Biological Forum – An International Journal **15(5a): 553-557(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Antimicrobial Analysis of *Centella asiatica* L. and *Hydrocotyle verticillata* Thunb.

S.M. Dhivya[1*], P. Vijayashalini[1], V. Sasmitha[2] and M. Dhivyasree[2]
[1]Assitant Professor, PG and Research Department of Botany,
Vellalar College for Women (Autonomous), Thindal, Erode (Tamil Nadu), India.
[2]M.Sc. Scholar, Botany PG and Research Department of Botany,
Vellalar College for Women, Erode (Tamil Nadu), India.

(Corresponding author: S.M. Dhivya)*
(Received: 30 March 2023; Revised: 26 April 2023; Accepted: 02 May 2023; Published: 15 May 2023)
(Published by Research Trend)

ABSTRACT: Microorganisms that were both susceptible to and resistant to antibiotics were used to assess the antibacterial activity of plant extracts and phytochemicals. The failure of the equipment, temperature, moisture, and antimicrobial agent potency are only a few of the variables that can have an impact on the outcomes of an antibiotic sensitivity test. The purpose of this research is to investigate the antimicrobial efficacy in *Centella asiatica* and *Hydrocotyle verticillata* against the gram positive (*Staphylococcus aureus, Bacillus subtilis)* and gram negative (*Escherichia coli, Klebsiella pneumoniae)* bacteria. The study revealed that the benzene and ethanol extracts of both *Centella asiatica* and *Hydrocotyle verticillata* inhibited the growth of all the tested bacteria. The benzene extract of *Centella asiatica* inhibits the bacteria *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Klebsiella pneumoniae* with a zone of inhibition of 9.7 ± 0.2mm, 8.6 ± 0.3mm, 7.8 ± 0.2mm and 6.2 ± 0.3mm, respectively and the same extract of *Hydrocotyle verticillata* showed an inhibition zone of 10.6 ± 0.2mm, 9.6 ± 0.3mm, 8.2 ± 0.2mm and 7.4 ± 0.3mm respectively. In the same way, the ethanol extract of *Centella asiatica* inhibits the bacteria *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Klebsiella pneumoniae* with a zone of inhibition of 11.1 ± 0.3mm, 10.0 ± 0.1mm, 8.2 ± 0.1mm and 6.4 ± 0.2mm respectively and the ethanol extract of *Hydrocotyle verticillata* showed an inhibition zone of 12.4 ± 0.3mm, 10.2 ± 0.1mm, 8.4 ± 0.1mm and 6.8 ± 0.2mm respectively. The results concluded that the study plants have effective antimicrobial properties.

Keywords: Antimicrobial, Staphylococcus aureus, inhibition and Ethanol.

INTRODUCTION

Despite tremendous progress in human medicine, many diseases caused by some microbes are still a major threat to public health. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the undesirable side effects caused by certain antibiotics. The undesirable side effects of certain antibiotics encourage the use of plants extracts as antimicrobial agents (Dash *et al.*, 2011). Medicinal plants and their uses in the indigenous medicine are well known to many Indian communities. The recent trend has been to blend the traditional knowledge with modern health care practices to provide effective health care services to wider population (Nag and Hasan 2011).

Plants are rich in secondary metabolites which have been used by humas for treating various types of diseases throughout the world since time immemorial. Traditionally, this treasure of knowledge has been passed on orally from generation to generation without any written document (Samy and Ignacimuthu 1998; 2000). A large number of people in developing countries depend on plants as their primary source for medications. The use of herbal medicines continues to grow throughout the world.

A thorough biological evaluation of plant extracts is vital to ensuring their efficacy and safety. These factors are of importance if plant extracts are to be accepted as valid medicinal agents. Many plants have been used because of their antimicrobial traits and the antimicrobial properties of plants have been investigated by a number of researchers worldwide (Ahmad *et al*., 2015).

Increased use of antibiotics, spurs increased resistance of bacteria to the antibiotic. The research should be developed for the discovery of new drugs that are derived from nature to prevent the occurrence of bacterial resistance to antibiotics (Chaudhary, 2010).

A riparian zone is the interface between land and a river or stream. Plant habitats and communities along river margins and banks are called riparian vegetation, which is characterised by hydrophilic plants. Riparian vegetation comprises plant communities that grow laterally along rivers and streams. Riparian vegetation is affected by both the flood process and the characteristics of landforms that are shaped by floods (Bendix and Hupp 2000).

The family Apiaceae **is** also called Umbelliferae, the parsley family, in the order Apiales, comprising about 434 genera and nearly 3,780 species of plants distributed throughout a wide variety of habitats, principally in the northern temperate regions of the world. The Apiaceae are a diverse group of plants that include vegetables, herbs and spices.

The plant *Centella asiatica* belongs to the family Apiaceae. It prefers relatively shady and damp habitats such as wetlands, riversides, ponds, wet meadows and forests from 300 to 1800m altitude (Gohil *et al.,* 2010; Lansdown, 2019; Parker, 2020). It is a creeping prostate herb. *Centella asiatica* is a prostrate or creeping aromatic herb with long branches (Fig. 1). Leaves alternate, arising from rhizomes, reniform and pale green in colour with palmatous venation. Petiole quite long, inflorescence axillary, umbel, peduncle long with pale green in colour. Flowers are bisexual, pedicellate a with pink coloured perianth and fruit cremocarp (Jelani *et al*., 1993).

The leaves resemble the structure of the human brain. It is a quite aromatic, perennial, stoloniferous herb with the height of up to 15 cm (Jayaprakash and Nagarajan, 2016). The plant is rich in flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins (Mubassara *et al.,* 2017). *Centella asiatica* has been used by Ayurvedic medical practitioners for almost 3000 years to treat wounds, mental and neurological disorders, artherosclerosis, microbial infections and cancer (Pokhrel and Neupane 2021). It has been used as a brain tonic for the mentally challenged. It has also been used traditionally and in Ayurvedic medicine for ailments of the central nervous system, including failing memory, insomnia, depression, stress and epilepsy. In South Africa, it was used to treat leprosy, wounds, cancer, fever and syphilis, while in Europe, the extract has been used for many years to treat wounds. The plant is also used to treat acne and allergies, as a psycho-physical regenerator and blood purifier (Suresh *et al*., 2010).

The plant *Hydrocotyle verticillata* Thunb. is commonly known as water pennywort, which comes under the family Apiaceae. It is cosmopolitan in origin and common habitats of the plant are South and North America and the West Indies. It usually grows in freshwater swamps, lagoons and along streams and rivers, sometimes partially submerged.

Hydrocotyle verticillata is a groundcover prostrate herb, stem creeping, rooting at nodes. Leaves are alternate, orbicular, serrate, blackish green, predominantly have long petiole. Inflorescence long, flowers in clusters, white coloured. Fruit cremocarp (Fig. 2). Its juice is used for the treatment of fevers. The poultice is used for the treatment of wounds and boils. The decoction of the plant is used for the treatment of abscesses, colds, coughs, hepatitis, influenza, prurities and sore throats (Umate and Deogade 2020).

The main objective of the present study is to investigate the antimicrobial activity of both study plants.

MATERIALS AND METHODS

Collection and identification of study plants. The plant *Centella asiatica* L. and *Hydrocotyle verticillata* Thunb. were collected from the bank of Bhavani river in Erode district, Tamil Nadu. The authenticity of the plants was confirmed by referring to the research articles.

Macroscopical analysis. Macroscopic characters of the simple determination technique, which includes organoleptic studies like shape, size, colour and odour of the plant and its extra features. The entire plants of *Centella asiatica* and *Hydrocotyle verticillata* were analysed (Gamble and Fischer 1915- 1936) in the field, photographed in their original environment and evaluated botanically.

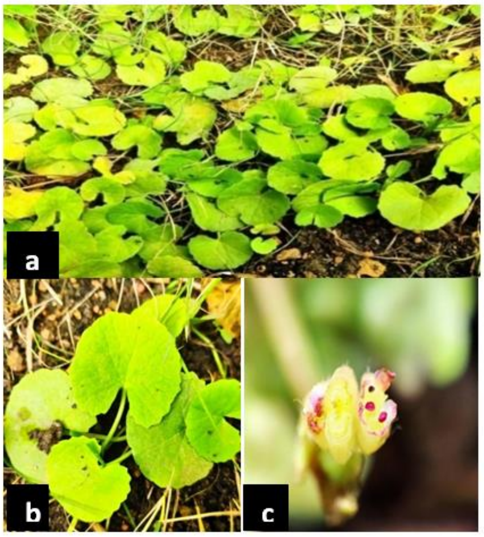


Fig. 1. Images of *Centella asiatica*; a- habit, b- leaves, c- inflorescence.

Fig. 2. Images of *Hydrocotyle verticillata*; a- Habit, b- Leaves, c- Inflorescence.

Preparation of plant powder. Fresh leaves of study plants (*Centella asiatica* and *Hydrocotyle verticillata*) were cleaned to remove adhering dust and then shade dried. The shade dried plant materials were mechanically ground to a coarse powder and used for further investigations (Fig. 3).

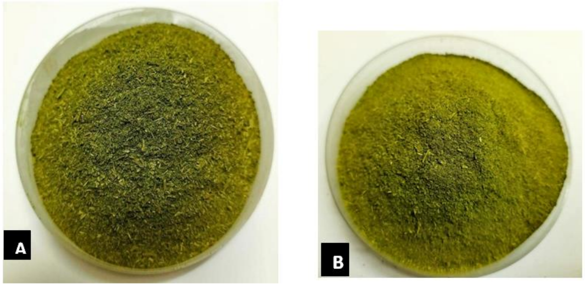


Fig. 3. Images showing plant powder of A. *Centella asiatica* and B. *Hydrocotyle verticillata*

Preparation of the extract. Coarsely powdered plant material was extracted using Benzene and Ethanol through the Soxhlet apparatus. The collected extracts were then used for testing against selected microorganisms.

Microorganisms:

The microorganisms used in the present study are *Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus.* The cultures were collected from the KMCH hospital at Erode in Tamil Nadu, India.

Composition of Bacterial Medium

Peptone - 5g/l
Sodium chloride - 5g/l
Yeast - 3g/l
Beef extract - 3g/l
pH - 5.4

Nutrient Agar medium

Beef extract - 5g/l
Casein hydrolysate - 1g/l
Starch - 1g/l
Agar - 3g/l
pH - 5.4

Preparation of Inoculum. Stock cultures were maintained at 4ºC on nutrient agar slants. Active cultures for experiments were prepared by transferring a loopful of culture to 10 ml of nutrient broth and incubating at 37ºC for 24 hours for bacterial proliferation.

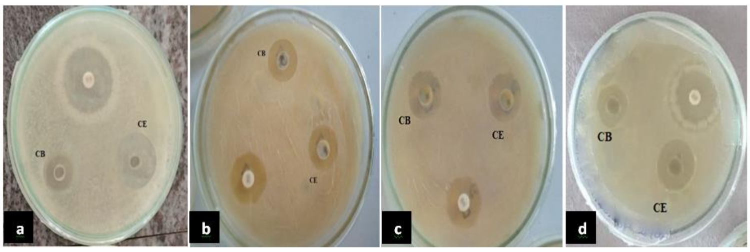
Antibacterial assay. The autoclaved media (Mueller-Hinton agar media) was poured into the petridishes. The test strain (0.2 ml) was inoculated into the media to an inoculum size of 108 cells/ml. The plant extracts were tested against *Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus* for antibacterial activity by using the agar well diffusion assay.

Agar-well diffusion method

Procedure. An Agar well bioassay was employed for testing the antibacterial activity of the extract. 100 µl of extract were taken for each study of activity. 24-hour old cultures of test organisms were seeded onto Mueller Hinton agar plates and uniformly spread with a spreader. Wells (5 mm) were made in the agar plate with a sterile cork borer. The extract was introduced into the well and the plates were incubated at 37ºC for 24 hours. The antibacterial activity of the extract was determined by measuring the diameter of the inhibition zone. The antibiotic disc is used as a control. The antibacterial assay for each of the extracts against all microorganisms tested was performed in triplicate.

RESULTS

Centella asiatica. The effects of benzene and ethanol extracts of *Centella asiatica* against the bacteria *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Klebsiella pneumonia* by using agar well diffusion method are shown in Table 1, Fig. 4, 6. The results clearly showed that the tested plant extracts were specific in their action against the growth of bacteria. The ethanol extract was significantly more active against all the tested bacteria than the benzene extract. The inhibitory zones of benzene extract against the bacteria *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Klebsiella pneumoniae* are 9.7 ± 0.2mm, 8.6 ± 0.3mm, 7.8 ± 0.2mm, 6.2 ± 0.3mm respectively. The inhibitory zones of ethanol extract against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Klebsiella pneumoniae* are 11.1 ± 0.3mm, 10.0 ± 0.1mm, 8.2 ± 0.1mm, 6.4 ± 0.2mm respectively.



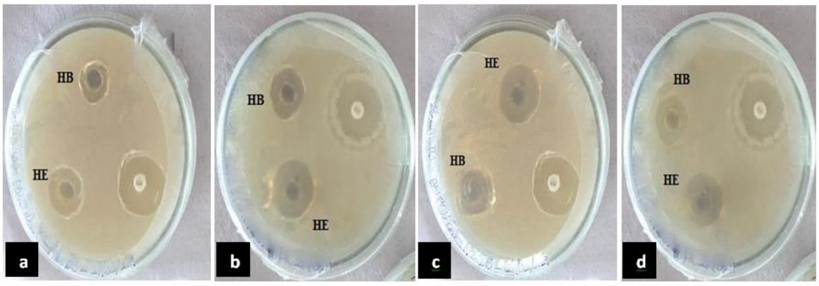
CB - *C. asiatica* Benzene extract CE - *C. asiatica* Ethanol extract

Fig. 4. Antibacterial activity of *Centella asiatica* against the bacteria; a- *Staphylococcus aureus*, b- *Bacillus subtilis*, c- *Escherichia coli*, d- *Klebsiella pneumonia.*

Table 1: Effect of Plant Extracts of *Centella asiatica* L. against Bacteria.

Content	Zone of inhibition (mm)				
	Samples	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Klebsiella pneumoniae*
Control	Chloramphenicol	18.5 ± 0.1	17.2 ± 0.1	15.0 ± 0.1	16.2 ± 0.1
Solvent Extract	Benzene	9.7 ± 0.2	8.6 ± 0.3	7.8 ± 0.2	6.2 ± 0.3
	Ethanol	11.1 ± 0.3	10.0 ± 0.1	8.2 ± 0.1	6.4 ± 0.2

Hydrocotyle verticillata. The benzene and ethanol extracts of *Hydrocotyle verticillata* showed significant inhibitory effects on gram positive and gram negative bacteria such as *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Klebsiella pneumoniae* which were depicted in Table 2, Fig. 5 and 6. The inhibitory zones of benzene extract against the bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* are 10.6 ± 0.2mm, 9.6 ± 0.3mm, 8.2 ± 0.2mm, 7.4 ± 0.3mm respectively. The inhibitory zones of ethanol extract against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Klebsiella pneumoniae* are 12.4 ± 0.3mm, 10.2 ± 0.1mm, 8.4 ± 0.1mm, 6.8 ± 0.2mm respectively.



HB – *H. verticillata* Benzene extract HE – *H. verticillata* Ethanol extract

Fig 5: Antibacterial activity of *Hydrocotyle verticillata* against the bacteria; a- *Staphylococcus aureus*, b- *Bacillus subtilis*, c- *Escherichia coli*, d- *Klebsiella pneumoniae*

Table 2: Effect of Plant Extract of *Hydrocotyle verticillata* Thunb. against Bacteria.

Content	Zone of inhibition (mm)				
	Samples	***Staphylococcus aureus***	***Bacillus subtilis***	***Escherichia coli***	***Klebsiella pneumoniae***
Control	Chloramphenicol	18.5 ± 0.1	17.2 ± 0.1	15.0 ± 0.1	16.2 ± 0.1
Solvent	Benzene	10.6 ± 0.2	9.6 ± 0.3	8.2 ± 0.2	7.4 ± 0.3
Extract	Ethanol	12.4 ± 0.3	10.2 ± 0.1	8.4 ± 0.1	6.8 ± 0.2

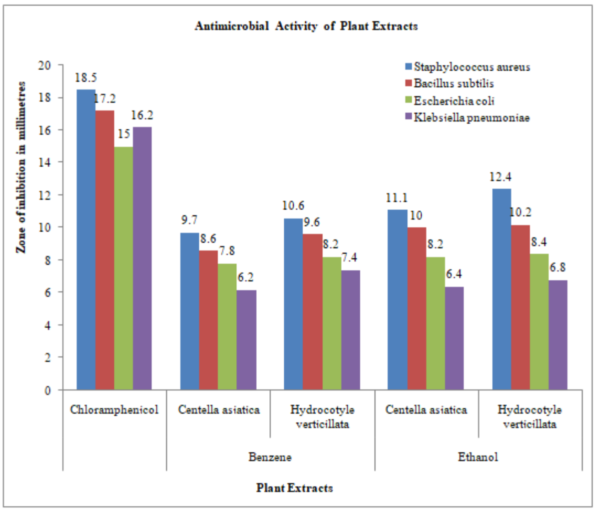


Fig. 6. Graph showing antimicrobial activity of plant extracts against different microorganisms.

DISCUSSION AND SUMMARY

The comparative analysis of *Centella asiatica* and *Hydrocotyle verticillata* through an antimicrobial approach is limited. The medicinal properties of the plants are due to the presence of various phytoconstituents. These are responsible for the antimicrobial activity of the plant extracts. The present study focused on the comparative analysis of *Centella asiatica* and *Hydrocotyle verticillata* on the basis of antimicrobial studies.

All the extracts of *Centella asiatica* and *Hydrocotyle verticillata* showed significant antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Klebsiella pneumoniae* at 100 µg/ml concentration. The ethanol extract of *Centella asiatica* showed more significant antimicrobial activity against all the tested bacteria when compared to the benzene extract. In *Hydrocotyle verticillata*, the ethanol extract showed a greater inhibition zone against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* than the benzene extract but the benzene extract showed more significant antimicrobial activity against *Klebsiella pneumoniae* than the ethanol extract. Similarly, Malaviya and Mishra (2011) reported Alcohol extract of *Malus domestica* (apple) was found to be most effective against the bacterial strain, *B. subtilis.*

Taemchuay *et al*. (2009) identified that ethanol was the most effective solvent for the *Centella asiatica* extraction. Bhagawati *et al*. (2021) reported that the alcohol extract showed the most significant antimicrobial activity when compared with the benzene extract against *Escherichia coli.* In the same way, Senthilkumar (2018) reported that the higher concentration of both benzene and ethanol extract showed significant antimicrobial activity against *Klebsiella pneumoniae* in *Centella asiatica.*

The ethanol extract of *Hydrocotyle javanica* exhibited a higher zone of inhibition against *Bacillus subtillus* and *Escherichia coli* (Sood and Yadav 2021). It is in accordance with the present study. The ethanol extract of *Hydrocotyle sibthorpioides* showed antimicrobial activity against *Klebsiella pneumoniae* (Khoo *et al*., 2014).

Thus, antimicrobial studies revealed that the plant *Hydrocotyle verticillata* shows more antimicrobial activity against the tested microbes than the plant *Centella asiatica*.

REFERENCES

Ahmad, T., Kamruzzaman, M., Ashrafuzzaman, M., Ahmad, A. & Ahmed, L. (2015). *In vitro* antimicrobial activity of different extracts of gotu kola and water spinach against pathogenic bacterial strains. *Curr. Res. Microbiol. Biotechnol.*, *3*(4), 663-669.

Bendix, J. & Hupp, C. R. (2000). Hydrological and geomorphological impacts on riparian plant communities. *Hydrological Processes*, *14*(16-17), 2977-2990.

Bhagawati, M., Hazarika, N. K. & Sarmah, R. (2021). Antimicrobial activity of the crude extracts of *Houttuynia cordata* and *Centella asiatica* on some human gut microflora. *International Journal of Ayurvedic Medicine*, *12*(1), 94-101.

Dash, B. K., Faruquee, H. M., Biswas, S. K., Alam, M. K., Sisir, S. M. & Prodhan, U. K. (2011). Antibacterial and antifungal activities of several extracts of *Centella asiatica* L. against some human pathogenic microbes. *Life Sciences and Medicine Research*, 1-5.

Gamble, J. S. & Fischer, C. E. Flora of the Presidency of Madras. 1915 – 1936, Vol I – III, Ad. Lord and sons limited, London.

Gohil, K. J., Patel, J. A. & Gajjar, A. K. (2010). Pharmacological review on *Centella asiatica*: a potential herbal cure-all. *Indian Journal of Pharmaceutical Sciences*, *72*(5), 546.

Jayaprakash, S. B. & Nagarajan, N. (2016). Studies on the bioactive compounds and antimicrobial activities of medicinal plant *Centella asiatica* (Linn). *J. Med. Plants Stud.*, *4*(5), 181-185.

Jelani, S., Jabeen, F., Prabhakar, M. & Leelavathi, P. (1993). Pharmacognostic studies on *Centella asiatica* (L) urban. *Anc. Sci. Life*, *12*(3-4), 439.

Khoo, K. S., Gwee, P. S., Beh, V., Chung, T. C., Tan, L. Y., Sit, N. W. & Ong, H. C. (2014). Antifungal and antibacterial properties of three medicinal plants from Malaysia. *Acta Hortic*, *1023*, 109-116.

Lansdown, R. V. (2019). *Centella asiatica*. *The IUCN Red List of Threatened Species*, 2.

Malaviya, A. and Mishra, N. (2011). Antimicrobial activity of tropical fruits. *Biological Forum – An International Journal*, *3*(1), 1-4.

Mubassara, S., Sarkar, A. K. & Huda, M. N. (2017). Antioxidative and antimicrobial activities of four important medicinal herbs of bangladesh. *Int. J. Ayurveda Pharma. Res*, *5*(10), 9-14.

Nag, K. and Hassan, Z. (2011). Uses of Wild Medicinal Herbs and Ecology of Gardens of District Bhopal, Madhya Pradesh (India). *Biological Forum — An International Journal*, *3*(1), 29-31.

Parker, R., Roberts, N. & Burke, M. (2020). You Against the World!–The Science Behind Skin and Skincare Products. *Discovering Cosmetic Science*, 109-154.

Pokhrel, S. & Neupane, P. (2021). Phytochemical analysis, antioxidant and antibacterial efficacy of methanol and hexane extract of *Centella asiatica*. *Bibechana*, *18*(2), 18-25.

Samy, R. P., Ignacimuthu, S. & Sen, A. (1998). Screening of 34 Indian medicinal plants for antibacterial properties. *J. Ethnopharmacol*, *62*(2), 173-181.

Samy, R. P. & Ignacimuthu, S. (2000), Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. *J. Ethnopharmacol*, *69*(1), 63-71.

Senthilkumar, M. (2018) Investigations on quantification of bioactive compounds and *in vitro* antibacterial property of important medicinal plant of *Centella asiatica* (L.) urban. *Int. J. Res. Pharm. Pharm. Sci*, *3*(1), 127-132.

Sood, K. & Yadav, R. N. S. (2021). Bioactivity guided isolation and characterization of antimicrobial principle from *Hydrocotyle javanica* Thunb. *Indian J. Tradit. Knowl*, *20*(4), 913-919.

Suresh, M., PK, R., Panneerselvam, A., Dhanasekaran, D. & Thajuddin, N. (2010). Anti-mycobacterial effect of leaf extract of *Centella asiatica* (Mackinlayaceae). *Res. J. Pharm. Technol*, *3*(3), 872-876.

Taemchuay, D., Rukkwamsuk, T., Sakpuaram, T. & Ruangwises, N. (2009). Antibacterial activity of crude extracts of *Centella asiatica* against *Staphylococcus aureus* in bovine mastitis. *Kasetsart Vet*, *19*(3), 119-28.

Umate, P. R. & Deogade, M. S. (2020). Pharmacognostic study of *Hydrocotyle verticillata* Thunb. *J. Indian Syst. Med.*, *8*(2), 122.

How to cite this article: S.M. Dhivya, P. Vijayashalini, V. Sasmitha and M. Dhivyasree (2023). Antimicrobial Analysis of *Centella asiatica* L. and *Hydrocotyle verticillata* Thunb. *Biological Forum – An International Journal*, *15*(5a): 553-557.