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Phytochemical Screening and Antibacterial Activity of Aqueous and Methanolic Leaf Extracts of Two Medicinal Plants against Bovine Mastitis Bacterial Pathogens

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Abstract

Spathodea campanulata P. Beauv is extensively used in Indian traditional and folklore medicines to cure various human ailments. *Tridax procumbens* Linn is a tropically distributed medicinal plant. Antimicrobial activity of aqueous and methanol extracts of two plants were investigated by agar disc and well-diffusion method against bovine mastitis bacterial pathogens. The plant extracts showed inhibitory activity against the tested organisms. Phytochemical screening of the plant revealed the presence of tannins, flavonoids, saponins and alkaloids. The study scientifically validates the use of plant in traditional and ethnoveterinary medicine.

Key words: *Spathodea campanulata*; *Tridax procumbens*; Antibacterial activity; *Bovine mastitis*; Ethnoveterinary medicine.

Introduction

Ever since the dawn of civilization man has used plants for his food, shelter and fodder for his animals. Plants were also identified for use to cure him from innumerable ailments which struck his physical being. They designated these plants as ‘medicinal plants’. In India, Ayurvedic system of medicine has

existed for over four thousand years. From ancient literature it is evidence that the various parts of the plants were used in Siddha, Ayurvedha and Unani medicine for the treatment of disease of human beings (Palaniswamy *et al*., 2008). In India specifically in Tamil Nadu ethnoveterinary practices are very common in villages. Most of the approaches of the farmers are based on empiric knowledge with significant results in cattle's. A short survey prior to this study was undertaken between known farmers about their interest in ethnobotany and treatment of their cattle sources. Most of them expressed a desire to learn more about the proper use and application of ethnoveterinary practices as these were economically, socially and culturally more acceptable for marginalized communities. Amongst cattle diseases bovine mastitis is a serious problem which affects the basic income of the farmers destroying their dairy sources. Mastitis is an inflammation of the udder. It adversely affects milk production whereby losses due to subclinical mastitis are more severe than those due to clinical cases. Controlling subclinical mastitis can reduce the losses in milk production substantially. Routinely, clinical and subclinical cases of mastitis are treated with antimicrobials both intramammarily and parenterally. The use of antimicrobials over long periods has triggered the development of multidrug resistant strains, which has resulted in the use of increasing doses of antimicrobials, causing the danger of increasing amounts of drug residues in milk, a potential biohazard.

In view of the dearth of all above information's, the present study was undertaken to investigate the effects of aqueous and methanolic extracts of leaves of *Spathodea campanulata* and *Tridax procumbens*. This study is to elucidate the antibacterial action of plant material against bovine mastitis causing pathogens.

Plant Description

Spathodea campanulata is a species belonging to the Bignoniaceae family, native from equatorial Africa. The Siddha/Tamil name of this species is Patadi and in folk it is popularly called as Ruugatuuraa. It is very commonly found and planted in the coffee estates of Munnar, South Tamilnadu and denoted by the name Malaria Maram (tree). In English the species is called as Syringe tree, Fountain tree, African Tulip tree, Flame-of-the-forest or Nandi Flame. It is a medium-size tree (15-25 m high), characterized by red garish flowers. It is often employed in gardening in tropical and subtropical areas including South America (Joly, 1985). Several phytochemical studies were performed with different parts of *S. campanulata*, including stem barks, flowers, leaves, and fruits (Ngouela *et al.,* 1988; Ngouela *et al.,* 1990; Amusan *et al.,* 1996). Flowers and stem bark extracts have shown molluscicidal activity. These are also employed in diuretic and anti-inflammatory treatments. Banerjee

and DE (2001) showed the presence of anthocyanins in flowers of *S. campanulata*.

The stem bark preparations are employed against enemas, fungus skin diseases, herpes, stomach aches and diarrhea (Mendes *et al.*, 1986; Jardim *et al.*, 2003). Hypoglycemic, anti-HIV and antimalarial activities were also observed in stem bark extracts (Makinde *et al.*, 1988; Niyonzima *et al.*, 1999). The leaves are used against kidney diseases, urethra inflammations and as an antidote against animal poisons. *In vitro* antimalarial activity against P*lasmodium falciparum* and antibacterial activity of bovine mastitis causing *S.aureus* were evaluated using leaf extracts of *S.campanulata* (Dhanabalan *et al.*, 2008). The leaves have been found to contain spathodol, caffeic acid, other phenolic acids and flavonoids (Ngouela *et al.*, 1991; Subramanian *et al.*, 1973; El-Hela, 2001a; El-Hela, 2001b). *In vitro* antibacterial activity of leaf extracts of this plant against standard strains was evaluated (Parek and Chanda, 2007). A qualitative fungitoxic activity of *S. campanulata* roots against *Cladosporium herbarum* CCT 0279 has been evaluated and reported (Pianaro *et al.*, 2007).

Tridax procumbens Linn (compositae) is a common grass found in tropical areas of all countries, growing primarily during rainy season. It is a common weed in Tamilnadu present along with economically important crops. It habitats waste places, road sides and hedges throughout India. It is denoted by different names; in English as Mexican Daisy, in Ayurvedic as Jayanti, in Siddha/Tamil as Vettukkaaya-thalai and in Folk as Akala Kohadi. The exomorphology and histomorphology of leaf, petiole, internode and root of this plant were studied (Suseela *et al.*, 2002). The extracts of *T. procumbens* have been reported to have various pharmacological effects including antimicrobial activity, wound healing property and immunomodulatory activity on the experimental animals (Diwan *et al.*, 1982; Diwan *et al.*, 1983, 1989; Udopa *et al.*, 1991; Babu *et al.*, 2003; Oladunmoye, 2006; Taddel and Rosas-Romero 2007). Flavones and glycosides have been isolated from the leaves of the plant (Raju and Davidson, 1994; Yadawa and Saurabh, 1998; Ali *et al.*, 2001).

MATERIALS AND METHODS

Plant collection

Fresh plant leaves were collected from the villages of Coimbatore district, Tamil Nadu, India. The taxonomic identities of plants were confirmed by Botanical Survey of India (Southern Circle), Coimbatore, Tamil Nadu, India and the voucher specimen of the plant was preserved in RVS College Microbiology Laboratory. Fresh plant material were washed with tap water, air dried, homogenized to a fine powder and stored in air-tight bottles.

Plant extraction

For aqueous extraction, 10 g of air-dried powder was mixed with 100 ml distilled water and kept at room temperature for 48 h. It was then filtered through muslin cloth and centrifuged at 5000 *g* for 10 min. The supernatant was collected and stored at 4ºC. For solvent extraction, 10 g of air dried powder was mixed with 100 ml of organic solvent (methanol) in a conical flask, plugged with cotton and then kept on a rotary shaker at 220 rpm for 24 h. After 24 h, it was filtered through muslin cloth and centrifuged at 5000 *g* for 10 min. The supernatant was collected and the solvent was evaporated using rotary vacuum pump and stored at 4ºC in air-tight bottles.

Phytochemical screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the phytoconstituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1989).

Bacterial strains

Bacterial strains used in this study were isolated from clinical cases of bovine mastitis such as coagulase positive *Staphylococcus aureus*, coagulase negative *Staphylococcus aureus* (CNS), *Escherichia coli*, *Streptococcus agalactiae*, *Streptococcus uberis* and *Klebsiella pneumonia*. All the strains were confirmed by cultural and biochemical characteristics (Klastrup, 1975) and maintained in slants for further use.

Antibacterial activity

The antibacterial assay of aqueous and methanolic extracts was performed by two methods. The agar disc diffusion method (Bauer *et al.*, 1966; Parekh and Chanda, 2006) and agar well diffusion method (Perez *et al.*, 1990; Nair and Chanda, 2005). The media (Mueller Hinton Agar No.2), along with the inoculum (10^8 cfu/ml) was poured into the Petri plate (Hi-Media). For the agar disc diffusion method, the disc (0.7 cm) (Hi-Media) was saturated with 100 µl of the test compound, allowed to dry and then placed on the upper layer of the seeded agar plate. For the agar well diffusion method, a well was prepared in the plates with a cup-borer (0.85 cm) and 100 µl of the test compound was pipetted directly into the well. The plates were incubated overnight at 37°C. Antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm) surrounding bacterial growth. For each bacterial strain, controls were included that comprised pure solvents instead of the extract (Parekh and Chanda, 2007). The experiments were repeated three times and the mean values are presented.

Results and Discussion

The results on antibacterial activity of *S. campanulata* and *T. procumbens* were shown in table 1. Methanol extracts of *S. campanulata* showed significant activity against *Streptococcus agalactiae* (7.6 ±0.547) followed by *Streptococcus uberis* (7.2±0.447), *Escherichia coli* (7.2±0.836), coagulase positive *Staphylococcus aureus* (7.0±1.0). Whereas only a moderate activity was observed against coagulase negative *S. aureus* (CNS) (5.4±0.547), and *Klebsiella pneumonia* (5.8±0.447), the aqueous extract of *S .campanulata* showed only a minimum antibacterial activity against the selected mastitis pathogens.

On the other hand the methanol extracts of *T. procumbens* showed significant activity against coagulase positive *S. aureus* (8.0±0.707). But only least antibacterial activity was observed on other selected bacterial strains. The aqueous extracts of T. *procumbens* showed no pronounced antibacterial activity against *Streptococcus uberis* and *K. pneumonia*.

The phytochemical screening revealed the presence of alkaloids, tannin, saponin, steroids, terpenoid and falvonoids (Table 2). Most of the secondary metabolites were identified in the polar (methanol and water) extracts. The concentration of polar metabolites is higher than non-polar metabolites in leaves of these species. Alkaloids are one of the characteristic secondary metabolites in leaves of this genus. 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 agents (Jamine *et al.*, 2007). Tannins (commonly referred to as tannic acid) are also known as antimicrobial agents. They are water-soluble polyphenols and precipitated proteins present in many plant foods. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein. The growth of many fungi, yeasts, bacteria, and viruses were inhibited by this compound (Prasad *et al*., 2008). They have been reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically, tannin-containing plants are used to treat nonspecific diarrhoea, inflammations of mouth, throat and slightly injured skins (Prasad *et al.*, 2008). New commercial synthetic antimastitis drugs can bring biohazards such as consumer health problem, bulk tank milk storage problem, emergence of multidrug resistant strains. Cow as a grazing animal can be directly fed with S. campanulata and *T. procumbens* with all phytoconstituents to the animal which has no side effect as it is commonly grazed along with grasses. This process will be a natural remedy to cure mastitis in dairy cows. Further studies may be necessary to elucidate the phytochemistry of the active principles in the leaf extract of the plant *S. campanulata* and *T. procumbens.*

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Table 1. Antibacterial activity of methanolic and aqueous extracts of *Spathodea campanulata-* and *Tridax procumbens* against bovine mastitis pathogens.

Mastitis isolates from different breeds of cows	**Zone of inhibition in (mm)**			
	Spathodea campanulata-		***Tridax procumbens***	
	Methanol	**Aqueous**	**Methanol**	**Aqueous**
Staphylococcus aureus (Coagulase positive)	7.0±1.0	4.6±0.894	8.0±0.707	4.8±0.836
Staphylococcus aureus ((CNS) Coagulase negative)	5.4±0.547	4.8±0.836	3.2±0.836	2.0±1.0
Streptococcus agalactiae	7.6±0.547	4.4±0.547	3.6±0.547	2.2±0.447
Streptococcus uberis	7.2±0.447	4.2±0.447	1.4±0.547	NA
Escherichia coli	7.2±0.836	4.6±0.547	4.2±0.447	2.2±0.447
Klebsiella pneumonia	5.8±0.447	4.2±0.447	1.2±0.447	NA

NA-No Activity; (±) Mean of three replicates

Table 2. Phytochemical screening of *Spathodea campanulata-* and *Tridax procumbens.*

Phytochemicals	*S. campanulata-*	*T. procumbens*
Alkaloids	+	+
Tannin	+	+

Saponin	+	+
Steriod	+	-
Phlobatannin	-	-
Terpenoid	+	-
Flavonoid	+	+
Cardic glycoside	-	-