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In vitro Phytochemical Screening and Antibacterial Activity of Aqueous and Methanolic Leaf Extracts of *Tridax procumbens* against Bovine Mastitis Isolated *Staphylococcus aureus*

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Abstract

Tridax procumbens Linn is a tropically distributed medicinal plant. Antimicrobial activity of aqueous and methanol extracts of this plant was investigated by agar disc and well-diffusion method against bovine mastitis causing *Staphylococcus aureus* strains. 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 this plant in traditional and ethnoveterinary medicine.

Key words: *Tridax procumbens*, *Staphylococcus aureus*, Ethnoveterinary medicine, Anti-mastitis and Flavonoids.

Introduction

Tridax procumbens Linn (compositae) is a common plant found in tropical areas of all countries, growing primarily during raining 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 was studied (Suseela *et al.,* 2002). The extracts of *T. procumbens* have been reported to have various pharmacological effects, antimicrobial activity, wound healing property and immunomodulatory activity on the experimental animals (Taddel A and Rosas-Romero AJ, 2007; Taddel and Rosas, 2000; Udopa *et al.,* 1991; Diwan *et al.,* 1982; Diwan *et al.,* 1983; Babu *et al.,* 2003 and M.K. Oladunmoye, 2006; Diwan *et al.,* 1989). Flavones and glycosides have been isolated from the leaves of the plant (Ali *et al.,* 2001, Yadawa and Saurabh, 1998 & Raju and Davidson, 1994).

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 *T. procumbens*. This study is to elucidate the mechanism of *in vitro* antibacterial action of plant material against mastitis isolated contagious *Staphylococcus aureus*. To our knowledge, no reports or studies exist relating to *in vitro* application of *T. procumbens* extracts in bovine mastitis works. This is the first report on *T. procumbens* antibacterial action against bovine mastitis isolated contagious pathogens.

MATERIALS AND METHODS

Plant collection

Fresh plant leaves were collected randomly from the villages of Coimbatore district, Tamilnadu, India. The taxonomic identities of plants were confirmed by Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu, 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 and then 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 stand at room temperature for 48 h. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000 *g* for 10 min. The supernatant was collected and stored at 4^{o}C. 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 190 - 220 rpm for 24 h. After 24 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 *g* for 10 min. The supernatant was collected and the solvent was evaporated using rotary vaccum pump and stored at 4^{o}C in air-tight bottles.

Bacterial strains

Bacterial strains used in this study were the multidrug resistant contagious bovine mastitis *S.aureus* isolated from the mastitis infected dairy cows of four different breeds (Jersey, Holstein-Friesian, Zebu and Cross breeds). All the strains were confirmed by cultural, biochemical characteristics (Klastrup, O, 1975) and screened for their antibacterial resistance (Bauer *et al.*, 1966). Among 21, only four multidrug resistant *S.aureus* resistant to Methicillin and other Penicillin derivatives but susceptible to Cloxacillin were selected. Each strain was denoted by J.*Sau*, HF.*Sau*, Z.*Sau* and CB.*Sau* as they were isolated from Jersey, Holstein-Friesian, Zebu and Cross breed cows respectively. An ATCC 25923 *S. aureus* was used as reference strain in the study.

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, 2007b). The experiments were repeated three times and the mean values are presented with ± Standard Deviation (SD).

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).

Results and Discussion

T. procumbens has shown significant antibacterial action against bovine mastitis isolated *S.aureus*. Our present investigation proved that the methanol extracts of this plant showed maximum activity (8.2 ± 0.836) against CB *Sau* followed by J *Sau* & Z.*Sau* (8.0 ± 0.707) and HF *Sau* isolates (7.8 ± 1.30) Table 1. The aqueous extract also showed antimastitis activity but lesser when compared to methanolic extract. The phytochemical screening revealed the presence of Alkaloids, Tannin, Saponin 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 *in vitro* effective antibacterial substances against a wide array of infectious agents (Jamine *et al.,* 2007). Tannins (commonly referred to as tannic acid) are also known 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. 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 (R. Naveen 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 *T. procumbens* with all phytoconstituents can be directly fed 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 *Tridax procumbens*.

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Table 1. Antibacterial activity of methanolic and aqueous extracts of *Tridax procumbens* against bovine mastitis *Staphylococcus aureus*

Mastitis isolates from different breeds of cows	**Antibacterial activity Zone of inhibition in (mm)**		
	Mean±SD		**Methicillin (5mcg/disc)**
	Methanol	**Aqueous**	
J.*Sau*	8.0±0.707	4.8±0.836	>17
HF.*Sau*	7.8±1.30	5.0±0.707	>17
Z.*Sau*	8.0±0.707	5.0±1.00	>17
CB.*Sau*	8.2±0.836	5.2±0.836	>17
ATCC 25923	19.67±1.52	19.33±1.52	>17

J.*Sau- Staphylococcus aureus* isolated from Jersey cow

HF.*Sau- Staphylococcus aureus* isolated from Holstein-Friesian cow

Z.*Sau- Staphylococcus aureus* isolated from Zebu cow

CB.*Sau- Staphylococcus aureus* isolated from Cross breed cow

Table 2. Phytochemical screening of *Tridax procumbens*.

Phytochemicals	**Presence/ absence**
Alkaloids	+
Tannin	+
Saponin	+
Steriod	-
Phlobatannin	-
Terpenoid	-

Flavonoid	+
Cardic glycoside	-