Phytochemical Investigation and Antimicrobial Activities of Leaf of *Centella asiatica* (L.) Urban

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Abstract

Centella asiatica (L.) Urban belongs to the family Apiaceae (formerly known as Umbelliferae). It is known as Indian penny wort in English and Myin Khwar in Myanmar. Morphological characters of *Centella asiatica* (L.) Urban were analyzed in the present investigation. Samples for this study were collected from Loikaw University Campus form June 2016 to February 2017. Phytochemical screening of *Centella asiatica* (L.) Urban revealed the presence of many therapeutically important classes of phytoconstituents such as alkaloids, glycosides, reducing sugars, saponins, carbohydrates, phenolic compounds, tannins, steroids, terpenoids, α-amino acids, flavonoids and base compounds. The leaf samples of *Centella asiatica* (L.) Urban were extracted with various solvents: pet-ether, acetone, chloroform, methanol, ethanol, ethyl acetate and water. The antimicrobial activities of the extracts were tested by the agar well diffusion method. In this case, the acetone extracts had the highest activity against *Staphylococcus aureus*. The present work aimed to inform the relevant phytochemical and antimicrobial data needed for the proper utilization of medicinal plants.

Keywords: *Centella asiatica* (L.) Urban, leaf, phytoconstituents, antimicrobial activities.

Introduction

Centella asiatica (L.) Urban is a perennial herb and it has been applied for various purposes since times immemorial. *Centella* is indigenous to tropical and subtropical regions. In Myanmar, it is commonly found in moist areas and in crop fields. It is used as whole plant or aerial parts. The drug is used internally and externally. According to the ancient literature of Charaka Chikitsa, the juice of *Centella asiatica* (L.) Urban promotes longevity and cures all diseases. It also provides strength, digestive power, complexion, voice and intellect. It is rejuvenating and used as a nervine tonic. In traditional Indian medicines, *Centella asiatica* (L.) Urban has been used for various pathological disorders and in particular for healing wounds and for leprosy for a long time. In the Ayurvedic system of medicine, it is also utilized in chronic diseases and as a "brain tonic" in various mental disorders (Bhavna D. & K. Jyoti, 2011).

The plants are being identified as power houses because of the presence of various phytochemicals. They can be denoted as medicinal plants. They are useful for therapeutic purposes or precursors for the manufacturing of drugs. Natural plants are locally used as primary health remedies in Asia, Latin America and Africa (Aruljothi S., *et al*., 2014).

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Phytochemical, a natural bioactive compound is found in plants such as vegetables and medicinal plants and plant parts such as fruits, flowers, leaves, stems and barks. It works with nutrients and fibers to provide immune system against diseases. Many plants are valuable sources of natural antimicrobial compounds (Saranya Babu Jayaprakash C.M. and N. Nagarajan, 2016).

To treat human pathogens against commonly used antibiotics, drug resistance has more and more developed so that new antimicrobial substances from living things including plants are essentially explored. *Centella asiatica* (L.) Urban is found to improve the mentally retarded in children and to treat rheumatic disorders. Moreover, it possesses a wide range of pharmacological effects including antimicrobial, antioxidant and anticancer etc. (Arumugam, T. *et al*., 2011).

In the present study, phytoconstituents of leaf of *Centella asiatica* (L.) Urban were analyzed and its antimicrobial activities were conducted. These can be applied as nutraceuticals and antibiotics.

Materials and Methods

Collection and Identification of *Centella asiatica* (L.) Urban

In the present study, the plant specimens were collected from Loikaw University Campus from June 2016 to February 2017 (Fig. 1). The morphological characters of the specimens were studied, measured and recorded. The plants were identified with the literatures: - Flora of China by Ho, Ting-naung & Robert Ornduff, 1995; Flora of Ceylon by Dassanayake, 1996.

Preparation of Powdered Samples

The samples were thoroughly washed with water and air-dried at room temperature for about two to three weeks. The dry samples were pulverized by grinding machine into powder and kept in air-tight container for further study.

Preliminary Phytochemical Examination of Leaf of *Centella asiatica* (L.) Urban

Phytochemical examination was conducted according to British Pharmacopoeia, 1968 and Trease and Evens, 1978.

(1.) Test for alkaloids

The leaves extracts were stirred with 2 ml of dilute hydrochloric acid separately and filtrate was tested with the following reagents;

1. Dragendroff's test: Dissolve a few mg of alcoholic or aqueous extract of dried leaf powdered sample in 5 ml of distilled water, add 2 M hydrochloric acid until an acid reaction occurs, then add 1 ml of Dragendroff's reagent an orange or orange-red ppt. is produced immediately (Central Council of Research in Unani Medicine, 1987).

2. Mayer's test: Add a few drops of Mayer's reagent to 1 ml of acidic aqueous extract of dried leaf powdered sample. A white or pale yellow ppt. is formed (Central Council of Research in Unani Medicine, 1987).

3. Wagner's test: Acidity 1 ml of alcoholic extracts of dried leaf powdered sample with 1.5 % V/ V of hydrochloric acid and adds a few drops of sodium picrate solution reagent. A yellow of brown ppt is formed (Central Council of Research in Unani Medicine, 1987).

(2.) **Test for α - amino acids**

Each extract 2 ml of dried leaf powdered sample was added with two drops of ninhydrin reagent. The formation of violet color appears due to the presence of α-amino acid (Marini Bettolo, *et al.,* 1981).

(3.) Test for carbohydrates

Each extract 2 ml of dried leaf powdered sample was introduced into a test tube and a few drops of 10% α-napthol were added and shaken. The test tube was then inclined at an angle of 45˚ and concentrated sulphuric acid was added slowly along the side of the tube. A red ring or violet ring was formed between the two layers, showing the presence of carbohydrate (Central Council of Research in Unani Medicine, 1987).

(4.) Test for flavonoids

In a test tube containing 0.5 ml of alcoholic extract of dried leaf powdered sample, add 5-10 drops of dilute hydrochloric acid followed by a small piece of zinc or magnesium. Boil the solution for a few minutes. In the presence of flavonoids, a pink, reddish pink or brown color is produced (Central Council of Research in Unani Medicine, 1987).

(5.) Test for glycosides

Dissolve a small amount of alcoholic extract of dried leaf powdered sample in 1 ml of water, and add sodium hydroxide solution. A yellow color indicates the presence of glycosides (Central Council of Research in Unani Medicine, 1987).

(6.) Test for cyanogenic glycoside

Two grams of dried leaf powdered sample were mixed with distilled water and a few drops of concentrated sulphuric acid were added. The sodium picrate paper was inserted with the cork at the top of the test tube. Then, the resulting mixture was gently heated by means of a spirit burner. If the sodium picrate paper turns pink, it indicates the presence of cyanogenic glycoside.

(7.) Test for phenolic compound

Each extract 2 ml of dried leaf powdered sample was added 2 drops of 4 % ferric chloride solution in a test tube separately. If formation of green or blue color which may indicate that the presence of phenolic compound (Central Council of Research in Unani Medicine, 1987).

(8.) Test for reducing sugar

Each extract 2ml of dried leaf powdered sample was added a few drops of Benedict's solution in a test tube separately solution appear green, yellow or red precipitate, which indicated that the presence of reducing sugar (Vogel, 1956).

(9.) Test for saponin

Each extract 2 ml of dried leaf powdered sample was added a few drops of distilled water. Then the mixture was vigorously shaken for a few minutes.

Observation was made to see if foaming took place, indicating that the presence of saponin (Marini Bettol, *et al.,* 1981).

(10.) Test for steroid and terpenoid

Each extract 2 ml of dried leaf powdered sample was added two drops of concentrate H_2SO_4. This formation of blue or green color which shows the presence of steroid and formation of deep red color, greenish color or blue color indicate that the presence of terpenoid (Central council for Research in Unani Medicine, 1987).

(11.) Test for tannin

Each extract 2ml of dried leaf powdered sample was added with a few drops of 1 % ferric chloride solution in a test tube separately. If yellowish brown precipitate or blue green color was resulted indicating that the presence of tannins (Central Council of Research in Unani Medicine, 1987).

(12.) Test for acid or base or neutral compound

Two grams of dried leaf powdered sample was boiled with distilled water for about 30 minutes and filtered. A few drops of bromocresol green were added. If no change in color was found, it indicates a neutral compound. If yellow color was found, it indicates acid compound. If blue color was found, it indicates base compound.

Antimicrobial Screening of Leaf of *Centella asiatica* (L.) Urban

The antimicrobial activities of leaf of *Centella asiatica* (L.) Urban were conducted at Pharmaceutical Research Department, Yangon.

Solvent and Extraction

Solvents: Pet-ether, Chloroform, Methanol, Acetone, Ethyl acetate, Ethanol and water were used for the extraction of leaf of *Centella asiatica* (L.) Urban Five grams of dried leaf powder were soaked in 50ml of different solvents for a week. The extracting solvents were filtered and the filtrates were concentrated by using water bath.

Test Microorganisms

The test microorganisms: - *Bacillus subtilis* (N.C.T.C – 8236), *Staphylococcus aureus* (N.C.P.C – 6371), *Pseudomonas aeruginosa* (6749), *Bacillus pumilus* (N.C.I.B – 8982), *Candida albicans* and *Escherichia coli* (N.C.I.B – 8134) were used for the determination of antimicrobial activity.

Preparation of plates for antimicrobial activity

The antimicrobial activities were performed by agar-well diffusion method. Nutrient agar was prepared according to the method described by Harley J.P. & L.M. Prescott (2002). Nutrient agar was boiled and 20-25ml of the medium was poured into a test tube. They were plugged with cotton wool and autoclaved at 121˚C for 15 minutes. Then they were cool down to 60˚C and poured into sterilized petridishes.

Then 0.1ml of spore suspension was added into the dishes and the agar was allowed to set for 30 minutes.

Agar well diffusion method

In agar well diffusion method, a cork borner was used to make a small well (10mm) in the nutrient agar. After that 0.15ml of crude extract was introduced into the agar well and the plates were incubated at 37˚C for 24 hrs. The inhibition zone appeared around the agar well indicated that the presence of antimicrobial activity.

Results

Fig. 1. Habit of *Centella asiatica* (L.) Urban

Taxonomoic Description

Scientific Name **:** *Centella asiatica* (L.) Urban

Synonyms **:** *Hydrocotyle asiatica* L.

Family **:** Apiaceae (Umbelliferae)

Local name : Myin Khwar

Common name : Indian penny wort

Flowering period : August to November

Preliminary Phytochemical Investigation of Leaf of *Centella asiatica* (L.) Urban

The various extracts of the plant showed the presence of alkaloids, glycosides, reducing sugars, saponins, carbohydrates, phenolic compounds, tannins, steroids, terpenoids, α-amino acids, flavonoids and base compounds (Table. 1).

Table 1. Preliminary Phytochemical Investigation of Leaf of *Centella asiatica* (L.) Urban

No.	Chemical constituent	Extract	Reagent used	Observation	Results
1.	Alkaloid	1% HCl	1. Mayer's reagent 2. Dragendroff's reagent 3. Wagner's reagent	white ppt. orange ppt. yellow ppt.	+ + +
2.	Glycoside	H_2O	10% lead acetate solution	white ppt.	+
3.	Reducing sugar	H_2O	Fehling solution	reddish ppt.	+
4.	Saponin	H_2O	Distilled water	Frothing	+
5.	Phenolic compound	H_2O	$FeCl_3$ solution	Deep brown	+
6.	Carbohydrate	H_2O	10% α-naphthol, Conc. H_2SO_4 acid	pink ring	+
7.	Cyanogenic glycoside	H_2O	Conc. H_2SO_4 acid, sodium picrate paper	no change in color	-
8.	Tannin	H_2O	1% gelatin solution	white ppt.	+
9.	Steroid	Pet – Ether	Acetic anhydride, Conc. H_2SO_4 acid	green	+
10.	Terpenoid	EtOH, $CHCl_3$	Conc. H_2SO_4 acid	reddish brown	+
11.	α-amino acid	H_2O	Ninhydrin solution	pink color	+
12.	Acid, Base, Neutral Compound	H_2O	Bromocresol solution	blue	**Base color**
13.	Flavonoid	MeOH	Conc. HCl acid, Mg turning	pink color	+

Fig. 2. Dried Leaves of *Centella asiatica* (L.) Urban

Fig.3. Leaves Powdered Sample of *Centella asiatica* (L.) Urban

Table 2. Sensory Characters of Leaves Powdered Sample of *Centella asiatica* (L.) Urban

Sensory Characters	Observations
Color	Green
Taste	Bitter
Odor	Pungent
Texture	Fibrous

Antimicrobial Activity of Different Solvent Extracts of Leaf of *Centella asiatica* (L.) Urban

The antimicrobial activity assays were conducted by using agar well diffusion method. The leaf samples of *Centella asiatica* (L.) Urban were extracted with various solvents: pet-ether, acetone, chloroform, methanol, ethanol, ethyl acetate and water. The antimicrobial activities of the extracts were tested by six test microorganisms. In this case, the acetone extracts had the highest activity against *Staphylococcus aureus*. The methanol extracts, acetone extracts and water extracts showed the moderate activity against *Staphylococcus aureus*. The pet-ether extracts, acetone extracts and water extracts showed the moderate activity against *Candida albicans*. The methanol extracts and acetone extracts showed the moderate activity against *Escherichia coli* (Table. 3), (Fig. 4 – A, B).

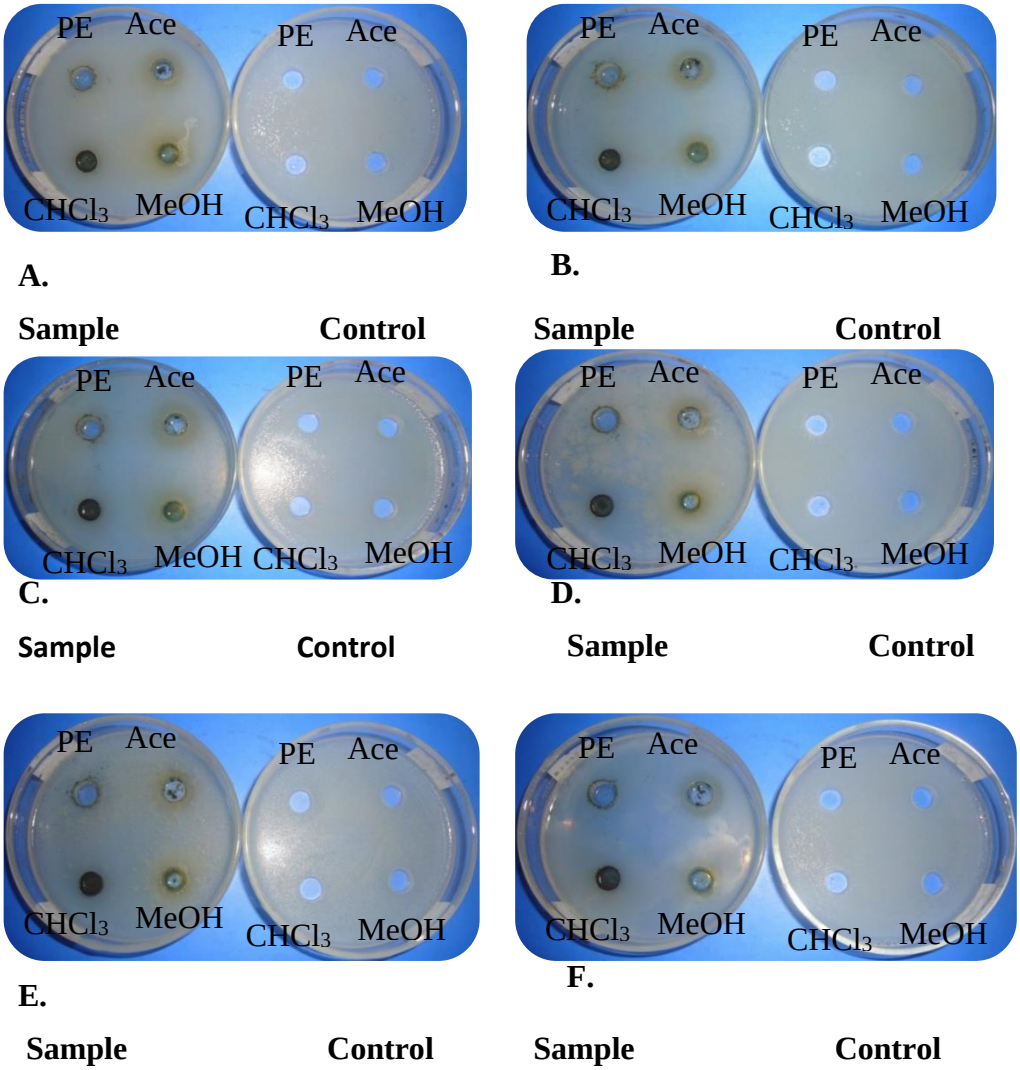

Fig. 4. A. Antimicrobial Activity of Different Solvent Extracts of Leaf of *Centella asiatica* (L.) Urban

(A.) *Bacillus subtilis*

(B.) *Staphylococcus aureus*

(C.) *Pseudomonas aeruginosa*

(D.) *Bacillus pumilus*

(E.) *Candida albicans*

(F.) *Escherichia coli*

Different Solvents:
PE – Pet-ether
Ace – Acetone
$CHCl_3$ – Chloroform
MeOH – Methanol

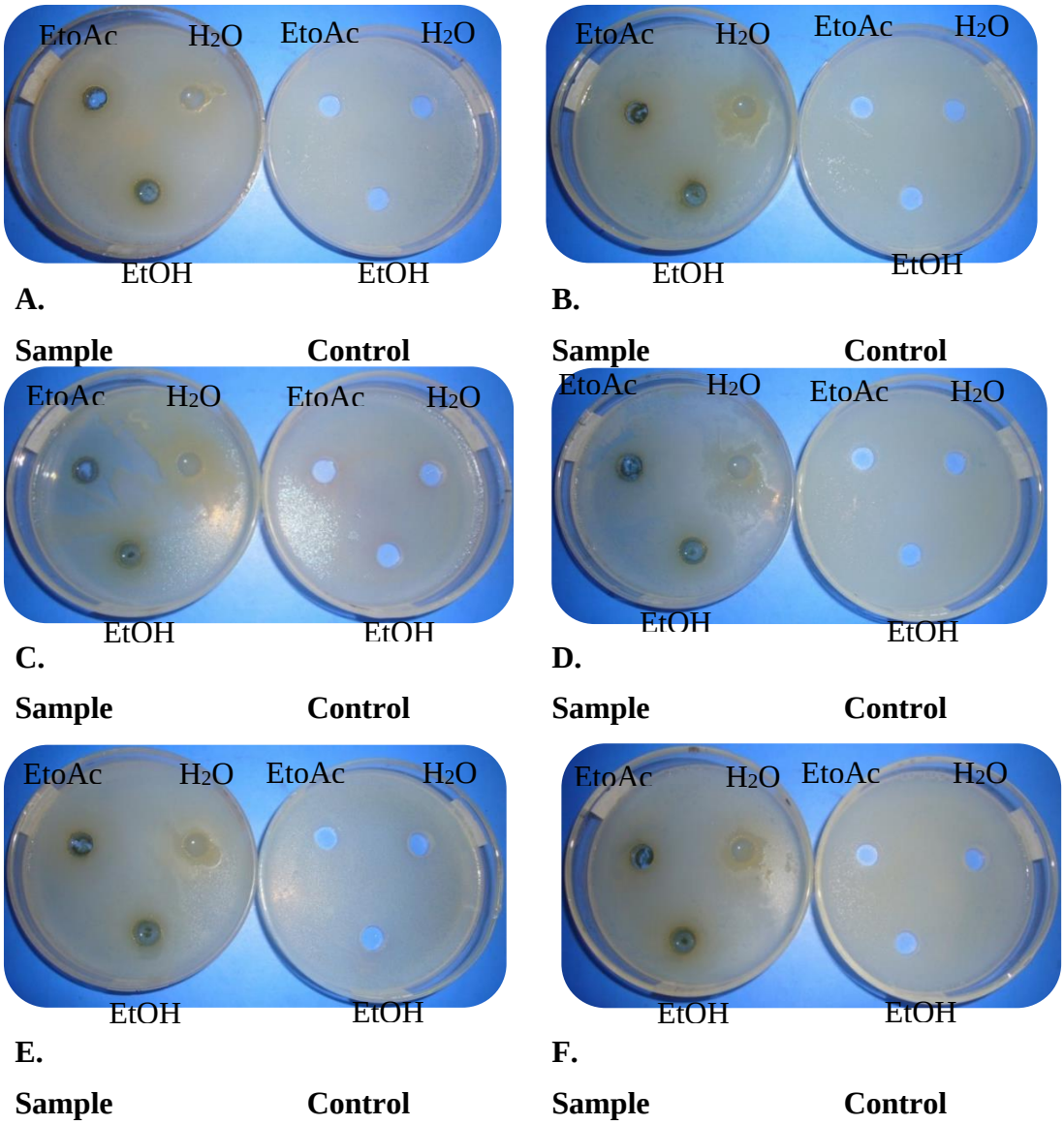

Fig. 4. B. Antimicrobial Activity of Different Solvent Extracts of Leaf of *Centella asiatica* (L.) Urban

(A.) *Bacillus subtilis*

(B.) *Staphylococcus aureus*

(C.) *Pseudomonas aeruginosa*

(D.) *Bacillus pumilus*

(E.) *Candida albicans*

(F.) *Escherichia coli*

Different Solvents:
EtoAc – Ethyl acetate
H_2O – Aqueous
EtOH – Ethanol

Table 3. Antimicrobial Activity of Seven Extracts of *Centella asiatica* (L.) Urban Leaf on Six Pathogenic Microorganisms (agar well diffusion method)

		Test Organisms					
Samples	Solvent	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*B. pumilus*	*C. albicans*	*E. coli*
Centella asiatica L.	Pet - ether	12 mm (+)	12 mm (+)	12 mm (+)	12 mm (+)	16 mm (++)	13 mm (+)
	$CHCl_3$	11 mm (+)	13 mm (+)	13 mm (+)	14 mm (+)	14 mm (+)	14 mm (+)
	MeOH	13 mm (+)	16 mm (++)	14 mm (+)	12 mm (+)	14 mm (+)	16 mm (++)
	Acetone	13 mm (+)	19 mm (++)	12 mm (+)	14 mm (+)	15 mm (++)	17 mm (++)
	EtoAc	11 mm (+)	-	14 mm (+)	13 mm (+)	-	-
	EtOH	11 mm (+)	11 mm (+)	13 mm (+)	12 mm (+)	12 mm (+)	11 mm (+)
	H_2O	12 mm (+)	15 mm (++)	11 mm (+)	11 mm (+)	15 mm (++)	12 mm (+)

Agar well – 10 mm

10 mm – 14 mm (+)

15 mm – 19 mm (++)

20 mm & above (+++)

Test Organisms

(1.) *Bacillus subtilis* (N.C.T.C – 8236)

(2.) *Staphylococcus aureus* (N.C.P.C – 6371)

(3.) *Pseudomonas aeruginosa* (6749)

(4.) *Bacillus pumilus* (N.C.I.B – 8982)

(5.) *Candida albicans*

(6.) *Escherichia coli* (N.C.I.B – 8134)

Discussion

In the present investigation, the morphological characters of *Centella asiatica* (L.) Urban were in agreement with those described by Ho, Ting-naung & Robert Ornduff, (1995); & Dassanayake, (1996), (Fig. 1). Phytochemical examination was carried according to British Pharmacopoeia, (1968) and Trease and Evens, (1978), (Table. 1).

In the present study, morphological characters of *Centella asiatica* (L.) Urban were agreed with those described by Singh S., *et al*., (2010).

Manisha N.T., *et al*., (2011) stated that *Centella asiatica* (L.) Urban, were chemically rich in saponins. Presence of carbohydrates and glycosides were also noted. Hanif U., *et al*., (2016) discussed that triterpenoid and saponins were the primary constituents of *Centella asiatica* (L.) Urban and they were responsible for its wide therapeutic actions. Glycosides, sterols, tannins, sugars, alkaloids, inorganic salts and amino acids were the constituents of *Centella asiatica* (L.) Urban.

Bhavna D. & K. Jyoti, (2011) reviewed that the leaves of *Centella asiatica* (L.) Urban (Indian penny wort) consisted of glycosides, alkaloids, flavonoids, sterols, tannins and amino acids. The chloroform extracts were found to be active against gram positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus*; gram negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and fungi: *Candida albicans*. Experimental studies demonstrated that preparations of *Centella asiatica* (L.) Urban were used in traditional and alternative medicine due to the wide spectrum of pharmacological activities. The plant had high medicinal value which made it socially desirable. It was economically affordable and sustainable.

Saranya Babu Jayaprakash C.M. and N. Nagarajan, (2016) stated that the presence of medicinally active constituents: alkaloids, flavonoids, saponins, phenols, steroids, glycosides, tannins, terpenoids and triterpenoids. They revealed that acetone and methanol crude extracts showed the maximum inhibition zone against fungal strains: *Fusarium oxysporum* and bacterial strains: *Proteus mirabilis*. *Bacillus subtilis* showed least zone of inhibition to the crude leaf extract. This is in agreement with the present study in which all the extracts of leaf of *Centella asiatica* (L.) Urban showed the least antimicrobial activity against *Bacillus subtilis*.

Arumugam, T. *et al*., (2011) mentioned that alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, saponins and reducing sugars were present in most of the tested extracts of leaf of *Centella asiatica* (L.) Urban. Methanol, acetone, chloroform and water extracts of leaf showed significant antibacterial activity against *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

In the present study, alkaloids, glycosides, reducing sugars, saponins, phenolic compounds, carbohydrates, tannins, steroids, terpenoids, α-amino acids, base compounds and flavonoids were present (Table. 1). It has been reported that the extract of the leaf of *Centella asiatica* (L.) Urban was found to be active against gram positive bacteria: *Staphylococcus aureus*, gram negative bacteria: *Escherichia coli* and fungi: *Candida albicans* (Table. 3); (Fig. 4 – A & B). These were in agreement with those described by Manisha N.T., *et al*., (2011), Hanif U., *et al*., (2016), Bhavna D. & K. Jyoti, (2011), Saranya Babu Jayaprakash C.M. and N. Nagarajan, (2016), Arumugam, T. *et al*., (2011). It can be noted that the leaf of *Centella asiatica* (L.) Urban contained various phytochemical constituents that were active against pathogenic microorganisms. Thus, they were able to be utilized for nutraceuticals and modern and traditional medicines.

Conclusion

It can be concluded that the majority of the information on the identity of the plant material can be obtained from its macroscopical characters. The present work was conducted to obtain some pharmacognostical standards for *Centella asiatica* (L.)

Urban The above studies provided information dealing with their identification and chemical constituents useful for pharmacognostical study and standardization for the medicinal plants.

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