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Inhibitory Effects of Acaciasides Isolated from the Funicles of *Acacia auriculiformis* on the growth of *Escherichia coli*

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ABSTRACT

The present study was carried out to establish the effect of acaciaside on Gram positive and Gram negative bacterial community especially to *Escherichia coli*. The inhibitory effect of acaciaside on growth of typical intestinal gram negative pathogen *E.coli* was identified. The degree of inhibition was measured by well disc assay method. In recent days, antimicrobial resistance has become a great global threat to public health systems worldwide. Bacteria pose the greatest threat to human health because of its growing resistance to antibiotics are the members of the enterobacteriaceae family, mainly *E.coli*. *E.coli* is an important contaminant of drinking, agricultural, industrial and recreational water which is a major environmental and public health concern. Acaciaside A and acaciaside B were isolated individually from the funicles of *Acacia auriculiformis*. The mixture of these two acylated triterpenoid bisglycoside saponins are known to have antihelminthic and antimicrobial activity. Here antibacterial activity of the individual compound has been investigated.

Due to continuously increasing number of infections caused by multidrug-resistance *E.coli* as they are transmitted through fecal-oral route among humans and from other environmental sources, the better understanding of the epidemiology of this strain and their mechanism of resistance are key components to cure against their infections. Acaciaside A inhibited the growth of *Escherichia coli, Salmonella typhimurium* and *Bacillus megaterium* at 200, 400 and 600 µg/ml, respectively whereas acaciaside B inhibited the growth of *Pseudomonas aeruginosa* at 600 µg/ml. The present investigation reveals the inhibitory effect produced by acaciaside A or in combination with acaciaside B in *E.coli*, and in comparison with other bacterial strain. By this inhibitory effect of acaciaside which acts as a natural product we can minimize the growth of several species of harmful bacteria. In conclusion, *E.coli* revealed a great deal for its presence in the environment, its diversity as well as its main role in the human microbiome and disease. This findings also outcomes its biology and ecology for better understanding of its growth inhibition.

KEY WORDS: ACACIASIDE A AND B, ANTIHELMINTHIC, ANTIMICROBIAL, INHIBITORY EFFECT, SAPONINS.

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INTRODUCTION

During ancient times, plants are the important source of natural products for maintaining normal human life; they are used as natural therapies. According to World Health Organization (Santos et al.1995) all medicinal plants around the world are of good source to obtain a variety of important drugs. *Acacia auriculiformis* is one of the important therapeutic plant from which many valuable compounds have been isolated. The active principle, which was isolated from the funicles of *A. auriculiformis* contains two triterpenoid saponins, acaciaside A and acaciaside B which have antifilarial activity against *Setaria cervi*. Its native origin is in Australia but came in India in 1946 in West Bengal (Kushalapa 1991). It is also important for making paper, furniture's etc. and has medicinal and forestry importance (Singh et al.2007). The growth of *A. auriculiformis* is very fast generally first year of its development, its growth rate is also high 2-3 m per year even in low fertility soil (Pinyopusarerk 1990) By adding nitrogen to soil and mixed with other species *A. auriculiformis* provides enhanced productivity in early thinning operations (CABI 2013).

Acacia auriculiformis is also known as ear leaf *Acacia* which is an important medicinal plant and widely distributed group of fabaceae (Gijasahnkar 2010). This plant acts as safe and effective substitutes for chemical control against several types of pathogens (Ouassat et al.2020). Many parts of the plant used in traditional medicine such as bark is used as a remedy from rheumatism in Australia, its seeds and roots are used in the treatment of skin diseases and sore eyes respectively (Singh et al. 2007). The plant is also useful as antimalarial drug in many parts of the world as in Nigeria (Okokon et al. 2010). It acts as an antifilarial (Ghosh et al. 1993, Mahato 1996), antioxidant (okokon et al. 2010), antidiabetic (Sathya et al. 2013), antimutagenic (Kaur et al. 2002) compound. Ethylacatate fraction of *A. auriculiformis* was found to be most potent extract which have protein kinase inhibitory activity and another ethylacetate fraction, 3, 4', 7, 8-tetrahydroxyflavone was isolated and it is also a potent inhibitor of DYRK1A and CDK9 which proves the plants anticancer and intiflammatory property (Ahmadu et al. 2019).

Acaciaside generally grows in humid areas and also rich in glucuronic acid, methylglucuronic acid, arabinose, rhamnose and galactose (Anderson 1978). Their antibacterial and antifungal activity was proved in several bacterial and fungal strains. In *Aspergillus ochraceous* and *Curvularia lunata* complete inhibition of conidial germination was recorded at 300 µg/ml or less but in case of *Salmonella typhimurium, Pseudomonas aeruginosa* and *Bacillus megaterium* 700 µg/ml or higher concentrations of the mixture was required for inhibition of their growth (Mandal et al. 2005). *E.coli* the gram negative bacteria which normally resides as intestinal flora in humans, and also acts as an indicator species of fecal contamination to assess the safety and quality of water (US EPA 1986).

Although *E. coli* maintains a friendly environment for its anaerobic neighbors by consuming oxygen that enters the gut (Chang et al. 2004), most of them are harmless to humans but certain strains are pathogenic and causes several types of fatal diseases such as bloody diarrhea, watery diarrhea, meningitis, urinary tract infection, and sepsis, which can lead to human death (Nataro et al. 1998, Gyles et al. 2007). *E.coli* also causes worldwide infections neonatal meningitis, bacteremia and also traveler's diarrhea (Peleg et al. 2010).Urinary tract infections is the most common in many countries causing community and hospital acquired urinary tract infections (gajdacs et al. 2019). A recent WHO report mentioned that medicinal plants around the world are one of the good sources of new important drugs (Efferth 2017). There are several examples of these compounds isolated from many plants that have been proved to be effective as antimicrobial agents.

In this research, antibacterial activity of acaciaside A and acaciaside B or their mixture were performed to investigate the inhibitory effect of the samples obtained from *Acacia* plant on the activity of drug-resistant *E.coli*. This research plays a foundation for the further development of antibacterial agents and also identify the least MIC (minimum inhibitory concentration) value for *E.coli* in comparison to other bacteria for sustainable normal human life.

MATERIAL AND METHODS

Plant material: *Acacia auriculiformis* A.Cunn (Mimosaceae) funicles collected from Santiniketan was authenticated by Department of Botany, Visva-Bharati University. The dried and powered leaf extracts of *Acacia auriculiformis* mixing up with 70% ethanol for 72 hours. Then it is vaccum at 38^{0}C and the extract was stored in a refrigerator at 4^{0}C for future use.

Use as traditional medicine: Different extracts of the plant material are used against filaridis and helminthes (Ghosh et al. 1993, Ghosh et al. 1996). It has also been examined for its spermicidal activity (Pakrashi et al. 1991) and also for fungicidal activities against *Aspergillus ochraceous* and *Curvularia lunata* (Mandal et al. 2005).

Experimental material: Acaciaside A, Acaciaside B and mixture of acaciaside A and acaciaside B obtained from *Acacia auriculiformis* was used in this experiment (Ghosh et al.1993).

Studied activity: Experiment was conducted to study antibacterial activity after treating with sublithal concentration of acaciaside A, acaciaside B and the mixture of acaciaside A and acaciaside B (Mandal et al. 2005).

Used microorganisms in the experiment: Four bacterial strains belonging to both Gram positive and Gram negative categories (Table 1) were procured from Microbial type Culture Collection, Institute of Microbial Technology, Chandigarh, India.

RESULTS AND DISCUSSION

Antimicrobial activity of acaciaside A and acaciaside B and the mixture of acaciaside A and acaciaside B against four bacteria are reported in (Table 1).

Acaciaside A and acaciaside B singly or in mixture produced inhibitory effect on the growth of bacteria tested. However, the minimum inhibitory concentration of the two saponins when tested against bacterial strains appears to differ. Acaciaside A completely inhibited *E.coli* at 200 µg/ml as did the mixture of two saponins, whereas acaciaside B inhibited completely at 600 µg/ml. Similar results were obtained in *S.typhimurium* and *B.megaterium* where acaciaside A singly or in combination of acaciaside B contributed as the major inhibitory substance. It appears from the results that bacteriostatic activity of saponins may be mediated by acaciaside A. Acaciaside B completely inhibited *P. aeruginosa* at 600 µg/ml as did the mixture of two saponins, which indicate that the bacterioststic effect of saponins on *P.aeruginosa* may rest with acaciaside B. Previuosly it was reported that the mixture of two saponins inhibited the growth of *P.aeruginosa* and *S.typhimurium* at 700 µg/ml (Mandal et al. 2005).

Table 1. MIC (minimum inhibitory concentration) value of acaciaside A and acaciaside B against different bacterial strains

Test Compound (µg/ml)	*E.coli* MTCC 68 Gram Negative	*P.aeruginosa* MTCC 741 Gram Negative	*S.typhimurium* MTCC 98 Gram Negative	*B.megaterium* MTCC 1684 Gram Positive
Acaciaside A	200	More than 1000	400	600
Acaciaside B	600	600	600	1000
Acaciaside A+B	200	600	400	600

Here it is suggested that saponins-induced bacteriostatic activity may be strain specific i.e. either Gram +ve or Gram –ve bacteria. In case of *E.coli* it was noticed that their minimum inhibitory treatment dose either by acaciaside A or their combinationis is only 200.When acaciaside A and B applied in combination or acaciaside A singly to the growing culture, they produced almost similar kind of effects on the inhibition of *B. megaterium* cells. Major deviation occurs due to stress induced by the compounds at early phases of sporogenesis process. Change of morphology of different Gram positive and Gram negative bacteria were reported due to change in nutritional conditions or stress in the growth environment an even in the growth phase dependent process (Rasanen 2002, Sawer et al. 2005).

Kumar et al, showed that the ethanolic, ethyl acetate, and water extracts with *Acaciaside* were found be active against certain bacteria such as *E.coli* and fungi. Many evidences till date shows that the extracts obtained from medicinal plants are effective as antimicrobial agents. But no reports have claimed to observe that bacteria developing resistance to plant-based antimicrobials (PBAs) (Cheesman et al. 2017). In the present investigation inhibitory effect produced by acaciaside A, or in combination with acaciaside B in some bacterial cells was clearly established. As inhibition of *P.aeruginosa* is not affected very much by saponins probably a different mechanism is operating in the process of inhibition. But in case of *E.coli* minimum inhibitory concentration was noticeable perfectly.

CONCLUSION

E.coli has a variety of strains that ranging from commensal residents of the gastrointestinal tract to mixed pathogens that are able to create several illnesses. The present investigation aims to discuss the least minimum inhibitory concentration *Acacia auriculiformis* on bacterial population particularly *E.coli*. This plant has many medicinally important phytoconstituents which prove their pharmacological activities. Inhibition of *E.coli* growth by acaciaside A or their combination insights a significant outcome and better understanding in medicinal purposes for future human life.

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