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Research Article

IN VITRO ANTIBACTERIAL EFFICACY OF AQUEOUS AND METHANOLIC EXTRACTS OF *DALBERGIA SISSOO* (SHISHAM) LEAVES AGAINST *E. COLI* ISOLATES FROM DIARRHOEIC CALVES

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ABSTRACT

The present study was undertaken to evaluate the *in vitro* antibacterial activity of the aqueous and methanolic extracts of *Dalbergia sissoo* leaves against *E. coli* isolated from faecal samples of diarrhoeic calves affected with colibacillosis. Antibacterial activity was determined by using agar cup method in terms of zone of inhibition measured as the diameter of the clear zone around well where no bacterial growth was observed. Results showed that both aqueous and methanolic extracts of *Dalbergia sissoo* leaves have significant antibacterial activity against *E. coli* but the activity of methanolic extract was found to be higher than that of aqueous extract at varying concentrations ranging from 1000 mg/ml to 62.5 mg/ml. The maximum zone of inhibition was observed of 24 mm diameter by using methanolic extract at concentration of 1000 mg/ml.

Keywords: Antibacterial activity, Colibacillosis*, Dalbergia sissoo, E. coli*

The demand for plant based therapeutics is increasing in both developing and developed countries due to the growing recognition of ethno veterinary medicine. As they are natural products, non-narcotic, easily biodegradable, minimum environmental hazards, have no adverse side effects and are easily available at affordable prices. *Dalbergia sissoo*, well known as Tali, Shisham and Indian rosewood-a rapid-growing, erect deciduous tree indigenous to the Indian subcontinent, has an important place in the Indian traditional systems of medicine and Ayurveda (Sharma, 2013). The previous studies revealed that *Dalbergia sissoo* possess diverse medicinal properties and is used to cure various ailments and diseases including diarrhoea and dysentry in animals and humans (Kalaskar *et al.*, 2010; Mehesare *et al.*, 2017 and Wankhede *et al.*, 2019). *Dalbergia sissoo* showed good antibacterial activity against causative organisms of diarrhoea *viz. E. coli*, *Salmonella* and *Shigella* spp. (Prasad *et al.*, 2014; Pandey, 2020 and Rathore, 2020). For pathogenic *E. coli* being one of the most common causes of diarrhoea in new born calves, the present study was conducted to comparatively evaluate the antibacterial activity of aqueous and methanolic extracts of *Dalbergia sissoo* (Shisham) leaves against *E. coli* isolates from diarrhoeic calves affected with colibacillosis.

MATERIALS AND METHODS

Collection, extraction and formulation of plant materials: Leaves of *Dalbergia sissoo* were collected from different places of Rajasthan and were identified and authenticated from Arid Zone Regional Centre (AZRC), Jodhpur of Botanical Survey of India vide reference no. BSI/AZRC/I.12012/Tech./2019-20(Pl.Id.)/712, dated 17.03.2020. Leaves were air dried, made powder and extracted by using water and methanol as solvent separately for 24 hours 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 aqueous and methanolic extracts were then stored in air tight jars at 4 ºC till further use.

***In vitro* antibacterial activity of *Dalbergia sissoo* leaves extracts:** The antibacterial activity of aqueous and methanolic extracts was evaluated by using agar cup method as described by Cruickshank *et al.* (1975).

Test bacteria and preparation of stock inoculums: The antibacterial activity of extracts of *Dalbergia sissoo* leaves was tested against *Escherichia coli* culturally isolated from the faecal samples of 32 diarrhoeic calves below one month of age affected with colibacillosis which were confirmed on the basis of multiplex polymerase chain reaction for presence of K99, Stx1 and eaeA genes. For stock inoculums, microorganisms were taken and streaked on Eosin Methylene Blue (EMB) sterile agar plates in such a manner that individual colony could develop. After incubation at 37 ºC for 24 hour, colonies of the test bacteria were taken in 5 ml of sterilized nutrient broth and incubated at 37 ºC for 8 to 12 hours to obtain log phase of *E. coli* for antimicrobial activity. The tubes showing obvious turbidity represented stock inoculums and were kept in refrigerator at 4 ºC for further use within permissible time limit of 12 hours.

Test dilution of herbal extracts: Dilution of sterilized aqueous and methanolic extracts were prepared by dissolving prepared extract in triple glass distilled water by serial dilution method to yield different concentration from 1000 mg/ml to 62.50 mg/ml.

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Preparation and inoculation of agar plates: Nutrient agar was used as media for testing of *in vitro* antibacterial activity of aqueous and methanolic extracts of leaves of *Dalbergia sissoo* alone against *Escherichia coli* isolates using standard agar cup method as described by Cruick Shank *et al.* (1975). Stock inoculums of test bacterium were swept over the nutrient agar plates using a sterile cotton swab, and plates were air dried for 5 minutes. Five equidistant wells of size 6 mm were cut into the agar. 100 µl of different concentration of extracts were poured into different wells. Plates were incubated at 37 ºC for 24 hrs and zones of inhibition were measured in millimeter (mm). The final values were taken as mean ± S.E of the recorded observations.

RESULTS AND DISCUSSION

The average zone of inhibition of aqueous extract of leaves of *Dalbergia sissoo* against *E. coli* were 21.75±0.32 mm, 17.75±0.32 mm, 15.5±0.20 mm, 11.88±0.24 mm and 7.62±0.24 mm at concentrations of 1000 mg/ml, 500 mg/ml, 250 mg/ml 125 mg/ml and 62.5 mg/ml, respectively (Fig. 1). The average zones of inhibition of methanolic extract were 24.50 ± 0.20 mm, 19.63 ± 0.24 mm, 16.63 ± 0.24 mm, 12.88 ± 0.13 mm and 8.25 ± 0.14 mm at concentrations of 1000 mg/ml, 500 mg/ml, 250 mg/ml 125 mg/ml and 62.5 mg/ml, respectively (Fig. 2). The maximum and minimum zone of inhibition for aqueous extract was ~21 and ~8 mm of diameter at concentrations of 1000 mg/ml and 62.5 mg/ml, respectively. The maximum and minimum zone of inhibition for methanolic extract was ~24 and ~8 mm of diameter at concentrations of 1000 mg/ml and 62.5 mg/ml, respectively.

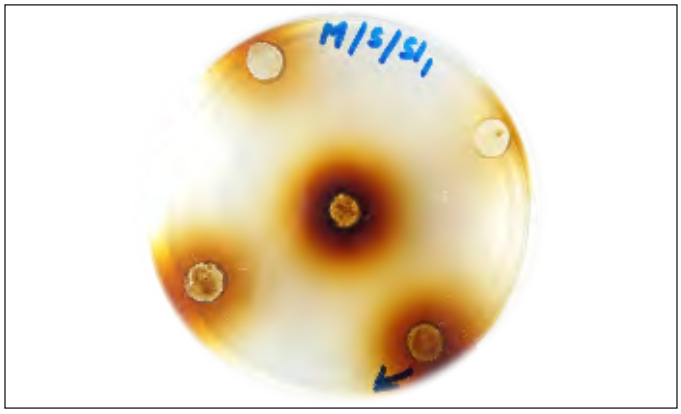

Fig. 1. Zone of inhibition of aqueous extract of *Dalbergia sissoo* against *E. Coli* isolate

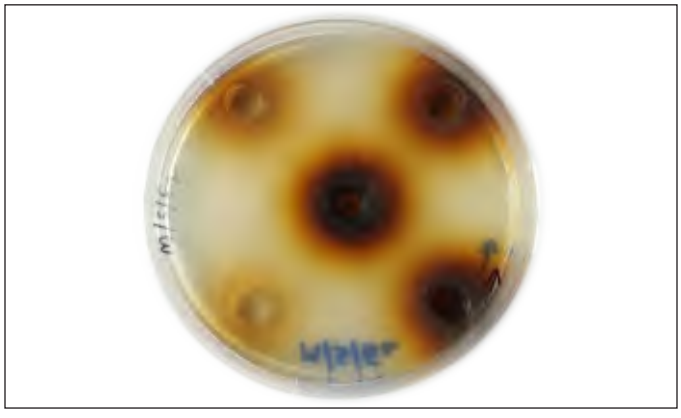

Fig. 2. Zone of inhibition of methanolic extract of *Dalbergia sissoo* against *E. coli* isolate

The result clearly showed that the extracts of *Dalbergia sissoo* leaves exhibited significant antibacterial activity against *E. coli* isolated from faecal samples of colibacillosis affected diarrhoeic calves. Findings in the present study were in accordance with Brijesh *et al.* (2006), Malik *et al.* (2011), Aly *et al.* (2013), Behera *et al.* (2013), Prasad *et al.* (2014), Parmar and Johari (2014), Al-Snafi (2016) and Al-Snafi (2017) who also reported significant antimicrobial activity of *Dalbergia sissoo* against *E. coli*. The *Dalbergia sissoo* leaves contain many functional, biological and pharmacological active compounds such as alkaloids, coumarins, flavonoids, carotenoids, terpenoids, phenolics and antioxidants. Behera *et al.* (2013) reported that chalcone [(E)-3-(3,4-dihydroxyphenyl)-1-(2,3,4-trihydroxyphenyl) prop-2-en-1-one] or okanin isolated from methanolic extract of *Dalbergia sissoo* leaves exhibited good antibacterial activity against *E. coli*.

Based on average zone of inhibition, at varying concentrations ranging from 62.5 mg/ml to 1000 mg/ml, the *in vitro* antibacterial activity of methanolic extract of *Dalbergia sissoo* leaves was found higher as compared to respective concentrations of aqueous extract against *E. coli* isolates from diarrhoeic calves. In the present study, the maximum zone of inhibition was 24 mm in diameter of methanolic extract at concentration of 1000 mg/ml. Our findings were in agreement with that of Prasad *et al.* (2014) who also reported higher antibacterial potential of alcoholic extract of the *Dalbergia sissoo* as compared to aqueous extract against pathogenic *Escherichia coli* of clinical origin. Essawi and Srour (2000) reported that methanolic extract was more effective as compared to aqueous extract because chemical constituents which are either polar or non polar can be effectively extracted only through the organic solvent medium.

Results of the present study are encouraging to evaluate the *in vivo* therapeutic efficacy of these extracts in clinical cases of colibacillosis in calves. Further, phytochemical studies for identification and elucidation of active constituents in the *Dalbergia sissoo* leaves could be used in the development of novel bioactive antimicrobial compounds.

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