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***In vitro* antibacterial activity of *annona squamosa* and cow urine ark against *Staphylococcus aureus* isolated from contaminated wounds of dogs**

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Abstract

In the present study, The *In vitro* antibacterial activity of *Annona squamosa* and cow urine ark was evaluated against *Staphylococcus aureus* isolated from contaminated wounds of dogs. The dried antimicrobial disc of *Annona squamosa* and cow urine ark along with known antibiotic Ciprofloxacin (Ci) were simultaneously placed on muller hinton agar plate that contain isolated *Staphylococcus aureus* for antibiotic sensitivity. *Annona squamosa* showed the zone of inhibition against the isolated *Staphylococcus aureus* but the zone of inhibition was lesser than standard antibiotic ciprofloxacin however, zone of inhibition was not shown by cow urine ark.

Keywords: *Annona squamosa*, cow urine ark, *Staphylococcus aureus*, Ciprofloxacin

Introduction

In various indigenous and traditional sources of medicine, plants have been extensively used for treatment. Various parts of plants such as leaves, fruits, bark, root and even the seeds are being used for preparation of medicine. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of these problematic bacterial infections (Iwu *et al.*, 1999)^[7]. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs (WHO, 2002)^[20]. Many plants have been used because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant (Medina *et al.*, 2005 and Romero *et al.*, 2005)^[11, 13]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Duraipandiyan *et al.*, 2006 and Djeussi *et al.*, 2013)^[5, 4].

Annona squamosa commonly known as "sitaphal" is also been extensively used as traditional medicine in various cultures. The leaves of the plants have been used as insecticide, anthelmintic, styptic and externally used as suppurant (Saha, 2011)^[14].

The cow (Kamadhenu) was adored and considered the 'best wealth' of mankind. In Sushrut Samhita, several medicinal properties of cow's urine have been mentioned and cow urine was known to cause weight loss and to cure leprosy, cardiac and kidney problems, indigestion, stomach ache, edema etc. This kind of alternative treatment is termed as 'panchgavya therapy' or 'cowpathy' (Kaviratna and Sharma, 1996)^[9]. Cow urine based preparations possess powerful antimicrobial, antiviral, antiallergic and antioxidant activities (Shivkumar *et al.*, 2011)^[17].

Material and Methods

The proposed work was conducted in the department of veterinary pharmacology and toxicology in collaboration with department of veterinary microbiology and teaching veterinary clinical complex (T.V.C.C), College of veterinary science and animal husbandry, N.D.V.S.U., Jabalpur (M.P).

The dogs presented with contaminated wound at TVCC, College of Veterinary Science and Animal Husbandry, Jabalpur were selected for the present study.

Preparation of antimicrobial disc

Sterile discs were soaked in aqueous extract of leaves of *Annona squamosa* (10 per cent) and cow urine ark for twenty four hours and dried in Laminar flow chamber. After drying, the

discs were used immediately for disc impregnation in the inoculated plates (Kirubaharan *et al.*, 1999).

Antibacterial test

The isolated bacterial inoculums were evenly spread on a sterile Mueller Hinton agar plate as per method described by Bauer *et al.* (1969)^[2]. The dried antimicrobial disc of *Annona squamosa* and cow urine ark along with known antibiotic Ciprofloxacin (Ci) discs were simultaneously placed on agar plate for antibiotic sensitivity. The petri plates were incubated at 37°C for 24 hrs. Result was recorded as positive (growth) or negative (no growth) and zone of inhibition of growth exerted by these impregnated discs.

Results and Discussion

Annona squamosa and cow urine ark were evaluated for *In vitro* antibacterial activity against *Staphylococcus aureus* isolated from contaminated wounds of dogs. At first, *Staphylococcus aureus* was isolated and identified from contaminated wounds of dogs presented at TVCC, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P). Further the antibacterial activity of indigenous plant *Annona squamosa* and cow urine ark was evaluated against isolated *Staphylococcus aureus*.

In vitro antibacterial activity

The *In vitro* antibacterial activity of *Annona squamosa* and cow urine ark was evaluated against isolated *Staphylococcus aureus* by antibiotic sensitivity test. Zone of inhibition was observed around the *Annona squamosa* impregnated disc which indicated that the *Annona squamosa* posses antibacterial activity against the isolated *Staphylococcus aureus* (Plate 01). The findings are in agreement with Patel and Kumar (2008)^[12]; Yushau *et al.* (2011)^[21] and Biba *et al.* (2013)^[3]. However, Gowdhami *et al.* (2014)^[6] reported *In vitro* antibacterial activity of various solvent extracts of leaves and seeds of *Annona squamosa* against *Escherichia coli*, *Vibrio cholera*, *Salmonella typhi*, *Salmonella paratyphi*, *Klebsiella pneumoniae* and *Proteus mirabilis*. Salman and Kumar (2015)^[15] reported the *In vitro* antibacterial activity of methanolic extract of *Annona squamosa* against *Streptococcus mutans* and *Streptococcus sobrinus*. Simon *et al.* (2016)^[18] observed the *In vitro* antibacterial activity of *Annona squamosa* against *Escherichia coli* and *Pseudomonas aeruginosa*.

In the present study, the zone of inhibition was not observed around the cow urine ark impregnated disc which indicated that the cow urine ark is not having antibacterial action against the isolated *Staphylococcus aureus*. The finding is in agreement with Shah *et al.* (2011)^[16]. However, Jarald *et al.* (2008)^[8] and Ahuja *et al.* (2012)^[1] tested cow urine and its distillate for *In vitro* antibacterial activity against *Staphylococcus aureus* and reported that fresh cow urine was comparatively better than its distillate. According to Stephany (1978)^[19], the possible reasons for resistance in antibacterial activity of cow urine distillate may be due to removal of toxic components from it or absence of biogenic volatile inorganic and organic compounds. It may also be due to presence of more cations and formation of nitrosoamines. The antibacterial properties of the active plants may be due to the presence of different bioactive chemical agents in the extracts, which are known to act by a different mechanism to exert an antibacterial action.

Finally, the results of this study clearly elucidate the

antibacterial potential of *Annona squamosa* and provide an evidence to support its use in folk medicine. The positive findings from this study provide a scientific basis for the traditional use of *Annona squamosa* for treatment of skin and wound infections.

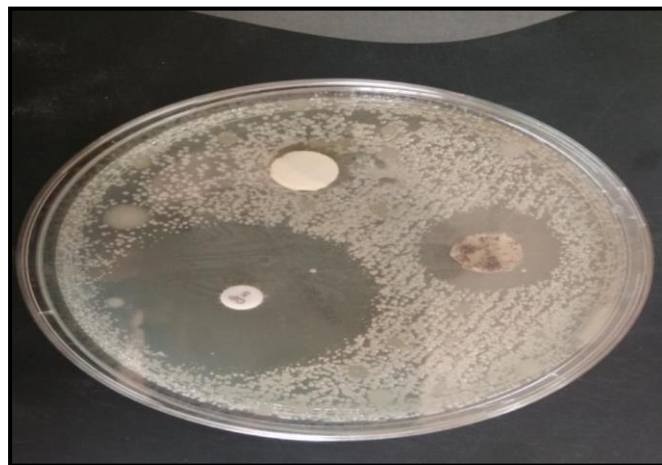


Plate 1: *In vitro* antibacterial activity of *Annona squamosa* and cow urine ark against bacteria isolated from contaminated wounds of dogs

Conclusion

In vitro antibacterial activity of *Annona squamosa* was observed against isolated *Staphylococcus aureus* but it was not shown by cow urine ark. *Annona squamosa* exhibited good antibacterial activity against isolated *Staphylococcus aureus*. However, cow urine ark did not show *In vitro* antibacterial activity.

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