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Antimicrobial Activity of Guggulsterone E and Z

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A B S T R A C T

Guggulsterone is a main active compound of gum guggul, which has commonly used as ant-inflammatory, hepatoprotective, muscle relaxing, anti-arthritic, hypolipidemic, hypocholestrolemic and anti-obesity. In the present study, its antimicrobial activity of guggulsterone E and Z against five microorganisms i.e. *Escherichia coli* (Wild), *E. Cole* (DH5α), *Micrococcus luteus*, *Staphylococcus aureus* and *Bacillus subtilis* were undertaken. Disc diffusion method was used for antimicrobial activity assessment of compound guggulsterone E and Z. The discs were impregnated with 100 and 200 µg per disc and exposed to culture bed of five test organism i.e. *E. coli* (Wild), *E. coli* (DH5α), *M. luteus*, *S. aureus* and *B. subtilis*. After 24 hours of incubation at 37 °C, it was observed that the guggulsterone E and Z did not show any inhibition zone against the all five test organisms.

Introduction

Recently, the continued emergence of bacterial strains resistant to antibacterial drugs has been a serious threat to human lives, these pathogens that are resistant to multiple drugs have been emerged around the globe (Walsh, 2003). Plants are the largest pharmaceutical stores ever known on Earth, being able to produce endless bioactive compounds (Abdallah, 2011). Plants of medicinal benefits are major sources of antimicrobial drugs (Sofowora, 1986). This has led to the screening of medicinal plants for their antimicrobial activities.

Bacterial infectious diseases represent an important cause of morbidity and mortality worldwide. An antibiotic resistant bacterium is a threat which is becoming increasingly common (Chartone-Souza, 1998). The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce

this problem, there is a need, to control the use of antibiotic, and so the antibiotic resistance, to carry out the R&D activities for better understanding of genetic mechanisms of resistance, and to continue studies to develop new drugs for taking care of future challenges (Cowan, 1999; Kadar *et al*., 2011).

Guggulsterone is a main active compound of gum guggul, which has commonly used as ant-inflammatory (Francis *et al.,* 2004), hepatoprotective (Al-Howriny *et al.,* 2004, 2005), muscle relaxing (Allam *et al.,* 2001), anti-arthritic (Chaturvedi and Singh, 1965; Sharma and Sharma, 1977), hy-polipidemic, hypocholestrolemic and anti-obesity (Tripathi *et al.,* 1968; Satyavati *et al.,* 1969; Bhatt *et al.,* 1995)

In the present study, its antimicrobial activity of guggulsterone E and Z against five microorganisms i.e. *Escherichia coli* (Wild), *E. Cole* (DH5α), *Micrococcus luteus, Staphylococcus aureus* and *Bacillus subtilis* were undertaken.

E. coli is a gram-negative, facultative anaerobic, rod-shaped bacterium. It is commonly found in the lower intestine and causes food poisoning by food contamination. *E. coli* is also used as a prokaryotic model organism in the fields of biotechnology and microbiology, where it's used as a host organism for the majority of recombinant DNA related works.

M. luteus belongs to the family *Micrococcaceae*, is a Gram-positive, obligate aerobe, non-motile, spherical, saprotrophic bacterium and found in soil, dust, water and air, mammalian skin, mouth, mucosae, oropharynx and upper respiratory tract. *S. aureus* is gram-positive coccal bacterium and commonly found in the human respiratory tract and on the skin and causes skin infections (boils), respiratory disease (sinusitis) and food poisoning. *B. subtilis* is a gram-positive and naturally found in soil and vegetation. It is rod-shaped with ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions.

Materials and Methods

Antimicrobial activity assay

Disc diffusion method was used for the assay of antimicrobial activity of guggulsterone E and Z (Bauer *et al.,* 1966; Sharma *et al.,* 2009, 2010). The NCCLS (1979) guidelines were followed for antimicrobial assay.*Escherichia coli* (Wild) (MTCC 443), *E. coli* (DH5α), *Micrococcus luteus*, *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* were used as a test organism.

Sample preparation

Guggulsterone E and Zwere procured from Chromadex, USA and used as a sample for antimicrobial activity assay. A stock solution of guggulsterone E and Zin the concentration of 5 mg/ml and 10 mg/ml was prepared in the Dimethylformamide (DMF). Sterile 5 mm diameter filter paper disc (prepared by Whatman filter paper grade 1) was aseptically impregnated with the sample.

In the preparation of guggulsterone E and Z impregnated discs, 20 µl of both the sample concentrations i.e. 5 mg/ml and 10 mg/ml was applied on the separate discs using a micro pipette. In this way, two types of sample discs were prepared having 100 µg and 200 µg of guggulsterone E and Z concentration. The impregnated disc was allowed to air dried aseptically and stored in the sterile micro-vials separately.

Preparation of Luria Bertani agar and broth medium

28 gm of dehydrated Luria Bertani agar medium (Difco), 25 g of the dehydrated Luria Bertani broth medium (Difco), 17 gm of dehydrated Nutrient agar medium (Hi-media) and 14 g of the dehydrated Nutrient broth medium (Hi-media) were suspended in one litre of double distilled water separately. Agar medium was autoclaved at 121 °C and 15 lbs pressure for 15 minutes, cooled to 50-60 °C and poured into sterilized petri-plates aseptically under laminar air flow hood. Broth medium was poured into a test tube and sterilized by autoclaving at 15 lbs pressure with 121 °C temperature for 15 minutes.

Preparation of Mueller Hinton agar medium

38 g of the dehydrated Mueller Hinton agar medium (Hi-media) was suspended in one litre of double distilled water and sterilized by autoclaving at 121 °C and 15 lbs pressure for 15 minutes. After media were cooled up to 50-60 °C it was poured into sterilized petri plates aseptically under laminar air flow.

Culture of bacterial strain

Both *E. coli* strains were maintained in the Luria Bertani agar/broth medium, while *M. luteus, S. aureus* and *B. subtilis* were maintained on Nutrient agar/broth medium. In the preparation of test organism culture plates, petriplates containing Mueller Hinton agar medium were seeded with 24 hours old culture of bacterial strains maintained in the broth medium.

Exposer of impregnated discs to the culture plate

The sterile impregnated discs of sample guggulsterone E and Z were placed aseptically on the surface of seeded plates using a sterile pair of forceps. The plates were incubated at 37 °C for 24 hours and zone of inhibition was observed.

Results and Discussion

Disc diffusion method was used for antimicrobial activity assessment of compound guggulsterone E and Z. The discs were impregnated with 100 and 200 µg per disc and exposed to culture bed of five test organism i.e. *E. coli* (Wild), *E. coli* (DH5α), *M. luteus, S. aureus* and *B. subtilis*. After 24 hours of incubation at 37 °C, it was observed that the guggulsterone E and Z did not show any inhibition zone against the all five test organisms (table 1, figure 1).

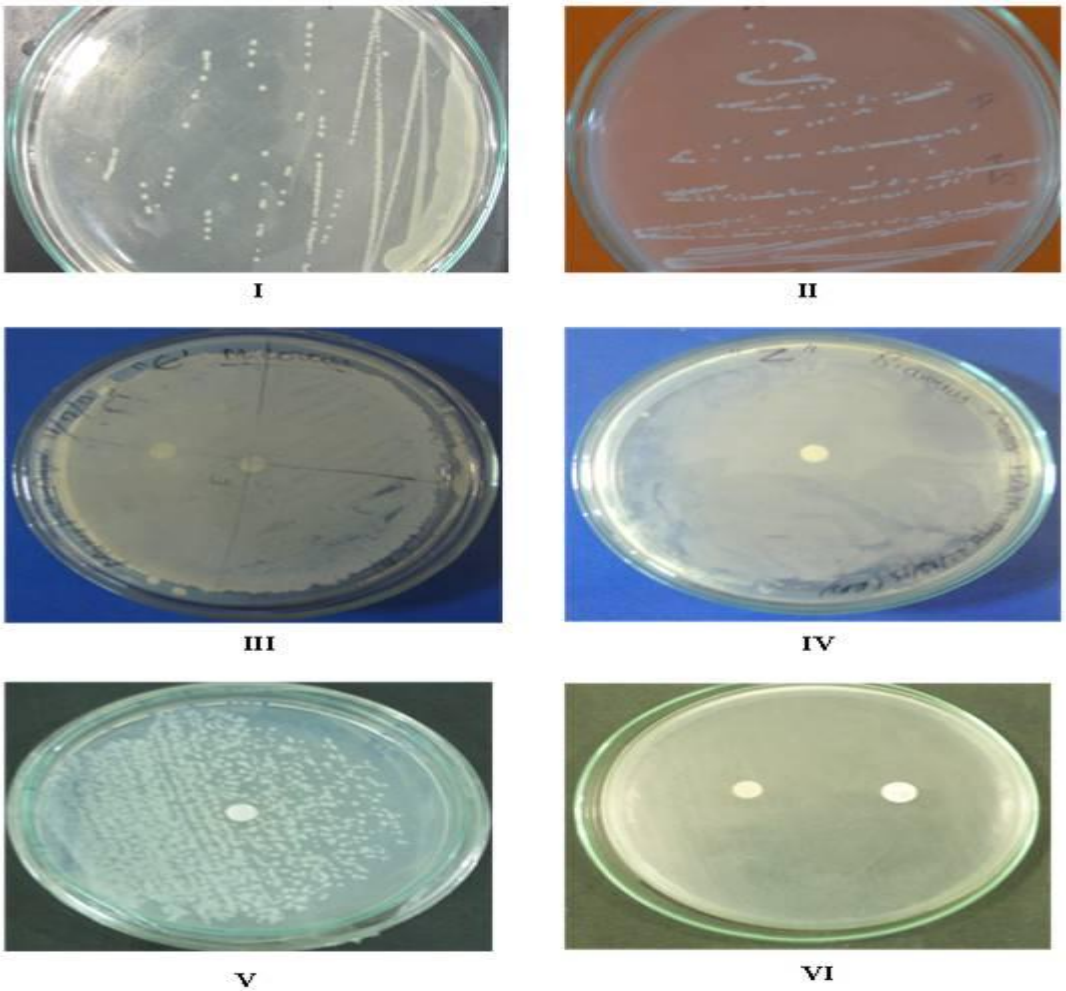
The present experiment revealed that guggulsterone E and Z had no antimicrobial activity against the test microorganisms (two strains of *E. coli* and one strain each of *M. luteus, S. aureus* and *B. subtilis*) even at maximum concentration of 200 µg/disc. This is the first report regarding antimicrobial activity of these compounds. Early reports were describes antimicrobial activity of gum-guggul and plant extract of *C. wightii*.

C. wightii, used traditionally for the treatment of tuberculosis, was assayed for antimycobacterial activity. The crude methanolic resin extract displayed significant antimycobacterial activity, with a minimum inhibitory concentration against *Mycobacterium aurum* (Newton *et al.*, 2002). The isolation and identification of muscanone from *C. wightii*, was found to be antifungal active against *Candida albicans* (Fatope *et al.*, 2003). A wide range of inhibitory activity against Gram-positive and Gram-negative bacteria were observed (Saeed and Sabir, 2004).

Table.1 Antimicrobial assay of guggulsterone E and Z against test organisms

SL	Test organism	Zone of inhibition (mm)			
		Guggulsterone E		Guggulsterone Z	
		100 µg/disc	**200 µg/disc**	**100 µg/disc**	**200 µg/disc**
1	*Escherichia coli* (Wild)	- -	- -	- -	- -
2	*E. coli* (DH5α)	- -	- -	- -	- -
3	*Micrococcus luteus*	- -	- -	- -	- -
4	*Staphylococcus aureus*	- -	- -	- -	- -
5	*Bacillus subtilis*	- -	- -	- -	- -

Fig.1 Antimicrobial assay of guggulsterone E and Z compound



Culture of *Escherichia coli* on LB agar medium; II. Culture of *Staphylococcus aureus* on NA agar medium; III. Antimicrobial susceptibility test of guggulsterone E on *Micrococcus luteus* Culture bad; IV. Antimicrobial susceptibility test of guggulsterone Z on *S. aureus* Culture bad; V. Antimicrobial susceptibility test of guggulsterone E on *E. coli* Culture bad; VI. Antimicrobial susceptibility of guggulsterone Z test on *Bacillus subtilis*Culture bad

Seven Gram negative strains, *Pseudomonas aeruginosa*, *Pseudomonas testosteroni*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Escherichia coli* and *Citrobacter freundii* and five Gram positive strains *Staphylococcus epidermidis*, *Bacillus cereus*, *Streptococcus fecalis*, *Streptococcus cremoris* and *Streptococcus agalactiae* were also screened for antimicrobial activity of ethanolic or aqueous extract of *C. wightii*. Out of which *C. wightii* was showed maximum antibacterial activity against *Streptococcus cremoris* (Nair and Chanda, 2006). Ethanolic or aqueous extract of *C. wightii*, showed considerable antibacterial activity against *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella typhimurium* bacteria (Nair and Chanda, 2007). The potential antibacterial efficacy of guggul gum was checked against six Gram-positive (*Bacillus cereus, Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Micrococcus luteus* and *Enterococcus faecalis*) and four Gram-negative (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi*) strains. Gram-positive bacterial strains were found to be the most susceptible organisms compare to Gram-negative towards guggul gum extract once (Ishnava *et al.,* 2010).

Sharma *et al.,* (2010) screened antibacterial activity of 20 Indian folkoric medicinal plants against nine environmental isolates of *Klebsiella pneumoniae* and found that ethanol extract of *C. wightii* exhibited best antibacterial activity at 5 mg/ml. Goyal *et al.,* (2010) reported a good antimicrobial activity of the extracts of *C. wightii* against six bacterial strains, including both Gram-negative and Gram-positive bacteria (*Escherichia coli, Salmonella typhi, Bacillus cereus, Bacillus subtilis, Streptococcus pyogenes* and *Staphylococcus aureus*). Stem extract of *C. wightii* were screened on seven gram negative strains, and five gram positive strains and out of them *Pseudomonas aeruginosa, Proteus mirabilis, Streptococcus cremoris, Bacillus cereus, Streptococcus fecalis* and *Streptococcus cremoris* shown antimicrobial property (Yadav and Khan, 2012).

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