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CHEMICAL PROPHYLAXIS AND ANTIBACTERIAL ACTIVITY OF METHANOLIC AND AQUEOUS EXTRACTS OF SOME MEDICINAL PLANTS AGAINST BOVINE MASTITIS

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ABSTRACT
Phytochemical analysis of methanolic and aqueous extracts of medicinal plants namely *Cymbopogon citrates, Punica granatum L*, *Pennisetum setaceum* and *Nerium oleander* revealed different bioactive compounds such as steroids, terpenoids, alkaloids, flavonoids, coumarins, saponins, tannins, phenols, catechin, anthraquinone and quinone. Antibacterial activity against bovine mastitis causing microorganism such as *Escherichia coli, Streptococcus uberi, Staphylococcus aureus,* and Coagulase-negative *staphylococcus aureus* by agar well diffusion method revealed that *Pennisetum setaceum* and *Nerium oleander* has no much antibacterial activity whereas *Cymbopogon citrates* and *Punica granatum L* has antibacterial activity with *Punica granatum L* having the highest potency.

KEYWORDS: methanol extracts, aqueous extracts, medicinal plants, bioactive compounds, bovine mastitis

INTRODUCTION

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. [16] 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 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]. This study is an effort towards finding some vital plant extracts which can further be used as drug molecules against bovine mastitis. Bovine Mastitis is an intramammary infection which is most common among the dairy cattle and reduces milk yield, producer's profits and milk product quality. [4] 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].

In regard to above information's, the present study was undertaken to investigate the effects of aqueous and methanolic extracts of *Cymbopogon citrates, Punica granatum L*, *Pennisetum setaceum* and *Nerium oleander a*gainst bovine mastitis.

MATERIALS AND METHODS

The leaves of all the plants were collected locally from Tumkur and Bangalore district, 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 by evaporation and stored at 4°C for further study [6].

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 lactose agar media at 37 ºC for 24hrs and maintained in LB agar slants at 4ºC for further use.

Antibacterial activity

The antibacterial assay of aqueous and methanolic extracts was performed by agar disc diffusion method [6,7]. 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.

Phytochemical analysis

Phytochemical analysis for major phytoconstituents of all the plant extracts were undertaken using standard

qualitative methods as described by various authors [8,9]. The plants extracts were screened for the presence of biologically active compounds like as steroids, terpenoids, alkaloids, flavonoids, coumarins, saponins, tannins, phenols, catechin, anthraquinone and quinone.

RESULT AND DISCUSSION

TABLE 1: Phytochemical analysis of *Cymbopogon citrates, Punica granatum L() Pennisetum setaceum* and *Nerium oleander*

	Cymbopogon citrates		Punica Granatum L.		Fountain grass		Nerium oleander	
Compounds	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water
Steroids	-	-	-	-	-	-	+	-
Terpenoids	-	+	-	+	+	+	+	+
Alkaloids	+	+	+	+	-	+	-	-
Flavonoids	+	+	+	+	-	+	-	-
Coumarins	-	-	-	-	+	-	-	-
Saponins	+	+	+	+	-	+	+	+
Tannins	+	-	-	-	-	+	-	-
Phenols	+	+	+	+	-	+	+	+
Catechin	-	+	-	-	-	-	-	-
Anthraquinone	-	-	-	-	-	-	-	-
Quinone	+	+	+	+	+	+	+	+

TABLE 2: Antibacterial activity of *Cymbopogon citrates, Punica granatum L*, *Pennisetum setaceum* and *Nerium oleander*

Organisms	ZONE OF INHIBITION(mm)							
	Cymbopogon citrates		Punica Granatum L.		Pennisetum setaceum L.		Nerium oleander L.	
	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water
S. aureus	20	16.2	36	36	0	0	0	0
S. uberis	16	13	25	25	0	12	12	0
E. coli	12	10	27	32	0	0	0	0
CONS	16	18	32	30	0	0	0	0

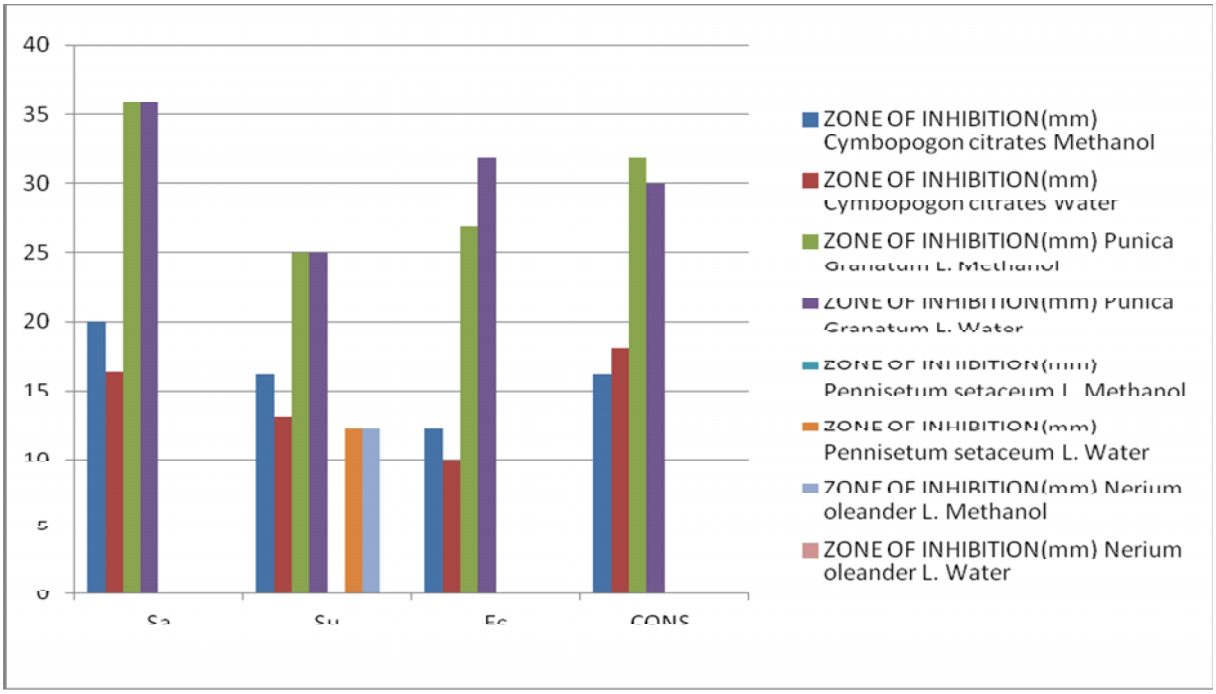

FIGURE 1. ZONE OF INHIBITON (mm) vs Organisms for all extracts obtained

Phytochemical analysis of the plants revealed the presence of different bioactive compounds such as steroids, terpenoids, alkaloids, flavonoids, coumarins, saponins, tannins, phenols, catechin, anthraquinone and quinone as described in table 1. 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 [10]. Phytotherapuetically, tannin containing plants are used to treat nonspecific diarrhea, inflammations of mouth, throat and injured skins [11]. 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 [12]. All plant pots synthesize some chemicals by themselves to perform their physiological activity. Table 2 shows the results of antibacterial activity. The most pronounced activity was observed by *Punica granatum L.* 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. Methanolic extracts of *Pennisetum setaceum L*. showed no effect against all the 4 organism which are supposed to cause bovine mastitis whereas water extracts showed inhibition only against streptococcus uberis with zone of inhibition around 12mm. Aqueous extracts of *Nerium oleander* showed no effect against isolated organism from bovine mastitis whereas water extracts showed inhibition only against streptococcus uberis (12 mm). Hence, with the traditional knowledge in the background these potential plants can be prospected to reach bioactive compounds which can be further formulated and used as vital compounds against bovine mastitis.

CONCLUSION

Cymbopogon citrates and Punica Granatum L has shown to have antibacterial effect against the causative organism of Bovine mastitis whereas *Pennisetum setaceum* and *Nerium oleander* have not much effect. These plants contain potent bioactive compounds which have antibiotic effect. But *Punica Granatum L*, is much more effective as compared to *Cymbopogon citrates.* Thus, we can say that *Punica Granatum L* is the most important of all the four. Hence, *Punica Granatum L* and *Cymbopogon citrates* may be further studied for extracting drug molecules to work against Bovine mastitis.

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