



Original Research Article

Preliminary Analysis of Two Medicinal Plants against causative organism of Bovine Mastitis

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Abstract

Bovine Mastitis is an intramammary infection which is most common among the dairy cattle and continues to be the most costly disease to the dairy farmers. Presently, antibiotics are used for treatment of mastitis leading to the development of antibiotic resistant strains and consumer health problem. The present study investigates *in vitro* antibacterial activity of leaves of two medicinal plants namely *Cymbopogon citratus* and *Punica granatum* L against causative organisms of bovine mastitis. Aqueous and methanol extracts of two plants were investigated by agar well-diffusion method. Methanol and aqueous extracts of leaves of *Cymbopogon citratus* and *Punica granatum* L. were found to possess potency against infectious pathogens *Staphylococcus aureus*, Coagulase negative *Staphylococcus aureus*, *Streptococcus uberis* and *Escherichia coli* isolated locally from clinical and sub clinical samples of Mastitis from Karnataka region whereas .Phytochemical screening of the plants revealed mainly the presence of alkaloids, tannin, saponin, terpenoids and flavonoids.

Keywords: *Cymbopogon citratus*, *Punica granatum* L, Aqueous extracts, methanol extracts, phytochemical screening, antibacterial activity

Introduction

Bovine Mastitis is an intramammary infection which is most common among the dairy cattle. This disease is seen to reduce milk yield, producers profits and milk product quality. Microbiological causes of mastitis are many and multiple factors involved in the management, housing, milking should be considered and continues to be the costliest disease in the dairy industry all over the world [1].The repeated use of antibiotics to treat Mastitis for a long period may cause multidrug resistivity in causative organisms which requires high doses of

antibiotics may leads to accumulation of large amount of antibiotics in milk and its products, again a potential hazard [2]. The World Health Organization (WHO) noted that the majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds. [17] Some medicinal plants have been used for a wide variety of purposes such as food preservation, pharmaceutical, alternative medicine, and natural therapies for many thousands of years. It is generally considered that compounds produced naturally, rather than synthetically, will be biodegraded more easily

and therefore be more environmentally acceptable. Positive response of plant based drugs might lie 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].

In regard to above information's, the present study was undertaken to investigate the effects of aqueous and methanolic leaf extracts of *Cymbopogon citrates* and *Punica granatum* L. This study is to compare the mechanism of *in vitro* antibacterial action of the two plant materials against bovine mastitis isolated pathogens.

Plant description

Punica granatum L. commonly known as Pomegranate belongs to the Family Punicaceae. The pomegranate is native to the Iranian Plateau, the Himalayas in north Pakistan and Northern India

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.[16]

Cymbopogon citrates, commonly known as lemon grass as well as oil grass is a native herb from India and is also cultivated in other tropical and subtropical countries. It is a tall perennial grass. It is consumed as an aromatic drink and used in traditional cuisine for its lemon flavor [4].

Materials and Methods

The leaves of both the plants were collected from Acharya Institute of Technology campus, Soladevanahalli, Bangalore, India in April 2011. The dried leaves were grinded into fine powder

and the total mass was subjected for extraction by hot percolation method with water and Methanol in soxhlet apparatus for 72 hrs. Each solvent extraction step was carried out for 24 hrs and after extraction the extracts were concentrated to form a thick solution by keeping in hot water bath and then stored at 4°C for further study[7].

Bacterial strains

Bacterial strains used in this study were isolated from clinical cases of Bovine mastitis, namely *Staphylococcus aureus*, *Streptococcus uberis*, *Escherichia coli*, and coagulase negative *Staphylococcus aureus* (CONS). All the strains were confirmed by cultural and biochemical studies [5]. These organisms were cultured on Nutrient agar at 37 °C for 24hrs and maintained in nutrient agar slants at 4°C for further use.

Antibacterial activity

The antibacterial assay of aqueous and methanolic extracts was performed by agar disc diffusion method [8]. 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 37°C. Microbial growth was determined by measuring the diameter of the zone of inhibition of each bacterial strain.

Phytochemical analysis

Phytochemical analysis for major phytoconstituents of both the plant extracts was undertaken using standard qualitative methods as described by various authors [9] [10]. The plants extracts were screened for the presence of biologically active compounds like alkaloids, phenolics, tannins, flavonoids, saponins phenols, quinone, anthraquinone, steroids etc.

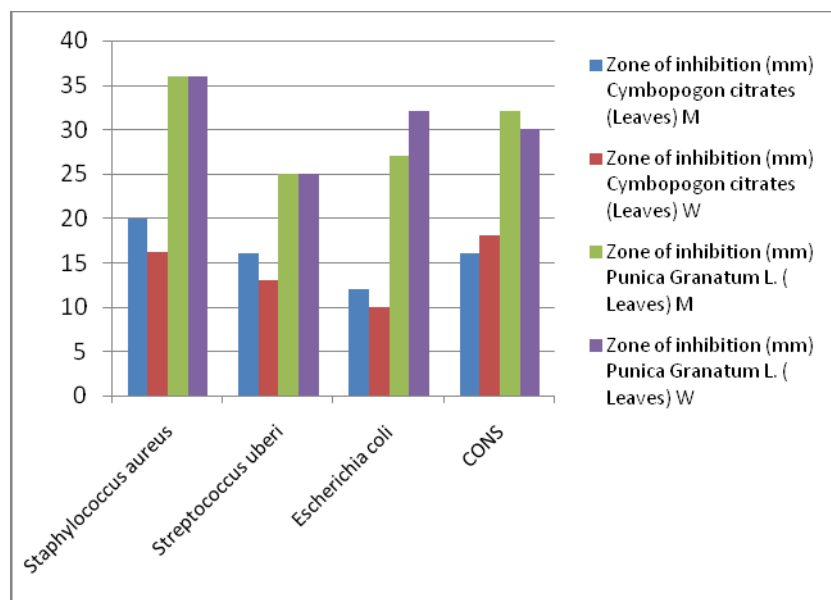
Results and Discussion

Table 1 represents the antibacterial activity of *Cymbopogon citrates* and *Punica Granatum L.* In *Punica Granatum L.* and *Cymbopogon citrates*, the most pronounced activity was shown by methanol as well as water extracts against *staphylococcus aureus* and minimum activity was observed in *streptococcus uberis* from aqueous and methanol extracts of the 2 plants. Figure 1 shows the values of zone of inhibition versus organism which cause bovine mastitis. Table 2 represents the phytochemical screening of the 2 plants. 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 [11].

Table 1: Antibacterial activity of *Cymbopogon citratus* and *Punica Granatum L.*

Organism	Zone of inhibition (mm)			
	<i>Cymbopogon citrates</i> (Leaves)		<i>Punica Granatum L.</i> (Leaves)	
	M	W	M	W
<i>Staphylococcus aureus</i>	20	16.2	36	36
<i>Streptococcus uberis</i>	16	13	25	25
<i>Escherichia coli</i>	12	10	27	32
CONS	16	18	32	30

Fig 1: ZONE OF INHIBITON (mm) vs Organisms for all extracts obtained



Phytotherapeutically, tannin containing plants are used to treat non specific diarrhea, inflammations of mouth, throat and injured skins [12]. 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].

Table 2: Phytochemical analysis of *Cymbopogon citratus* and *Punica Granatum L.*

Compounds	<i>Cymbopogon citrates</i> (Leaves)		<i>Punica Granatum L.</i> (Leaves)	
	Methanol	Water	Methanol	Water
Steroids	-	-	-	-
Terpenoids	-	+	-	+
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Coumarins	-	-	-	-
Saponins	+	+	+	+
Tannins	+	-	-	-
Phenols	+	+	+	+
Catechin	-	+	-	-
Anthraquinone	-	-	-	-
quinone	+	+	+	+

All plant pots synthesize some chemicals by themselves to perform their physiological activity. Methanolic extracts of *Cymbopogon citrates* revealed the presence of alkaloids, flavonoids, saponins and phenols. Along with these terpenoids and catechin were present in the aqueous extracts of *Cymbopogon citrates*. Alkaloids, flavonoids, saponins and phenols are present in methanolic extracts of *Punica granatum L* whereas along with these terpenoids are seen in water extracts of *Punica granatum L*. 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 [14] [15] describes today's traditional medicine as undoubtedly the oldest form of medicine and probably had evolved simultaneously with the evolution of human beings. Results suggest that extraction with water is shown to possess many bioactive compounds than methanol extraction. Thus, Water extraction is a better option for our analysis [3, 6]. With the traditional knowledge in the background potential plants can be prospected to reach bioactive compounds which can be further formulated.

Conclusion

More bioactive compounds have been seen in aqueous extract of leaves suggesting water extracts to be more powerful solvent for extraction. Leaves of *Cymbopogon citrates* and *Punica Granatum L* have shown to have same effect against the causative organism of Bovine mastitis and contain potent bioactive compounds which have antibiotic effect. But *Punica Granatum L*, is much more effective as compared to *Cymbopogon citrates*. Hence, these plants extracts may be used for extracting drug molecules to work against Bovine mastitis.

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