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IN VITRO ANTIBACTERIAL ACTIVITIES OF CRUDE LEAF EXTRACTS OF *GMELINA ASIATICA* L.

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ABSTRACT

To examine the *in vitro* antibacterial activities of the crude extracts and essential oil extracted from *Gmelina asiatica* leaf for the control of human pathogenic bacteria such as *Actinomyces howelli*, *Bacillus circulans*, *Staphylococcus aureus*, *Streptococcus pyogenes Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. Hydro distilled essential oils and aqueous, petroleum ether, chloroform, ethanol and acetone extracts of *G. asiatica* were tested for antibacterial activity, which was determined by disc diffusion method. All the crude extract have moderate activity against all the tested pathogens and essential oils had very less antibacterial activity against *Actinomyces*, *Bacillus* and *Proteus* and did not display any potential of antibacterial activity against *Staphylococcus*, *Streptococcus*, *Escherichia* and *Pseudomonas* and the activity was compared with standard antibiotic kanamycin. The findings led to the conclusion that *G. asiatica* leaf extract may be regarded as an antibacterial agent for the treatment of various bacterial diseases.

KEYWORDS: *Gmelina asiatica*, antibacterial activity, agar disc diffusion method, standard antibiotic.

INTRODUCTION

In most of the developing countries nearly 80% of total population relies on traditional medicines, mainly plant drugs for their health. It has been estimated that about 45,000 plant species are present in the Indian subcontinent and 7500-8000 wild plants are used in medicine. Only 1500 plants are utilized in Sidda, Unani and Ayurveda.[1] In the last few decades medicinal plants form the backbone of traditional medicine and a major subject for pharmacological studies. The valuable sources and new compounds of medicinal plants are

used in the drug development. The antimicrobial activities of crude extracts and essential oils in many plants function as alternative sources in natural therapies.[2] Microorganisms such as bacteria, fungi, viruses etc are pathogenic and cause deadly diseases to all populations and a serious problem all over the world. Medicinal plants represent a rich source of antimicrobial agents.[3-7] Many researchers have proved the crude extracts and essential oils of plants possess antibiotics and biologically active compounds.[8] Generally plants have secondary metabolites which constitute an important source of microbicides, pesticides and pharmaceutical agents.[9-11]

Gmelina asiatica L. (Syn: *Gmelina parvifolia* Roxb.), is a deciduous large sized bush or shrub belonging to the family Verbenaceae which comprises about 35 species and 2 subspecies spread over in tropical and temperate regions of Asia. It is commonly called "Asiatic Bush Beech" and "Nilakumizh" in Tamil. The whole plant is medicinally important and well documented as a source of bioactive components with medicinal properties such as antimicrobial[12-16] anti-inflammatory[17], antioxidant[18,19], antihyperglycemic and hypoglycemic[20], hepatoprotective[18], antipyretic[21], nematicidal[22] and anticancer activity.[23-25] The aerial parts and roots are used in traditional medicine for the treatment of jaundice, rheumatism, syphilis, gonorrhea, burning sensation of eyes, fever, dysuria, wounds, dandruff, diabetes, hepatic diseases, catarrh of the bladder, blood purifier and also to reduce body heat.[26-31] Hence the present study is focused to evaluate the potential antimicrobial activities of crude extract and essential oils of *G. asiatica* leaves on four gram positive bacteria (*Actinomyces howelli*, *Bacillus circulans*, *Staphylococcus aureus* and *Streptococcus pyogenes*) and three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*).

MATERIALS AND METHODS

Collection of plant materials: Leaves of *G. asiatica* were collected from Scott Christian College Campus, Nagercoil, Kanyakumari District, South Tamilnadu, India and identified using Gamble and Fisher.[32]

Extraction of plant materials: The freshly collected healthy mature leaves are thoroughly washed with distilled water and kept in shade at room temperature for about two weeks to dry. They were made into powdered with the help of a mechanical grinder and sieved. The dried powdered sample (100 g) of *G. asiatica* was extracted with 1000 mL of solvents such as aqueous, petroleum ether, chloroform, ethanol and acetone by a Soxhlet apparatus

separately. The resultant filtrate was concentrated in powdered form by evaporation of the solvents using Rotary evaporator. The solid residue was designated as the extract, which was stored in a refrigerator at 4°C until further analysis.

Bacterial strains and culture preparation: Seven human pathogenic bacterial strains were taken into consideration, four Gram-positive (*Actinomyces howelli* MTCC-3048, *Bacillus circulans* MTCC-9720, *Staphylococcus aureus* MTCC-3160 and *Streptococcus pyogenes* MTCC-1927) and three Gram-negative (*Escherichia coli* MTCC-9721, *Pseudomonas aeruginosa* MTCC-1688 and *Proteus vulgaris* MTCC-7299) bacteria were selected for antibacterial activity assay. These species were collected from Microbial Type Culture Collection (MTCC), Chandigarh, India. The test organisms were sub cultured at 37°C for 24 hrs and maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

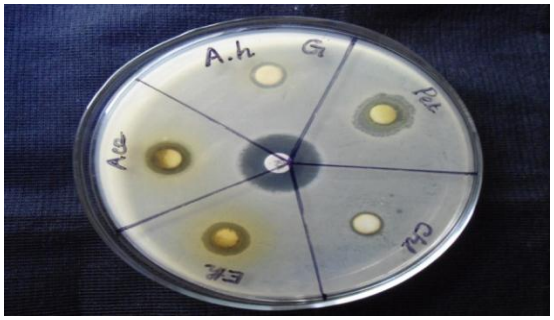
Preparation of standard inoculums, filter paper discs and plates: The microorganisms were inoculated in Muller Hinton Broth (MHB) and incubated at 37°C for 24h. The resulting suspension was diluted with MHB to match with 1 McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0×10^8 CFU/ mL, equivalent to 0.5 McFarland standards. The dried plant extracts were dissolved in 10% aqueous dimethylsulfoxide (DMSO) to a final concentration of 200 mg/mL and sterilized by filtration. Antimicrobial tests were carried out by the disc diffusion method using the inoculums containing 10^6 bacterial cells to spread on Muller-Hinton agar plates (1 mL inoculum/plate). The discs (diameter 6 mm) were impregnated with 50 µL of extract (10 mg/disc) at a concentration of 200 mg/mL.

Screening for antimicrobial activities: Antibacterial activities of *G. asiatica* leaf extracts were carried out by disc diffusion method using the Kirby-Bauer technique.[33] All the bacterial strains were maintained in nutrient agar medium (NA). Pure culture was inoculated into (Muller Hinton Agar) MHA plate and subcultured at 37ºC for 24 h. Standardized inoculum was transferred and spread evenly on a MHA plate to yield a lawn culture. Sterile Whatman No.1 filter paper discs were impregnated with plant extracts (50µg/disc) and inoculated in MHA plates. They were allowed to diffuse for 30 min at 4ºC and incubated at 37ºC for 24 h. Kanamycin (10µg) served as a positive control. The plates were observed for the presence of inhibition of bacterial growth which was indicated by the clear zone around the disc. The size of the zone inhibition (excluding disc) was measured in millimetres. The

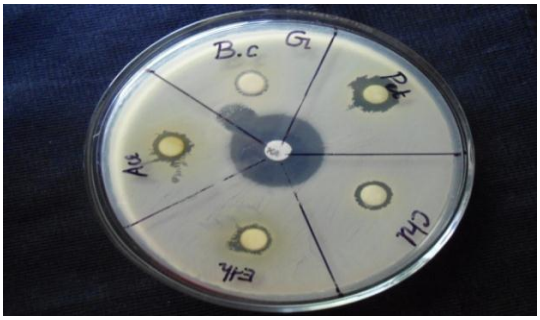
absence of zone inhibition was interpreted as the absence of activity. All the experiments were carried out in triplicates under strict aseptic conditions and the zone of inhibition around each disc was measured for sensitivity or resistance. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) ± standard deviation (S.D) produced by the plant extract.

RESULTS

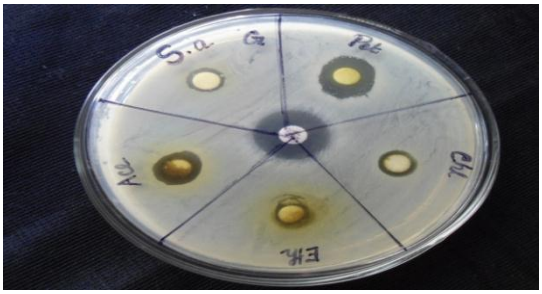
Antibacterial activity of *Gmelina asiatica* extracts: In the present study, the results of antibacterial activity of five solvent extracts (aqueous, petroleum ether, chloroform, ethanol and acetone) of *G. asiatica* leaves against different pathogens are shown in Table 1.



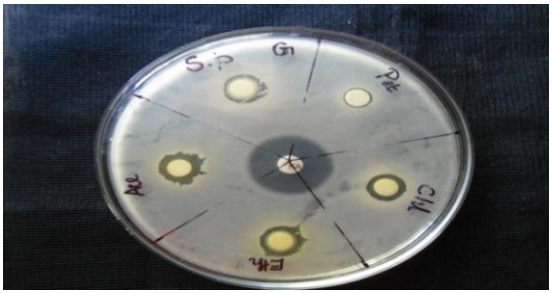
Growth of inhibition on *A. howelli*



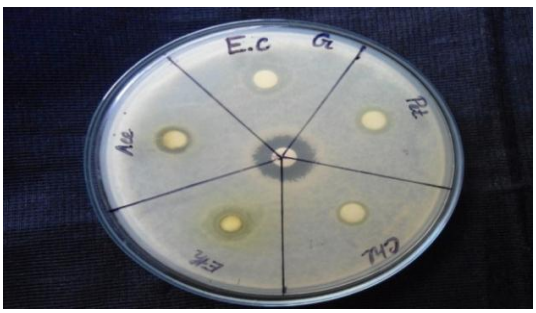
Growth of inhibition on *B. circulans*



Growth of inhibition on *S. aureus*

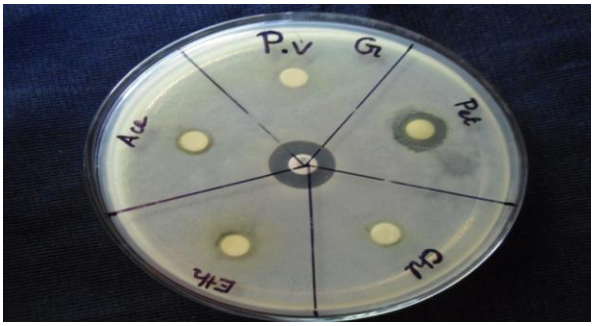


Growth of inhibition on *S. pyogenes*



Growth of inhibition on *E. coli*

Growth of inhibition on *P. aeruginosa*



Growth of inhibition on *P. vulgaris*

Plate 1: Antibacterial activity of crude leaf extracts of *Gmelina asiatica*

The gram positive strains were found to be the most susceptible to growth inhibition by the plant extracts forming zones of inhibition ranging from 1.33 ± 0.471mm to 7.66 ± 0.471mm. All the crude extract has moderate activity against all the tested pathogens. The aqueous extract revealed the most potent antibacterial activity against *S. pyogenes* (4.33 ± 0.471mm); *A. howelli* (4.00 ± 0. 816mm) and least potent against *B. circulans* (2.99 ± 0.471mm) and *S. aureus* (2.33 ± 0.471mm). The petroleum ether extract was most potent against *A. howelli* (9.66 ± 0.471mm), moderate activity against *S. aureus* (7.00 ± 0. 816mm); *B. circulans* (6.66 ± 0.471mm) and least potent against *S. pyogenes* (1.33 ± 0.471mm). The chloroform extract revealed the least potent antibacterial activity against all tested positive bacterial strains such as *B. circulans* (2.66 ± 0.471mm); *S. aureus* (2.66 ± 0.471mm); *S. pyogenes* (2.66 ± 0.471mm) and *A. howelli* (2.33 ± 0. 471mm). The ethanolic extract was most potent against *A. howelli* (5.66±0.471mm); *S. pyogenes* (5.33 ± 0.471mm) and least potent against *B. circulans* (3.66 ± 0. 942mm) and *S. aureus* (3.33 ± 0.471mm). The acetone extract revealed most potent antibacterial activity against *S. aureus* (7.66 ± 0.471mm), *S. pyogenes* (6.66 ± 0.816mm), *B. circulans* (5.00 ± 0.861mm) and *A. howelli* (4.66 ± 0. 471mm) in the leaf extracts of *G. asiatica*. The standard drug Kanamycin showed most potent activity against *B. circulans* (17.00 ± 0.816mm) followed by *S. pyogenes* (16.00 ± 0.816mm); *A. howelli* (13.66 ± 0. 471mm) and *S. aureus* (11.00 ± 0.816mm). Among the all plant extracts tested, the zone of inhibition of kanamycin activity was more when compared to aqueous, petroleum ether, chloroform, ethanol and acetone extracts. Among the pathogens *A. howelli* was more susceptible to leaf extracts of the plant followed by *S. aureus, S. pyogenes* and *B. circulans*.

The gram negative strains were more sensitive to the plant extracts when compared to the gram positive strains, forming zones of inhibition ranging from 0.66 ± 0.47 to 3.66 ± 0.47 mm. The aqueous extract was most potent against *E. coli* and there is no inhibitory activity

against *P. aeruginosa* and *P. vulgaris*. The petroleum ether extract was most potent against *P. vulgaris* (4.66 ± 0.471mm) and moderate potent against *E. coli* (2.00 ± 0.816mm) and least potent against *P. aeruginosa* (0.66 ± 0.471mm). The chloroform extract was most potent against *P. aeruginosa* (2.33 ± 0.471mm) followed by *E. coli* (2.00 ± 0.816mm) and *P. vulgaris* (1.33 ± 0.471mm). The ethanolic extract was most potent against *P. vulgaris* (2.66 ± 0.471mm) followed by *P. aeruginosa* (2.33 ± 0.471mm) and *E. coli* (2.00 ± 0.816mm) whereas, the acetone extract was more potent against *P. aeruginosa* (2.00 ± 0.816mm) followed by *E. coli* (2.00 ± 0.816mm) and *P. vulgaris* (1.66 ± 0.471).

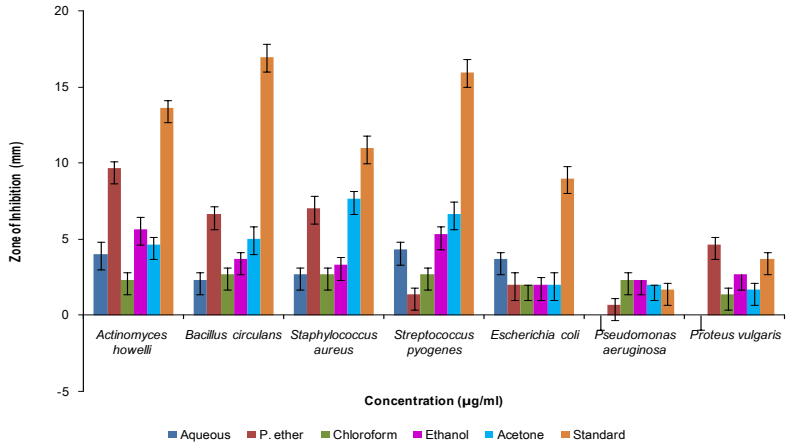


Fig.1: Antibacterial activity of crude leaf extracts of *Gmelina asiatica*

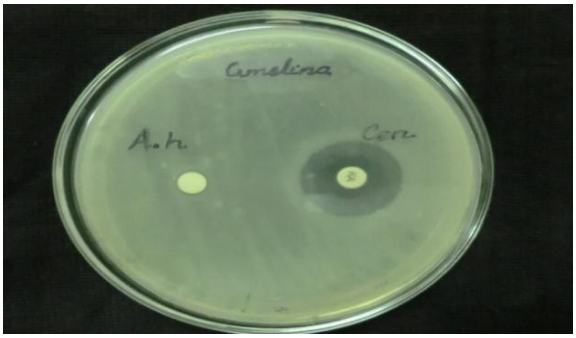
Table 1: Antibacterial activity of crude leaf extracts of *Gmelina asiatica*

Zone of inhibition in diameter (mm) (n=3)						
Micro organisms	**Aqueous**	**Pet. Ether**	**Chloroform**	**Ethanol**	**Acetone**	**Standard**
Gram Positive bacteria						
Actinomyces howelli	4.00 ± 0.816	9.66 ± 0.471	2.33 ± 0.471	5.66 ± 0.471	4.66 ± 0.471	13.66 ± 0.471
Bacillus circulans	2.33 ± 0.471	6.66 ± 0.471	2.66 ± 0.471	3.66 ± 0.942	5.00 ± 0.861	17.00 ± 0.816
Staphylococcus aureus	2.66 ± 0.471	7.00 ± 0.816	2.66 ± 0.471	3.33 ± 0.471	7.66 ± 0.471	11.00 ± 0.816
Streptococcus pyogens	4.33 ± 0.471	1.33 ± 0.471	2.66 ± 0.471	5.33 ± 0.471	6.66 ± 0.816	16.00 ± 0.816
Gram Negative bacteria						
Escherichia coli	3.66 ± 0.471	2.00 ± 0.816	2.00 ± 0.00	2.00 ± 0.816	2.00 ± 0.816	9.00 ± 0.816
Pseudomonas aeruginosa	NI	0.66 ± 0.471	2.33 ± 0.471	2.33 ± 0.471	2.00 ± 0.00	1.66 ± 0.471
Proteus vulgaris	NI	4.66 ± 0.471	1.33 ± 0.471	2.66 ± 0.471	1.66 ± 0.471	3.66 ± 0.471

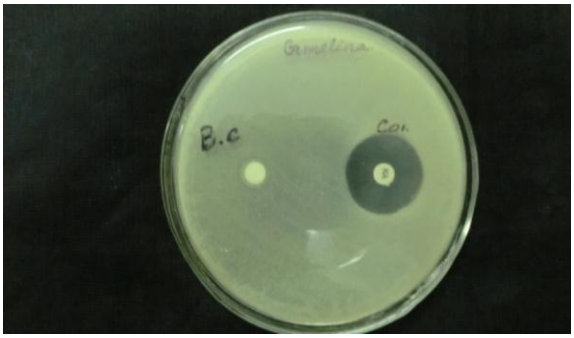
Antibacterial activity of essential oil: The results revealed that the essential oil exhibited very less antimicrobial activity against *P. aeruginosa*, *A. howelli* and *P. vulgaris* and no inhibitory effect against the *S. pyogenes*, *S. aureus* and *E. coli* was recorded (Fig. 2; Table 2; Plate 2).

Table 2: Antibacterial activity of essential oil of *Gmelina asiatica* leaves

Zone of inhibition in diameter (mm)			
Micro organisms	**MTCC No.**	**Control**	**Oil**
Gram positive bacteria			
Actinomyces howelli	3048	17.33 ± 0.471	0.66 ± 0.471
Bacillus circulans	9720	17.0 ± 0.816	3.33 ± 0.471
Staphylococcus aureus	3160	16.0 ± 0.942	NA
Streptococcus pyogenes	1927	23.0 ± 0.816	NA
Gram negative bacteria			
Escherichia coli	9721	16.66 ± 0.471	NA
Pseudomonas aeruginosa	1688	8.66 ± 0.471	NA
Proteus vulgaris	7299	23.0 ± 0.816	1.33 ± 0.471



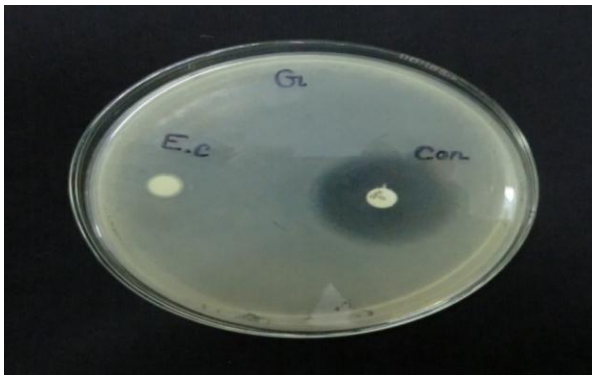
Growth of inhibition on *A. howelli*



Growth of inhibition on *B. circulans*

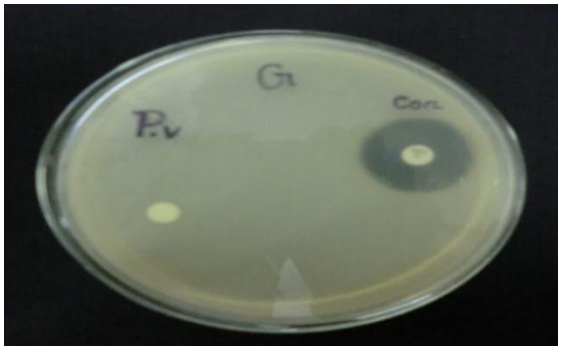
Growth of inhibition on *S. aureus*

Growth of inhibition on *S. pyogenes*



Growth of inhibition on *E. coli*

Growth of inhibition on *P. aeruginosa*



Growth of inhibition on *P. vulgaris*

Plate 2: Antibacterial activity of essential oil of *Gmelina asiatica* leaves

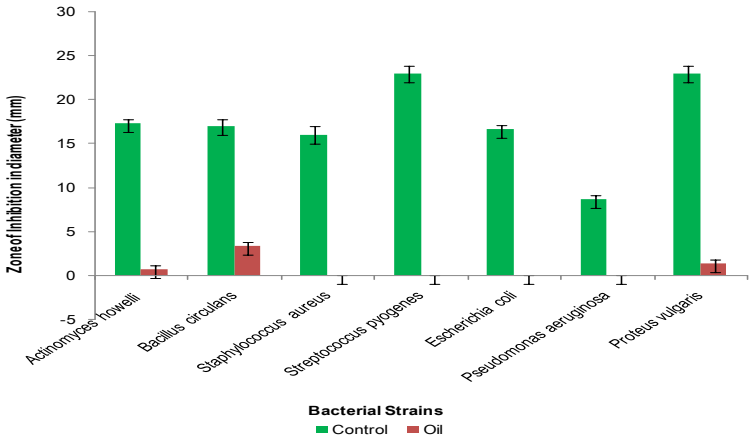


Fig. 2: Antibacterial activity of essential oil of *Gmelina asiatica* leaves

DISCUSSION

In recent years, infections have increased to a greater extent and resistance to antibiotics has become an ever-increasing therapeutic problem.[8] The development of new antimicrobial agents for the treatment of bacterial infections is of increasing interest.[34,35]

Sensitivity of test strains is given in decreasing order: *Actinomyces howelli*< *Staphylococcus aureus*< *Bacillus circulans*< *S. pyogenes*< *P. vulgaris*< *E. coli*< *P. aeruginosa.* Antibacterial activity obtained in this study varied with inhibition pattern, solvents used for extraction and the microorganism tested for susceptibility assay. In the present investigation, maximum inhibition was observed against *A. howelli* (9.66mm), *B. circulans* (6.66mm) and *S. aureus* (7.00mm) for petroleum ether extract. Similar to the present study, Merlin *et al.* noticed that the petroleum ether, chloroform, ethyl acetate and ethanol extract of aerial parts of *G. asiatica* showed maximum antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Escherichia coli* and *Micrococcus*

luteus.[14] Chloroform extracts of *G. asiatica* showed minimum antibacterial activity against all the tested strains with the inhibition zone ranging from 1.33-2.66mm. Similar studies conducted by Shibu and Dhanam on chloroform leaf, stem and root extract of *G. asiatica* showed minimum antibacterial activity against *S. aureus, E. coli* and *P. aeruginosa*.[16] Ethanolic leaf extract of *G. asiatica* showed moderate activity and the zone of inhibition varied between 3.33-5.66mm against all the tested bacterial strains. Similar studies by Sudhakar *et al*. exhibited moderate antibacterial activity to the ethanolic root extracts of *G. asiatica* against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus vulgaris*.[12] The growth of *Pseudomonas aeruginosa* and *Proteus vulgaris* were not inhibited at 50µg/ml concentration of aqueous leaf extract. Generally, it was also noted that organic solvent extracts had greater inhibitory effect than aqueous extract, for extracting active compounds from the plant.[36] These results are in parallel to the findings of Parekh *et al*. on the aqueous extracts of *G. asiatica* leaf which showed no inhibitory activity against *P. vulgaris*, which implies that, these organisms are resistant to aqueous.[29]

Generally, plant extracts are more effective against gram positive than gram negative bacteria.[37,38] In the present study also gram positive bacteria were more effective than the gram negative bacteria which may be due to the fact that gram negative bacteria have an outer membrane consisting of lipoprotein and lipopolysaccharide, which is selectively permeable and thus regulates accesses to the underlying structures.[39-41] The results are in agreement with the studies of Bakkiyaraj and Pandiyaraj and it showed that the gram positive bacteria were more sensitive than the gram negative in *G. asiatica* leaf extract.[15] As reported earlier, secondary metabolites like tannins, saponins[42-45], flavonoids[46], terpenoids[47] and fatty acids[48-50] are likely responsible for the observed antibacterial activity of plants.

Antibacterial activity of essential oil

The results revealed that the essential oil exhibited very less antimicrobial activity against *P. aeruginosa*, *A. howelli* and *P. vulgaris* and no inhibitory effect against the *S. pyogenes, S. aureus* and *E. coli* was recorded (Fig. 2; Table 2; Plate 2). Essential oil extracted from *Lippia nodiflora* leaves showed no antibacterial activity against *B. cereus, S. aureus* and *E. coli*[51]; this is in agreement with the current results which demonstrated that *G. asiatica* showed no inhibitory activity against *S. aureus, S. pyogenes, E. coli* and *P. aeruginosa*. Similarly, *Vitex agnus-castus* oil had no effect on *Listeria monocytogenes* and *E. coli*.[52] The reason may be

attributed to the low amount of oils, terpenes and (E)-9-Octadecanoic acid (fatty acid) of *G. asiatica* leaf.

CONCLUSION

In conclusion, the results of this work suggest that the extracts of *G. asiatica* leaf extracts have moderate antibacterial activity and essential oil has less antibacterial activity. According to our knowledge, this is the first study to provide data on the evaluation of essential oils against the tested bacterial strains. However further studies are required to understand more about the underlying properties of the plant for proper elucidation of its biological activities.

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