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Chemical profiling and antibacterial activity of *Punica granatum* L. against pathogens causing Bovine Mastitis

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ABSTRACT

The antibacterial activity of aqueous and methanol extracts of Punica granaatum L was investigated against bovine mastitis causing bacterial pathogens Streptococcus agalactiae, Staphylococcus aureus, Streptococcus uberis, Escherichia coli, Streoptococcus dysagalactiae and coagulase negative Staphylococcus aureus by agar disc diffusion method isolated locally from clinical cases. The results obtained in the present study scientifically proved and suggest the use of Punica Granatum for treating mastitis causing organism. The phytochemical screening of the plant revealed the presence of alkaloids, tannins, carbohydrates, flavanoids, phytosterols, phenols, sterols, terpenes, and volatile oils. The proximate analysis was carried out.

Key words: *Punica Granatum*, Bovine mastitis pathogen, Disc Diffusion method, Solvents, Antibacterial activity, Phytochemical screening.

INTRODUCTION

Bovine Mastitis, the inflammation of mammary gland associated with bacterial infection continues to be the costliest disease to the dairy industry all over the world [1]. Financial losses due to Mastitis occur for both subclinical and clinical stages of the disease and leads to spoilage of milk and milk yield. World wide, Mastitis is associated with economic losses of 35 billion dollars annually. Clinical and sub clinical cases of mastitis usually treated with antibiotics intramammarily and parenterally. The continuous use of antibiotics for a long period may lead to

multi drug resistance in causative organisms which has resulted in the use of high doses o f antibiotics and leads to the danger of increasing amounts of antibiotics residues in milk, a potential hazard [2]. Antimicrobials of plant origin have enormous therapeutic potential and have been used from ancient times. They have been proved effective in the treatment of infectious diseases without any side-effects which are often associated with synthetic antibiotics. Positive response of plant based drugs might lies in the structure of natural products which react with toxins and / or pathogens in such a way that less harm is then to other important molecules or physiology of the host. It is because of this reason drug designing studies now-a-days having come up as a new field of research [3]. The aim of this work was to collect ethno veterinary information about plants used in the prevention and control of Bovine mastitis in Karnataka region. In this context, to investigate the effect of aqueous and methanolic extracts of Punica granatum was selected. There were no reports available relating to in-vitro applications of Punica granatum L. extracts in Bovine mastitis studies

About the plant

Punica granatum Linn commonly known as Pomegranate belongs to the Family Punicaceae. *Punica granatum* is a shrub or small tree with several upright, thorny stems, the leaves are elliptic, roughly 2x1 inches. The plant has also been used as an antispasmodic and antihelmintic.

EXPERIMENTAL SECTION

All the solvents and reagents used in the study were analog grade sourced from Hi media.

2.1 Collection and Extraction of plant material

The plant was collected in the month of March-2011 from Acharya Institute of technology campus, soladevanhalli, Bangalore. The plant with leaves was rinsed with sterilized water and leaves were removed and separated. The leaves were air dried for 3 weeks and then crushed with mortar and pestle and kept in air tight glass container at 4^0C until further use.

2.2 Proximate analysis

Proximate analysis was carried out to determine the moisture content, total ash value, acid insoluble ash value, and alcohol and water extractive values.

2.3 Preparation of crude extracts

Aqueous extract was prepared by using 500g of crushed leaves and 500 ml of distilled water in soxhlet apparatus and the apparatus was allowed to run for 10 hours. Similarly the methanol extract was prepared[15].

2.4 Bacterial strains

Bacterial strains used in this study were isolated from clinical cases of BM namely *Streptococcus agalactiae, Staphylococcus aureus, Streptococcus uberis*, *Escherichia coli*, *Streoptococcus dysagalactiae and* coagulase negative *Staphylococcus aureus.* All the strains were confirmed by cultural and biochemical studies [4] and maintained in nutrient agar slants at 4ºC for further use.

2.5 Antibacterial activity

The antibacterial assay of aqueous and methanolic extracts was performed by agar disc diffusion method [5] [6]. The molten Mueller Hinton agar was inoculated with 100µl of the inoculums (1*106 CFU/ml) and poured into the petriplate (Himedia). For agar disc diffusion method, the disc (0.7cm), (Himedia) was saturated with 100µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at 37ºC. Microbial growth was determined by measuring the diameter of the zone of inhibition of each bacterial strain[14].

2.6 Phytochemical analysis

Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described by various authors [7] [8]. The plants extracts were screened for the presence of biologically active compounds like glycosides, alkaloids, phenolics, tannins, flavoniond, saponins and steroids

RESULTS AND DISCUSSION

The proximate analysis showed 6.5% moisture content,22 water soluble extractive value, 26% alcohol soluble extractive value,4.9% total ash value and 0.63% Acid insoluble ash value(Table - 1)

Table 1: Proximate analysis of *Punica granatum L.*

Leaf	
Parameters	Values (%)
Moisture content	6.5
Water soluble extractive value	22
Alchohol soluble extractive value	26
Total ash value	4.9
Acid insoluble ash value	0.63

Results obtained in the present study revealed that the tested plant, *Punica granatum* extract possess potential antibacterial activity against *Streptococcus agalactiae, Staphylococcus aureus, Streptococcus uberis, Escherichia coli,* Coagulase negative *staphylococcus aureus* and *streptococcus dysagalactieae* (Table 2).

Table 2: antimicrobial activity – zone of inhibition
(M- Methanol, W- Water)

Organisms	Zone of inhibtion (mm)	
	M	W
Streptococcus agalactiae	29.2	29
Staphylococcus aureus	36	36
Streptococcus uberis	25	25
Escherichia coli	27	32
Coagulase negative *staphylococcus aureus*	32	32
Streptococcus dysagalactiae	28	28

Table 3: Phytochemical analysis of *Punica granatum* L.

Phytoconstituents	M	W
Alkaloids	-	+
Tannins	+	+
Carbohydrates	-	+
Flavonoids	+	+
Phtoysterols	+	+
Phenols	+	+
Saponins	-	-
Sterols	+	+
Terpenes	+	+
Volatile oils	+	-

The most pronounced activity with inhibition zones of more than 36 mm was shown by methanol as well as water extracts against *staphylococcus aureus.* The minimum activity was observed in *streptococcus uberis* from aqueous and methanol extracts. The phytochemical screening revealed the presence of alkaloids, tannins, carbohydrates, flavanoids, phytosterols, phenols, sterols, terpenes, and volatile oils (Table 3). Most of the secondary metabolites were identified in the polar extracts. Alkaloids are one of the characteristic secondary metabolite in leaves of this genus found in aqueous extract. 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. Many microorganisms [9]. Phytotherapuetically, tannin containing plants are used to treat non specific diarrhea, inflammations of mouth, throat and injured skins [10]. Flavonoids are known to be synthesized by plants in response to microbial infection. Hence it should not be surprising that they have been found to be effective as antibacterial substances against a wide array of infectious agent[13]. All plant pots synthesize some chemicals by themselves to perform their physiological activity. In the present study, Punica granatum exhibited different kinds of secondary metabolites. The medicinal value of these secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these substances include carbohydrates, phenols, phytosterols, sterols, terpenes and volatile oil for cell growth, replacement, and body building [11],[12] describes today's traditional medicine as undoubtedly the oldest form of medicine and probably had evolved simultaneously with the evolution of human beings. With the traditional knowledge in the background potential plants can be prospected to reach bioactive compounds which can be further formulated. Further studies are suggested to explore the specific phytoactive compounds in the leaf extract of plant *Punica Granatum.*

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