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Research Article

Effect of *Centella Asiatica* Extracts on *Shigella Dysenteriae* and *Bacillus coagulans* Compared to Commonly Used Antibiotics

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ABSTRACT
Centella asiatica is known for its antibacterial property from time immemorial. A comparative study on the action of its crude extract on *Shigella dysenteriae* and *Bacillus coagulans* was performed to see the difference of its effects on the two bacteria. As one of them is a pathogen and the other one is useful for human hosts. The study revealed that 1 mg of the extract dissolved in 1ml 4% DMSO inhibits *Shigella. dysenteriae* with zone of inhibition of 12±1 mm but could not act on *Bacillus coagulans* in same concentration. However, known antibiotics act on both the organisms. The action of acetone, chloroform and methanol extracts of *Centella asiatica* on *Shigella dysenteriae* did not vary significantly. The GC-MS analysis of the methanol extracts with peaks > 90% similarity index with NIST library revealed bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methylene; caryophyllene; 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester; 1h-3a,7-methanoazulene, octahydro-1,4,9,9-tetramethyl.

Keyword: Guts flora; *Centella asiatica*; extracts; inhibition; pathogen

INTRODUCTION

Centella asiatica (Linn) is one of the ethanomedicinal plants used for the ailment of human guts irritation and disorders. In Assam; a state of India, it is known as 'Manimuni' and widely been used as antimicrobial agent during guts infections. It is also described as Mandukaparni in Ayurvedic System of medicine [1]. *Centella asiatica* is claimed to possess a wide range of pharmacological effects, such as wound healing [2], fungicidal antimicrobial [3], antioxidant and anticancer [4, 5]. Besides these, it has also been reported to be useful in the treatment of various problems like inflammation, diarrhoea, skin lesions, tuberculosis, asthma, leprosy etc. [6]. The herb is used as vegetable by the people of Assam (India) [7]. *Centella asiatica* is also of considerable importance in China, Nepal, Bangladesh, Malaysia, Indonesia, and Sri Lanka [8,9]. The human digestive-tract or gastrointestinal (GIT) associated microbes are referred to as the gut microbiome. It was reported that human gut consists of more than 50 bacterial phyla [10] dominated by Bacteriodes and Firmicutes. The number of bacterial species present in human

gut vary widely but it is generally accepted that individuals harbor more than 1000 microbial species level phylotypes [11-13]. Favourable microenvironment of gut harbor mostly the genera *Bacteriodes, Bifidobacterium, Streptococcus, Enterococcus, Clostridium, Lactobacillus* and *Ruminococcus* as the luminal community[14]. Whereas *Clostridium, Lactobacillus* and *Enterococcus* were detected in the mucus layer and epithelial crypts of the small intestine as well [14]. It is well established that microbiome of human guts play manifold function for human health such as enhancing metabolism, synthesizing vitamins [15-18], protecting host from pathogenic microorganisms by competitive-exclusion effect, synthesizing bacteriocin, competing for attachment sites on epithelial cells and competing for resources [18].
On infection by pathogenic microorganisms such as *Shigella dysenteriae* causing shigellosis, antibiotics viz Ampicillin, Cefixime, Ciprofloxacin, Nalidixic acid are generally recommended. These antibiotics have side effects and may have effect on non pathogenic microbiome .On the other hand *Centella asiatica* has antimicrobial properties.Therefore an effort has been made to ascertain i) if the above mentioned antibiotics have effect on non-pathogenic microorganism with reference to *Bacillus coagulans* and ii) whether it is possible to replace antibiotics mentioned above by using *Centella asiatica.*

MATERIAL AND METHODS

Collection and Identification of Plant Material

The plant specimen *Centella asiatica* was collected from the Agricultural Research Institute, Kahikushi, Guwahati, Assam India and cultivated in the laboratory. The identity of the plant was confirmed by the Department of Botany Gauhati University on the herbarium submitted to it having accession No. 18243.

Preparation of crude extract

Matured Leaves of the plant were collected and air dried for a week. Dried leaves were grinded, sieved and stocked. The powdered plant leaves weighing 200 g were subjected for extraction in different solvent viz methanol, acetone and chloroform, for six hours at 62 ^{0}C using soxhlet apparatus followed by evaporation of the solvents and drying by rotator evaporator (BUCH 1 TYPE IRA). The final products were stored in sterile screwed capped bottles at – 4 ^{0}C.

Collection and isolation of bacterial strains

The pathogen *Shigella dysenteriae* and *Bacillus coagulans* were collected from various clinical specimens at Bacteriology Laboratory, Microbiology Department of Gauhati Medical College. These organisms were cultured in blood agar and after identification the pure cultures were maintained in Muller Hinton agar medium. Biochemical tests including gram staining were performed for their identification. The identification of the organisms was done by observing their colony characteristics and performing various biochemical tests including KB2 kit, HiMedia. The stock samples were preserved at -20 ^{0}C [19].

Comparative analysis of Antimicrobial activity of the plant extract and known antibiotics

Shigella dysenteriae and *Bacillus coagulans* were evenly cultured in the Muller Hinton agar media by swabbing technique. The zone of inhibition was assayed by well diffusion method. Wells of size 6mm diameter was prepared after inoculation of the organisms to incorporate 50 µg of the 1 mg of extract in 1 ml of 20% Dimethyl sulfoxide (DMSO). A well for control was filled with 20% DMSO alone as control so as to determine neutral effect of the DMSO. Similarly antibiotic discs containing 10µg of Ampicillin, 5µg Ciprofloxacin, 30µg Nalidixic acid and 5µg Cefixime (CFM) were used for evaluation and their effects on both the organisms. The extracts that showed inhibitory effects were further evaluated through MIC (Minimum Inhibitory Concentration) using microtitre plate based assay with resazurin dye.

Minimum Inhibitory Concentration (MIC)

Preparation of Resazurin Dye Solution: To 100 ml distilled water 0.5 gm of the resazurin dye was added, mixer was vortexed to get homogenous mixture of resazurin dye solution.

Resazurin based Microtitre Dilution Assay (RMDA)

100 µl of Luria Bertani (LB) broth was added to all microtitre plate wells that were to be tested. To the broth of 100 µl of drug prepared in different solvent was transferred from column 1

to column 6 by two fold serial dilution technique. The experiments for each solvent were triplicated. To each of the well 10µg of resazurin dye was added. Finally 10µg of bacterial inoculums was added to each well to achieve a final concentration of $5x10^6$ CFU/ml each of the plate had a set of 2 controls (a) a column with all solution except the bacterial solution and (b) a column with all solution except the studied plant extracts. The plates were then incubated at 37^0C and results were observed after 24 hours. The experiment was performed under aseptic condition in the laminar air flow. After 24 hours the colour change was observed. The colour of the wells changed from blue to pink which is considered to be positive result. The lowest concentration of the plant extract at which the colour change occurred was taken as the MIC values. Form the triplicates the average value was considered to be final.

Thin Layer Chromatography (TLC)

TLC of *Centella asiatica* leaf extract was done using different solvent systems which confirmed the presence of the different phytochemicals. Two different solvent systems were prepared and used for separation of the compounds in the extract. One consisted of the mixture of chloroform and methanol and the other mixture of chloroform, glacial acetic acid and methanol. The separation of the compounds were done as described by *Biradar et.al.* [20].

GC – MS

Thin layer Chromatography (TLC) followed by Gas chromatography - Mass spectrometry (GC – MS) was performed for predicting the compounds present in the extracts.

Statistical Analysis

All statistical analysis were performed using MS excel.

RESULTS

The biochemical tests revealed that the organism which was colourless, circular, convex, hemolytic colony moderately translucent with smooth surfaces and entire edges was identified to be *Shigella dysenteriae*. The organism with convex, entire margined and smooth surfaced colonies, white to cream in colour and did not grow in 7% NaCl containing media was identified to be *Bacillus coagulans* (Table 1).

S. dysenteriae and *B. coagulans* were grown evenly covering the entire media before application of the plant extracts. It has been observed that the extracts of *C. asiatica* inhibit *S. dysentria* forming a zone of inhibition ranging from 11-12 mm in size (Table 2, Fig 1A). Minimum Inhibitory Concentration of the extracts of *C. asiatica* was analyzed in case of the *S. dysentria* (Table 3) ignoring *B. coagulans* as no inhibition was observed for it. The *S. dysenteriae* was inhibited by Ciprofloxasin and Nalidixic acid with a zone on inhibition of 25 and 32 mm respectively (Table 2, Fig 1B). *B.coagulans* was susceptible to all of the antibiotics used in the study with zone of inhibitions 11, 31, 23, 24 mm on application of antibiotics Ampicillin, Ciprofloxasin, Nalidixic acid and CFM respectively. The minimum inhibitory concentration of the extracts required for inhibiting *S. dysentria* was recorded to be 2.5, 1.25 and 2.08 for extract in solvents acetone, methanol and chloroform respectively. The extract which had shown better inhibitory effects was then subjected for Thin Layer Chromatography (TLC) followed by Gas Chromatography – Mass Spectrometry) GC-MS analysis.

Thin Layer Chromatography (TLC) was done using solvent system chloroform and methanol in 9:1 proportion with different leaf extracts of *C. aciatica* reveals three compounds, Rf values of the experiment is shown in Table 4. When used solvent system chloroform glacial acetate and methanol also revealed three compounds and four compounds were present in methanol extract, Rf values of the experiment is shown in Table 4.

Plant extract were subjected for thin layer chromatography for separation followed by GC-MS (Gas Chromatography Mass spectrometry). The fractions obtained from TLC were scrapped and dissolved in DCM (Dichloromethane) filtered and centrifuged and finally subjected for GC – MS analysis (Fig. 2). Those peaks matching similarity index greater than 70% in the NIST library were assigned. The library search of the peaks which revealed the following compounds were present in the plant extracts. Bicycle [7.2.0] undec-4-ene,4,11,11-trimethyl-8-methylene; caryophyllene; 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester; 1h-3a,7-methanoazulene, octahydro-1,4,9,9-tetramethyl.

Table 1: Showing the results of the various test performed for identification:

Sl. No	Tests	Results	
		S. dysenteriae	***B. Coagulans***
01	Gram Staining	Gram Negative	Gram Positive
02	Mobility	Negative	Positive
03	Indole	Negative	Negative
04	Methyl red	Positive	Positive
05	Voges- proskaver	Negative	Variable
06	Citrate	Negative	Negative
07	Catalase	Positive	Positive
08	Oxydase	Negative	Negative
09	Nitrate Reductase	Positive	Negative
10	Sugar	Glucose + Lactose	-

Table 2: Antimicrobial activity of the plant extracts and antibiotics on the organisms:

	Different types of extracts with diameter of inhibition in mm						
Organism	**Acetone**	**Chloroform**	**Methanol**	**Ampicilin**	**Ciprofloxacin**	**Nilidixic**	**CFM**
S. dysentreae	11	11	12	R	32	25	R
	10	10	13		31	23	
	10	12	11		33	26	
	0.527(sd)	1(sd)	1(sd)		1(sd)	1.527(sd)	
Lactobacillus sp	NA	NA	NA	10	30	25	26
				11	33	23	24
				13	30	22	22
				1.527(sd)	1(sd)	1.527(sd)	2(sd)

sd is standard deviation, NA is no activity, R is resistant, CFM is Cefixime

Table 3: Minimum inhibitory concentration of the plant extracts against *S. dysenteriae*

Extracts	Minimum Inhibitory Concentration (in mg)			Mean
	1	2	3	
Acetone	2.5	2.5	2.5	2.5
Methanol	1.25	1.25	1.25	1.25
Chloroform	1.25	2.5	2.5	2.08

Table 4: ANOVA within antibiotics activity against the organisms:

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	49	1	49	19.6	0.047421	18.51282
Within Groups	5	2	2.5			
Total	54	3				

SS is Sum of Squares, df is degree of freedom, MS is Mean Square, F is F test,
P-Value is probability value, F crit is F test critical value

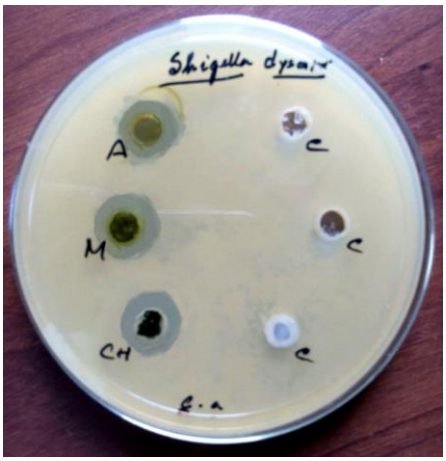

Figure 1A: the inhibition zones formed due to application of various plant extracts on S. *dysenteriae. A – Acetone extract, M – Methanol extract, CH – Chloroform extract, C – control*

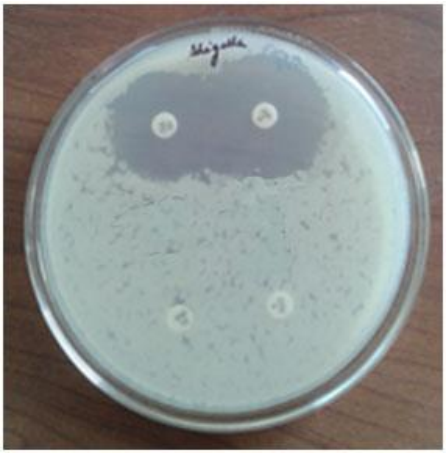

Figure 1B: the inhibition zones formed due to application of various antibiotics on *S. dysenteriae*

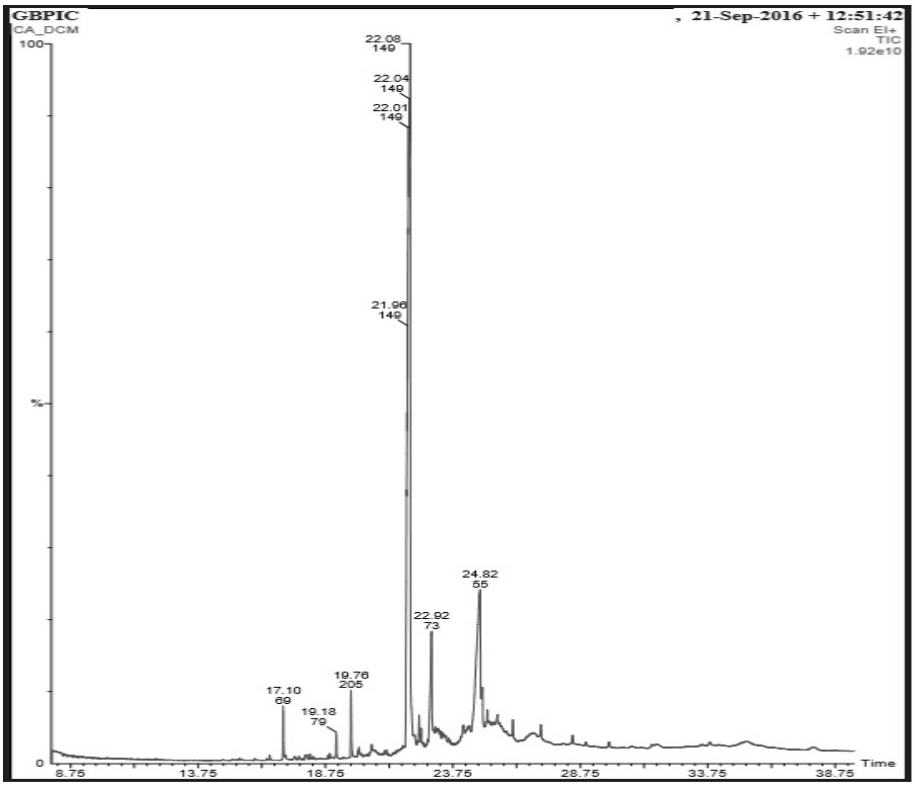

Fig.2: GC-MS peaks showing the probable compounds

DISCUSSIONS

The variation in the size of zone of inhibition was non-significant revealing all of the extracts obtained using different solvents were showing almost equal size of zone of inhibition. However maximum sized was recorded in case of the extracts obtained using methanol as a solvent. It was however, interesting to notice that plant extracts when applied on *B. coagulans,* no zone of inhibition were formed. The *S. dysenteriae* was resistant to Ampicillin and Cefixime (CFM) but inhibited by ciprofloxasin and Nalidixic acid where as *B.coagulans* was susceptible to all of the antibiotics used in this study. Analysis of

variance shows that *S. dysenteriae* and *B. coagulans* were inhibited by Ciprofloxacin and Nalidixic acid with no significant difference (p-value 0.047421). However, the zone of inhibition formed by these antibiotics varies significantly (p – value 0.727834) when compared among the actions of the different antibiotics (Table – 5, 6). The plant extract although produced smaller sized zone against *S. dysenteriae* showing its antimicrobial activity and significantly (P – value 0.985654) varies (Table - 7) when compared between the activity of various plant extracts and antibiotics Ciprofloxacin and Nalidixic acid. The capability of the extracts may be improved by increasing its concentration. The results also reveal that plant extracts did not have any inhibitory activity against *B. coagulans,* which is most interesting to be noted. This specificity of the plant extracts enabling it to be used targeting pathogenic *S. dysenteriae* specifically, without harming the beneficial microorganism (*B. coagulans*) present in the host. The antibacterial effect of the plant extracts on *S. dysenteriae* seems to be correlating with the result of Tanvir et al 2015 [21], where the zone of inhibition was 16mm on application of 16 mg/ml of plants extracts, which in this study is about 11-12 mm on application of 5mg/ml that may be because of the plant growth and synthesis of the metabolites as well as variation in the experiments performed. In this study the activity of the acetone, chloroform and methanol extracts did not significantly vary on the contrary some of the literature reported maximum sized zone of inhibition in case of chloroform extract. The study of ethanolic extract of *C. asiatica* was reported to have no action against *S. dysenteriae* [22], however, after three repetition of the experiment with methaonolic extract this study revealed inhibition as illustrated above (Table 2 – 3). The antimicrobial properties of *C. asiatica* which was observed in this study is well supported by several studies [23, 24, 25].

Table 5: Results of Thin layer chromatography of the leaf extracts:

Leaf extracts	Sol(CM)			Sol(CGAM)			
	R_f1	R_f2	R_f3	R_f1	R_f2	R_f3	R_f4
Acetone	0.88	0.82	0.78	0.95	0.71	0.47	-
Chloroform	0.86	0.78	0.74	0.89	0.63	0.52	0.36
Methanol	0.84	0.78	0.72	0.89	0.67	0.47	-

Sol (CM) is solvent Chloroform and methanol, Sol (CGAM) is Solvent Chloroform, Glacial Acetate and Methanol

Table 6: ANOVA between antibiotics activity against the organisms:

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4	1	4	0.16	0.727834	18.51282
Within Groups	50	2	25			
Total	54	3				

SS is Sum of Squares, df is degree of freedom, MS is Mean Square, F is F test, P-Value is probability value, F crit is F test critical value

Table 7: ANOVA between various plant extracts and antibiotics against the *S. dysenteriae*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.8	2	1.4	0.014468	0.985654	3.885294
Within Groups	1161.2	12	96.76667			
Total	1164	14				

SS is Sum of Squares, df is degree of freedom, MS is Mean Square, F is F test, P-Value is probability value, F crit is F test critical value

CONCLUSION

Centella asiatica is as important herb used as vegetable as well as ethno-medicine for gasteroenteric diseases since time immemorial. The present study reveals that the extract of the plant likely to be effective against *S. dysenteriae* (pathogen) without acting upon *B. coagulans* (useful bacteria). The compounds of *C. asiatica* may be established as pathogen targeting rather than host beneficial bacteria by further comparative studies. However, the present study reveals that *Centella asiatica* may be used as an alternative to antibiotics or along with antibiotics against *S. dysenteriae*. As the present and many of the studies have shown that the extracts of the plant have wound healing and antibiotic property the plant may be of high value. Further, bioinformatics approach of targets identification and molecular dynamics studies may throw insights of interactions.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. Nadkarni KM. Indian materia medica. Bombay,India: Popular Prakashan; 1996, p 662.
2. Suguna L, Sivakumar P, Chandrakasan G. Effects of *Centella asiatica* extract on dermal wound healing in rats. Indian J Exp Biol 1996; 34(12): 1208-1211.
3. Oyedeji OA, Afolayan AJ. Chemical composition and antibacterial activity of the essential oil of *Centella asiatica*. Growing in South Africa. Pharm Biol 2005; 43(3): 249-252.
4. Jayashree G, Muraleedhara GK, Sudarslal S, Jacob VB. Anti-oxidant activity of Centella asiatica on lymphoma-bearing mice. Fitoterap 2003; 74(5): 431-434.
5. Babu TD, Kuttan G, Padikkala, J. Cytotoxic and anti-tumour properties of certain taxa of Umbelliferae with special reference to *Centella asiatica* (L.) Urban. J Ethnopharmacol 1995; 48(1): 53-57.
6. Zheng C J, Qin L P. Chemical components of *Centella asiatica* and their bioactivities. J Integ Trad Chinese Western Med 2007; 5(3): 348-351.
7. List of Vegetables used in Assamese cuisine: Available from: https://en.wikipedia.org /wiki/List_of_vegetables_used_in_Assamese_ cuisine 03 Nov 2017.
8. Singh S, Gautam A, Sharma A, Batra A. *Centella asiatica* (L.): a plant with immense medicinal potential but threatened. Int J Pharm Sci Rev Res 2010; 4(2): 09-17.
9. Peiris KHS, Kays SJ. Asiatic Pennywort-*Centella asiatica* (L.) Urb.: A little-known vegetable crop. Horttechnol 1996; 6(1):13-18.
10. Schloss PD, Handelsman J. Status of the microbial census. Microbiol Mol Biol Rev 2004; 68(4): 686-691.
11. Claesson MJ, O'Sullivan O, Wang Q, Nikkilä J, Marchesi J R, Smidt H, Willem M, de Vos R, Paul R, O'Toole PW. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. PloS one 2009; 4(8): e6669.
12. Xu J, Gordon J I. Honor thy symbionts. Proceed Nat Academy Sci 2003; 100(18): 10452-10459.
13. Lozupone CA, Stombaugh J I, Gordon J I, Jansson J K, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature 2012; 489(7415): 220-230.
14. Swidsinski A, Weber J, Loening-Baucke V, Hale L P, Lochs H. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. J Clin Microbiol 2005; 43(7): 3380-3389.
15. Vyas U, Ranganathan N. Probiotics, prebiotics, and synbiotics: gut and beyond. Gastroenterol Res Pract 2012; 1-16.
16. Cummings J, Pomare EW, Branch W J, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 1987; 28(10): 1221-1227.
17. Koropatkin NM, Cameron EA, Martens EC. How glycan metabolism shapes the human

gut microbiota. Nature Rev Microbiol 2012; 10(5): 323.
18. Guarner F, Malagelada JR. Gut flora in health and disease. Lancet 2003; 361(9356): 512-519.
19. Collee JG, Mackie TJ, McCartney JE. Practical medical microbiology. New York: Churchill Livingstone; 1989.
20. Biradar Sanjay R, Bhagyashri D Rachetti. Extraction of some secondary metabolites & thin layer chromatography from different parts of *Centella asiatica* L. American J Life Sci 2013; 1(6):243-247.
21. Ahmad T, Kamruzzaman M, Ashrafuzzaman M, Ahmad A, Ahmed L. In vitro Antimicrobial Activity of different extracts of Gotu Kola and Water Spinach against pathogenic Bacterial Strains. Current Res Microbiol Biotechnol 2015; 3(4): 663-669.
22. Ferdous N, Rahman M, Alamgir ANM. Investigation on phytochemical, cytotoxic and antimicrobial properties of ethanolic extracts of *Centella asiatica* (L.) Urban. J Med Plants 2017; 5(2): 187-188.
23. Shetty BS, Udupa SL, Udupa AL, Somayaji SN. Effect of *Centella asiatica* L (Umbelliferae) on normal and dexa-methasone-suppressed wound healing in Wistar Albino rats. Int J Lower Extrem Wound 2006; 5(3):137-143.
24. Etkin NL. Edible Medicines: An Ethnopharmacology of Food. Arizona: University of Arizona Press; 2008.
25. Ogle BM, Tuyet HT, Duyet HN, Xuan DNN. Food, feed or medicine: the multiple functions of edible wild plants in Vietnam. Econom Bot 2003; 57(1):103-117.

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