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In Vitro Antibacterial Activity of Two Medicinal Plants against Bovine Udder Isolated Bacterial Pathogens from Dairy Herds

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Abstract

Bovine mastitis continues to be the most costly disease to the dairy farmers. In Tamil Nadu dominates as one of the most prevalent diseases in dairy cattle among the dairy farms. Mastitis treatment with antibiotics leads to the development of antibiotic resistant strains and consumer health problem. The present study is an *in vitro* antibacterial activity of two medicinal plants against bovine udder isolated bacterial pathogens. Aqueous and methanol extracts of two plants were investigated by agar disc and well-diffusion method. Methanol extracts of *Tridax procumbens* and *Spathodea campanulata* showed significant activity against coagulase positive *Staphylococcus aureus* (8.0 ± 0.70) and *Streptococcus agalactiae* (7.6 ± 0.54) respectively. Phytochemical screening of the plants revealed the presence of alkaloids, tannin, saponin, steroids, terpenoids and falvonoids.

Keywords: *Spathodea campanulata*; *Tridax procumbens*; Antibacterial activity; Bovine Mastitis; Ethno-Veterinary Medicine (EVM).

Introduction

Mastitis continues to be the most costly disease to the worldwide dairy industry and research efforts continue toward development of improved methods for the control and elimination of this disease from dairy herds (Salmon *et al*., 1997). 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. Medicinal and aromatic plants have played an important role in the socio-cultural, spiritual and healthcare needs of the rural and tribal people and their live stocks in the emerging and developing countries. In many developing countries, a large section of population relies on traditional system of medicines derived from medicinal and aromatic plants to meet not only their own healthcare needs but for their live stocks also. Traditional medicine has existed since pre-historic times and flourishes today as the primary form of human and animal medicine for perhaps as much as 80% of the world's population. 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 and animals (Palaniswamy *et al*., 2008). Specifically in Tamil Nadu ethno-veterinary practices are very common in villages and most of the approaches of the farmers treating cattle diseases are based on empiric knowledge with significant results in cattle's. Proper use and application of ethno-veterinary practices are economically, socially and culturally more acceptable for marginalized communities.

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

Plant Description

Spathodea campanulata P.Beauv 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). The stem bark preparations are employed against enemas, fungus skin diseases, herpes, stomachaches, diarrhea (Jardim *et al.*, 2003), Hypoglycemic, anti-HIV and antimalarial activities were also observed in stem bark extracts (Niyonzima *et al.*, 1999; Makinde *et al.*, 1988; Rangasamy Dhanabalan *et al.*, 2008). Several phytochemical studies were performed with different parts of *S. campanulata*, including stem barks, leaves, flowers and fruits (Ngouela *et al.*, 1990; Amusan *et al.*, 1996). The leaves have furnished spathodol, caffeic acid, other phenolic acids and flavonoids (Ngouela *et al.*, 1991; El-Hela, 2001a; El-Hela, 2001b). Banerjee and DE (2001)

showed the presence of anthocyanins in flowers of *S. campanulata*.

Tridax procumbens Linn (Compositae) is common grass found in tropical areas of all countries, growing primarily during raining season. It habitats waste places, road sides and hedges throughout India. It is a common weed in Tamilnadu present along with economically important crops. 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 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 and Rosas-Romero, 2007; Udopa *et al.,* 1991; Babu *et al.,* 2003; Diwan *et al.,* 1989). Flavones and glycosides have been isolated from the leaves of the plant (Ali *et al.,* 2001, Yadawa and Saurabh, 1998).

MATERIALS AND METHODS

Plant collection

Fresh plant leaves of *S. campanulata* and *T.procumbens* were collected randomly from the gardens and 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. The collected plants were washed with running tap water, air dried, homogenized to a fine powder and stored in air-tight bottles at 4°C.

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°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 vacuum pump and stored at 4°C in air-tight bottles.

Bacterial strains

Bacterial strains used in this study were the isolated pathogens 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 Mueller Hinton Agar media, along with the inoculum (10^8 cfu/ml) was poured into the petri plate. 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

Phytochemical screening was carried out on both the plant extract to identify the phyto-constituents (Trease and Evans, 1989).

Results and Discussion

The traditional ethno-veterinary medicinal practices are being followed by the ruralities through which a number of veterinary diseases are managed in the developing countries. The use of antibiotics and other chemical products are banned for animal healthcare in a number of countries because of human healthcare. The World Health Organization (WHO) states that 74% of the plants derived medicines have a modern indication that correlates with their traditional, cultural (and sometimes ancient) uses (Wynn, 2001). The results of antibacterial activity of *S.campanulata* and *T.procumbens* shown in table 1. Methanol extracts of *S.campanulata* showed significant activity against *Streptococcus agalactiae* (7.6±0.547) followed by *Escherichia coli* (7.2±0.836), *Streptococcus uberis* (7.2±0.447), coagulase positive *Staphylococcus aureus* (7.0±1.0). Whereas only a moderate activity was observed against *Klebsiella pneumonia* (5.8±0.447) and coagulase negative *Staphylococcus aureus* (CNS) (5.4±0.547). The aqueous extract of *S.campanulata* showed only a moderate antibacterial activity against the tested pathogens.On the other hand the methanol extracts of *T.procumbens* showed

significant activity against coagulase positive *Staphylococcus aureus* (8.0±0.707). But only least antibacterial activity was observed on tested strains. The aqueous extracts of *T.procumbens* showed no pronounced antibacterial activity against *Streptococcus uberis* and *Klebsiella pneumonia*.

The phytochemical screening revealed the presence of alkaloids, tannin, saponin, steroids, terpenoids and falvonoids (Table 2). 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 have been reported to prevent the development of microorganisms by precipitating microbial protein and have been reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic and antiparasitic effects (Naveen Prasad *et al.*, 2008). Wynn (2001) describes the today's traditional medicine, as undoubtedly the oldest form of medicine and probably evolved simultaneously with the evolution of human beings. EVM has been a mainstay of developing countries that lack access to conventional medicines for veterinary health care, often only unaffordable means to poor farmers.

The EVM practices could be an effective approach for tackling problems like mastitis, bovine viral diarrhea and many deficiency disorders. With the traditional knowledge in the background potential plants can be prospected to reach the active fraction or molecule(s), which can be further formulated, also the dried plant material itself could be utilized by premixing it with the fodder of cattle feed while utilizing the pure molecule as a marker to maintain the product quality control. Further studies may be necessary to elucidate the specific phytoactive compounds in the leaf extract of the plant *S. campanulata* and *T.procumbens.*

References

1. Ali,M., Rawinder,E., Ramachamdram, R., 2001. A new flavonoid from the aerial parts of *Tridax procumbens*. *Fitoterapia* **72 (3)**, 313-5.
2. Amusan, O. O. G., Adesogan, E. K., Makinde, J. M., 1996. Antimalarial active principles of *Spathodea campanulata* stem bark. *Phytotherapy Research* **10 (8)**, 692-693.
3. Babu, G., Sanjeeva., Bairy, K.L., 2003. Effect of *Tridax Procumbens* on burn wound healing. *Indian Drugs* **40 (8)**, 488-491.
4. Banerjee, A.; DE, B., 2001. Anthocyanins in some flowers of West Bengal. *Journal of Medicinal and Aromatic Plant Science* **23**, 600-604.
5. Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by

a standardized single disk method. *Am. J. Clin. Pathol* **45**, 493-496.
6. Dhanabalan, R., Doss, A., Jagadeeswari, M., Balachandar, S., Kezia, E., Parivuguna, V., Reena Josephine C.M., Vaidheki, R and Kalamani, K., 2008. *In vitro* Phytochemical Screening and Antibacterial Activity of Aqueous and Methanolic Leaf Extracts of *Tridax procumbens* against Bovine Mastitis Isolated *Staphylococcus aureus*
7. Diwan,P.V., Karwande Margaret, I., Sattur, P.B., 1989. Pharmacology and biochemical evaluation of *Tridax Procumbens* on infl ammation. *Ind. J. Pharmac* **21**, 1-7.
8. El-Hela, A. A., 2001a. Phenolics from *Spathodea campanulata* Beauv. Leaves. *Al-Azhar Journal of Pharmaceutical Sciences* **27**, 152-162.
9. El-Hela, A. A., 2001b. A new iridoid glucoside from *Spathodea campanulata* Beauv. leaves. *Al-Azhar Journal of Pharmaceutical Sciences* **27**, 115-120.
10. Jamine.R., Daisy.P and Selvekumar. B.N., 2007. *In vitro* Efficacy of Flavonoids from *Eugenia jambolana* Seeds Against ESL-Producing Multidrug-Resistant Enteric Bacteria. *Research Journal of Microbiology* **2 (4)**, 369–374.
11. Jardim, M. A., Mendonca, L. F. R., Ferreira, M.M. D., 2003. http://www.mdic.gov.br/tecnologia/revistas/artigos/200112pa/mariojardim.pdf.
12. Joly, A. B., 1985. Introdução à taxonomia vegetal. 7. ed. Sao Paulo: Ed. Nacional, 503.

13. Klastrup, O., 1975. Scandinavian recommendations on examination of quarter milk samples, In: Dodd, F.H. (Ed.), Proc. IDF Seminar on Mastitis Control. *Int. Dairy Fed* **85**, 49-52.

14. Makinde, J. M., Amusan, O. O. G., Adesogan, E. K., 1988. The antimalarial activity of *Spathodea campanulata* stems bark extract on *Plasmodium berghei berghei* in mice. *Planta Medica* **54(2)**, 122-125.

15. Nair, R., Chanda, S., 2005. Antibacterial activity of *Punica granatum* in different solvents. *Ind. J. Pharm. Sci* **67**, 239-243.
16. Naveen Prasad,R., Viswanathan.S., Renuka Devi,J., Vijayashree Nayak., Sweth,V.C., Archana Parathasarathy, N and Johanna Rajkumar., 2008. Preliminary phytochemical screening and antimicrobial activity of *Samanea saman*. *Journal of Medicinal Plants Research* **2(10)**, 268-270.
17. Ngouela, S., Nyasse, B., Tsamo, E., Sondengam, B. L., Connolly, J. D., 1990. Spathodic acid: a triterpene acid from the stem bark of *Spathodea campanulata. Phytochemistry* **29(12)**, 3959-3961.
18. Ngouela, S., Tsamo, E., Sondengam. B. L., Connolly, J. D., 1991. Spathodol, a new polyhydroxysterol from the leaves of *Spathodea campanulata. Journal of Natural Products* **54(3)**, 873-876.

19. Niyonzima, G., Laekeman, G., Witvrouw, M., Van Poel, B., Pieters, L., Paper, D., Clercq, E., Franz, G., Vlietinck, A. J., 1999. Hypoglycemic, anticomplement and anti-HIV activities of *Spathodea campanulata* stem bark. *Phytomedicine* **6 (1)**, 45-49.

20. Palaniswamy, M., Pradeep, B.V., Sathya, R., Angayarkanni, J., 2008. In vitro anti-plasmodial activity of *Trigonella foenum–graecum* L. eCAM **30**, 1 - 5.

21. Parekh, J., Chanda, S., 2006. *In vitro* antimicrobial activities of extracts of *Launaea procumbens Roxb*. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). *Afr. J. Biomed. Res* **9**, 89-93.

22. Parekh, J., Chanda, S., 2007b. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *Afr. J. Biol. Res* **10**, 175-181.

23. Perez, C., Paul, M., Bazerque, P., 1990. An antibiotic assay by the agar well diffusion method. *Acta Biol. Med. Exp* **15**, 113-115.

24. Rangasamy Dhanabalan., Asirvatham Doss., Muthusamy Jagadeeswari., Rajamanickam Karthic., Muthusamy Palaniswamy and Jeyaraman Angayarkanni., 2008. Preliminary Phytochemical Screening and Antimalarial Studies of *Spathodea campanulatum* P. Beauv Leaf Extracts. *Ethnobotanical Leaflets* **12**, 811-19.

25. Salmon, S.A., Watts, J.L., Aarestrup, F.M., Pankey, J.W and Yancey,R.J., 1997. Minimum Inhibitory Concentration for Selected Agents against Organisms Isolated from the Mammary Glands of Dairy heifers in New Zealand and Denmark. *J Dairy Sci* **81**, 570-578.

26. Taddel, A., Rosas-Romero, A.J., 2007. Bioactivity studies of extracts from *Tridax procumbens*. *Phytomedicine* **7(3)**, 235-8.

27. Trease, G.S., and Evans, H.C., 1978. Textbook of Pharmacognosy. 9 th edition. Bailiar Zindall And Co., London.

28. Udopa, S.L., Udopa, A.L and Lalkarni, D.R., 1991. Influence of *Tridax procumbens* on lysl oxidase activity and wound healing. *Planta Med*. **57(4)**, 325-7.

29. Wynn, G. S., 2001. Herbs in Veterinary Medicine. Alternative Veterinary Medicine. http://www.altvetmed.com/articles/herbs.html as retrieved on 9 Oct 2001 **21(47)**, 38.

30. Yadawa, R.N., Saurabh, K., 1998. A new flavones glycoside: 5,7,4 –Trihydraxy – 6, 3 –

dimethasey Falavone 5-0 alpha –L- rhamnopyramoside from the leaves of *Tridax procumbens* Liun. *J. Asian Nat. Prod. Res.* **1(2)**, 147-52.

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	**Antibacterial activity Zone of inhibition in (mm) Mean±**SD			
	Spathodea campanulata-		***Tridax procumbens***	
	Methanol	**Aqueous**	**Methanol**	**Aqueous**
Coagulase positive *Staphylococcus aureus*	7.0±1.0	4.6±0.894	8.0±0.707	4.8±0.836
(CNS) Coagulase negative *Staphylococcus aureus*	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

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

Phytochemicals	*S. campanulata-*	*T. procumbens*
Alkaloids	+	+
Tannin	+	+
Saponin	+	+
Steriod	+	-
Phlobatannin	-	-
Terpenoid	+	-
Flavonoid	+	+

Cardic glycoside	-	-