Antibacterial and Antifungal Activities of Several Extracts of *Centella asiatica* L. against Some Human Pathogenic Microbes

***BK Dash[1], HM Faruquee[1], SK Biswas[1], MK Alam[1], SM Sisir[2], UK Prodhan[3]**

[1]Department of Biotechnology and Genetic Engineering, Faculty of Applied Science and Technology, Islamic University, Kushtia-7003, Bangladesh.

[2]Department of Pharmacy, Rajshahi University, Bangladesh.

[3]Department of Food Technology and Nutritional Science, Mawlana Bhashani Science and Technology University, Santosh, Tangail, Bangladesh.

*Correspondence to: Biplab Kumar Dash, biplab.iu@gmail.com

Accepted: June 21, 2011; Published: July 30, 2011

Abstract

An experiment was carried out to study the antimicrobial activity of petroleum ether, ethanol, chloroform, n- hexane and water extracts of *Centella asiatica* herb by agar well diffusion assay. The tested bacterial strains were *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* and fungal strains were *Aspergillus niger* and *Candida albicans*. Zone of inhibition produced by different extracts against the selected strains was measured and compared with standard antibiotic ciprofloxacin (10µg) and ketocanozole (10µg). The present study demonstrated that the petroleum ether, ethanol and chloroform extracts of *Centella asiatica* have higher antimicrobial activities (average 12-19 mm zone of inhibition) than n-hexane and water extracts (average 8-14 mm zone of inhibition) whereas n-hexane extract showed no activity against *E. coli*. All the extracts showed better results against the tested fungal strains comparing with ketocanozole (10µg). The results obtained in the present study suggest that the different extracts of *Centella asiatica* revealed a significant scope to develop a novel broad spectrum of antibacterial and antifungal herbal formulations.

Keywords: *Centella asiatica*; antibacterial activity; antifungal activity; disc diffusion assay.

1. Introduction

Despite tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance [1]. During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics [2] has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages [3]. Current research on natural molecule and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses [4]. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries [5]. A wide range of medicinal plants parts is used to extract as raw drugs and they possess varied medicinal properties. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw materials for many herbal industries [6]. Clinical microbiologists have great interest in screening of medicinal plants for new therapeutics [7]. The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds [8]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants [9]. Hence the sensitivity study of bacterial strains to the plant *Centella asiatica* was evaluated.

Centella asiatica (L) urban belonging to the family Umbeliferae is a common perennial herbaceous creeper flourishing abundantly in moist areas and distributing widely in tropical and subtropical countries including Bangladesh. Various chemical constituents are reported in *Centella asiatica* like asiaticoside, madecassoide, madecassicacid, asiatic acid, glucose, rhamnose, terpenoids, sitosterol, stigmasterol, fatty oils consist of glycerides of palmitic acid, stearic acid, linoleic acid, linolenic acid vitamins like ascorbic acid. It also contains calcium, iron, and phosphate [10, 11]. *C. asiatica* is claimed to possess a wide range of pharmacological effects,

being used for human wound healing, mental and neurological disorders, atherosclerosis, fungicidal, antibacterial, antioxidant and anticancer purposes. *C. asiatica* has also been reported to be useful in the treatment of inflammations, diarrhea, asthma, tuberculosis and various skin lesions and ailments like leprosy, lupus, psoriasis and keloid [12]. In addition, numerous clinical reports verify the ulcer-preventive and antidepressive sedative effects of *C. asiatica* preparations, as well as their ability to improve venous insufficiency and microangiopathy [13]. Therefore, the present investigation attempts to isolate and investigate the antimicrobial activities of *C. asiatica* extracts.

2. Methods

2.1. Plant material

The aerial part of plant of *Centella asiatica* was collected from local area of University campus region of Kushtia in September 2010. It was then botanically identified.

2.2. Preparation of the extracts

The aerial part of *Centella asiatica* was cleaned with deionized water and dried in shade and pulverized into fine powdered substance by a grinding machine. Each twenty gram of powder of *Centella asiatica* was weighted with the electric balance and transferred into five separate 100ml conical flasks. Then each 40ml of petroleum ether, ethanol, chloroform, n-hexane and water is added in the flasks respectively. The conical flasks were closed by foil paper and placed on a shaker at 37°C temperature for 24 hours. The crude extracts were then filtered by passing the extracts through Whatmann No. 1 filter paper and then concentrated under vacuum at 40°C by using a rotary evaporator. The residual extracts were stored in refrigerator at 4°C in small and sterile plastic bottles.

2.3. Tested microorganisms

Antibacterial activity of *Centella asiatica* powder extracts was investigated against two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative (*Escherichia coli* and *Proteus vulgaris*) registered bacterial isolates and two fungal strains (*Aspergillus niger* and *Candida albicans*), which were obtained from the Microbial Type Culture Collection (MTCC) of Microbiology Laboratory of the Biotechnology and Genetic Engineering Department, Islamic University, Kushtia, Bangladesh. The tested bacteria were cultured on Nutrient Agar (HiMedia, Mumbai) and the fungal strains on Potato Dextrose Agar (PDA) at 37°C for 24 h. The cultures were sub cultured regularly (every 30 days) and stored at 4°C.

2.4. Inoculum preparation

Ten ml of distilled water was taken into the screw cap tube and pure colony of freshly cultured bacteria and fungi were added into the tube and vortex was done. The OD (optical density) was measured with the colorimeter and microbial population was confirmed to be within in 10^7 ml^{-1} to 10^8 ml^{-1} and then plated out as inoculums [14].

2.5. Antimicrobial bioassay

The *in vitro* antimicrobial activities of the test samples were carried out by disc diffusion method [15, 16]. In this method, nutrient agar was used as culture media and the discs were placed aseptically over the bacterial culture on nutrient agar plates. Cups cut in medium using sterile cork borer about 8 mm in diameter are done for fungal culture. The cut agar discs were removed by vacuum device. Then, standard antibiotics ketocanozole (10μg) and dilution of the petroleum ether, ethanol, chloroform, n-hexane and water extracts were placed in appropriate position on the plate. After incubation at 37°C for 24 hours, the zone of inhibition around the discs was measured by millimeter scale. Discs were impregnated with each treatment and control was assayed on duplicate agar medium plate. The experiment was replicated two times to confirm the reproducible results. Sterile, blank paper discs impregnated with only sterile solvents served as negative control each time. Standard Ciprofloxacin (10μg/disc) was used as positive control for comparison of the antibacterial activity.

2.6. Statistical evaluation

The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of two replicates.

3. Results

3.1. Antimicrobial activities

Among all tested extracts, petroleum ether, ethanol and chloroform extracts were found to be most active and significant than corresponding organic extracts (Table 1, 2). Except n-hexane extract (which showed no activity against *E. coli*), all the extracts were found to be active against four tested bacteria (*P. vulgaris, S. aureus, B. subtilis* and *E. coli*) and two tested fungi (*A. niger* and *C. albicans*). Petroleum ether extract was found to have maximum zone of inhibition against both *P. vulgaris* and *B. subtilis* (17 mm) while the minimum zone of inhibition was against *E. coli* (13 mm). The fungal strains *A. niger* and *C. albicans* shown zone of inhibition 14 mm and 13 mm respectively. The ethanol extract was most effective against *B. subtilis* (19 mm) (Figure 1) and it was significantly effective against both *P. vulgaris* and *S. aureus* (17 mm) and *E. coli* (16 mm) and the fungal strains *A. niger* (16 mm) and *C. albicans* (15 mm). Chloroform extract was found to be very active against all tested bacterial and fungal strains. The n- hexane extract was found to be moderately effective against five of the tested strains whereas it is inactive against *E. coli.* The aqueous extract was relatively found to be less effective. It showed less effectiveness against *S. aureus* (8 mm) and both *B. subtilis* and *C. albicans* (9 mm) but moderate result against *P. vulgaris* (14 mm) and both *E. coli* and *A. niger* (11 mm). Negative control (disc containing only solvent) showed no zone against any microorganism. The positive controls (ciprofloxacin and ketocenozole) produce zone of inhibition against the tested microorganisms (Table 1, 2).

Table 1: Antibacterial activity of different extracts of *C. asiatica.*

Sample Bacteria	Diameter of Zone of Inhibition (mm)					
	Petroleum Ether	Ethanol	Chloroform	n-Hexane	Aqueous	Ciprofloxacin (10µg)
P. vulgaris	17	17	14	11	14	24
S. aureus	15	17	16	12	8	22
B. subtilis	17	19	12	10	9	26
E. coli	13	16	14	0	11	20

Table 2: Antifungal activity of different extracts of *C. asiatica.*

Sample Fungi	Diameter of Zone of Inhibition (mm)					
	Petroleum Ether	Ethanol	Chloroform	n-Hexane	Aqueous	Ketocanozole (10 µg)
A. niger	14	16	13	13	11	12
C. albicans	13	15	15	11	9	10

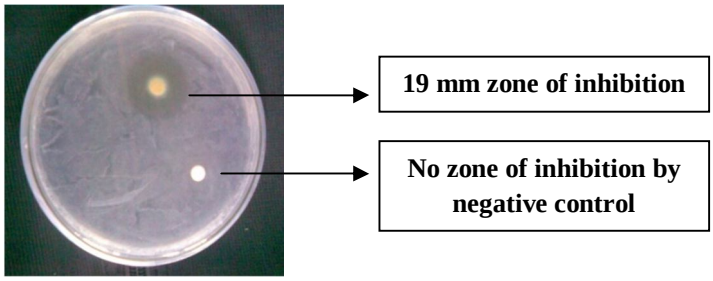

Figure 1: Ethanol extract of *Centella asiatica* produced highest zone of inhibition (19 mm) against *Bacillus subtilis.*

4. Discussion

Microorganisms are the concealed enemies to the mankind. There are a lot of antimicrobial drugs of which some are discovered or established and over 250,000 undiscovered flowering plants with medicinal properties exist worldwide [17]. Hence, the last decade witnessed an increase in the investigations on plants as a source of human disease management [18-21] and more natural antimicrobials have driven scientists to investigate the effectiveness of inhibitory compounds such as extracts from plants [22]. There are several reports of antibiotics resistance of human pathogens to available antibiotics [23, 24]. The main objective of this work is to increase the utilization of biomass from herb in order to isolate new biologically active compounds.

This study deals with four deadly pathogenic bacterial and two fungal strains. In the present work, the antibiotic potential of five different extracts of *C. asiatica* has been determined against different microorganisms i.e., *P. vulgaris, S. aureus, B. subtilis E. coli, A. niger* and *C. albicans.* In this study, crude petroleum ether, ethanol and chloroform extracts are found to be very effective in inhibiting the growth of all the tested microorganisms ranging from 12-19 mm zone of inhibition which are satisfactory comparing with ciprofloxacin (10µg) and absolutely better comparing with the commercial antifungal disc used. On the other hand, crude n-hexane and aqueous extracts showed somehow better antimicrobial activities ranging from 9-13 mm zone of inhibition against *A. niger* and *C. albicans* comparing with ketocanozole (10µg). Only *E. coli* was not sensitive to the n-hexane extract but the rest showed zone of inhibition ranging from 10-12 mm. Aqueous extract showed lowest inhibitory activity against the tested bacteria ranging from 8-14 mm zone of inhibition. Blank disc produced no zone of inhibition indicating that the solvents themselves did not possess any antimicrobial effect.

5. Conclusion

The extracts of *Centella asiatica* were found to be effective antibacterial agents against human pathogens**.** This study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity with the required minimum inhibitory concentration (MIC). Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect to identify the active ingredients which can be used in drug development program for safe health care services.

Competing Interests
The authors declare that they have no competing interests.

Authors' Contributions
All authors contributed equally during the collection of plant material, experimental data analysis and in the preparation of manuscript.

Acknowledgement

We wish to thank the Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia-7003, Bangladesh for providing the fund and contributing the materials essential for this study.

References

1. Zampini IC, Cuello S, Alberto MR, et al., 2009. Antimicrobial activity of selected plant species from the Argentine puna against sensitive and multiresistant bacteria. Journal of Ethnopharmacology, 124: 499-505.

2. Okemo PO, Bais HP, Vivanco JM, 2003. *In vitro* activities of *Maesa lanceolata* extracts against fungal plant pathogens. Fitoterapia, 74: 312-316.

3. Bouamama H, Noel T, Villard J, et al., 2006. Antimicrobial activities of the leaf extract of two Moroccan *Cistus* L species. Journal of Ethnopharmacology, 104: 104-107.

4. Arora DS, Kaur GJ, 2007. Antibacterial activity of some Indian medicinal plants. Journal of Natural Medicine, 61: 313-317.

5. Hashim H, Kamali EL, Mohammed Y, 2010. Antibacterial activity and phytochemical screening of ethanolic extracts obtained from selected Sudanese medicinal plants. Current Research Journal of Biological Science, 2(2): 143-146.

6. Uniyal SK, Singh KN, Jamwal P, et al., 2006. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal. Western Himalayan Journal of Ethnobiology and Ethnomedicine, 2: 1-14.

7. Kumar PA, Rajkumar, Kanimozhi M, 2010. Phytochemical screening and antimicrobial activity from five Indian medicinal plants against human pathogens. Middle-East Journal of Scientific Research, 5(6): 477-482.

8. Lai PK, Roy J, 2004. Antimicrobial and chemopreventive properties of herbs and spices. Current Medicinal Chemistry, 11: 1451-1460.

9. Erdogrul OT, 2002. Antibacterial activities of some plant extract used in folk medicine. Pharmaceutical Biology, 40: 269-273.

10. The Wealth of India: a dictionary of Indian raw materials and industrial products; CSIR, Vol III: 428-429.

11. Trease GE, Evans WC, 1966. Textbook of Pharmacognosy. The Williams & Wilkins Company, Baltimore 2, Maryland, p. 130, 303,480.

12. Ullah MO, Sultana S, Haque A, et al., 2009. Antimicrobial, cytotoxic and antioxidant activity of *Centella asiatica*. European Journal of Scientific Research, 30(2): 260-264.

13. Zheng CJ, Qin LP, 2007. Chemical components of *Centella asiatica* and their bioactivities. Chinese Journal of Integrative Medicine, 5(3): 348-351.

14. Gur S, Turgut-Balik D, Gur N, 2006. Antimicrobial activities and some fatty acids of turmeric, ginger root and linseed used in treatment of infectious diseases. World Journal of Agricultural Sciences, 2(4): 439-442.

15. Bauer AW, Kirby WM, Sherris JC, et al., 1966. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 45: 493-496.

16. NCCLS, 2000. Performance standards for antimicrobial disk susceptibility tests: Approval standard M2-A7 7th edition. Pennsylvania: Clinical and Laboratory Standards Institute.

17. Madureira MDC, 2008. Rediscovering traditional medicine. Spore, 136: 16-17.

18. Aiyelagabe OO, 2001. Antibacterial activity of *Jatropa multifida* roots. Fitoterapia, 72: 544-546.

19. Prashanth D, Asha MK, Amit A, 2001. Antibacterial activity of *Punica granatum*. Fitoterapia, 72: 171-173.

20. Mounishwamy V, Davimani S, Gunasegaran R, 2002. Antibacterial activity of Gossyptein isolated from *Hibiscus saddriffa*. The Antiseptic, 99: 81-82.

21. Woldemichael GM, Wachter G, Singh MP, 2003. Antibacterial diterpenes from *Calceolaria pinifolia*. Journal of Natural Products, 66: 242-246.

22. Nasar-Abbas SM, Halkman AK, 2004. Antimicrobial effect of water extract of sumac (*Rhus coriaria* L.) on the growth of some food borne bacteria including pathogens. International Journal of Food Microbiology, 97: 63-69.

23. Ganguly R, Mishra P, Sharma A, 2001. Microbes and infection. Indian Journal of Microbiology, 41: 211-213.

24. Di Martino P, Gagniere H, Berry H, et al., 2002. Antibiotic resistance and virulence properties of *Pseudomonas aeruginosa* strains from ventilated patients with pneumonia in intensive care units: comparison with imipenem resistant extra-respiratory tract isolates from uninfected patients. Microbes and Infection, 4: 613–620.