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## MEDICINAL CHEMISTRY | RESEARCH ARTICLE

# Evaluation of *in vitro* antibacterial activity of *Caralluma lasiantha* for scientific validation of Indian traditional medicine

Sireesha Malladi<sup>1,2</sup>, Venkata Nadh Ratnakaram<sup>3\*</sup>, K. Suresh Babu<sup>4</sup> and T. Pullaiah<sup>5</sup>

**Abstract:** *Caralluma lasiantha* is used as a traditional medicine in India to heal body heat and inflammations. In order to find out a scientific validation for the Indian traditional knowledge, antibacterial activity of *C. lasiantha* extracts was studied against inflammation causing bacteria (viz., *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus Sp.*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*) along with other Gram-positive and Gram-negative bacteria. Solvents with different polarity were used for extraction from dry roots and stems. Minimum inhibitory concentrations (MIC) were also studied. Differential antibacterial activity was exhibited by extracts and higher inhibition potential against Gram-positive bacteria was explained. The observed antibacterial activities were correlated with the chemical structures of phytochemicals present in *C. lasiantha*. Anti-inflammation activities are related to *C. lasiantha* extracts through their antibacterial activities.

**Subjects:** Pharmacology; Allied Health; Health & Society

**Keywords:** Indian traditional medicine; inflammation causing bacteria; *Caralluma lasiantha*; antibacterial activity; scientific validation

### 1. Introduction

Nowadays, an increase in awareness on traditional medicine is observed due to development of resistance by micro-organisms toward many synthetic antibiotics. The healing potential of plants is very well known from primeval times. The secondary metabolites produced in plants are responsible for their pharmacological activity (1). As plants provide a number of drugs, herbal products have been used in the healing of an array of ailments (2). The need of hour is the screening of local medicinal plants in view of their richness in antimicrobial agents (3).



Sireesha Malladi

### ABOUT THE AUTHOR

Sireesha Malladi has completed MSc (2005) and MPhil (2008) in Chemistry from Acharya Nagarjuna University, Guntur and Periyar University, Salem, respectively. She is an assistant professor in Chemistry since 2005 in the Department of S&H, Vignana's University, India. Currently, she is on academic leave and pursuing PhD from JNTU, Anantapur in the research area of Natural Products and Organic Synthesis.

### PUBLIC INTEREST STATEMENT

Now a days, plant-based products are gaining much importance due to the reason that micro-organisms are showing resistance day by day toward several synthetic antibiotic drugs. In addition to this, lot of side effects are there due to use of synthetic drugs. *Caralluma* is one of the genus of *Asclepiadaceae* family possessing good pharmacological importance. One of the species of *Caralluma* genus namely *Caralluma lasiantha* was selected to carry out its antibacterial activities to prove the scientific sanctity of its usage in Indian traditional medicine.

*Caralluma* is one of the prominent genus out of 200 genera and 2500 species of Asclepiadaceae family (4, 5). It grows well in dry regions like India, Africa and the Middle East (6). In folkloric medicine, as well as in Unani and Ayurvedic systems of medicine, the plants of *Caralluma* are being used for the treatment of diabetic patients and rheumatism (7). Tribals consider some of them as food during famines (8, 9) and also as a part of traditional medicinal system (10). A spectrum of biological activities of *Caralluma* species can be expected due to the existence of pregnane glycosides (11), stigmaterol and other phytochemicals (12) in them.

*Caralluma lasiantha* (syn. *Boucerosia lasiantha*) belongs to the family Asclepiadaceae and its local name is Sirumankeerai (in Tamil) / Kundeti Kommulu (in Telugu) (13). *C. lasiantha* is succulent in habit and is used as an indoor ornamental plant (14). It grows wild in Anantapur, Chittoor, and surrounding places of Andhra Pradesh, India. This plant is medicinally important as it is rich in pregnane glycosides, which may possess different biological activities (15) including anti-hyperglycemic effect (16). In India, 10 grams of fresh root less plant is taken as such twice a day for a period of 3 days to reduce the body heat and inflammations (17).

However to the knowledge of authors, no scientific validation was conducted for this Indian traditional knowledge of using *C. lasiantha* for the above-discussed medicinal usage. Hence, inflammation causing bacteria (viz., *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* Sp., *Bacillus subtilis*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*) as well as other bacteria are selected in the present investigation in order to determine their susceptibility toward extracts obtained using different solvents having varying polarities. Steroidal glycosides (15, 18), C<sub>21</sub> pregnane steroid (19) and C<sub>27</sub> steroid (20) were isolated using alcohol, chloroform, and n-hexane, respectively, as extracting solvents. The observed antibacterial activity of *C. lasiantha* extracts against those micro-organisms is very well substantiated by relating with different phytochemicals present in it, and also, relation between *C. lasiantha* extracts and anti-inflammation activity was discussed.

## 2. Materials and methods

Analytical Reagent grade chemicals of Merck India Co. Ltd. were used in the present study. Wherever it is necessary, they were purified according to the standard procedures (21). Geological location (22), season (23), and plant collection time (24) influence the chemical composition or active constituents of the plants which play a key role in exhibiting the biological activity by plant extracts. Fresh whole plants of *C. lasiantha* (Asclepiadaceae) were collected from Gooty, Anantapur District, Andhra Pradesh, India in February 2012. Voucher specimen of the plant was deposited in Herbarium, Department of Botany, Sri Krishna Devaraya University, Anantapur. Libermann–Burchard test (25), Molisch test and Shinoda test (26) were used, respectively, to test the presence of steroids, steroidal glycosides, and flavanoids in the extracts of *C. lasiantha*. Bacteria used of the present study were provided by Institute of Microbial Technology (IMTECH), Chandigarh. Antibacterial activity was carried out on four each Gram-positive and Gram-negative bacteria. Gram-positive bacteria are *S. aureus* (MTCC 3103), *B. subtilis* (MTCC 1305), *Streptococcus* Sp., (MTCC 9724), and *Bacillus megaterium* (MTCC 9554). Gram-negative bacteria are *E. aerogenes* (MTCC 8358), *K. pneumoniae* (MTCC 10309), *E. coli* (MTCC 9537), and *Pseudomonas aeruginosa* (MTCC 10636).

### 2.1. Preparation of extract

Roots and stems were dried separately under shade, powdered, sieved (sieve No.14), and stored in air tight containers. The weighed quantity (200 g) of dried powder was subjected to successive solvent extraction method in Soxhlet extractor using solvents with varying polarity (n-hexane, chloroform, and methanol). All the extracts were concentrated and last trace of solvent was removed by applying vacuum (27, 28). The crude extracts were purified by recrystallization.

### 2.2. In vitro screening of antibacterial activity

The *in vitro* antibacterial activity was evaluated for the root and stem extracts of the *C. lasiantha* by agar well diffusion method (29, 30). The medium (prepared by mixing peptone-5.0 g, sodium chloride-5.0 g, beef extract-1.5 g, agar-15.0 g in 1 l of distilled water) (31) was poured in to petridishes

under aseptic conditions in a laminar flow chamber. When the medium in the plates was solidified, 0.1 mL of 24 h old culture of test organism (Gram-positive bacteria such as *Streptococcus* Sp., *B. subtilis*, *S. aureus*, *B. megaterium* and Gram-negative bacteria such as *E. aerogenes*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*) was inoculated. After inoculation, cups were scooped out with 6-mm sterile cork borer and the lids of the dishes were replaced. Different concentrations of extracts (1000, 750, 500, 250 µg/mL) were used for study. Dimethylsulfoxide (DMSO) is maintained as negative control and inhibitions shown by it are negligible. Chloramphenicol and Ampicillin were used as positive controls. The plates were kept in an incubator at 37 °C for 24 h. Inhibition zones were measured to nearest millimeter. MIC was also determined for each bacteria tested (32). The antibacterial activities were carried out in triplicate and average values were compiled.

### 3. Results and discussion

Antimicrobial activity of all the *C. lasiantha* extracts was studied against Gram-positive (*B. subtilis*, *S. aureus*, *Streptococcus* Sp., *B. megaterium*) and Gram-negative (*E. aerogenes*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*) bacteria (Tables 1 and 2; Figures 1 and 2). The salient features of antibacterial analysis are summarized and given below.

- A. Highlights of antibacterial activity of stem / root extracts against Gram-positive bacteria
  - i. *B. megaterium* exhibits inhibition only at higher concentration like 1000 µg/mL
  - ii. Order of antibacterial activity is:  
*Staphylococcus aureus*  $\cong$  *Streptococcus* Sp., > *Bacillus subtilis*  $\geq$  *Bacillus megaterium*
- B. Highlights of antibacterial activity of stem / root extracts against Gram-negative bacteria
  - i. At higher concentration of extracts, order of antibacterial activity is:  
*Enterobacter aerogenes*  $\cong$  *Klebsiella pneumoniae*  $\cong$  *Pseudomonas aeruginosa*  $\cong$  > *Escherichia coli*
  - ii. However compared to other bacteria, *E. coli* is more effective at low concentration like 250 µg/mL
  - iii. *E. aerogenes* is effective only at higher concentrations like 750 and 1000 µg/mL
- C. Effect of polarity of solvent used extraction on antibacterial activity

Against *B. subtilis*, *Streptococcus* Sp., *E. aerogenes* and *E. coli*, the order of biological activity is: n-hexane extract > chloroform extract > methanol extract. Against *S. aureus*, *K. pneumoniae* and *P. aeruginosa*, the order of biological activity is: n-hexane extract < chloroform extract < methanol extract.

- D. Minimum inhibitory concentration (MIC):

These studies were carried out at different concentrations in order to find out MIC (Minimum inhibitory concentration) (Tables 3 and 4) of extracts for antibacterial activity. MIC values for stem extracts are as follows: 250 µg/mL for *B. subtilis* / *S. aureus* / *Streptococcus* Sp., (n-hexane, chloroform, and methanol extracts); 500 µg/mL for *P. aeruginosa* (n-hexane, chloroform, and methanol extracts); 750 µg/mL for *B. megaterium* (n-hexane extract), *E. aerogenes*/*K. pneumoniae* (n-hexane, chloroform, and methanol extracts); 1000 µg/mL for *B. megaterium* (chloroform and methanol extracts). MIC values for root extracts are as follows: 250 µg/mL for *B. subtilis* / *S. aureus* / *E. coli* (n-hexane, chloroform, and methanol extracts), *Streptococcus* Sp (n-hexane, chloroform extracts), *P. aeruginosa* (chloroform extract), 500 µg/mL for *P. aeruginosa* (n-hexane and methanol extracts), *E. aerogenes* (chloroform extract), *K. pneumoniae* (chloroform extract); 750 µg/mL for *B. megaterium* (n-hexane extract), *E. aerogenes* (n-hexane and methanol extracts), *K. pneumoniae* (n-hexane and methanol extracts); 1000 µg/mL for *B. megaterium* (chloroform and methanol extracts).

- E. Inhibition exhibited by plant extracts against Gram-positive bacteria is higher compared to that of Gram-negative bacteria.

**Table 1. Antibacterial activity of crude extracts of *Caralluma lasiantha* Stems**

Solvent used for extraction/ Control	Concentration of extract (µg/ml)	Zone of Inhibition (mm)*									
		Gram-positive organisms					Gram-negative organisms				
		<i>Bacillus subtilis</i> (MTCC 1305)	<i>Staphylococcus aureus</i> (MTCC 3103)	<i>Streptococcus Sp.</i> (MTCC 9724)	<i>Bacillus megaterium</i> (MTCC 9554)	<i>Enterobacter aerogenes</i> (MTCC 8358)	<i>Klebsiella pneumoniae</i> (MTCC 10309)	<i>Escherichia coli</i> (MTCC 9537)	<i>Pseudomonas aeruginosa</i> (MTCC 10636)		
n-Hexane	1,000	20	19	23	14	18	18	17	18	17	18
	750	18	17	18	12	15	16	17	16	17	15
	500	15	15	17	0	0	0	14	0	14	13
	250	13	12	13	0	0	0	12	0	12	0
Chloroform	1,000	17	23	22	18	19	19	16	19	16	19
	750	17	20	18	0	15	10	15	10	15	15
	500	16	17	14	0	0	0	15	0	15	14
	250	12	15	12	0	0	0	12	0	12	0
Methanol	1,000	16	20	20	16	19	15	16	15	16	17
	750	15	19	20	0	16	12	14	12	14	17
	500	13	19	12	0	0	10	14	10	14	15
	250	11	16	10	0	0	0	12	0	12	0
Chloramphenicol	1,000	15	13	14	12	14	15	14	15	14	20
	750	15	12	13	0	10	13	14	13	14	19
	500	0	12	10	0	0	0	10	0	10	0
	250	0	0	0	0	0	0	0	0	0	0
Ampicillin	1,000	20	30	22	16	17	15	22	15	22	50
	750	20	20	18	14	16	15	22	15	22	45
	500	15	20	16	0	15	14	20	14	20	20
	250	0	17	12	0	0	0	18	0	18	0

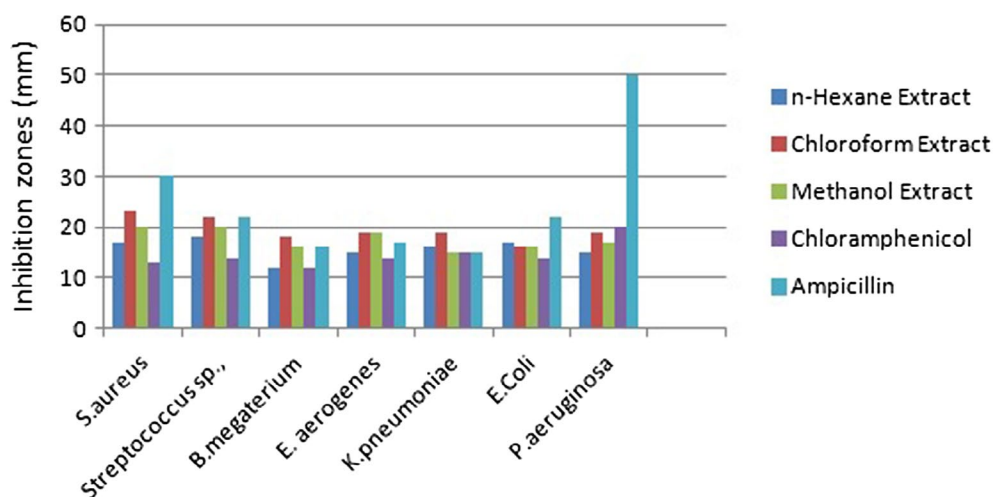
\*indicates average of triplicate.

**Table 2. Antibacterial activity of crude extracts of *Caralluma lasiantha* Roots**

Solvent used for extraction/ Control	Concentration of extract (µg/mL)	Zone of Inhibition (mm)*									
		Gram-positive organisms					Gram-negative organisms				
		<i>Bacillus subtilis</i> (MTCC 1305)	<i>Staphylococcus aureus</i> (MTCC 3103)	<i>Streptococcus Sp.</i> (MTCC 9724)	<i>Bacillus megaterium</i> (MTCC 9554)	<i>Enterobacter aerogenes</i> (MTCC 8358)	<i>Klebsiella pneumoniae</i> (MTCC 10309)	<i>Escherichia coli</i> (MTCC 9537)	<i>Pseudomonas aeruginosa</i> (MTCC 10636)		
n-Hexane	1,000	19	19	18	14	20	17	17	19		
	750	18	19	15	0	20	16	17	17		
	500	14	18	13	0	0	0	16	14		
	250	12	13	0	0	0	0	14	0		
Chloroform	1,000	18	15	25	18	20	23	15	18		
	750	16	13	19	0	14	20	14	15		
	500	16	13	14	0	10	12	14	13		
	250	14	11	10	0	0	0	10	10		
Methanol	1,000	18	21	22	14	19	20	18	17		
	750	17	20	19	10	14	16	14	15		
	500	18	15	12	0	0	0	14	12		
	250	15	13	0	0	0	0	13	0		
Chloramphenicol	1,000	15	13	14	12	14	15	14	20		
	750	15	12	13	0	10	13	14	19		
	500	10	12	10	0	0	0	13	0		
	250	0	0	0	0	0	0	0	0		
Ampicillin	1,000	20	20	22	16	22	15	22	50		
	750	20	20	18	14	17	15	22	45		
	500	15	20	16	14	15	14	20	20		
	250	0	17	12	0	0	0	18	0		

\*Indicates average of triplicate.

Figure 1. Antibacterial activity of crude extracts of *Caralluma lasiantha* Stems.



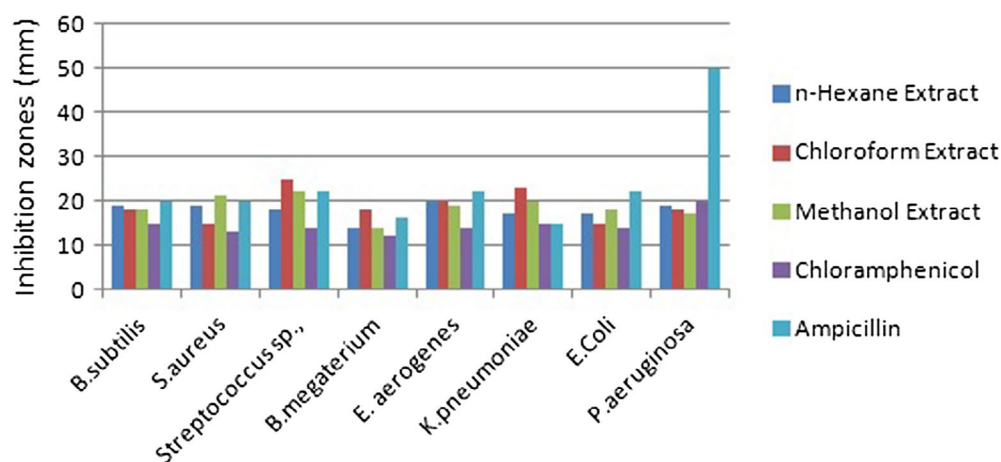
F. Statistical analysis of Figures 1 and 2 was carried out (excel sheets are attached). From the ANOVA test the following conclusions were drawn.

On a particular bacterium, variation in the effect of type of extract is negligible as  $F_{cal} < F_{crit}$  at 5% level of significance and hence, null hypothesis ( $H_0$ ) can be accepted. However, variation in the effect of type of extract on a particular bacteria is not negligible as  $F_{cal} > F_{crit}$  at 5% level of significance and hence, null hypothesis ( $H_0$ ) can be rejected.

### 3.1. Pharmacological activities and phytochemicals of *Caralluma lasiantha*

*C. lasiantha* is a part of Indian traditional medicinal usage to reduce body heat which is an indication of the antibiotic nature (17). So, it can be expected that extracts of *C. lasiantha* may exhibit activity against pathogenic bacteria. In the present study, the crude extracts of stem and roots of *C. lasiantha* were tested against infectious bacteria. The rationality in exhibiting medicinal properties by the extracts of *C. lasiantha* (Tables 1 and 2) can be ascribed to active constituents existing in the extracts. The active constituents isolated from *C. lasiantha* by earlier researchers are steroidal glycosides (lasianthoside-A (Figure 3), lasianthoside-B (Figure 3)) and flavone glycoside (Luteoline-4-O-neohesperidoside Figure 4) (15, 18) using polar solvents like alcohols. In the present study, existence of steroids in the *C. lasiantha* extracts is verified from appearance of to observe red color in Libermann-Burchard test (25). Similarly, existence of steroidal glycosides and flavanoids is confirmed from positive Molisch test and Shinoda test (26) to observe violet color and no reaction,

Figure 2. Antibacterial activity of crude extracts of *Caralluma lasiantha* Roots.



**Table 3. MIC of antibacterial activity of crude extracts of *Caralluma lasiantha* Stems**

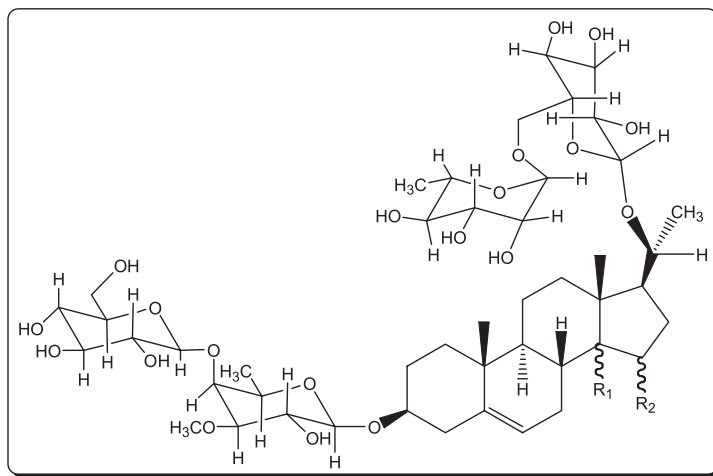
Solvent used for extraction/ Control	MIC of extract (µg/mL)							
	Gram-positive organisms				Gram-negative organisms			
	<i>Bacillus subtilis</i> (MTCC 1305)	<i>Staphylococcus aureus</i> (MTCC 3103)	<i>Strepto coccus</i> Sp. (MTCC 9724)	<i>Bacillus megaterium</i> (MTCC 9554)	<i>Enterobacter aerogenes</i> (MTCC 8358)	<i>Klebsiella pneumoniae</i> (MTCC 10309)	<i>Escherichia coli</i> (MTCC 9537)	<i>Pseudomonas aeruginosa</i> (MTCC 10636)
n-Hexane	250	250	250	750	750	750	250	500
Chloroform	250	250	250	1,000	750	750	250	500
Methanol	250	250	250	1,000	750	500	250	500
Chloramphenicol	750	500	500	1,000	750	750	500	750
Ampicillin	500	250	250	750	500	500	250	500



**Table 4. MIC of antibacterial activity of crude extracts of *Caralluma lasiantha* Roots**

Solvent used for extraction/ Control	MIC of extract (µg/mL)							
	Gram-positive organisms				Gram-negative organisms			
	<i>Bacillus subtilis</i> (MTCC 1305)	<i>Staphylococcus aureus</i> (MTCC 3103)	<i>Strepto coccus</i> Sp. (MTCC 9724)	<i>Bacillus megaterium</i> (MTCC 9554)	<i>Enterobacter aerogenes</i> (MTCC 8358)	<i>Klebsiella pneumoniae</i> (MTCC 10309)	<i>Escherichia coli</i> (MTCC 9537)	<i>Pseudomonas aeruginosa</i> (MTCC 10636)
n-Hexane	250	250	500	1,000	750	750	250	500
Chloroform	250	250	250	1,000	500	500	250	250
Methanol	250	250	500	750	750	750	250	500
Chloramphenicol	750	500	500	1,000	750	750	500	750
Ampicillin	500	250	250	750	500	500	250	500

**Figure 3. Lasianthosides A and B from *Caralluma lasiantha*.**



Lasianthoside-A- $R_1$ ,  $R_2 = \Delta^{14-15}$  and lasianthoside-B- $R_1 = \beta$ -OH,  $R_2 = H$

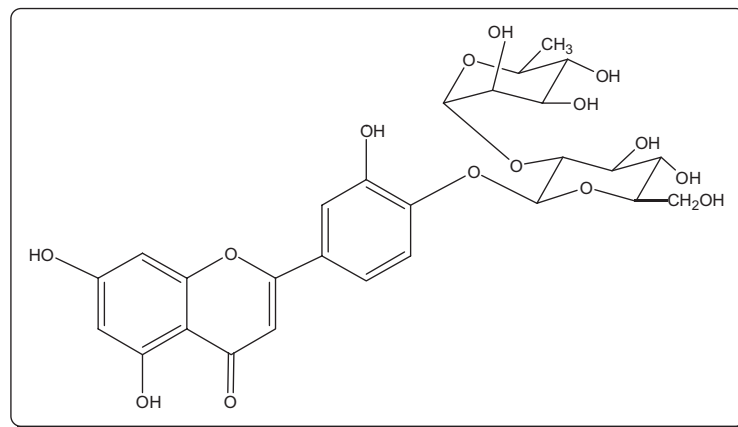
respectively. Based on the polarity, different solvents are capable of extracting different phytochemicals (33). Hence in the present study, different solvents (non-polar to polar) were used.

Nowadays, *Caralluma* is gaining a great importance from scientific community due to exhibition of immunostimulating activities as active constituents like saponins and flavonoids are present in it (34). The major bioactive constituents of Ayurvedic medicine are saponins, flavonoids, and polyphenols (35). Previous investigations on essential medicinal plants disclosed that saponins exhibit good anti-inflammatory activity (36) and antimicrobial activity (37). Literature survey reveals that other species of *Caralluma* show antimicrobial activity due to the existence of tannins, flavonoids, and sterols in them (38). For example, aqueous extracts of *Caralluma adscendens* were efficient against *S. typhi*, *E. coli* and *P. aeruginosa* (39), and petroleum ether extract was effective against *S. aureus* and *E. coli* (40). Antimicrobial activity can be explained based on interactions of saponins present in *C. lasiantha* with the membrane of cells which causes variation in the permeability of cells (37). In addition, the presence of sugar moiety in saponins indicates a higher hemolytic activity (41). Hence, the observed antimicrobial activity of extracts in the present study can be explained based on the existence of steroidal glycosides (lasianthoside-A (Figure 3), lasianthoside-B (Figure 3) in *C. lasiantha* (15).

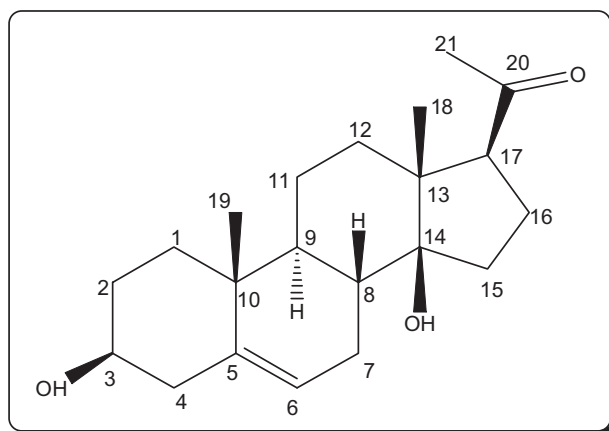
### 3.2. Antibacterial activity due to steroids

In our previous publications, we have reported the isolation and characterization of one  $C_{21}$  pregnane steroid, 3 $\beta$ ,14 $\beta$ -dihydroxy-14 $\beta$ -pregn-5-en-20-one (Figure 5) from chloroform extracts (19) and

**Figure 4. Flavone glycoside (Luteoline-4-O-neohesperidoside).**



**Figure 5.** 3 $\beta$ ,14 $\beta$ -dihydroxy-14 $\beta$ -pregn-5-en-20-one.



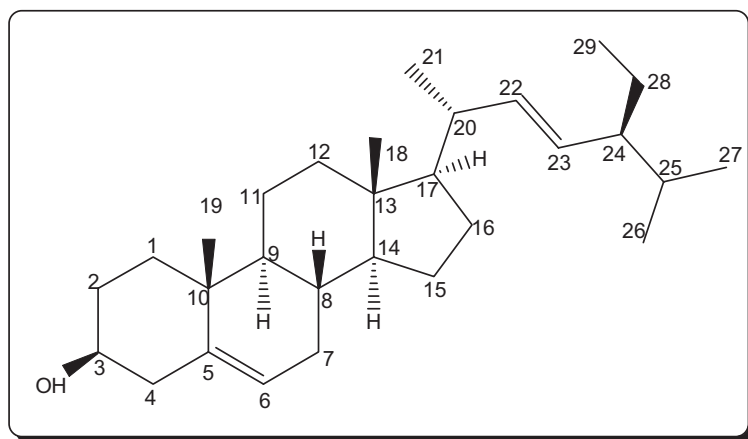
stigmasterol (Figure 6) from n-hexane extracts (20) of *C. lasiantha*. In addition, Qiu et al. (15) and Ramesh et al. (18) reported the isolation of steroidal glycosides from alcohol extracts. Hence from the above observations mentioned under “effect of polarity of solvent used extraction on antibacterial activity”, higher activity observed against *B. subtilis*, *Streptococcus* Sp., *E. aerogenes* and *E. coli* by n-hexane extract can be attributed to stigmasterol. Similarly, steroidal glycosides of methanol extract might be active against *S. aureus*, *K. pneumoniae* and *P. aeruginosa*.

The difference in reactivities of epimers of sterols can be established based on type of conformation of the hydroxyl group located at position-3. Equatorial 3-hydroxyl groups experience low shielding effect compared to axial groups. Hence, esters of equatorial hydroxyl groups hydrolyze easily and hence high reactivity is noticed. Epimers containing equatorial hydroxyl groups form stable complexes and are willingly sorbed on various carriers (42, 43) due to formation of hydrogen bonding. As hydroxyl group is present at position-3 in both C<sub>21</sub> pregnane steroid (3 $\beta$ ,14 $\beta$ -dihydroxy-14 $\beta$ -pregn-5-en-20-one) and stigmasterol, their contribution toward antibacterial activity can be understood as sterols are integrated into biological membrane by forming complexes with primary phospholipids of the membrane (44). The plausible mechanism is depicted in Figure 7.

### 3.3. Antibacterial activity due to flavonoids and flavone glycosides

It is well known that many traditional medicinal plants synthesize bioactive aromatic compounds like saponins, flavonoids, and polyphenols which form the basis for Ayurvedic medicine (35). Flavonoids, flavones, and flavonols demonstrate antimicrobial activity against a range of micro-organisms as these phytochemicals are synthesized in plants due to microbial infection (45). Moreover, antibacterial activity of saponins can be explained by release of strong antibiotic compounds after

**Figure 6.** Stigmasterol.



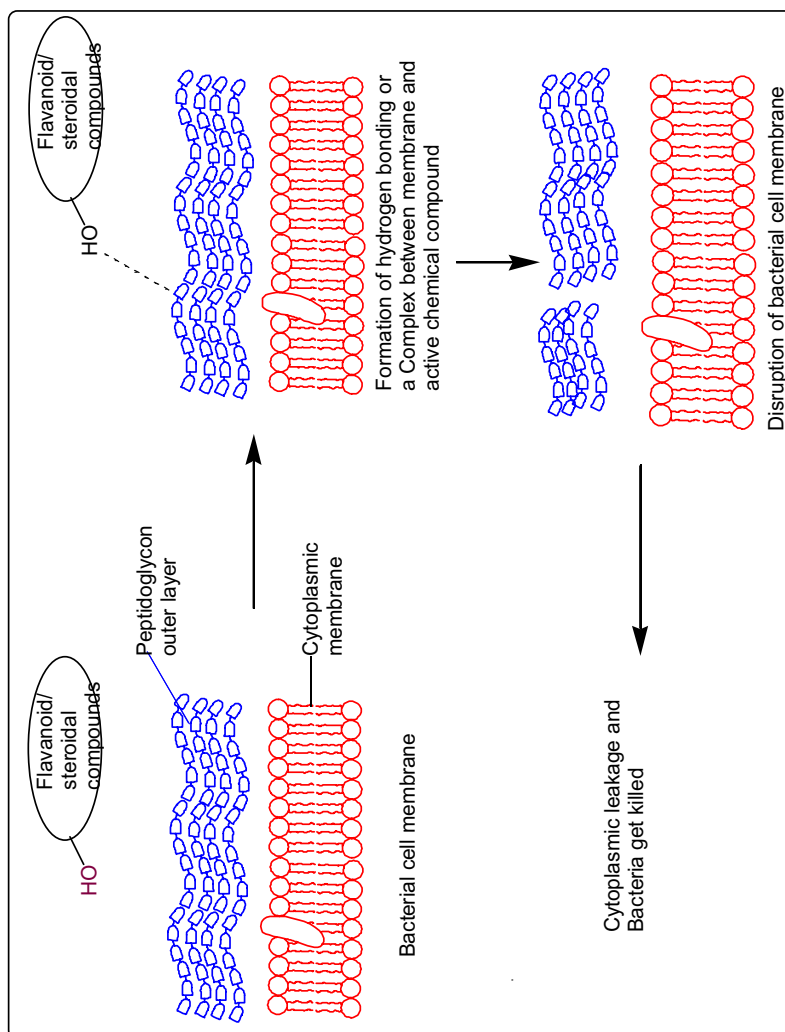


Figure 7. Plausible mechanism for antibacterial activity.

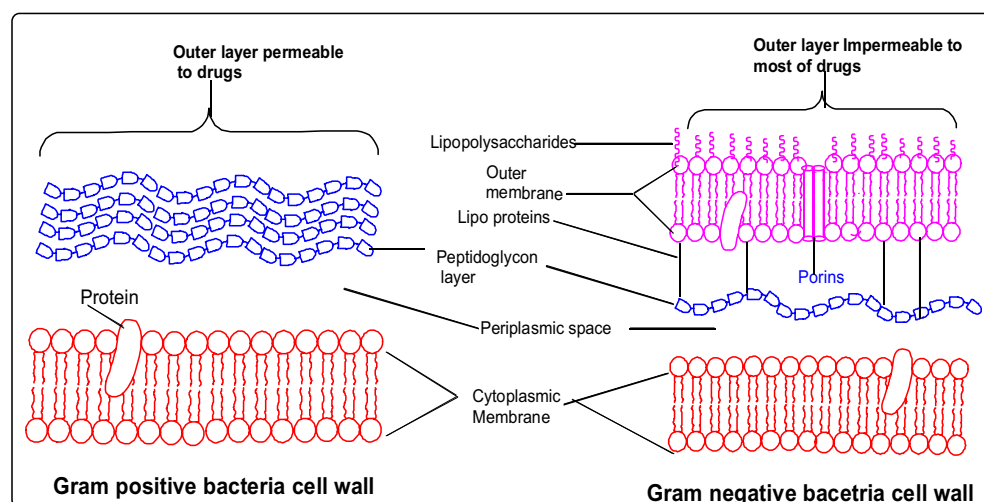
the hydrolysis of saponins (38, 46). As flavonoids are phenolic compounds, they form irreversible complexes with extracellular/soluble proteins (33) with involvement of hydroxyl group (47) which restrain protein synthesis in bacteria (48) and hence antibacterial activity is exhibited by them (2, 49). Other postulates on antibacterial activity of flavonoids are (1) disruption of microbial membranes by lipophilic flavonoids (50) (2) release of hydrogen peroxide by some flavonoids in aqueous media at physiological pH (51), (3) inhibition of enzymes and precipitation of proteins in micro-organisms due to interference of glycosylated flavonoids (52, 53), and (4) inhibition of phosphor kinases and ATP by flavonoids (54). Hence, antibacterial activity of *C. lasiantha* can be explained based on the presence of flavone glycoside (Luteoline-4-O-neohesperidoside) (15, 18).

### 3.4. Higher activity of plant extracts toward Gram-positive bacteria

In the present study, it is observed that inhibition displayed by all stem/root extracts against all the selected Gram-positive bacteria is higher than Gram-negative bacteria (Tables 1 and 2). It is similar to the previous reports which suggested a higher susceptibility of food-borne pathogenic Gram-positive bacteria to tea flavonoids in comparison with Gram-negative bacteria (55, 56). Higher activity against Gram-positive bacteria can be explained in terms of specificity of saponins toward them. Literature survey shows that saponins present in *Medicago* species exhibited high efficacy against Gram-positive bacteria (like *B. subtilis*, *S. aureus*, and *Bacillus cereus*) whereas unsuccessful against Gram-negative bacteria (41). The differential activities of plant extracts against these two types of bacteria can be elucidated taking into account of their cell outer layers. In the case of Gram-positive bacteria, cell outer barrier is made up of peptidoglycan layer which is ineffective and permeable. Hence, drug constituents are permeable through the cell wall of Gram-positive bacteria. However, in Gram-negative bacteria, cell wall is made up of multilayered peptidoglycan and phospholipid membrane which is impermeable to drug constituents (57). In addition, glycosylated flavonoids selectively inhibit topoisomerases in Gram-positive bacteria which shows a negative effect on replication and transcription mechanics (58). Hence, *C. lasiantha* extracts have preferable activity against Gram-positive bacteria (Figure 8).

The observed differential antibacterial activity of *C. lasiantha* extracts against Gram-positive and Gram-negative bacteria can also be explained based on the existence of flavone glycoside (Luteoline-4-O-neohesperidoside) in it (18). It is a well-known fact that presence of flavonoids is one of the reasons for antibacterial activity of plant extracts (2, 49) due to formation of a complex between extracellular protein on bacteria and carbonyl group on flavonoids (33). For example, (+)-catechin, a monomeric flavan sub-unit is capable of linking with the lipopolysaccharide present on the bacterial cell surface (59). Hence, all those polyphenols of plant origin can display various biological activities including anti bacterial activities (60).

Figure 8. Higher activity of plant extracts toward Gram-positive bacteria.



### 3.5. Relation between anti-inflammation activity and *Caralluma lasiantha* extracts

In India, *C. lasiantha* powder is used to reduce body heat, a characteristic of inflammation (17) and methanolic extracts of *C. lasiantha* exhibit anti-inflammatory activity (61). Moreover, anti-inflammatory action is exhibited by polyphenols of plant origin (60). Hence in the present study, bacteria were chosen which cause inflammation. For example, local inflammation is created by *S. aureus* as it replicates in metaphyseal capillary loops (62). About 70 to 80% of urinary tract infections (UTI) are due to *E. coli* infection (63) and *Streptococcus* Sp., (64). *B. subtilis* causes Endocarditis (inflammation of the endocardium) (65). *E. aerogenes* causes respiratory tract nosocomial infections (66). *K. pneumoniae* is responsible for *pneumoniae* (the destructive lung inflammation disease) (67). Exceptional antimicrobial activity against *E. coli* by lower concentrated *C. lasiantha* extracts helps to visualize their potential to cure inflammation of urinary bladder. This is supported by the fact that Ramesh et al., (7) reported the anti-inflammatory activity by flavone glycosides which are also present in *C. lasiantha* (15). As *C. lasiantha* extracts exhibit exceptional growth inhibition against these bacteria, the anti-inflammation activity of *C. lasiantha* can be substantiated.

### 4. Conclusion

From the present *in vitro* studies, it can be concluded that the crude plant extracts of *C. lasiantha* exhibit good antimicrobial activity against the selected bacteria due to the presence of steroids and flavones in plant extracts. Anti-inflammation activities are related to *C. lasiantha* extracts through their antibacterial activities. Hence, further *in vivo* studies have to be carried out using pure phytochemicals to review their usage in the treatment of infectious diseases caused by these micro-organisms and to establish the exact mechanism of action.

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