymbopogon* were selected for this study based on their traditional practices by Indians. The plant material was collected from local area in Dist-Pune, Maharashtra, India.⁹

Test Strains

The pure cultures of human pathogenic bacteria of two gram-negative bacteria *E-coli*, *Pseudomonas aeruginosa* and two gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* was selected.

Preparation of Extract

Initially peels of *Punica granatum* Linn and leaves of *Cymbopogon* were dried and converted into fine powder. Crude methanolic extract of pomegranate and lemon grass was prepared by using maceration process. Solid drug material was taken in a container having stopper, with about 750 ml of the menstrum and allowed to stand for 7 days in warm place with frequent shakings. The mixture of crude drug containing solvent was filtered until most of the liquid drains off. The filtrate and the washing were combined to produce 1000 ml of the solution.¹⁰

Anti-Microbial Screening

The reference strain of bacterial were maintained on nutrient agar slant (16), sub cultured regularly on Agar medium, incubated at 37°C for 24 h (every 30 days) and stored in at 4°C.

Preparation of Phosphate Buffer Solution (PBS)

2.38 g of disodium hydrogen phosphate, 0.1 g of potassium dihydrogen phosphate and 8 g of sodium chloride was dissolved in sufficient water to produce 1000 ml. pH is adjusted at 7.0-7.4.¹¹

Agar-well Diffusion Method

An agar-well diffusion method was employed for determination of antimicrobial activities. The stored pomegranate extract and Lemon grass extract samples were dissolved in phosphate buffered saline (PBS, pH 7.0-7.4). All bacteria were suspended in sterile water and diluted to 10 CFU/ml. The suspension (100 µL) was spread on to the surface of nutrient agar medium. Wells (4.6 mm in diameter) were cut from the agar with a sterile borer and 60 µL extract solution was delivered into them. The inoculated plates were incubated at 37°C for 24 h. Antimicrobial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. DIZ was expressed in millimeters.¹²

RESULT

The result of screening plant extracts for antimicrobial activity was summarized in Table 3 and Figure 1. Antimicrobial activity was produced to different extents by methanolic extracts of *Punica granatum* Linn and leaves of *Cymbopogon*. The antimicrobial study has been performed against two gram-negative bacteria; *E-coli* and *Pseudomonas aeruginosa* and two gram-positive bacteria; *Staphylococcus aureus* and *Bacillus subtilis*. It had shown that the zone of inhibition of bacteria by plant extracts has changed between 0.6 to 1.7 mm (Table 3). The Phytochemical investigation was found within the standard limits as mentioned in the IP (Table 2). All the extracts were acidic in nature (pH values ranging between 3-5). The acidity combined with bioactive components has enhanced the antimicrobial activity of the extracts especially against the bacteria. It was proven that the Pomegranate and Lemon grass has individual antimicrobial activity. Mixture of lemon grass and pomegranate extract showed the more antimicrobial activity than individual.

Table 1: Positive chemical test of lemon grass and pomegranate peels

S. No.	Chemical Test	Pomegranate	Lemongrass
1	Carbohydrate	+	+
2	Flavonoids	+	+
3	Alkaloids	-	+
4	Tannins	+	+
5	Phenolic Compound	+	-
6	Volatile Oils	+	+

Table 2: Phytochemical Investigation

S. No.	Parameters	w/w %
1	Ash Value	
	a) Pomegranate	25
	b) Lemon grass	35
2	Extractive Value	
	a) Pomegranate	15.5
	b) Lemon grass	25
3	Loss on Drying	
	a) Pomegranate	0.97
	b) Lemon grass	0.90
4	Foreign Material	Nil

Table 3: Anti-microbial Test

S. No.	Microorganism	Zone of Inhibition (mm)		
		Lemon grass	Pomegranate	Mixture
1	<i>Bacillus subtilis</i>	1.3	1.5	1.7
2	<i>Pseudomonas aeruginosa</i>	1.2	1.4	1.6
3	<i>Staphylococcus aureus</i>	1.0	1.2	1.5
4	<i>E. coli</i>	0.70	0.60	0.60

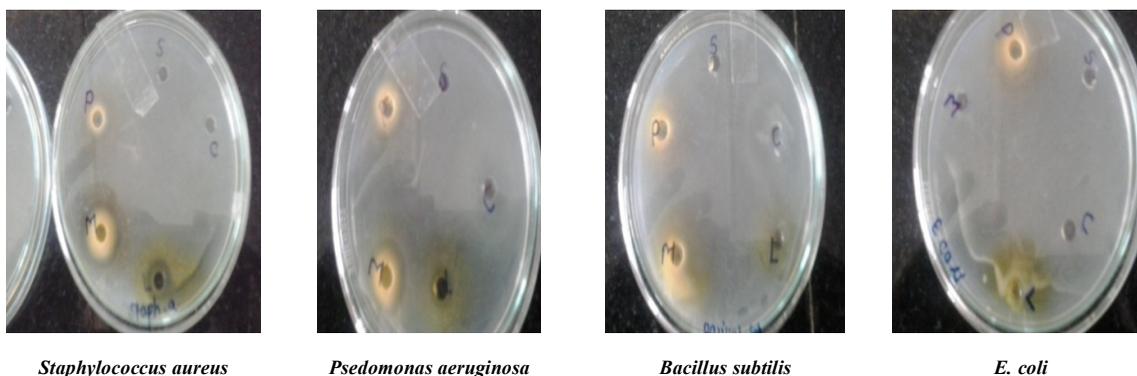


Figure 1: Zone of Inhibition

DISCUSSION

The present study was designed to obtain preliminary information of the antimicrobial activity of mixture of methanolic extract of the *Punica granatum* Linn and *Cymbopogon*. The main aim of the study was to evaluate the synergistic action of mixture peels of *Punica granatum* Linn and leaves of *Cymbopogon*. The *Punica granatum* Linn and *Cymbopogon* exhibited significant antimicrobial activity. The Agar-well diffusion method was preferred to be used in this study. Two gram-negative bacteria; *E. coli*, *Pseudomonas aeruginosa* and two gram-positive bacteria; *Staphylococcus aureus* and *Bacillus subtilis* were used for this study. The preliminary phytochemical tests have shown the presence of Carbohydrate, Flavonoids, Phenolic compound, Volatile oils and Tannins in Pomegranate Peels. The Carbohydrate, Flavonoids, Alkaloids, Volatile oils and Tannins were present in the leaf of lemon grass. The zone of inhibition of two Gram positive and Gram negative strains was significantly increased in the mixture of extract of Pomegranate and lemon grass as compared to their individual effect (Table 3).

CONCLUSION

The peel's of pomegranate and lemon grass leaves of these plants use since many years as decoctions or infusions prepared in water to treat various other ailments. Thus this study has provided a scientific basis for the use of methanolic plant extracts as home remedy; their possible application in treatment of gastrointestinal disorders and as an anticancer drug. Further studies may lead to their use as safe alternatives to synthetic antimicrobial drugs.

REFERENCES

1. Aviram M, M Rosenblat, D Gaitini, S Nitecki, A Hoffman, L Dornfeld, N Volkova, D Presser, J Attias, H Liker and T Hayek. Clin Nutr, Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation 2004; 23(3): 423-433.
2. Ahmad I and Beg AZ. Antimicrobial and photochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. Ethnopharmacol 2001; 74: 113-123. [http://dx.doi.org/10.1016/S0378-8741\(00\)00335-4](http://dx.doi.org/10.1016/S0378-8741(00)00335-4)
3. Hong MY, Seeram NP and Heber H. Pomegranate Poly phenols down-regulate expression of androgen-synthesizing genes in human prostate cancer cells over expressing the androgen receptor. J. Nutr. Biochem 2008; 19: 848-855. <http://dx.doi.org/10.1016/j.jnutbio.2007.11.006> PMID:18479901 PMCid:PMC2610864
4. Hugo WB and Bloomfield SF. Studies on the mode of action of the phenolic antimicrobial agent fenticlor against *Staphylococcus aureus* and *Escherichia coli*. The effect of fenticlor on the metabolic activities of *Staphylococcus aureus* and *Escherichia coli*. J. Appl. Bacteriol 1971; 34(3): 579-591. <http://dx.doi.org/10.1111/j.1365-2672.1971.tb02320.x>
5. Kulkarni PA, Aradhya SM. Chemical changes and antioxidant activity in pomegranate arils during fruit development. Food Chem 2005; 87: 319-324. <http://dx.doi.org/10.1016/j.foodchem.2004.09.029>
6. Kulkarni AP and Aradhya SM. Chemical changes and antioxidant activity in pomegranate arils during fruit development. Food Chem 2005; 93: 319-324. <http://dx.doi.org/10.1016/j.foodchem.2004.09.029>
7. Blatter E, Cains JF and Mhaskar KS. Indian Medicinal Plants 2nd ed. Uttranchal, India: Oriental Enterprises 2001; 5: 1506-1513.
8. Hussain A, Virmani OP and Popli SP. Dictionary of Indian Medicinal Plants. Lucknow, India: CIMAP, Jurenka, J.M.T. Therapeutic Applications of Pomegranate (*Punica granatum* L.): A Review. Alternative Medicine Review 2008; 13: 128-144.
9. Khandelwal KR. Practical pharmacognosy Techniques and Experiment Nirali Prakashan; 2008. p. 149-160.
10. Rangri Vinod. Pharmacognosy and Phytochemistry, Part 1, First Edition Carrer Publication; p. 8, 130.
11. I.P. Published by Indian Pharmacopoeia Commission, Gaziabad; 2007. p. 80.
12. Wayne. NCCLS (National Committee for Clinical Laboratory standard), Performance Standards for Antimicrobial Susceptibility Testing, 9 International activities. Supplement. M100-S9; 1999.

Cite this article as:

R.Y.Patil, P. N. Jagtap, Lad Meenal, A.C. Sankh, A.V.Patil, O. R. Walwadkar. Antimicrobial study of methanolic extract of Punica granatum Linn and Cymbopogon. J Biol Sci Opin 2013;1(3):190-192 <http://dx.doi.org/10.7897/2321-6328.01311>

Source of support: Nil; Conflict of interest: None Declared