Antibiotic potential of *Punica granatum* extract against *Listeria monocytogenes* and standardization with some commercial antibiotics Augmentin (Au) and Ticarcillin(Ti)

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Abstract

In the present investigation the *Punica granatum* belonging to family Punicaceae has been used to determine its antibiotic potential under laboratory condition. In the present study the antibiotic potential have explored against the most causing organism *Listeria monocytogenes* The ethanolic and methanolic 70% extract were used and the antimicrobial and antibiotic activity has been determined in both the control sets and experimental sets. The ethanolic extract is showing the 13.11mm zone of inhibition while methanolic extract is showing the 16.24 mm zone of inhibition against the causing microorganism *Listeria monocytogenes*. Thus the treatment of methanolic extract is found to be very effective to control the growth of causing microorganisms indicating the presence of some secondary bioactive compound in the plant extract as found in the commercial antibiotic like Augmentin (Au) and Ticarcillin (Ti).

KEYWORDS: - *Punica granatum, Listeria monocytogenes,* Augmentin (Au) and Ticarcillin (Ti)

Introduction:

India has a very great diversity of flora and fauna particularly there is a great biodiversity of medical plants. From region to region and season to season there is a great change in the biodiversity of flora of India. The biodiversity of Indian flora has been well explored by the pharmaceutical and medicine companies. The importance and the utility of the medicinal plant have been well presents in our traditional Ayurvedas. This Ayurveda is the important key of the medical pharmacology and we have given a name like the Naturo -therapy.

The herbs and spices are used for the treatment of various diseases since ancient time and found to be useful today also as therapeutic agent against many pathological infections (Gull et al., 2012). These medicinal plant spices have a unique aroma and flavour due to the presence of Phytochemicals or secondary metabolites present in the plants.(Avato et al.,2002; Melvin et al., 2009).among that ***Punica granatum*** commonly called as Pomogranate belonging to family Lythraceae is one the important the medicinal plant having the grate medicinal potential . Badreldin et al. (2008) has observed and stated that the medicinal plants has many folds of pharmacological actions such as immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions. It is a strong anti-oxidant preventing generation of free radicals.

The uncontrolled and excess usage of antibiotics has induced microorganisms to acquire resistance against number of medicinal drugs, which is the challenge of the present days (Abimbola et al., 1993). As a result there is an urgent need to find some eco-friendly alternative solutions of plants origin which are easily available, cheap and considerably less side effects (Khulbe & Sati, 2009). Due to prevalent resistance of micro organism to drugs and other therapeutic agent this investigation may provide some natural alternative for the treatments of some pathogenic organism. Shirin and Prakash (2011) has analyzed the extract of ginger in different solvents and recorded the different antioxidant compounds such as Polyphenols, flavonoids, total tannin vitamin C, β carotene and Ash, minerals like iron, calcium, phosphorous, zinc, copper, chromium and manganese.

Many plant-source materials have been used in traditional Chinese medicine (TCM) for many centuries in China and other Asian countries. In recent years, TCM has been gradually accepted by the western world. In the USA, TCM is studied and used as a complementary and alternative medicine (CAM) as well as a dietary supplement. By the year 2000, more than 35,000 plant-source materials were screened for potential medical use by the National Cancer Institute (NCI) and the United States Department of Agriculture (Yuan. et al, 2000). Scientific research on the use of TCM has increased with thousands of functional compounds being isolated from TCM (Zhou. et al, 2011) and the Punica granatum is one of the sorce of TMC. Research has been conducted to identify antimicrobial compounds contained in these plants. Phenolic compounds, such as essential oils, gallotannins and flavonoids, are the major compounds with antimicrobial activity found in these plants (Jiao et al. 2012).

Several scientists have reported the potential of medicinal plants as antimicrobial agent, antitumor agent (Khalil et al., 2005; Akroum et al., 2009; Omoya and Akharaiyi, 2012), anti-inflammatory and anti-necrotic agent (Lin and Huang, 2000).

Material and methods:

Medicinal profile of Punica granatum:

As per the previous literature the Punica granatum has several medicinal and therapeutic use such as immune-stimulatory, anti-oxidant, anti-inflammatory, anti-diabetic, Anti-cancerous treating including leukemia, breast, prostate and colon cancer. Prevention of atherosclerosis, natural blood thinners, Arthritis prevention, help in erectile dysfunction, prostate cancer and heart disease, cardiovascular disease, diabetes, , dysentery, diarrhea, excessive bleeding, removal of intestinal worms and parasites. **Stomachache, Hyperacidity, Poor appetite, digestive disorders ,Nausea and morning sickness.**

Profile of micro-organism Listeria:

Listeria is also called as an opportunistic pathogen it is a gram-positive rod-shaped bacterium that can grow under either anaerobic (without oxygen) or aerobic (with oxygen) conditions. Of the six species of *Listeria*, only *L. monocytogenes* causes disease in humans. These bacteria multiply best at 30-37 degrees C. but also multiply better than all other bacteria at refrigerator temperatures

Chemical profile of Punica granatum:
Over the past decade, significant progress has been made in establishing the pharmacological mechanisms of pomegranate and the individual constituents responsible for them. Extracts of all parts of the fruit appear to have therapeutic properties and some studies reported that the bark, roots, and leaves of the tree have medicinal benefit as well. These current research seems to indicate the most therapeutically beneficial pomegranate constituents are ellagic acid ellagitannins (including punicalagins), Punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonols and flavones besides that it also having the content like Moisture - 78.0% , Calcium - 10 mg, Protein - 1.6%, Phosphorus - 70 mg, Fat - 0.1% Iron - 0.3 mg Minerals - 0.7% , Vitamin C - 16 mg, Carbohydrates - 14.5%, Small amount of Vitamin B Complex Fibre - 5.1% Calorific Value – 65 .

Extraction of plant:
For the extraction of plant the Olayemi and Opaleye (1999) method was adopted. Extraction of peel was carried out by measuring 20 gm of fine grounded powder of *Punica granatum* peel with a digital balance. This was dispensed into two beakers, each containing 50 ml of ethanol and methanol separately. These were soaked for 3-5 days after which the solution was carefully filtered with muslin cloth into a sterilized conical flask of 100 ml and the filtrates obtained were stored in the refrigerator at 4C° a temperature

Phytochemical screening
The preliminary Phytochemical was conducted for the presence of bioactive compounds such as alkaloids, glycosides, tannins, saponins, flavonoids ,protein, carbohydrates and vitamin C as per the guidelines of Harbourne (1984) ,Hebert and Kenneth (1984), Raman (2006), Basset et al, (1985) Kokate et al. (1990) .

Preparation of bacterial suspension: Bacterial suspension was prepared by taking a loop full pure culture from already identified slant. The culture was added in nutrient broth (Hi-media) at 37 ºc for 24 hours (overnight) and an active culture of uniform suspension was obtained. Then this suspension was spread on nutrient Agar plate (Hi-media) for assay of antimicrobial activity.

Preparation of inoculums and plates: The bacterial cultures were pre-cultured in nutrient broth in a rotary shaker at 37 °C at 120 rpm overnight. It was used as test culture inoculums for antimicrobial activity of plant extracts after incubation. The inoculums were flooded on the surface of Agar plates by spread plate technique and it was made ready to use for assay of antimicrobial activity.

Antimicrobial Screening: The antimicrobial potential was determined by measuring the zone of inhibition of the extract against the test microorganism using the agar plate well diffusion method. The sterile cork borer of 6 mm in diameter was used to make bore well in the plate. The wells were filled with 0.20 ml of *Punica grantum* ethanolic and methanolic extract and left for incubation 24 hrs. at 37 c^o and the zone of inhibition was measured by using high media scale in mm. For testing the potential of ethanolic and methanolic peel extract the ethanol and methanol were used as control respectively and distilled water was used as a control to observe the potential of commercial antibiotics in control set.

Results and discussion:

The Preliminary phytochemical analysis *of Punica* granatum peel extract indicating the presence of important secondary bioactive metabolites such as glycosides, tannins, , Flavonoids ,protein, carbohydrates and vitamin C while alkaloids and saponins were found absent. According to earlier studies Hamid Reza et. al.(2012), Sharrif and Kashani (2016),the Peels of Punica granatum contain the polyphenols, tannins, flavonoids and anthocyanins (Cyanidins, delphinidins) as bioactive compounds. (Table -01)

The present study reveals that ethanolic extract is showing the 13.11 mm zone of inhibition while methanolic extract is showing the 16.24 mm zone of inhibition against the causing microorganism *Listeria monocytogenes*. Thus *Listeria monocytogenes* is found most susceptible to the methanolic extract than ethanolic extract.(Table -02 ,Fig. -01).

Similarly the commercial antibiotic Ticarcillin showing the 31.33 mm zone of inhibition and the Augmentin (Au) is showing 22 mm of zone of inhibition against the *Listeria monocytogenes* Thus the commercial antibiotic Ticarcillin (Ti) is found effective than the Augmentin (Au) and the *Punica granatum* peel extract is also showing the antibiotic zone of inhibition in both the solvent similar to the commercial antibiotic. This observation may confirm the presence of similar antibiotic potential as good as the commercial antibiotic. Thus the present study reveals that there is extensive scope to use the *Punica granatum* extract as antibiotic agent for the treatment of pathogenic microorganism *Listeria monocytogenes*.

Salgado et.al.(2009) has stated that *Punica granatum* extract can be use as therapeutic agents, additives, alimentary preservatives or biological agents for agricultural diseases. He has evaluated the antimicrobial potential against bacterial species: *Pseudomonas aeruginosa, Escherichia coli, Enteroccocus faecalis, Enterobacter aerogenes, Staphyloccocus aureus, Micrococcus luteus* and *Bacillus* sp. and found very effective to control the bacterial growth.

Al-Zoreky (2009) has studied the antimicrobial activity of pomegranate peel extract against some food-borne pathogens both *in vitro* (agar diffusion) and *in situ* (food) methods. The 80% methanolic extract of peels (WME) was a potent inhibitor for *Listeria monocytogenes, S. aureus, Escherichia coli* and *Yersinia enterocolitica.* The minimum inhibitory concentration (MIC) of WME against *Salmonella enteritidis* was the highest (4 mg/ml). WME afforded > 1 $\log_{10}$ reduction of *L. monocytogenes* in food (fish) during storage at 4 °C.

Shaza and Fadelesults (2013) has also studied the alcoholic extract of *P. granatum* against the sheep lungs infecting organism *P. haemolytica* and recorded the high antibacterial potential with pericarp extract whereas the water and ether petroleum extracts had no antibacterial effectiveness against resistant *P. haemolytica.* Jahir Khan and Sonali Hanee (2011) have evaluated the antimicrobial activity of *Punica granatum* pericarp in Hot aqueous, methanolic and ethanolic extracts *against E.coli, P.aeruginosa* and *S.aureus* using agar well diffusion method and observed an average inhibitory zone diameter of 23.3, 22.3 and 24.5mm respectively which indicates that ethanolic extract shows best result having greater zone than that of the standard antibiotic Tetracycline

(20.1mm). Ehanolic extract of Punica Granatum has lowest MIC of 1.45 µg/ml showing that it is most effective as compared to the other extracts

Sangeetha and Jayaprakash, A.(2016) investigated antimicrobial activity of Punica granatum Linn. With acetone peel extracts and tested against pathogenic bacterial strains *Bacillus cereus, B. subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* and the antibacterial activity was measured in the range of 7-35 mm. Tianchai et. al (2012) has evaluated the antibacterial potential of pomegranate fruit peels and arils with seeds against four food-related bacteria such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli,* and *Salmonella typhimurium* and recorded the high levels of phenolics compounds and exhibited antibacterial activity against all bacteria tested.

The present work is found similar to the above works confirming the Antimicrobial potential of *Punica granatum* peel extract.

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Table-01 phytochemical screening of *Punica granatum* extract

Phytochemical compounds	Test	Results
Alkaloids	Hager's Test	-ve
Glycosides	Keller Killiani Test Bromine water test	+ve +ve
Flavonoids	Ferric chloride Test Alkaline reagent test lead acetate test	+ve +ve +ve
Saphonin	Forth Foam Test	-ve
Tannins	Gelatin Test	+ve
Vitamin C	Dinitrophenyl hydrazine test	+ve
Carbohydrate	Benedict's test	+ve
Free Amino Acids	Ninhydrin Test	+ve
Proteins	Biuret Test	+ve

Table-02 Antibiotic activity of *Punica granatum* extract and commercial antibiotics against *Listeria monocytogenes*.

Extract/ Antibiotics	solvent	Control (mm)	Experimental (mm)
Ethanolic extract	Ethanol	7.66	13.11
Methanolic extract	Metahnol	6.24	16.24
Augmentin(Au)	Dist.Water	00	22.00
Ticarcillin(Ti)	Dist.Water	00	31.33
Test of Significance		Significant	Significant

Fig-1 Antibiotic activity of *Punica granatum* extract and commercial antibiotics against *Listeria monocytogenes*