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In-vitro antibacterial potential of aqueous and ethanolic extract of *Aegle marmelos* and *Murraya koenigii* leaf on the bacterial strains isolated from endometritic cows

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Green medicines of plant origin have emerged nowadays as an alternate therapy. *Aegle marmelos* (*bel*) and *Murraya. koenigii* (*curry*), medicinal plants have currently drawn many research attention for *in-vitro* antibacterial effect against a number of Gram positive and negative bacteria obtained from pure laboratory culture (Abirami *et al*. 2014, Yesaswini *et al*. 2014) coincidentally reported to be associated with bacterial endometritis in cattle (Bhat *et al*. 2014). However, to the best of our knowledge, no single study is available to examine the antibacterial effect of extract of both the leaf on the bacteria strains isolated from the endometritic cows. Therefore, the present study was designed to investigate, the *in-vitro* antibacterial effects of the extract of both the leaf either alone or in combination on the bacterial species isolated from the cows suffering from endometritis.

Preparation of herbal powder: Fresh green leaves of *Aegle marmelos* and *Murraya koenigii* were collected from their natural habitat in and around IVRI campus and shade-dried followed by powdering in a mixer grinder and packaged in plastic bags at room temperature with proper labels indicating plant name, month and place of collection.

Preparation of extract: Aqueous and ethanolic extract (250 mg/ml) were prepared as per the Firdaus *et al*. (2014) and Akinnibosun and Umufo (2015), respectively.

In-vitro *antibacterial sensitivity assay:* The antibacterial activity was tested by agar-well diffusion method (Baskaran *et al*. 2011). An antibiotic disc of ciprofloxacin (Hi-media) was used as control to compare the antibacterial effect with both the extracts used.

The present study demonstrates the *in-vitro* antibacterial potential of the aqueous and ethanolic leaf extracts of *A. marmelos* and *M. koenigii* alone and their combination against the bacterial species that were isolated from bovine endometritis. Extract either individually or in combination have shown zone of inhibition (ZI) against the bacteria isolated from uterus in different degree based on the diameter of ZI (Table 1). The ZI observed against the *E. coli, Corynebacterium* spp., *Staphylococcus* spp., *Bacillus* spp. and *Salmonella* spp. originating from clinical samples of this study indicated that both the extracts are also effective similar to pure culture (Abirami *et al*. 2014, Savitha and Balamurugan 2014, Akinnibosun and Umufo 2015).

A number of previous studies investigated the aqueous extract of the *Aegle marmelos* (Senthilkumaran *et al*. 2014) and *Murraya koenigii* (Baskaran *et al*. 2011) for *in-vitro* antibacterial potential against the similar bacterial spp. examined in the present study. However, the amount and concentration of the aqueous extract used in those studies were different and thereby limiting the comparison of results. The *in-vitro* ZI found after using the ethanolic extract of *M. koenigii* leaf against *Staphylococcus* spp. and *E. coli* of this study appeared to be similar as shown in previous report (Akinnibosun and Umufo 2015). However, the ZI showed against the *Salmonella* spp. of this study was lesser than the above study. In contrast to the previous report, the ethanolic extract of the said plant leaf, however, showed a ZI against *Bacillus* spp.

In the present study, combination of both the leaf extracts showed a higher ZI than the single leaf in both aqueous as well as ethanolic extracts. The higher ZI as observed in this study perhaps because of the synergistic effect of the bio-active compounds present in both the leaves as observed in reports using *Murraya koenigii* and *Telfaria occidentalis* (Akinnibosun and Umufo 2015). Further, the present result pertaining to the synergistic effect as a result of using 2 plant leaves together also supports the similar views reported earlier (Adwan *et al*. 2010). Therefore, enhanced anti-bacterial effect as evidenced by the increased ZI of

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Table 1. *In- vitro* antibacterial activity of different extracts of *Aegle marmelos* and *Murraya koenigii* against various bacteria isolated from endometritic cows

Organism (s)	Zone of inhibition (mm)						
	Aqueous extract			Ethanolic extract			Ciprofloxacin
	AM	MK	(AM+MK)	AM	MK	(AM+MK)	
Bacillus spp.	6	8	10	10	12	14	22
Corynebacterium spp.	-	6	9	10	14	13	20
E. coli	-	6	10	9	11	14	26
Staphylococcus spp.	6	7	13	10	17	16	26
Salmonella spp.	-	-	-	4	7	9	18

AM, *Aegle marmelos* extract; MK, *Murraya koenigii* extract; (AM+MK), *Aegle marmelos* and *Murraya koenigii* extract in combination.

this study is attributed to the additive and synergistic effects of phytochemicals present in the combined extracts (Matchimuthu *et al*. 2008).

The antibiotic (ciprofloxacin) used for comparison showed higher zone of inhibition against all the bacteria as compared to aqueous as well as ethanolic extracts of both the herbal plants either alone or in combination in the study (Table 1). However, when compared with positive control (ciprofloxacin), the ethanolic extract of both the leaves in combination showed 63.3, 65, 53, 61.5 and 50% equivalent activity to ciprofloxacin during *in-vitro* antibacterial sensitivity test.

In conclusion our result suggested that combination of *A. marmelos* and *M. koenigii* leaf extract showed better *in-vitro* antibacterial effect than the single irrespective of the type of extract used. The ethanolic extract showed a better response than the aqueous extract, either alone or in combination. Further, ethanolic extract of leaves in combination showed 50 to 65% equivalent activity to ciprofloxacin during *in-vitro* sensitivity test. Based on the *in-vitro* anti-bacterial sensitivity, the present study also shows rays of hope for the future *in-vivo* antibacterial potential of *A. marmelos* and *M. koenigii* for the treatment bacterial endometritis in bovine.

SUMMARY

The objective of the present study was to investigate the *in-vitro* antibacterial effect of 2 different extracts of *A marmelos* and *M. koenigii* either alone or in a combination against the bacteria isolated from the cows suffering from endometritis. Uterine swabs were taken from 12 cows suffering from endometritis, and isolates were positive for bacterial infection. The ethanolic and aqueous extract of *A marmelos* and *M. koenigii* were prepared to evaluate the in-vitro bacterial sensitivity against the isolated bacteria. Extract of both the plants showed antibacterial activity against the isolated bacteria, either alone or in combination, in a various degree. The ethanolic extract of both plant leaves showed better efficacy than the aqueous extract and also extract from combination of both plant leaves showed better and synergistic effect. The results demonstrated the potential antibacterial effect of *A. marmelos* and *M. koenigii* leaf extract on the bacterial species isolated from endometritic cows.

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