

Antibacterial Effect of Methanolic Extract of *Oroxylum indicum* Leaves Against Bacterial Isolates from Bovine Mastitis

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Abstract

Present research was aimed to find out the antibacterial effect of methanolic extract of *Oroxylum indicum* leaves on *E. coli*, *Klebsiella sp.* and *Staphylococcus aureus* isolates from bovine mastitis samples. Phytochemical analysis of methanolic leaf extract of *Oroxylum indicum* showed the presence of alkaloids, phenolic compounds, tannins and flavonoids. Methanolic leaf extract of *Oroxylum indicum* alone when used in disc diffusion test at a concentration of 100mg/ml, did not show any antibacterial activity against *E. coli*, *Klebsiella sp.* and *Staphylococcus aureus*. When the extract was added to antibiotic discs containing Tetracycline, Oxytetracycline and Cephalexin, moderate anti-bacterial potentiating activity was noticed against *Klebsiella sp.* and potentiating antibacterial activity was observed against *Staphylococcus aureus* whereas no antibacterial activity was observed against *E. coli*. From the present study it was concluded that the methanolic leaf extract of *Oroxylum indicum* can be used as a promising antibacterial potentiating agent along with Tetracycline, Oxytetracycline and Cephalexin to treat mastitis caused by *Staphylococcus aureus*.

Keywords: Antibacterial Effect, Bovine Mastitis, Methanolic Extract, *Oroxylum indicum* Leaves

Introduction

Mastitis is the most common disease of dairy cattle causing economic loss to the farmer. The economic losses due to mastitis occur mostly due to reduction in milk yield, the cost of veterinary services, the cost of discarded milk, cost of culled animals and due to the extra labour used. According to Lakshmi (2016), dairy industry in India experiences an annual loss of 2.37 thousand crores rupees due to mastitis. Etiological agents of mastitis can be infectious or non-infectious. In India, *Staphylococcus*, *Streptococcus* and *E. coli* generally cause 90-95% of all cases of mastitis (Dinesh *et al.*, 2016). A meta-analysis report from India for the period from 2005 to 2016 indicates *Staphylococcus sp.* (45%) to be most predominant mastitis pathogen followed by *E. coli* (14%) and *Streptococcus sp.* (13%) (Krishnamoorthy *et al.*, 2017). *Staphylococcus aureus* is resistant to many antimicrobials that are routinely used in the treatment of mastitis (Freitas *et al.*, 2005). Kulangara *et al.* (2017) found that the udder of dairy cattle in Thrissur District, Kerala were highly infected with virulent strains of antibiotic resistant bacteria. Frequent and inappropriate use of antibiotics for a long time leads to the development of antibiotic resistant strains of bacteria and consumer health problems. It is a serious problem affecting the global health today (Breijyeh *et al.*, 2020). In order to tackle this issue, several studies using different ethno-veterinary medicines have been conducted. However, no significant advancements in alternative treatments against bacterial mastitis have been achieved. Therefore, research using more plant extracts and novel solutions are still needed to treat antibiotic resistant mastitis bacteria in dairy cows.

Oroxylum indicum or Broken bones plant, Indian calosanthus, Indian trumpet flower or Midnight horror (Vernacular name in Malayalam- Palakappayyani) is native to the Indian subcontinent, in the Himalayan foothills with a part extending to Bhutan and southern China, in Indo-China and the Malaysia ecozone (Deka *et al.*, 2013). According to Gokhale and Bansal (2005) *Oroxylum indicum* (L.) is also known as Shivnak, Shyonaka, Sonapatha or Midnight horror. This plant is an active ingredient of well-known Ayurvedic formulations like Chyavanprash and Dashmularistha (Dev *et al.*, 2010). Its stem, leaf, bark and root are used in Ayurveda for treating snake bite. Leaves are used externally to treat enlarged spleen and also to alleviate headaches and ulcers. Reghu *et al.* (2013) cited about anti-cancer, antiulcer, anti-oxidant and anti-inflammatory properties of various compounds derived from *Oroxylum indicum*. Sowjanya *et al.* (2019) cited that various parts of *Oroxylum indicum* possess anti-inflammatory, hepatoprotective, nephroprotective, antihyperlipidemic, antidiabetic, anthelmintic, anti-cancer, antibacterial, analgesic, immunomodulatory, gastroprotective and antimutagenic activities. The present study aims to evaluate the antimicrobial effects of methanolic extract of *O. indicum* against bacteria isolated from the milk samples of mastitis affected cows.

Materials and Methods

Fresh and clean leaves of *O. indicum* were procured locally, shade dried; powdered coarsely and methanolic extract was prepared in Soxhlet apparatus. After extraction, the liquid portion was evaporated in a rotary evaporator; the extract was dried and stored at 4°C in sterile containers. The qualitative analysis of phytochemical constituents in the extract was done as per Harborne (1991). *E. coli*, *Klebsiella sp.* and *S. aureus* isolated from mastitis samples were procured from the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy. The antibacterial potentiating effect of methanolic extract of *O. indicum* leaves on resistant *E. coli*, *Klebsiella sp.* and *S. aureus* isolates in combination with Cephalexin, Oxytetracycline and Tetracycline were studied by Disc diffusion or Kirby-Bauer assay (Hudzicki, 2009).

Qualitative analysis of phytochemical constituents in methanolic extract was done as per Harborne (1973). For Kirby-Bauer disk diffusion susceptibility test, transferred the bacterial organisms (*E. coli*, *Klebsiella sp.* and *S. aureus*) to a broth (sterile saline). Took a swab, dipped it in the broth and inoculated Mueller Hinton agar (MH agar) by swabbing in three different directions. Placed antibiotic discs impregnated with Tetracycline, Cephalexin and Oxytetracycline on the agar. Inverted and incubated the agar plate for 16-18 hours at 37°C. During incubation, drug diffused into agar. Zones were measured to determine whether the organism is susceptible, intermediate, or resistant to the drug. Antibacterial activity of methanolic extract (20µl, 100mg/ml) of *O. indicum* alone and in combination with antibiotic discs containing Tetracycline, Cephalexin and Oxytetracycline was studied. 20µl of autoclaved distilled water was used as negative control. Biofilm production of bacteria was studied by Congo red assay. Congo red agar was prepared as per the method described by Mariana *et al.* (2009).

Results and Discussion

Phytochemical Analysis

Present research was mainly aimed to find the antibacterial effect of methanolic extract of *O. indicum* leaves on resistant *E. coli*, *Klebsiella sp.* and *S. aureus* isolates from bovine mastitis samples. Phytochemical analysis of methanolic leaf extract of *O. indicum* showed positive reaction for the presence of alkaloids, phenolic compounds, tannins and flavonoids whereas negative reaction for the presence of steroids, glycosides, diterpenes, triterpenes and saponins was observed. These findings are in agreement with that of Asmaliyah *et al.* (2016) who reported that the aqueous extract of leaves of *O. indicum* had high tannin content and hence have antiseptic, antimicrobial and antiviral potentials. Ramaswami *et al.* (2014) reported that the preliminary phytochemical screening of crude extracts of leaf and petiole of *O. indicum* revealed many bioactive substances and hence can be used in preventing many major diseases.

Kirby-Bauer Disk Diffusion Susceptibility Test

When 20 μ l of methanolic leaf extract of *O. indicum* alone at a concentration of 100mg/ml was used in disc diffusion test, zone of inhibition could not be identified against *E. coli*, *Klebsiella sp.* and *S. aureus* (Fig. 1). This indicated that methanolic leaf extract of *O. indicum* alone did not have antibacterial activity against *E. coli*, *Klebsiella sp.* and *S. aureus*. When 20 μ l of methanolic leaf extract of *O. indicum* at a concentration of 100mg/ml was added to antibiotic discs containing Tetracycline, Oxytetracycline and Cephalexin, no zone of inhibition was noticed against *E. coli* (Fig. 2) indicating that along with antibiotics also the extract had no antibacterial activity against *E. coli*. The zone of inhibition shown by Tetracycline, Oxytetracycline and Cephalexin against *Klebsiella sp.* was 16mm, 06mm and 18mm, respectively.

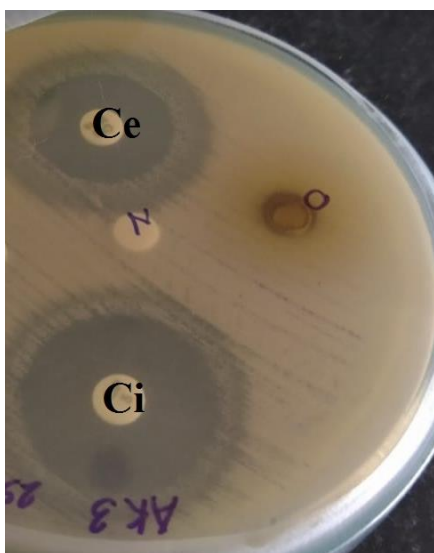


Figure 1: *Oroxylum indicum* extract (O) alone showing no antibacterial activity against *S. aureus*. N- Negative control, Ci- Ciprofloxacin, Ce-Cephalexin

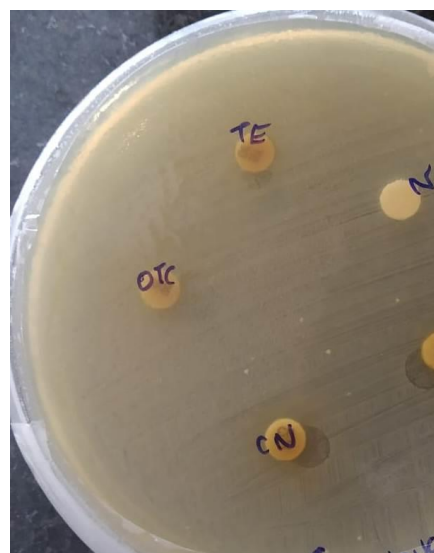


Figure 2: *Oroxylum indicum* (O) extract with antibiotics showing no antibacterial activity against *E. coli*. N- Negative control, CN- Cephalexin, OTC- Oxytetracycline, TE-Tetracycline

But when 20 μ l of methanolic leaf extract of *O. indicum* at a concentration of 100mg/ml was added to antibiotic discs containing Tetracycline, Oxytetracycline and Cephalexin with a micropipette, the zone of inhibition increased to 25mm, 10mm and 12mm, respectively (Fig. 3). This indicated that the methanolic leaf extract of *O. indicum* has moderate anti-bacterial potentiating activity against *Klebsiella sp.* when used along with Tetracycline and mild anti-bacterial potentiating activity against *Klebsiella sp.* when used along with Oxytetracycline. The zone of inhibition shown by the antibiotics *viz.* Tetracycline, Oxytetracycline and Cephalexin against *S. aureus* was 13mm, 12mm and 26mm, respectively. When disc diffusion test was done using 20 μ l of methanolic leaf extract of *O. indicum* at a concentration of 100mg/ml along with antibiotics *viz.* Tetracycline, Oxytetracycline and Cephalexin, the zone of inhibition against *S. aureus* was increased to 20mm, 20mm and 34mm, respectively (Fig. 4). This indicated that methanolic leaf extract of *O. indicum* has potentiating antibacterial activity against *S. aureus* when used along with

antibiotics *viz.* Tetracycline, Oxytetracycline and Cephalexin.

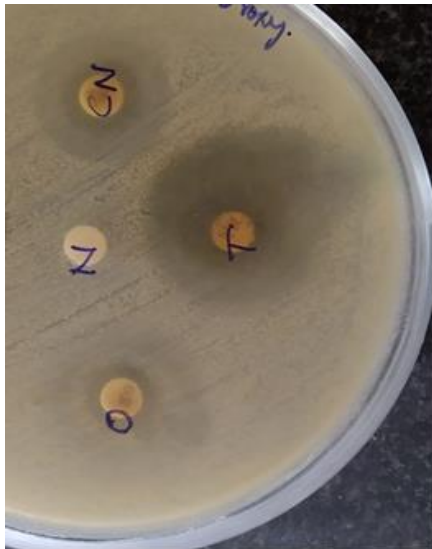


Figure 3: *Oroxylum indicum* extract with antibiotics showing mild antibacterial activity against *Klebsiella* sp. N- Negative control, CN- Cephalexin, O- Oxytetracycline, T- Tetracycline, A- Amoxicillin

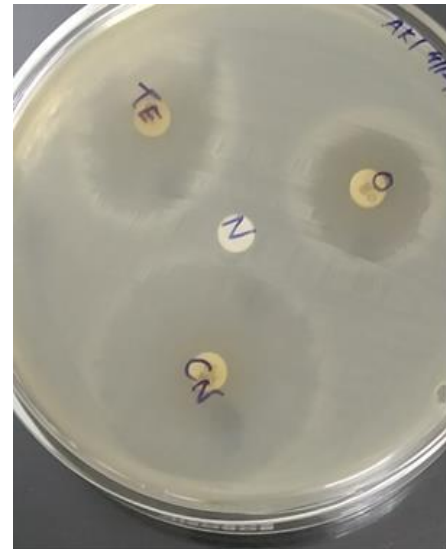


Figure 4: *Oroxylum indicum* extract (O) with antibiotics showing potentiating antibacterial activity against *S. aureus*. N- Negative control, CN- Cephalexin, O- Oxytetracycline, TE-Tetracycline

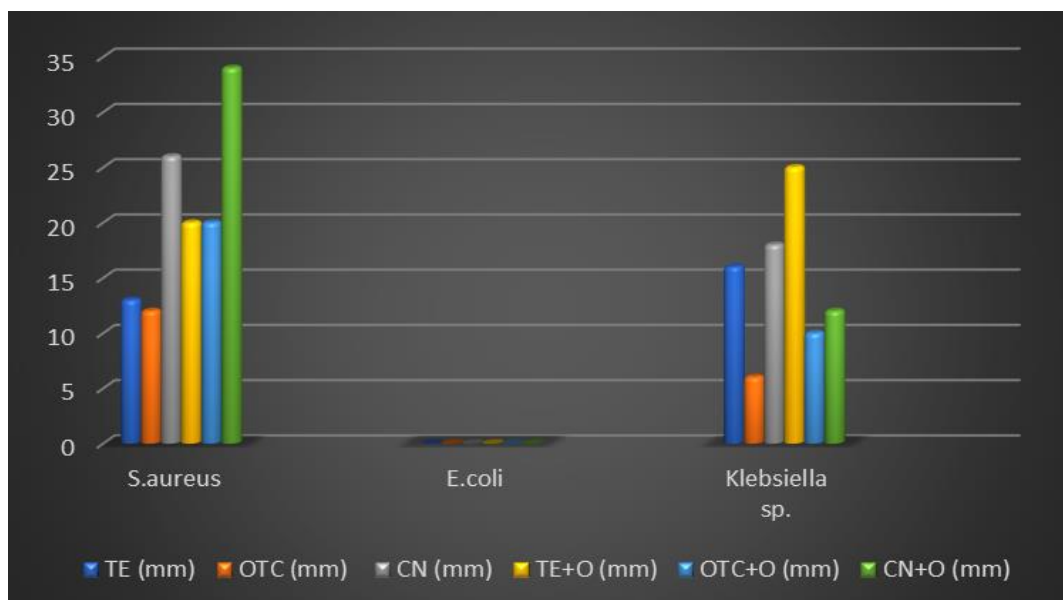


Figure 5: Graph showing antibacterial effect of antibiotics alone and combination of antibiotic with *Oroxylum indicum* extract against bacterial isolates. TE-Tetracycline, OTC-Oxytetracycline, CN-Cephalexin, O- *Oroxylum indicum* extract

Findings of the present study are in partial agreement with those of Zaghoul *et al.* (2015) who observed that hydro distilled essential oil of fresh leaves of *O. indicum* L. showed weak antimicrobial activity against Gram-positive bacteria *viz.* *S. aureus* and *Bacillus subtilis*, and Gram-negative bacteria *viz.* *E. coli*. Sabran (2016) found that the methanolic solvent extracts of *Oroxylum indicum* leaves exhibited antimicrobial activity and could be used in the treatment of highly resistant bacteria by increasing the concentration of leaves extract. Similar to the results of present study, Amancharla and Priyadarsini (2017) reported that the methanolic and ethanolic extracts from *O. indicum* leaves showed antimicrobial effects against clinically isolated bacteria, *S. aureus* MTCC 737 at the concentration of 1000 mg/mL with an inhibition zone of 15.9 mm and 14.1mm, respectively. Eswari *et al.* (2018) noticed that methanolic extract from the leaves of *Oroxylum indicum* were active against *P. aeruginosa*, a Gram-negative microorganism and *B. subtilis*, Gram-positive bacteria and can be used as a potential antibacterial agent.

Congo Red Assay

Biofilm production was not detected and the bacterial isolates containing *E. coli*, *Klebsiella sp.* and *S. aureus* produced pink colonies.

Conclusion

In the present study, methanolic leaf extract of *Oroxylum indicum* alone when used in disc diffusion test at a concentration of 100mg/ml, did not show any antibacterial activity against *E. coli*, *Klebsiella sp.* and *Staphylococcus aureus*. When the extract was added to antibiotic discs containing Tetracycline, Oxytetracycline and Cephalexin, moderate anti-bacterial potentiating activity was noticed against *Klebsiella sp.* and potentiating antibacterial activity was observed against *Staphylococcus aureus* whereas no antibacterial activity was observed against *E. coli*. Hence, it can be concluded that methanolic leaf extract of *O. indicum* can be used as a promising antibacterial potentiating agent along with Tetracycline, Oxytetracycline and Cephalexin in the treatment of mastitis caused by *S. aureus*.

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Conflict of Interests

There is no conflict of interest.

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