IJPSR (2022), Volume 13, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 January 2022; received in revised form, 02 April 2022; accepted, 27 April 2022; published 01 September 2022

IN-VITRO SCREENING OF EXTRACTS OF *CENTELLA ASIATICA* (LINN) URBAN FOR ANTIBACTERIAL PROPERTIES

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Keywords:

Centella asiatica, Antibacterial activity, Acetone extract, Aqueous extract, Ethanol extract

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ABSTRACT: *Centella asiatica* (Linn) Urban, a herb of immense medicinal properties, has been widely used by traditional healers in the treatment of skin diseases, mental fatigue, anxiety, rheumatism, eoilepsy and leprosy. In the present investigation, the antibacterial activity of ethanol, acetone and aqueous extracts of *Centella asiatica* were tested *in-vitro* against seven bacterial species *viz*. *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella paratyphi*, *Klebsiella* sp., *E. coli*, *Bacillus* sp. and *Pseudomonas* sp. by well diffusion method. The ethanol extract inhibited the growth of *Bacillus* sp. and *S. aureus*, maximally (14 mm each), followed by that of *E. coli* (12 mm) and *Klebsiella* sp. (11 mm). The extract unaffected the growth of Salmonella typhi, Pseudomonas, and Salmonella paratyphi. The acetone extract inhibited the growth of *Bacillus* sp. (10 mm); the other six bacteria remained unaffected. Likewise, the aqueous extract also inhibited the growth of none of the test bacteria in this study.

INTRODUCTION: *Centella asiatica (L) Urban*, a perennial herbaceous creeper, is an important medicinal herb belonging to Umbelliferae. The plant is commonly known as 'mandukparni', 'Indian pennywort', and jalabrahmi'. *Centella asiatica* is found in moist, damp places in tropical and subtropical countries, including parts of India, Pakistan, Sri Lanka, southern United States of America, Central America, South America, Australia, Venezuela, Madagascar and South Africa. The plant is sensitive to biological and chemical pollutants in water which may be absorbed into the plant [1, 2, 3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(9).3668-74

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(9).3668-74

The plant's stem is slender and green in color; it has creeping stolons connecting the plants to each other. The leaves are borne on pericardial petioles. They are orbicular, crenate, palmately nerved, deeply cordate, with an angular sinus and long petiole. Stipules are scarious. The rootstock consists of rhizomes growing vertically down. Flowers are sessile, white or pinkish to red in color, simple, axillary, few-flowered umbels. Petals are minute, ovate, acute and imbricate. Each flower bears 5 stamens and 2 styles. The fruits are densely reticulate, laterally compressed and with thick pericarp.

The plant can spread to form a dense ground cover. The plant is harvested manually [3, 4, 5]. *C. asiatica* abode a wide range of secondary metabolites, contained in the aerial parts of the plants and in the roots and rhizomes. Many of these phytochemicals possess a plethora of medicinal properties [5].

The bioactive compounds in the roots and leaves of *C. asiatica* have been evaluated and validated for a wide array of therapeutic applications in the areas of antimicrobial, anti-inflammatory, anticancer, antidiabetic, hepatoprotective, neuroprotective, antioxidant and wound healing activities [1]. It has been used for centuries in India and oriental countries for the treatment of large array of disorders such as fatigue, anxiety, epidermal wound, eczema, and other skin diseases, leprosy, rheumatism, inflammation, syphilis, mental illness, epilepsy, hysteria, dehydration and diarrhea [1, 5].

C. asiatica help reduce swelling and improve circulation in individuals with venous condition such as varicose veins and venous insufficiency. It is one of the herbs commonly used for revitalizing the nerves and brain cells and also to treat emotional disorders. *C. asiatica* is widely used as a blood purifier; also used for treating high blood pressure, memory enhancement and promoting longevity. The plant is highly effective as ulcer preventive, antidepressant and sedative [6, 7, 8].

C. asiatica is reported to have strong antimicrobial activity. Broad-spectrum antibacterial and antifungal activities of extracts of *C. asiatica* have previously been reported against various species of bacteria and fungi [9, 10, 11]. The present study was designed to experiment with the antibacterial activity of C. asiatica and compare the efficacy of aqueous, acetone and ethanol extracts of *C. asiatica* in inhibiting the growth of different bacteria.

MATERIALS AND METHODS:

Collection of Sample: Fresh plants of *Centella asiatica* (Linn) Urban were collected from Kottayam District, Kerala, India. In this study the *C. asiatica* plants were used in whole for preparing the extracts.

Bacterial Strains Used: Bacterial cultures used in this study were obtained from the culture collections of the School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India.

The bacterial strains used in this study included *Staphylococcus aureus, Salmonella typhi, Salmonella paratyphi, Klebsiella* sp., *E. coli, Bacillus* sp. and *Pseudomonas* sp. The bacterial strains were maintained on nutrient agar plates. The cultures were stored at 4 °C till use.

Surface Cleaning and Sterilization of *C. asiatica:* Surfaces of plant parts carry a wide range of microbial contaminants such as bacteria and fungi. The plant parts must be surface sterilized in disinfectant solutions to avoid microbial contamination. In this study, the whole plant of *C. asiatica* was surface-sterilized following the modified procedure of Aneja [12]. The plants were separately washed in running tap water for 10 minutes, followed by a detergent wash in 10% Extran (Merck) for 10 min. The samples were washed again in running tap water for 10 minutes and spread out in clean trays for oven drying.

Preparation of Extracts: Fresh sterile samples of *C. asiatica* were oven-dried at 60 °C, continuously, for 7 days. The dried samples were powdered using a clean grinder and stored in air-tight containers at room temperature. A fixed weight of 20 g of the powdered material was weighed out in aseptic condition and was transferred to a clean cheese cotton cloth. The cheesecloth with the dried plant material was tied tightly and was transferred to the Soxhlet apparatus. A volume of 250 ml of each solvent (water/acetone/ethanol) was taken in a 500 ml round bottom flask, and the temperature of the heating panel was adjusted to the required temperature. The aqueous extracts were prepared at an extraction temperature of 100 °C, and the acetone and ethanol extract were at 60 °C each. The Soxhlet extraction was carried out continuously for 8 hrs after which each extract was concentrated by evaporation and made up to a final volume of 10 ml. The extracts were stored at room temperature in sterile screw-capped containers till use.

Media Used: Nutrient agar (HiMedia, Mumbai) was used for the culture and maintenance of the test bacterial cultures. Peptone water was used for preparing the bacterial culture suspensions. Mueller Hinton agar (MHA from HiMedia, Mumbai) was used as a base medium for screening of antibacterial activity of *C. asiatica* extracts using the well diffusion assay.

Well-Diffusion Assay for Determination of Antimicrobial Activity: Pure isolated colonies of each bacterium on nutrient agar were inoculated into peptone water and incubated at 37 °C for 48 h. These broth cultures were used as inoculums for assaying the antibacterial activity of the aqueous,

acetone and ethanol extracts of *C. asiaticum* using well diffusion assay. For the assay, about 15 to 20 ml of MHA was poured into sterile Petri dishes and allowed to solidify. Using sterile cotton swab, 0.2 ml of 24 hrs old broth cultures of the test bacteria were inoculated evenly onto the surface of MHA to make a lawn culture. Three wells of 7 mm diameter were dug into the MHA plate using a sterile gel borer out of which two were loaded with 100 µl each of the extracts and the remaining one with the respective control (solvent in which the extract was prepared). The experiment was performed in duplicates. The plates were incubated at 37 °C for 24 h. The antibacterial activity of the extracts was assayed by measuring the diameter of zone of inhibition to the nearest mm, against each of the test bacteria. The results were recorded and compared.

RESULTS: In this study, the ethanol extract of *C. asiatica* alone was comparatively effective in inhibiting the growth of four of the test bacteria. The maximal inhibition of growth for the ethanol extract was observed for *Bacillus* sp. and *S. aureus* with a diameter zone of inhibition of 14 mm each **Table 1, Fig. 4**. The growth of *E. coli* and *Klebsiella* were also considerably affected by the crude ethanol extract with growth inhibition zones of 12 mm and 11 mm, respectively. However, the growth of other bacteria was not inhibited by the bioactive compounds contained in the ethanol extract.

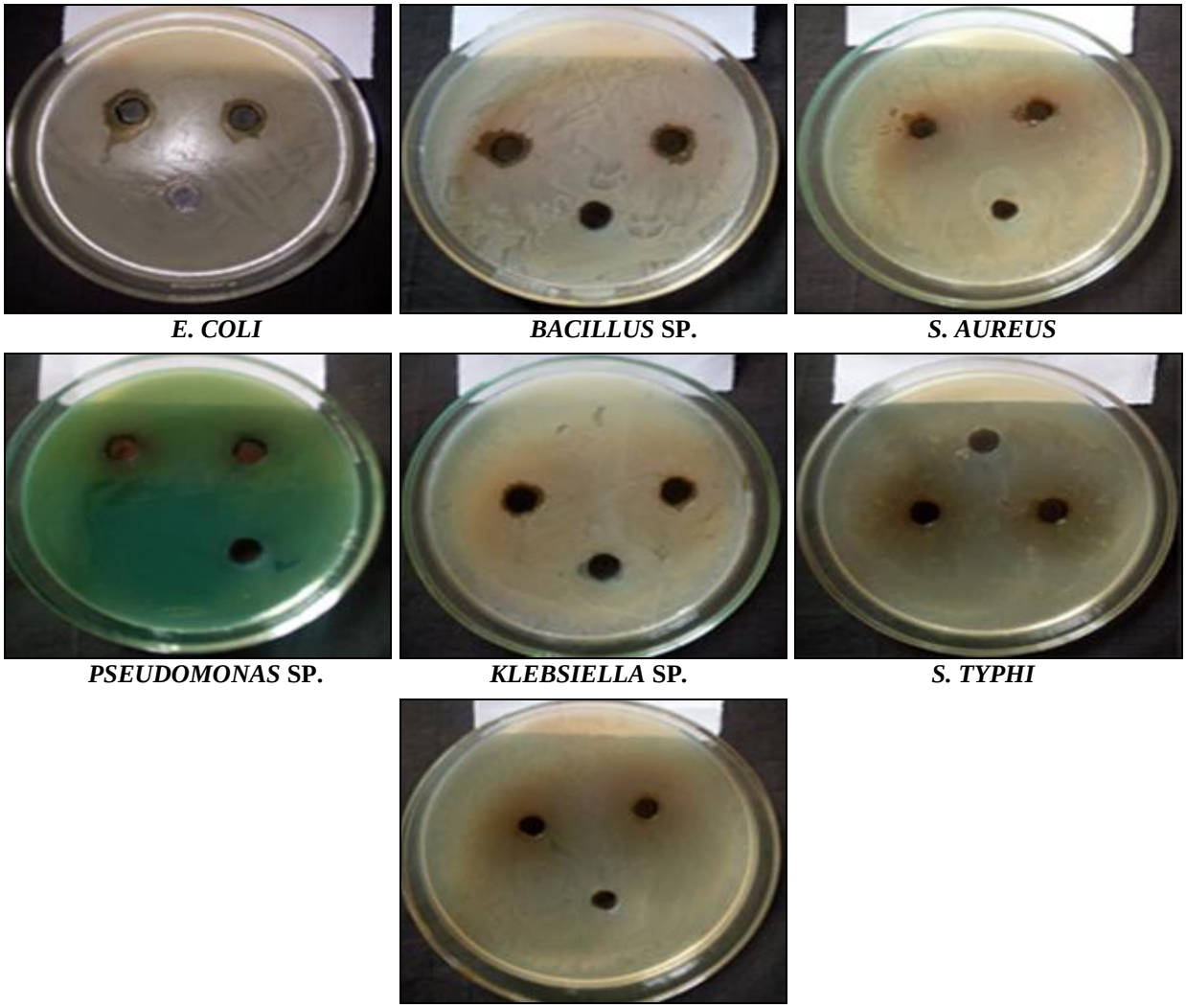
The acetone extract of *C. asiatica* also inhibited the growth of *Bacillus* sp. for which a zone of 10 mm of growth inhibition was obtained in the current study **Table 1, Fig. 5**. The aqueous extract had no such activity on the growth of *Bacillus* sp. The other six test bacteria were found to be resistant to both acetone and aqueous extracts of the plant **Table 1, Fig. 5**. This study shows that ethanol is perhaps one of the suitable solvents for extracting bioactive compounds of *C. asiatica*. It is noteworthy that the growth of three bacterial species *viz*. *Pseudomonas* sp., *S. typhi*, and *S. paratyphi*, was affected by neither of the extracts and were found resistant to the antibacterial action of the bioactive compounds present in *C. asiatica*.

TABLE 1: ANTIBACTERIAL ACTIVITY OF EXTRACTS OF *CENTELLA ASIATICA* BY WELL DIFFUSION METHOD

Name of the test bacteria	Average Diameter of Zone of Inhibition in mm		
	Ethanol	Acetone	Aqueous
Bacillus sp.	14	10	0
S. aureus	14	0	0
Pseudomonas sp.	0	0	0
Klebsiella sp.	11	0	0
S. typhi	0	0	0
S. paratyphi	0	0	0
E. coli	12	0	0

FIG. 1: THE HERBACEOUS CREEPER *CENTELLA ASIATICA*

FIG. 2: OVEN DRIED SAMPLE

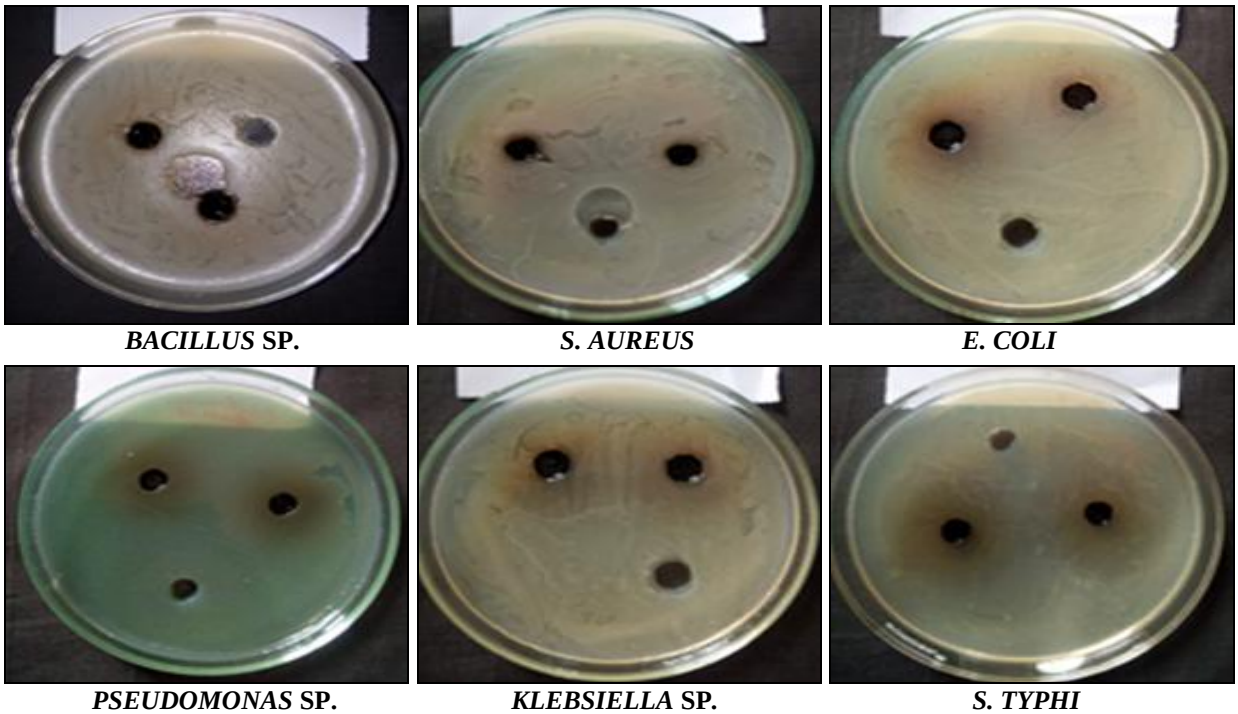


E. COLI ***BACILLUS* SP.** ***S. AUREUS***

PSEUDOMONAS* SP.** ***KLEBSIELLA* SP.** ***S. TYPHI

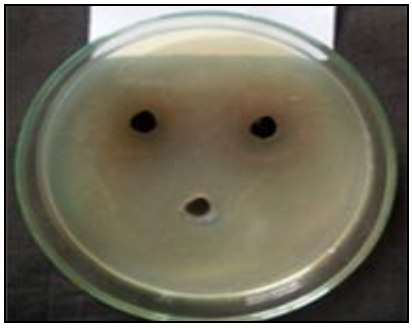
S. PARATYPHI

FIG. 3: ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF *CENTELLA ASIATICA* (LINN.)



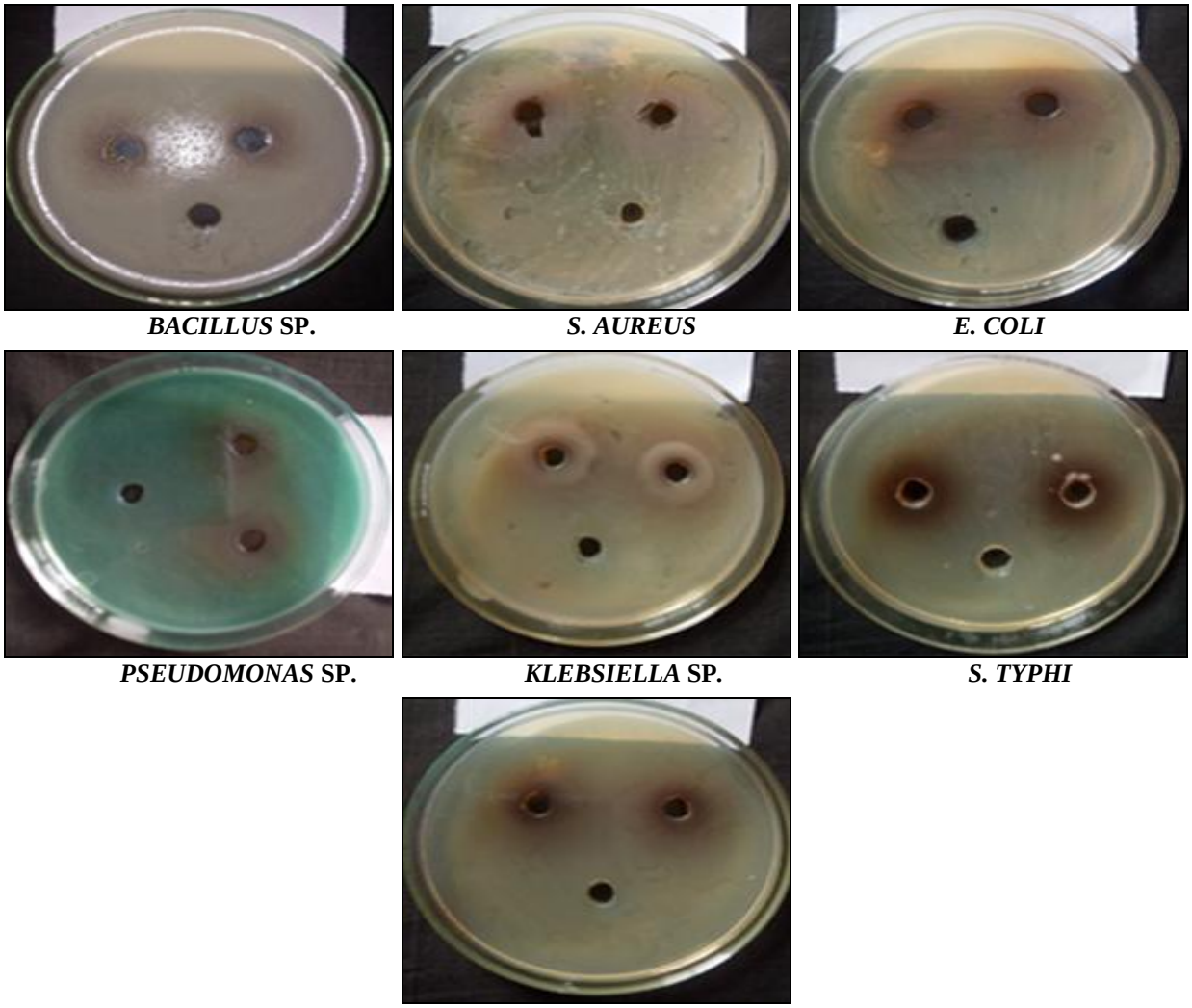
BACILLUS* SP.** ***S. AUREUS ***E. COLI***

PSEUDOMONAS* SP.** ***KLEBSIELLA* SP.** ***S. TYPHI



S. PARATYPHI

FIG. 4: ANTIBACTERIAL ACTIVITY OF ACETONE EXTRACT OF *CENTELLA ASIATICA* (LINN.)



BACILLUS* SP.** ***S. AUREUS ***E. COLI***

PSEUDOMONAS* SP.** ***KLEBSIELLA* SP.** ***S. TYPHI

S. PARATYPHI

FIG. 5: ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACT OF *CENTELLA ASIATICA* (LINN.)

DISCUSSION: In this study, the ethanol extract showed considerable antimicrobial activity against four of the test bacteria, the maximal being recorded for *S. aureus* and *Bacillus.* Taemchuay et al has formerly reported that the ethanol extract of *C. asiatica* has potent anti-*Staphylococcal* activity [11]. The antibacterial activity of methanolic and dichloromethane extracts of *C. asiatica* against *S. aureus*, *Bacillus subtilis* and *Bacillus cereus* has also been reported earlier [13, 14]. In the current study, the antibacterial activity of ethanol extract of *C. asiatica* was more pronounced against *S. aureus* and *Bacillus* sp. **Table 1**. There has also been a concordant study reporting an extensive zone of antibacterial activity obtained for the ethanol root extract of *C. asiatica* against *S. aureus* and *E. coli* [10]. Furthermore, reports on the plant's intense antibacterial activity of crude extracts have been

registered against *P. aeruginosa*, *E. coli*, and *S. aureus* [15]. The current study also ascertains that the growth of *E. coli* and *Klebsiella* sp. are fairly inhibited by the ethanol extract of *C. asiatica*; however, the growth of *S. typhi* remained unaffected. Conversely, Samy *et al.* has observed antibacterial activity of methanol and dichloromethane extracts of *C. asiatica* against *S. typhi* [14].

In this study, the acetone extract of *C. asiatica* exhibited no antibacterial activity; except for *Bacillus* sp. Concordant results have been reported for acetone extracts of *C. asiatica* by Arumugam *et al.* [16]. However, there are previous studies reporting the bactericidal activity of acetone extract against several bacterial species with varied inhibition rates [10, 13]. It has been reported earlier that the aqueous extract of *C. asiatica* has bactericidal effects against *S. aureus*, *E. coli*, *Pseudomonas* sp. and *Xanthomonas campestris* [17, 18]. However, this study shows that the aqueous extract of *C. asiatica* has no inhibitory effect on bacterial growth, at least with respect to the test bacteria concerned. The results of antibacterial activity of *C. asiatica* by Soyingbe et al agree with this study [13].

CONCLUSION: In this study, the antibacterial activity of ethanol, acetone, and aqueous extracts of *Centella asiatica* were tested *in-vitro* against seven bacterial species. The present study establishes the antibacterial activity of ethanol extract of *C. asiatica*; however, the acetone and aqueous extracts were less effective against the test bacteria. The latter report needs to be further investigated so as to obtain more promising results that will ascertain the findings made in this study.

ACKNOWLEDGEMENTS: The authors acknowledge the School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India, for providing the bacterial cultures.

CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest for the publication of this research paper in this Journal.

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How to cite this article:
Varghese LS and Suresh K: *In-vitro* screening of extracts of *Centella asiatica* (Linn) urban for antibacterial properties. Int J Pharm Sci & Res 2022; 13(9): 3668-74. doi: 10.13040/IJPSR.0975-8232.13(9).3668-74.