RESEARCH ARTICLE
JIVA 18(3) December 2020

Received: 28.05.2020
Accepted: 09.08.2020

METHANOLIC EXTRACTS OF *TECTONA GRANDIS* AND *BIOPHYTUM SENSITIVUM* EXTRACTS POTENTIATE THE EFFECT OF TETRACYCLINE ON BACTERIAL ISOLATES FROM BOVINE MASTITIS

Arya Mohan, Nisha A.R.*, Keerthika V. and Usha P.T.A.

Department of Veterinary Pharmacology and Toxicology,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur
**Corresponding author: nisha@kvasu.ac.in*

ABSTRACT

Extensive and indiscriminate use of antibiotics in treatment and control of mastitis may lead to emergence of antibiotic resistant bacteria and transfer of resistant genes. Among various pathogens, *E. coli* and *Staphylococcus* contributes to major occurrence of mastitis in dairy animals and also show antimicrobial resistance. Combination of phytochemicals with antimicrobial agents is shown to be effective in overcoming antimicrobial resistance. Methanolic extracts of *Tectona grandis* and *Biophytum sensitivum* are phytochemicals used in this study in combination with tetracycline in *Escherichia coli, Klebsiella sp* and *Staphylococcus aureus* isolates from mastitis. In the present study disc diffusion antimicrobial assay by Kirby Bauer assay was conducted in *E. coli, Klebsiella sp* and *S. aureus* isolates from mastitis with tetracycline and methanolic extracts of *Tectona grandis* and *Biophytum sensitivum* combination at different doses and compared their efficacies.

Key words: Antimicrobial activity, *Biophytum sensitivum, Tectona grandis*, Phytochemical screening

INTRODUCTION

'Antimicrobial resistance' is a serious concern worldwide as there are millions of human deaths occurring due to unsuccessful chemotherapy. Contrary to deaths due to antimicrobial resistance in humans, deaths in animals are nowhere documented, although huge economic loss to dairy sector is imperative. Mastitis is the most common disease in dairy industry in which multidrug resistance often results in therapeutic failure. The desirable antimicrobial activity against resistance pathogen is a most challenging task today. However, phytochemicals offers a promising alternative as in the case of pre-biotic, where they accounted most in curing many diseases (Basha and Sudarshanam, 2011; Raj *et al.*, 2013) Among the various

phytochemicals, breakthrough in the history of medicine was contributed by alkaloids *viz.,* atropine, morphine, xanthine, digitalis, strychnine, nicotine etc, these alkaloids served as 'drug like' molecules in the discovery of various synthetic drugs in the treatment of infectious diseases.

Escherichia coli (*E. coli*), a Gram negative organism of the family *Enterobacteriaceae* commonly cause environmental mastitis in dairy cattle, while *Staphylococcus aureus* (*S. aureus*), a Gram positive organism of family *Staphylococcaceae* causes contagious mastitis in dairy cattle. *Klebsiella sp.,* a Gram negative organism of *Enterobacteriaceae* family is also a common cause for mastitis in dairy cattle.

Biophytum sensitivum (*B. sensitivum*) is an ethnomedicinal plant used in folklore medicine (Mary and Raj, 2017). *Tectona grandis* (*T. grandis*), found in tropical region also finds its use in medicine (Nayeem and Karvekar, 2011). The antibacterial activity of methanolic extracts of these two plants are not yet explored. Hence the research was carried out to compare the antibacterial efficacies of methanolic extracts of two plants *T. grandis* and *B. sensitivum* in potentiating tetracycline against *E. coli, S. aureus* and *Klebsiella sp*.

MATERIALS AND METHODS

Collection of plant materials

The plant *B. sensitivum* and leaves of *T. grandis* were collected from College of Veterinary and Animal Sciences, Mannuthy, Thrissur. The collected plant materials were shade dried, powdered and used for extraction.

Methanolic extracts of phytochemicals

Methanolic extract of the phytochemicals were extracted from the plants - *B. sensitivum* (whole plant) and *T. grandis* (leaves) by Soxhlet Apparatus extraction procedure. The obtained extracts were measured and percentage yield of whole plant extract of *B. sensitivum* and leaf extract of *T. grandis* were 38.15 per cent and 36.8 per cent, respectively.

Phytochemical screening

The crude methanolic extracts of *B. sensitivum* and *T. grandis* were analysed for the presence of various phytochemicals by the procedure described by Narendran *et al.* (2016). The isolates of *E. coli, Klebsiella* and *S. aureus* isolates procured from Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, were used for the study. Isolates were subjected to different treatments with the combination of antibiotic tetracycline (30mcg, SDO37,

M/s Himedia labs, Mumbai, India) and the methanolic extracts of plant materials in various dilutions like 100mg/L, 200mg/L and 400mg/L as described in Table 1. The values obtained were compared with the group in which tetracycline was used alone. All the treatments were triplicated.

Antibiotic disc diffusion test (ABST)

The antibiotic susceptibility testing was carried out for the isolates of *E. coli, Klebsiella sp* and *S. aureus* by Kirby Bauer disc diffusion assay. The bacterial culture was prepared by growing the bacteria in Muller Hinton broth (M/s Himedia labs, Mumbai, India). A loopful of culture was taken from the primary culture plate and added to the broth which was incubated at 37^{o} C for 24 hours. Then the culture was evenly spread on Muller- Hinton (MH) agar using sterile cotton swab. Antibiotic discs of tetracycline (30mcg) were placed on MH agar plates with gentle pressure. Methanolic extracts of plants and antibiotic combination were prepared by dissolving methanolic extracts in sterile distilled water and 20µL of the extract were added on the antibiotic disc at concentration of 100, 200 and 400 mg/mL. The plates were incubated at 37^{o} C for 24 hours. The diameter of zones of inhibition was measured for each treatment. The obtained data was compared with interpretative chart furnished by manufacturer to grade the isolates as resistant, sensitive or intermediate resistant.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of the methanolic whole plant extract of

Table 1. Various treatment groups employed in ABST

Group	Treatment of bacterial culture
Treatment I	Tetracycline alone
Treatment II	100mg/mL *B. sensitivum* alone
Treatment III	100mg/mL *T. grandis* alone
Treatment IV	A combination of 100mg/mL *B. sensitivum* and tetracycline
Treatment V	A combination of 100mg/mL *T. grandis* and tetracycline
Treatment VI	A combination of 200mg/mL *B. sensitivum* and tetracycline
Treatment VII	A combination of 200mg/mL *T. grandis* and tetracycline
Treatment VIII	A combination of 400mg/mL *B. sensitivum* and tetracycline
Treatment IX	A combination of 400mg/mL *T. grandis* and tetracycline

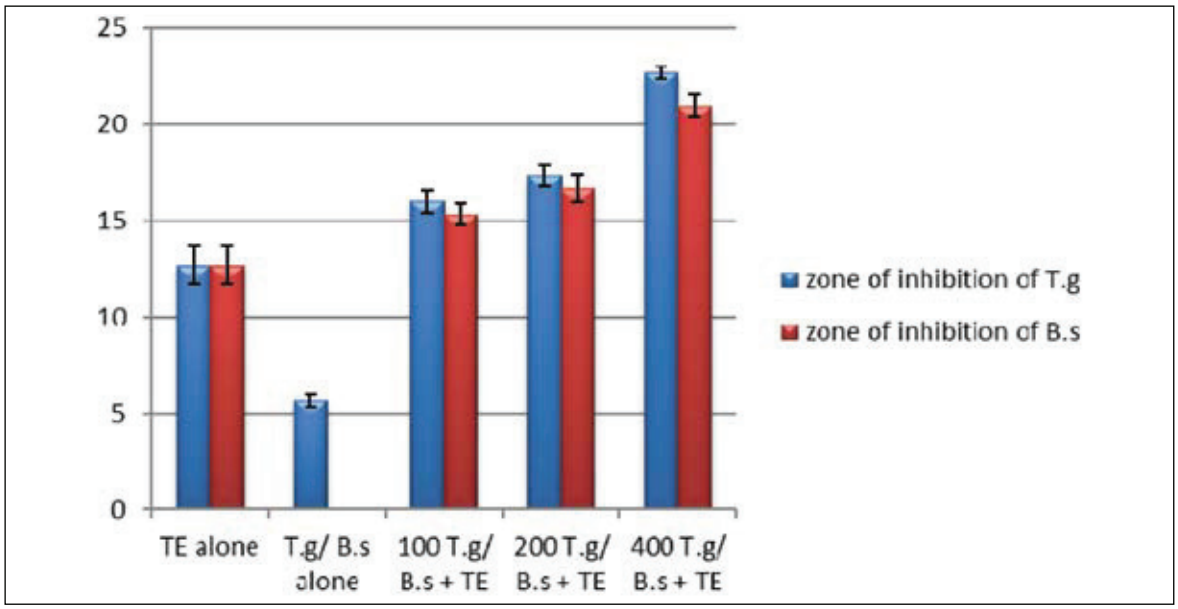

Fig. 1. Comparison of zone of inhibition of plant extracts

B. sensitivum showed the presence of alkaloids, tannins, flavonoids and saponins and that of methanolic leaf extract of *T. grandis* showed the presence of tannins and alkaloids.

Disc diffusion assay

The methanolic extracts of *B. sensitivum* and from leaves of *T. grandis* were made by hot extraction process. Antimicrobial sensitivity was done using combination of antibiotic tetracycline and methanolic extracts of *B. sensitivum* and *T. grandis*. Study revealed that plant extracts alone did not have any antimicrobial activity, but when used along with the antibiotic tetracycline; both the plant extracts could potentiate the activity of tetracycline (Table 2). Among the three bacterial isolates tested,

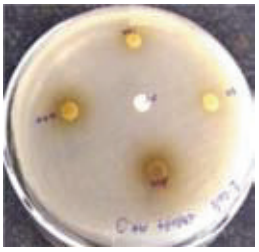

Fig. 2. Disc diffusion assay on *E. coli*

Fig. 3. Disc diffusion assay on *S. aureus* (TE + *T. grandis*)

Fig. 4. Disc diffusion assay on *S. aureus* (TE + *B. sensitivum* 400mg/mL)

Table 2. Results of ABST

Treatment groups	Diameter of zone of inhibition (mm) for *S. aureus*			Mean ± SE
	I	II	III	
Treatment I	12	13	13	12.67 ± 0.57
Treatment II	0	0	0	-
Treatment III	5	6	6	5.67 ± 0.33
Treatment IV	15	16	15	15.33 ± 0.33
Treatment V	15	17	16	16.00 ± 1.00
Treatment VI	16	17	17	16.67 ± 0.33
Treatment VII	17	18	17	17.33 ± 0.33
Treatment VIII	20	22	21	21.00 ± 0.33
Treatment IX	21	22	22	22.67 ± 0.33

the resistant Gram positive *S. aureus* gave the positive results. The synergistic action was not seen in the case of Gram negative isolates *E. coli* and *Klebsiella sp.* The study also revealed that the plant extracts at the higher concentration of 400mg/mL did not show any activity against *E. coli* and *Klebsiella sp*. Figures 1-5 shows the comparison of potentiating antibacterial activity of *B. sensitivum* and *T. grandis* against *S. aureus*. The methanolic extracts of plant materials at concentrations 100mg/mL and 200mg/mL showed intermediate sensitivity. The whole plant extract of *B. sensitivum* and *T. grandis* at 400mg/mL showed a zone of inhibition of 21mm and 22.67mm respectively. *B. sensitivum* extract showed similar results against *S. aureus* in accordance with that of Natarajan *et al.* (2010) and Augustine *et al.* (2016). Leaf extract of *T. grandis* also showed similar results as that of Mittal *et al.* (2012) and Purushotham and Sankar, (2013) against *S. aureus*. Resistance mechanism shown by *E. coli and Klebsiella* sp may be due to mutations in the target site of drugs or over expression of efflux pumps.

SUMMARY

In the present study, methanolic extracts of *T. grandis* and *B. sensitivum* enhanced the zone of inhibition of tetracycline and hence it could be concluded that efficacy of tetracycline against *S. aureus* was potentiated in the presence of methanolic plant extracts. The result of present investigation highlights the antibacterial potential of the methanolic leaf extracts of *T. grandis* and methanolic whole plant extract of *B. sensitivum* along with the antibiotic tetracycline. The plant extracts can be used as an adjuvant along with the antibiotics. There is wide scope for further exploiting the results of the study in overcoming antimicrobial resistance with appropriate clinical modelling.

REFERENCES

Augustine, R., Augustine, A., Kalarikkal, N. and Thomas, S. 2016. Fabrication and characterization of biosilver nanoparticles loaded calcium pectinate nano-micro dual-porous antibacterial wound dressings. *Prog. Biomater.* **5**(3-4): 223-235.

Basha, S.K. and Sudarshanam, G. 2011. Evaluation of antibacteial activity of some Medicinal plants used by Sugali tribe of Yerramalais forest of Andhra Pradesh. *J. Pharm. Res.* **4**(7): 2067-2068.

Mary, L. and Raj, T. 2017. Antibacterial activity of root extract of *Biophytum sensitivum* (L.) DC. *Int. J. Biol. Res*. **2**(3): 16-19.

Mittal, S., Rao, N. and Menghani, E. 2012. Phytochemical and antimicrobial activity of *Tectona grandis*. *Res. J. Sci. Tech*. **4**(5): 188-191.

Narendran, R.T., Ramavarma, S.K.M., Mesna, A. and Pongathara, R.A. 2016. A study on antimicrobial and antioxidant potential of *Biophytum sensitivum* in three different solvents. *South Ind. J. Biol. Sci*. **2**(1): 1-8.

Natarajan, D., Shivakumar, M.S. and Srinivasan, R. 2010. Antibacterial activity of leaf extracts of *Biophytum sensitivum* (L.) *J. Pharm. Sci. Res*. **2**(11):717-720.

Nayeem, N. and Karvekar, M.D. 2011. Antimicrobial and anti-oxidant properties of the isolated compounds from the methanolic extract from the leaves of *Tectona grandis*. *J. Bas. Clin. Pharm*. **2**(4): 163-165.

Purushotham, K.G. and Sankar, L. 2013. Screening of *in vitro* antibacterial activity of *Tectona grandis* on burn pathogens. *Int. J. Pharm. Biol. Sci.* **3**(3): 488-492.

Raj, S., Berthomier, M., Babu, M.A., Karthikeyan, S., Sivakumar, A. and Gothandam, K.M. 2013. Antioxidant, Antibacterial and Anti-Proliferative Activity and Phytochemical Analysis of Selected Medicinal Plants from Dasapushpam of Kerala. *Int. J. Pharm. Sci*. **23**(1): 172-179.