Haryana Vet. (June, 2023) **62(1),** 138-140 ISSN 0033-4359 (P) **Research Article**

IN VITRO ANTIBACTERIAL ACTIVITY OF AQUEOUS AND HYDRO-ALCOHOLIC EXTRACTS OF FRUITS OF *PHYLLANTHUS EMBLICA* AGAINST BACTERIAL ISOLATES OBTAINED FROM SUBCLINICAL MASTITIS AFFECTED CATTLE

S. GAUTAM*, S.R. GUPTA, A.P. SINGH, J.P. KACHHAWA and S. CHAUDHARY
Department of Clinical Veterinary Medicine, Ethics and Jurisprudence,
College of Veterinary and Animal Science, RAJUVAS, Bikaner-334 001, Rajasthan

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ABSTRACT

The present study was undertaken to evaluate *in vitro* antibacterial activity of the aqueous and hydroalcoholic extracts of *Phyllanthus emblica* fruits against common bacterial isolates from natural cases of subclinical mastitis affected cattle. Antibacterial activity was determined in terms of zone of inhibition measured as the diameter of the clear zone around well in which there was no bacterial growth. Results showed that both aqueous and hydroalcoholic extract of *Phyllanthus emblica* fruit has significant *in vitro* antibacterial property against bacterial isolates in subclinical mastitis affected cattle. It is found that *in vitro* antibacterial activity of *Phyllanthus emblica* was higher against gram-positive bacteria than the gram-negative bacteria at different concentrations. Based on average zone of inhibition, *in vitro* antibacterial activity of hydro-alcoholic extract of *Phyllanthus emblica* against bacterial isolates was found to be more as compared to aqueous extract at varying concentrations ranging from 25 mg/ml to 300 mg/ml.

Keywords: Antibacterial activity, Cattle, *Phyllanthus emblica*, Subclinical mastitis

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Mastitis is a global problem as it adversely affects animal health, quality of milk and economics of milk production and every country including developed ones suffers huge financial losses related to culling, decreased fecundity, decreased production and treatment costs (Seegers *et al.*, 2003). The irregular and repeated use of antibiotics to treat bovine mastitis for a long period may cause multi drug resistance in causative organisms (White and McDermott, 2001). Medicinal plants can be used as an alternative therapeutic option or as an adjunct agent in managing bovine mastitis (Mushtaq *et al.*, 2018). *Phyllanthus emblica* (syn. *Emblicaofficinalis*), commonly known as Indian gooseberry or Amla. *Phyllanthus emblica* rich source of vitamin C with presence of tannins, phyllembelic acid, phyllemblin, rutin, curcuminoids, emblicol, and some phenolic compounds (Zhang *et al.*, 2000).

In the past, different studies reported that extracts *Phyllanthus emblica* has antimicrobial property to counter different bacterial pathogens by phytochemical analysis (Ahmad *et al.*, 1998). It also posess antioxidant (Chatterjee *et al.*, 2011) and anti-inflammatory properties (Golechha *et al.*, 2011). The present study was therefore designed to analyse the beneficial use of ethno-veterinary medicine in sub clinical mastitis treatment.

*Corresponding author: sandeepgautam373@gmail.com

MATERIALS AND METHODS

Collection, extraction and formulation of plant materials

The *Phyllanthus emblica* fruits were collected and were identified and authenticated by a botanist. Air dried powder of fruits was extracted with water and ethanol for 24 h in Soxhlet apparatus. The solvents were removed in rotary evaporator and the crude extracts were dried at room temperature in a steady air-current. The dried extracts of the plant materials were stored in air-tight jars at 4°C for microbial analysis.

In vitro antibacterial activity of *Phyllanthus emblica* fruits against common bacteria causing mastitis

In vitro antibacterial activity of aqueous and hydro-alcoholic extracts of *Phyllanthus emblica* fruits against bacterial pathogens isolated from subclinical mastitis affected cattle was done by agar cup method as described by Cruickshank *et al.* (1975). The antibacterial activity of *Phyllanthus emblica* fruits extracts was tested against *Staphylococcus aureus*, *Escherichia coli*, *Klebseilla pneumoniae* and *Streptococcus uberis* isolated from natural cases of bovine subclinical mastitis. The isolated organisms were used for the preparation of stock inoculums. Firstly, microorganisms were taken and streaked on sterile agar plates in such a manner that individual colonies could develop. These plates were incubated at 37ºC for 24 hours. After incubation, colonies of the test bacteria were picked up and emulsified in 5 ml of

sterilized nutrient broth. These broth tubes were incubated overnight at 37º C. The tubes showing obvious turbidity represented stock inoculums. Then, these tubes were kept in refrigerator at 4º C for further use. Different dilutions of aqueous and hydro-alcoholic extracts of *Phyllanthus emblica* fruits were prepared by dissolving in triple glass distil water by serial dilution method to yield different concentration *viz*. 300 mg/ml, 200 mg/ml, 150 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml. About 25 ml of nutrient agar was poured in one petri-plate and allowed to solidify. Stock inoculums of test bacterium were swept over the agar plate with the help of a sterile cotton swab. These plates were dried for 5 minutes before use then wells of size 6 mm were cut into the agar plate at equal distance. Then 100 µl of different concentrations of extract was poured into different well. A total three replicates for each bacterial isolate were prepared. Then Plates were incubated at 37º C for 18-24 hours. After incubation, the diameter (in mm) of zones of inhibition were measured. The final values of zones of inhibition were taken as mean ± S.E. of the recorded observations.

RESULT AND DISCUSSION

In the present study *in vitro* antibacterial activity of the aqueous and hydroalcoholic extracts of *Phyllanthus emblica* fruits against bacterial isolates of *Staphylococcus aureus*, *Escherichia coli*, *Klebseilla pneumoniae* and *Streptococcus uberis* from subclinical mastitis affected cattle. In this Zone of inhibition is recorded of different extract at different concentration. In present study mean ± SE values of zone of inhibition of aqueous extract and hydroalcoholic extracts of *Phyllanthus emblica* against common bacteria isolated from sub-clinically affected mastitic cows with bacterial isolated at different concentration has been presented in table 1 and table 2. The average zone of inhibition in table 1 and fig. 1 for *Staphylococcus aureus* was maximum 22.40 ± 0.12 mm to minimum 10.13 ± 0.03 mm, average zone of inhibition for *E. coli* was maximum 15.20 ± 0.06 mm to minimum 8.20 ± 0.06 mm, average zone of inhibition for *Klebsiella pneumoniae* was maximum 14.20 ± 0.06 mm to minimum 7.33 ± 0.15 mm, average zone of inhibition for *Streptococcus uberis* was maximum 19.20 ± 0.06 mm to minimum 9.33 ± 0.09 mm in recorded. The average zone of inhibition in Table 2 and fig. 2 for *Staphylococcus aureus* was maximum 25.03 ± 0.12 mm to minimum 13.22 ± 0.16 mm, average zone of inhibition for *E. coli* was maximum 16.70 ± 0.06 mm, to minimum 9.67 ± 0.06 mm, average zone of inhibition for *Klebsiella pneumoniae* was maximum 16.13 ± 0.06 mm to minimum 10.70 ± 0.15 mm, average zone of inhibition for *Streptococcus uberis* was maximum 20.13 ± 0.06 mm to minimum 10.20 ± 0.09 mm

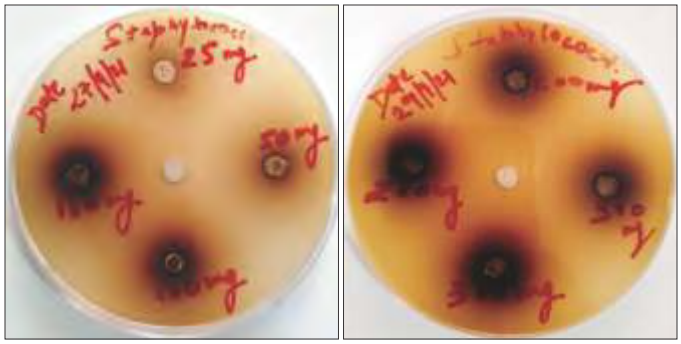

Fig. 1. Zones of inhibition of aqueous extract of *Phyllanthus emblica* at different concentrations against *Staphylococcus aureus*

Fig. 2. Zones of inhibition of hydro-alcoholic extract of *Phyllanthus emblica* at different concentrations against *Klebsiella* spp.

as we move concentration from 300 mg/ml, 200 mg/ml, 150 mg/ml, 100 mg/ml, 50 mg/ml and 25mg/ml, respectively. These finding were in agreement with previous similar studies by Mandal *et al.* (2010), Nain *et al.* (2012), Gautam and Shukla (2017), Khurana *et al.* (2019), Singh *et al.* (2019) and Gupta (2021). The findings of Sharma *et al.* (2012) shows close comparison with present study who recorded zone of inhibition 8.00 mm, 12.5 mm, 12.0 mm, 7.5 mm, 8.0 mm, 7.0 mm, 9.5 mm, 12.5 mm, and 9.0 mm against *Escherichia coli* ATCC 632, *Klebsiella aerogenes* ATCC 9621, *Klebsiella pneumoniae* ATCC 31488, *Salmonella typi* ATCC 13311, *Pseudomonas aeruginosa* ATCC 13525, *Bacillus cereus* ATCC 128263 *Staphylococcus aureus* ATCC 12600, *Streptococcus faecium* ATCC 8043 and *Listeria seeligeri* ATCC 35967, respectively for ethanolic extract of *Phyllanthus emblica* at 20 mg/mlconcentration. The present study describes the effectiveness of Amla plant extracts against, isolated pathogenic organisms. In present investigation it is found that antibacterial activity of *Phyllanthus emblica* was higher against gram positive bacteria than the gram-negative bacteria at different concentrations on comparative analysis of zone of inhibition against various microorganisms and based on mean ± SE values zone of inhibition. *In vitro* antibacterial activity of hydro-alcoholic extract of *Phyllanthus emblica* against bacterial isolates was found to be more as compared to aqueous extract at varying concentrations

Table 1. Mean±SE values of zones of inhibition (mm) of aqueous extract of *Phyllanthus emblica* at different concentration against common bacteria isolated from subclinical mastitis affected cattle

S.No.	Bacterial isolates	Zone of inhibition (mm)					
		25mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	300 mg/ml
1.	*Staphylococcus aureus*	10.13 ± 0.03	12.46 ± 0.18	12.67 ± 0.12	14.27 ± 0.12	18.30 ± 0.12	22.40 ± 0.12
2.	*Escherichia coli*	8.20 ± 0.06	10.27 ± 0.09	11.13 ± 0.03	12.10 ± 0.06	12.60 ± 0.06	15.20 ± 0.06
3.	*Klebseilla pneumonia*	7.33 ± 0.15	9.27 ± 0.09	11.23 ± 0.15	12.77 ± 0.09	13.70 ± 0.12	14.20 ± 0.06
4.	*Streptococcus uberis*	9.33 ± 0.09	11.27 ± 0.12	12.60 ± 0.12	15.10 ± 0.06	16.10 ± 0.06	19.20 ± 0.06

Table 2. Mean±SE values of zones of inhibition (mm) of hydro-alcoholic extract of *Phyllanthus emblica* at different concentration against common bacteria isolated from subclinical mastitis affected cattle

S.No.	Bacterial isolates	Zone of inhibition (mm)					
		25mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	300 mg/ml
1.	*Staphylococcus aureus*	13.22 ± 0.16	15.71 ± 0.27	18.30 ± 0.12	19.67 ± 0.12	20.67 ± 0.21	25.03 ± 0.12
2.	*Escherichia coli*	9.67 ± 0.06	11.27 ± 0.09	12.63 ± 0.03	13.20 ± 0.06	14.80 ± 0.06	16.70 ± 0.06
3.	*Klebseilla pneumonia*	10.70 ± 0.15	11.30 ± 0.09	12.30 ± 0.15	13.17 ± 0.09	14.10 ± 0.12	16.13 ± 0.06
4.	*Streptococcus uberis*	10.20 ± 0.09	13.60 ± 0.12	15.47 ± 0.12	17.47 ± 0.06	18.57 ± 0.06	20.13 ± 0.06

ranging from 25 mg/ml to 300 mg/ml. The result of this study shows that different extracts of amla has effective antibacterial property and can be use as alternative for mastitis management in cattle.

CONCLUSION

The result of this study indicates that different extracts of *Phyllanthus emblica* fruits has effective *in vitro* antibacterial property against bacterial isolates of subclinical mastitis affected cattle. In future researches can be use as alternative therapeutic approaches for subclinical mastitis management in cattle.

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