International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X
Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 3; Issue 1; January 2018; Page No. 127-132

Investigations on quantification of bioactive compounds and *in vitro* antibacterial property of important medicinal plant of *Centella asiatica* (L.) urban

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Abstract
The present study deals with the investigation to assess the preliminary phytochemical constituents and *in vitro* antibacterial activity of *Centella asiatica*. The different solvent extracts such as hexane, chloroform, ethyl acetate and ethanol were used for extracting bioactive compounds present in leaf. The phytochemical tests were revealed the presence of alkaloids, total phenols, glycosides, terpenoids, steroids, flavonoids, tannins, saponins and reducing sugars. The ethanolic extract showed maximum yield of bioactive compounds than the other solvent extracts. Proteins, antheroquinones and amino acids were absent in all the extracts. Ethanol and ethyl acetate extracts were yielded maximum percentage on nine bioactive compounds than hexane and chloroform extracts yielded six compounds rich. The results were validating the traditional uses of this plant in treatment of various diseases. Five gram-positive (*Bacillus cereus*, *Bacillus subtilis*, *Streptococcus cremoris*, *Streptococcus fecalis*, *Staphylococcus aureus*) and five gram-negative (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella dysentriae*) bacterial strains were tested. The maximum *in vitro* antibacterial activity were recorded 30.7 mm in *Bacillus cereus* at 250 µl concentration followed by *S. aureus* (28.1 mm), *S. cremoris* (27.2 mm), *Streptococcus fecalis* (24.6 mm), *B. subtilis* (22.3 mm), *E. coli* (20.6 mm), *P. aeruginosa* (19.2 mm), *Shigella dysentriae* (18.3 mm) and *Proteus vulgaris* (17.5 mm) and least inhibition was observed in *Klebsiella pneumonia* (16.2 mm). Moderate activity was observed in ethyl acetate and chloroform extracts. Minimum activity was observed in hexane extract at different concentration tested. Compared to synthetic antibiotic Ampicillin (50 mg), solvent extracts showed significant antibacterial activity. The present findings revealed that the presence of bioactive compounds and their activity against selected pathogenic bacteria. These results will support to the traditional knowledge of the medicinal plants to the local users and these plants used as therapeutic agents for treat several diseases.

Keywords: *Centella asiatica*, bioactive compounds, pathogenic bacteria, antibacterial activity

1. Introduction

Centella asiatica (L.) Urban belongs to the family Apiaceae (Umbelliferare) is a significant medicinal herb employed based on the familiarity, which is very popular in most tropical and subtropical countries [1]. It is commonly known as Asiatic pennywort containing 20 species grows fastly in most parts wet-rocky and higher elevations [2]. The whole plant parts are used as medicinal values. The stem, leaves, and aerial parts are used to the traditional drug formulations to decrease blood pressure, cure the fresh wound, heal bruised and diuretic [3]. The leaves are extensively utilized as a blood purifier, memory enhancement and for treating elevated blood pressure and prevent ageing [4]. The herb contains many types of active compounds, Terpenes or Terpenoids [5, 6]. In Ayurvedic system of medicine, the whole plant is one of the main herbal constituents for invigorating the nerves and brain cells. The plant has been used traditionally as brain tonic in ayurvedic medicine. This plant is used as brain tonic, and to treat chronic diseases and mental disorders. The plant contains several valuable bioactive compounds viz., centellasaponin, asiaticoside, madecassoside and sceffoleoside, pectin, castilliferol 1 and castillicetin 2 [7]. Further, the plant used to treat depression, wound inflammation, gastric ulcer, epilepsy, leprosy, cognitive disorders and venomous bites [8-11]. In Asiatic countries, *C. asiatica* is used as an ingredient in traditional systems of medicine such as Ayurveda, Siddha and Unani. In accordance with its potential wound healing property, several report described the remarkable protective effect of the plant against several diseases of central nervous system [12, 13].

Biological effects of *C. asiatica* have been attributed to the existence of major triterpene derivatives including asiatic acid, madecassic acid, asiaticoside, madecassoside, and brahmic acid [14, 15]. The occurrence of several important flavonoid derivatives including quercetin, kaempferol and several important phenolic compounds has also been reported [16]. A bitter principle vellarine, pectic acid and resin present in the leaf and root; asiaticoside and oxyasiaticoside shown to be active in the treatment of leprosy and tuberculosis. The fatty oil isolated from the plant consists of glycerides of oleic, linolic, centoic, linolenic, lignoceric, palmitic, and steric acids; the leaves contain triterpene madasiatic acid as well as 3-glycosyl quercetin, 3-glycosyl kaempferol and 7-glycosyl kaempferol [17]. Asiaticoside is one of the prime triterpene saponin found in leaves in large amount is utilized commercially as a wound healing agent due to its potent anti-inflammatory effect and showed the potential use as anti-gastric ulcers drugs [18].

Many plants were found to contain compounds, which are used as natural medicines to treat common bacterial infections. Medicinal plants are regularly used in various system of medicine because of minimal side effect and cost effectiveness. The potential for developing antimicrobials from higher plants appears rewarding as it may lead to the development of phytomedicine against microbes. Phytochemicals are plant derived chemicals, which may protect human from a host of numerous diseases. They are non-nutritive plant derivatives that are capable to prevent various dreaded diseases [19]. In general, the plant manufactures these active chemicals to protect itself. However the current research suggests that these phytochemicals can defend human diseases. Phytochemicals are secondary metabolites obtain from the leaves, stem, roots, fruits and whole herbs, which synthesize different quantity of bioactive compounds based on the habitat and adaptation [20]. These medicinal compounds are safe and eco-friendly. According to WHO, about 75% of the world's inhabitants relies on conventional medicine for their chief health care [21]. In the customary system of medicine, most of the plant extracts are helped to cure a diversity of the non-communical and communicable diseases [22].

Centella asiatica is one of the important plant shows antibacterial activity against wide variety of bacteria [23]. Several reports showed that the crude extraction of *C. asiatica* by 95% ethanol exhibited antimicrobial activity against *Bacillus cereus* and *Listeria monocytogenes* [24, 25]. Lee and Vairappan [26] studied antioxidant, antibacterial and cytotoxic activities of essential oils and ethanol extracts of selected South East Asian herbs. One of the selected South East Asian herbs was *C. asiatica*. Ethanolic extract of *C. asiatica* showed antibacterial activity against *Salmonella enteritidis* and *Salmonella typhimurium* while essential oil of *C. asiatica* showed no inhibition at same concentration. The present study is aimed to assess the phytochemical constituents in methanol, hexane and aqueous extracts of *C. asiatica* and qualtity assessment of active constituents such as alkaloids and saponins in leaves and stem of the plant. Therefore, the present study has been carried out to evaluate the preliminary screening of bioactive compounds and its *in vitro* antibacterial activity present in leaves of *C. asiatica*.

2. Materials and Methods

2.1 Plant Materials

The plant leaves of *Centella asatica* were collected during ethnobotanical survey studies in Vathal Hills in November 2016. Specimen was labeled, numbered, annotated with the date of collection, the locality and their medicinal uses. The voucher specimens were then identified, and deposited in the herbarium of PG and Research Department of Botany, Government Arts College, Dharmapuri, Tamil Nadu, India for future reference. After authentication leaves were collected in bulk, washed, shade dried and extracted with different solvents such as hexane, chloroform ethyl acetate and ethanol for 48 hrs in a soxhlet assembly.

2.2 Preparation of plant extract

Fresh leaves were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. They were ground into coarse powder by using mechanical pulveriser. The leaf powder, about 100 g were weighed and extracted repeatedly with hexane, chloroform, ethyl acetate and ethanol in a 500 mL round bottom flask containing 250 mL solvent individually. The reflux time for each solvent was varying with 25 to 40 cycles for complete extraction in soxhlet apparatus [27-29]. The filtrate was collected and concentrated by using rotary evaporator under controlled condition of temperature and pressure. The extracts were concentrated to dryness to yield crude residue. These residues were stored at 4°C, used for preliminary phytochemical screening of secondary metabolites and *in vitro* antibacterial activity assay. The presence of different chemical constituents in crude drugs can be detected by subjecting them to successive extraction using solvents in the order of increasing polarity. In the present study were therefore, subjected to extraction followed by qualitative chemical tests in order to know the phytoprofiles on a preliminary basis.

2.3 Preliminary phytochemical screening

Preliminary phytochemical screening of the *C. asiatica* leaf extract was carried out for the detection of the various plant constituents. Shaded dried and powdered of aerial part of plant samples were successively extracted with hexane, chloroform, ethyl acetate and ethanol. The extracts were filtered and concentrated using vaccum distillation. The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedure [30].

2.4 Antimicrobial Activity

Ten human pathogenic bacterial species were used in this present study, they were five gram-positive (*Bacillus cereus*, *Bacillus subtilis*, *Streptococcus cremoris*, *Streptococcus fecalis*, *Staphylococcus aureus*) and five gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*) obtained and identified specimens obtained from the Government medical college Hospital, Dharmapuri. All the bacterial strains were maintained on nutrient agar medium at ambient temperature. They were cultured in nutrient broth for 24 hours and the fresh inoculums were taken for the test. The antibacterial screening of the extract was carried out by determining the zone of inhibition using disc diffusion method.

2.5 Culture media and inoculums

Muller Hinton (MH) media (Hi-media Pvt. Ltd; Bombay, India) was used for growth of bacteria.

The inoculum for bacteria was prepared by transferring a large number of bacteria from fresh culture plates to tube containing 10mL of liquid media (DIFCO, Bacto: dehydrated nutrient broth) and incubating over night at 37°C. The tubes were shaken occasionally to aerate and promote growth.

2.6 Antibacterial Screening

The agar well diffusion method [31] was employed for the determination of antibacterial activity of the extracts. The petriplates containing 20 ml of Muller Hinton Agar medium (Himedia) were seeded with 24 h culture of the microorganisms. Sterilized cotton swabs were dipped in the bacterial culture in nutrient broth and then swabbed on the agar plates. Wells of equal size were cut with proper gaps in the medium and the plant extracts were added into it. The wells (6 mm in diameter) were cut from the agar and the extract solutions in different concentration (50 µl, 100 µl, 150 µl, 200 µl and 250 µl) were delivered into them. The control well of Ampicillin was used at 50 µl concentration. The plates were incubated at 37°C for 24 h. Clear inhibition zones around the wells indicated the presence of antibacterial activity. After incubation time, the zone of inhibition was measured precisely in millimeters (mm). The same procedure was followed for standard antibiotics ampicillin (50 µl) to compare the efficacy of extracts against test organisms. Each experiment was repeated three times, and the average values were calculated. The ampicillin stock solution was prepared at the concentration of 1mg/mL. The controls were prepared using the same solvents employed to dissolve the extracts. The inoculated plates with the test and standard discs on them were incubated at 37ºC for 24 h.

2.7 Statistical Analysis

Phytochemical estimation and quantification were performed in three replicates under standard procedures to ensure consistency of all conclusions. Data of all experiments were statistically analysed and expressed as Mean ± Standard Deviation.

3. Results

The present study revealed that the presence of bioactive compounds in leaves of *Centella asiatica*. The presence of phytochemical compounds in the leaf extracts of *C. asiatica* was evaluated by hexane, chloroform, ethyl acetate and ethanol. This investigation carried out through cold percolation as well as Soxhlet extraction methods have showed the presence of bioactive compounds in varying concentrations. The concentrated extracts of *C. asiatica* were carefully stored and analyzed. The colours of the extracts were pale green colour to yellow colour particularly chloroform extract was bright green in colour. The percentage yield of these extract were also measured, and it was the ethanolic extract 52.47 % maximum yield in comparison with other solvent extracts. All the extracts were sticky semisolid in their consistency. The qualitative phytochemical screening of ethanol, ethyl acetate, chloroform, and hexane extracts of leaves of *C. asiatica* and its percentage of yielded the secondary metabolites were shown in Table-1. The results were clearly demonstrated that the ethanol and ethyl aceate extracts yielded maximum nine bioactive compounds such as carbohydrates (78.54 %, 64.56 %), glycosides (65.32 %, 48.69 %), alkaloids (58.63 %, 46.35 %), terpenoids (42.12 %, 30.25 %), phenols (36.54 %, 27.36 %), anthraquinones (20.20 %, 15.23 %), amino acid (18.54 %, 14.56 %), reducing sugars (28.31 %, 22.10 %) and proteins (12.55 %, 10.21 %) respectively, these compounds were yielded minimum in hexane and chloroform extracts. Similarly, other six compounds were yielded high in hexane and chloroform extracts such as tannins (86.20 %, 62.24 %), flavonoides (82.31 %, 63.32 %), steroids (74.21 %, 58.32 %), saponins (70.11 %, 55.55 %) and terpenoids (62.17 %, 42.31 %) respectively.

In vitro antibacterial activity results of leaf extracts of *C. asiatica* showed excellent activity against the five gram-positive and five gram-negative bacteria. The result of leaves extracts are presented in Table 2&3. The maximum zone of inhibition was observed on *B. cereus* (30.7 mm) in ethanol 250 µl concentration followed by *S. aureus* (28.1 mm), *S. cremoris* (27.2 mm), *Streptococcus fecalis* (24.6 mm), *B. subtilis* (22.3 mm), *E. coli* (20.6 mm), *P. aeruginosa* (19.2 mm), *Shigella dysentriae* (18.3 mm) and *Proteus vulgaris* (17.5 mm) and minimum zone of inhibition observed on *Klebsiella pneumonia* (16.2 mm). The positive control, ampicillin (50 µl) had shown zone of inhibition are presented in Table 2. The *in vitro* antibacterial activity revealed that the ethanol extract had significant activity against all the microorganisms tested, mainly *B. cereus*, *S. aureus*, *S. cremoris*, *S. fecalis*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *Shigella dysentriae* and *Proteus vulgaris* (zone of inhibition >30 mm) but inactive in lower concentration (50 µl) on *Klebsiella pneumonia*. Moderate activity was observed in ethyl acetate and chloroform extracts. Minimum activity was observed in hexane extract at different concentration tested. Compared to synthetic antibiotic Ampicillin (50 mg), solvent extracts showed significant antibacterial activity. The ethyl acetate extracts possessed moderate activity against all microorganisms tested, *B. cereus*, *S. aureus*, *S. cremoris*, *S. fecalis*, *B. subtilis*, *E. coli*, *P. aeruginosa* and *Shigella dysentriae* (zone of inhibition >25 mm) but was inactive against *Proteus vulgaris* and *Klebsiella pneumonia*. The chloroform extracts possessed moderate activity against all microorganisms tested, *B. cereus*, *S. aureus*, *S. cremoris*, *B. subtilis*, *S. fecalis*, *E. coli*, and *P. aeruginosa* (zone of inhibition >20 mm) but was inactive against *Shigella dysentriae*, *Proteus vulgaris* and *Klebsiella pneumonia*. The hexane extract exhibited only weak activity against *B. cereus*, *S. aureus*, *S. cremoris*, and *S. fecalis* (zone of inhibition >15 mm) but was inactive against *B. subtilis*, *E. coli*, *P. aeruginosa*, *Shigella dysentriae P. vulgaris*, and *K. pneumonia*. The moderate inhibition activity was observed in antibiotic ampicillin against *B. cereus*, *S. fecalis*, *S. aureus*, *E. coli* and *K. pneumonia* these results showed the similar effect in 100 µl of ethanol extract and 150 µl of chloroform extract.

Table 1: Quantative Phytochemical analysis of different solvent extracts of *Centella asiatica* leaves.

Bioactive compounds	Hexane extract	Chloroform extract	Ethyl acetate extract	Ethanol extract
Alkaloids	23.21 %	34.62 %	46.35 %	58.63 %
Carbohydrates	36.21 %	42.31 %	64.56 %	78.54 %
Glycosides	12.58 %	28.79 %	48.69 %	65.32 %
Steroids	74.21 %	58.32 %	36.01 %	22.15 %
Proteins	6.21 %	8.33 %	10.21 %	12.55 %
Flavonoides	82.31 %	63.32 %	44.25 %	25.98 %
Tannins	86.20 %	62.24 %	45. 62 %	35.11 %
Anthraquinones	9.21 %	12.30 %	15.23 %	20.20 %
Terpenoids	62.17 %	42.31 %	37.21 %	30.23 %
Saponins	70.11 %	55.55 %	41.65 %	28.64 %
Amino acid	8.21 %	12.31 %	14.56 %	18.54 %
Phenols	8.69 %	15.24 %	27.36 %	36.54 %
Terpenoids	10.26 %	18.64 %	30.25 %	42.12 %
Reducing sugars	12.01 %	17.65 %	22.10 %	28.31 %

Table 2: *In vitro* Antibacterial activity of Hexane and chloroform extracts of *Centalla asiatica* by well diffusion method.

Bacterial organisms	Amp (50 µl)	Zone of inhibition (mm)									
		Concentrations									
		Hexane extract					Chloroform extract				
		50 µl	100 µl	150 µl	200 µl	250 µl	50 µl	100 µl	150 µl	200 µl	250 µl
B. cereus	25.2	11.4	13.4	15.2	20.8	24.8	10.2	12.5	14.5	22.0	26.1
S. aureus	22.4	12.3	15.9	18.8	22.3	17.2	10.2	12.6	16.2	19.5	24.4
S. cremoris	18.7	-	11.6	15.7	18.6	21.1	-	12.2	17.5	21.6	23.3
S. fecalis	17.5	-	10.1	12.4	15.8	18.2	-	-	10.7	15.2	20.1
B. subtilis	15.3	-	-	-	12.4	16.4	-	-	10.4	13.2	18.3
E. coli	23.1	-	-	11.2	14.5	22.6	-	-	10.0	13.5	16.6
P. aeruginosa	16.7	-	-	-	10.6	13.2	-	-	10.5	12.4	15.5
S. dysentriae	13.2	-	-	-	11.6	12.8	-	-	10.2	14.5	14.7
P. vulgaris	13.6	-	-	-	10.3	12.2	-	-	-	10.4	14.3
K. pneumonia	18.2	-	-	-	10.2	11.4	-	-	-	11.1	13.5

(Zone of inhibition = values are expressed in millimeter (mm), - = Negative results)

Table 3: *In vitro* Antibacterial activity of ethyl acetate and ethanol extracts of *Centella asiatica* by well diffusion method.

Bacterial organisms	Amp (50 µl)	Zone of inhibition (mm)									
		Concentrations									
		Ethyl acetate extract					Ethanol extract				
		50 µl	100 µl	150 µl	200 µl	250 µl	50 µl	100 µl	150 µl	200 µl	250 µl
B. cereus	25.2	10.6	14.8	20.8	25.2	28.1	13.2	20.5	22.4	26.4	30.7
S. aureus	22.4	11.3	13.5	18.4	21.5	26.4	13.0	15.8	20.4	23.1	28.1
S. cremoris	18.7	-	14.0	19.6	23.4	25.6	10.7	16.4	21.3	25.2	27.2
S. fecalis	17.5	-	-	12.9	17.0	22.1	-	10.2	14.5	19.6	24.6
B. subtilis	15.3	-	-	11.3	15.4	20.5	-	10.1	14.0	19.4	22.3
E. coli	23.1	-	-	11.6	15.5	18.0	-	10.3	13.1	17.2	20.6
P. aeruginosa	16.7	-	-	10.7	14.1	17.3	-	-	12.7	16.1	19.2
S. dysentriae	13.2	-	-	11.0	13.4	16.7	-	11.1	13.6	15.4	18.3
P. vulgaris	13.6	-	-	-	11.4	15.2	-	-	10.2	13.4	17.5
K. pneumonia	18.2	-	-	-	12.4	14.5	-	-	10.5	14.3	16.2

(Zone of inhibition = values are expressed in millimeter (mm), - = Negative results)

4. Discussions

Among the four solvents used, ethanol extracts were yielded maximum bioactive compounds followed by ethyl acetate extract, chloroform extract and minimum amount of compounds present in hexane extract. The result of preliminary phytochemical analysis of *C. asiatica* leaf extracts were revealed the presence bioactive components namely alkaloids, amino acids, cardiac glycosides, phytosterols, triterpenoids, reducing sugars, steroids, saponins, flavonoids, phenols, tannins, anthraquinones in different concentrations except carbohydrates. *Centella asiatica* is considered as an enriched source of different active compounds presented in Table 1. The main active compounds are pentacyclic triterpenes (Asiatic acid, madecassic acid, asiaticoside and madecassoside). The main active principle compounds are triterpenoids, glycosides such as asiatic acids. Carbohydrates and falvonoides were present in all four extracts in varying concentrations. Hexane and chloroform extracts were yielded similar bioactive compounds such as alkaloids, carbohydrates, steroids and flavonoids. Ethyl acetate extract yield the compounds are carbohydrates, flavonoids, tannins, saponins, phenols, terpenoids and reducing sugars. Ethanol extracts showed the presence of all compounds of ethyl acetate extract and additionally glycosides. Proteins, antheroquinones and amino acids were absent in all the extracts. Further, the ethyl acetate and ethanol extracts were showed the absence of anthraquinones. In the present study, most of the biologically active compounds such as flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoids, cardiac glycosides and tannins were found to be present in the ethanolic extracts of *Centella asiatica* leaves. The medicinal properties of *C. asiatica* leaf extracts may be due to the presence of above mentioned phytochemicals. Studies on the efficiency of medicinal plants with respect to the control of infectious diseases are more essential to know their therapeutic value and hence in pharmaceutical arenas.

Tannins is a phenolic compounds that are acting as principal antioxidants or free radical scavengers. Since these phenolic compounds were originated to be present in the extracts, it might be accountable for the potent antioxidant capacity of *C. asiatica*. These phytochemicals of medicinal plants have primarily reported for their medicinal value, which can be valuable for therapeutic index. For instance, saponins proved as hypotensive and cardiodepressant properties [32], which are helpful for the treatment of congestive heart failure and cardiac myopathy [33]. The occurrence of saponins in ethanol extracts of leaves of *C. asiatica* might play a role in the cardioprotective potential. Alkaloids and tannins have the potential of hypoglycemic and anti-inflammatory activities [34]. Moreover, the terpenoids have also been revealed to decrease blood sugar level in animal studies. In addition, the steroids and triterpenoids demonstrated the analgesic properties and central nervous system activities [35, 36]. Hence the preliminary phytochemical investigations are actually obliging in finding chemical ingredients in the plant that may help to their quantitative evaluation and also in locating the source of pharmacologically active principle. The reducing power of ethanolic extract was also significantly higher in CA-4 compared to other samples. It has been reported that there is a positive correlation between the total phenolic content and antioxidant activity [37].

Dash *et al*, [38] was observed the *n*-hexane, carbon tetrachloride, chloroform soluble fractions of methanol extract from the plant *Centella asiatica* showed antibacterial activity against 5 gram positive bacteria and 8 gram negative bacteria. Ethanolic extract of *Centella asiatica* shows significantly higher rate of sensitivity against various bacteria strains, while petroleum ether extract shows moderately sensitivity and water extract showed that least sensitivity against these strains [39]. Wei *et al*, [40] was observed that methanol extract of *C. asiatica* whole plant showed inhibition zone against *V. alginolyticus*, *V. vulnificus* and *Streptococcus* sp while inhibition zone was found in *C. freundii* and all *Vibrio* sp. except *V. vulnificus* against aqueous extracted *C. asiatica* whole plant.

Our results were coincide with the hexane, dichloromethane, ethyl acetate, diethyl ether and methanol extracts of *C. asiatica* showed antibacterial activity against *B. subtilis*, *K. aerogenes*, *P*. v*ulgaris* and *S. aureus* species. But they did not show antibacterial activity against *Escherichia coli* and *Pseudomonas aerogenes* [41]. Similarly Zaidan *et al*, [42] was also observed the antibacterial activity of *Centella* plant extracts by using diffusion method. In contrastingly, ethanolic and ethyl acetate extracts of *C. asiatica* plant leaves did not showed antibacterial activity against some gram positive and negative bacteria [43]. Water extract of *Centella* did not show antimicrobial activity against *E. coli*, *Staphyloccoccus aureus* and *Klebsiella pneumonia* [44].

5. Conclusion

Nowadays, there are increasing trends of using natural products. From the discovery that effective life span of any antibiotic is limited. Scientists try to discover alternatives. One of the sources is from plants. *C. asiatica* has been mentioned in Ayuravedic record. It can be used to treat leprosy, insanity, asthma, ulcers, eczema, skin tuberculosis, wounds, stomach aches, arthritis, varicose veins and high blood pressure. By that, this plant seems to be interesting candidate for investigation. Thus obtain data of antibacterial activity profile against different microbial strains under different extraction conditions that would provide a basic knowledge of solvent selection in extracting bioactive compounds, could also serve as scientific proved data as the stepping stone to develop and promote *C. asiatica* as herbal medicine.

6. Acknowledgments

The author is greatly thankful to the Principal and Head of the Department, PG and Research Department of Botany, Government Arts College, Dharmapuri, for providing the laboratory facilities.

7. References

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