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Original Research Article

Phytochemicial Screening and Antibacterial Property of *Centella Asiatica* (Linn).

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A B S T R A C T

The use of medicinal plants as a source for relief from illness can be traced back over five millennia from start of human civilization. Medicinal plant represents a rich source of antimicrobial agents. Plants are used as also a source of many potent and power full drugs. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied properties. The plant used for the present study was *Centella asiatica*. Which is a very common plant and was traditionally used for various purposes, especially as single medicinal therapy. The present study mainly focused on the phytochemical, antibacterial property of the plant *Centella asiatica*. The leaf and root of the plant is used for the analysis. Methanol and petroleum ether were used as solvents for preparing the extracts. The phytochemical analysis revealed that the plant *Centella asiatica* contains most of the important phytochemicals such as carboxylic acid, flavanoids, saponin, resin, Xanthoprotein, coumarins etc. These plant extract were also tested against three bacterial strains *Pseudomonas aeroginosa*, *Klebisiella pneumonia*, and *Staphylococcus aureus*. Most of the extract showed good inhibitory activity. The methanol leaf and petroleum ether root showed maximum inhibitory activity. Among the pathogens *Staphylococcus aureus* was most effectively inhibited by the plant extract.

Keywords: C.Asiatica, Xanthoprotein, K.Pnuemonia, Antibacterial, MIC, Umbeliferae

A R T I C L E I N F O

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1. Introduction

Plants have been an integral part of human society since the start of civilization. India is rich in its plants diversity, a number of plants have been documented for their medicinal potential which are in use by the traditional healers, herbals folklorists and in Indian systems of medicine namely, Ayurveda, Unani, Siddha apart from a Homeopathy and Electropathy.

Different national and international pharmaceutical companies are utilizing such plant based formulations in treatment of various diseases and disorders world around (Singh & Gautam, 1997; Khan *et al*, 2002). It is estimated that there are 250,000 to 500,000 species of plants on Earth (Borris, 1996). A relatively small percentage (1 to 10%) of these is used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes (Moerman, 1996).

Different extracts and essential oils from traditional medicinal plants have been tested to identify the source of therapeutic effects. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action (Kaur and Kaur, 2010). A number of compounds extracted from various species of higher plants have shown antimicrobial activity (Kloucek *et al*, 2005). Examples included tannins, flavones, alkaloids, that displayed *in vitro* activity against numerous microorganisms.. It has been suggested that selection of plant on the basis of ethnomedical considerations gives a higher hit rate than screening programmes of general synthetic products.(Vanden Berghe *et al* ,1991). *Bacopa monneri* has been used in conditions like epilepsy, insanity, nervous disorders, *Hypercicum hookerianum* in anxiety and inflammation, *Usnea complanta* and *Tagetes minuta* for bacterial infections, *Santolina chamaecyparissus* as a stimulant, vermifuge(Hobbs, 1986).

The WHO indicated that as many as 80 percent of all people living in the world make use of herbal medicines as main source of healthcare, from treating common cold to controlling blood pressure and cholesterol. Patel (2007) reported that plant based drugs can be used in the form of tablets. As modern medicine developed a large number of pills are take today have their origins in those humble herbs gathered from waysides, steam-beds.

Phytochemicals are natural and non-nutritive bioactive compounds produced by plants that act as protective agents against external stress and pathogenic attack (Chew *et al*, 2005). Basic phytochemical investigation of plant extracts for their phytoconstituents were also vital. Based on their biosynthetic origin, phytochemicals can be divided into several categories: phenolics, alkaloids, steroids, terpenes, saponins, *etc*. Phytochemicals could also exhibit other bioactivities such as antimutagenic, anticarcinogenic, antioxidant, antimicrobial, and anti-inflammatory properties (Yen *et al*,1993). These plant-derived phytochemicals with therapeutic properties could be used as single therapeutic agent or as combined formulations in drug development.

Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Considering the vast potentiality of plants as sources for antimicrobial drugs, a systematic investigation was undertaken to screen the common medicinal plant *Centella asiatica* for antimicrobial and antioxidant property.

Centella asiatica

Botanical description

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 (Trease and Evans, 1966).

Scientific Classification

Kingdom-Plantae
Division-Angiospermae
Class-Dicots
Order-Umbeliferae
Genus-Centella
Species-Asiatica

Physiological effects

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 (Ullah *et al*, 2009). 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 (Zheng and Qin, 2007)

Medicinal value

1. Cytotoxic and anti tumor
2. Memory enhancing
3. Neuro protective
5. Cardio protective
6. Hepato protective
7. Antioxidant
8. Anti-inflammatory
9. Anti-fertility
10. Antiulcer activity
11. Skin nourishment
12. wound healing

Centella asiatica is a reputed medicinal plant used in the treatment of various skin diseases in the Indian system of medicine. It has been used for centuries as traditional medicine in India and oriental countries for treatment of mental fatigue, anxiety, epidermal wound, eczema and leprosy (Guo *et al* ,2004). The plant is cooling, alternative, seductive to nerve stomachic, carminative, improve appetite, antiseptic, diuretic, tonic to memory and nerve. In homoeopathic medicine it is used for skin disease associated with itching and swelling (P.S Varrier 1992). Total triterpenoids fraction extracted from C.asiatica increased the percentage of collagen in cell layer fibronectin and thus may help in promoting wound healing (Tenni R *et al*, 1988). The most prominent group of biologically active compound is the triterpenes (wijeweera et al, 2006) which consist of Asiatic acid, madecassic acid and asiaticoside. Scientific studies have proven a variety of biochemical components, ie secondary metabolites have been found in C.asiatica. The chemical constituents of the plant have a very important role in neutraceuticals application and it is believed due to its biologically active compounds of triterpenes saponins (Loiseau.A *et al* 2000). According to Zainol *et al* (Centella asiatica, 2008) the highest concentration of phytochemicals was found in the leaves relative to the petioles and the roots .The flavanoids and tannin posses antimicrobial activity(Cowan,1999) . The antimicrobial activity of flavanoids is due to their ability to complex with cellular and soluble protein and to complex with bacterial cell wall while that of tannin may be related to their ability to inactivate microbial adhesion, enzymes and cell envelop protein. Most of the phytochemical studies concentratedon leaves and the constituents vary depending upon the geographical distribution (Chong and Aziz, 2011) It also inhibit growth of *Staphylococcus Spp* and reduce inflammation (Department of pharmaceutical botany,1996). The essential oil extracted from the plat exhibited a broad spectrum of antibacterial activity against gram positive and negative bacteria.

The crude extract of C.asiatica particularly with water had a promising antibacterial effect against gram positive bacterias and also water extract of this plant leaves shows activity against E.coli and Shigella (Taemchuay *et al*, 2008). C.asiatica is one of the important plant shows antibacterial activity against wide variety of bacteria(Jagtap *et al*,2009). On different extract plant shows different activity. The alcoholic extract of plant shows bacterial action within 2hours against Vibrio cholera (Mamtha *et al*, 2004). Methanolic extract of Centella was showed antibacterial activity against Vibrio species such as V.harveyi, V.alginolyticus, and V.parahaemolticus but acetone, chloroform and hexane extract was not showed antibacterial activity against these species (Sankar *et al*, 2008).

2. Materials and Methods

MATERIALS USED IN THE STUDY

a) Collection of plant samples: *Centella asiatica (L.),*

The leaves and roots of this experimental plant were collected from alappuzha and evaluated for preliminary phytochemical analysis and antimicrobial activity.

b) Extraction of the plant materials

1. **Solvents used** – Organic solvents such as petroleum ether and methanol were employed for the extraction of platnt parts under investigation.

2. **Extraction** – The various parts of the plants such as leaves and roots were collected and subsequently washed to remove adhering dust particles. The leaves and roots were cut into small pieces separately to conduct phytochemical as well as antimicrobial studies.

3. **Microorganisms used**

Bacterial strains: *Streptococcus aureus, pseudomonas aeroginosa* and *klebsiella pneumonia* were used

4. Preparation of Inoculum

Inoculum was prepared using nutrient broth .5ml of nutrient broth was inoculated with a loopful culture and was incubated at a temperature of 37^0 C for overnight until a turbidity was obtained. The same procedure was repeated for all the strains and was used as inoculum for further studies

5. Culture Media –Nutrient agar used for bacteria.

Nutrient broth

Peptone	-	5gm
Beef extract	-	3gm
Sodium chloride	-	3gm
PH	-	7.2
Distilled water	-	1000 ml

Nutrient Agar

Peptone	-	5gm
Beef extract	-	3gm
Sodium chloride	-	3gm
Agar	-	15gm
PH	-	7.2
Distilled water	-	1000ml

6. Preparation of herbal antibiotic discs – Sterile filter paper discs of 6 mm diameter (WhatmannNo. 1) was used for the present study. In each sterile disc, 200µl of the plant extract were incorporated, using a 10µl pipette. Precautions were taken to prevent the flow of the plant extract from the disc's outer surface. This was obtained by applying the condensed extracts in small quantities and the discs were allowed for air-drying.

7. Antibacterial assay:

The antibacterial activity was assessed using the disc diffusion assay (Kirby et al., 1966). 10ml of sterile nutrient broth were aseptically inoculated with test cultures and incubated at 37 ± 0.5°C for 18 hours. After incubation, the test bacterial cultures were spread on air-dried nutrient agar plates using a sterile cotton swab. Using a sterile forceps, sterile disc (Hi-Media) of different concentration (25%, 50%, and 100%) loaded with plant extract and was placed on the surface of Nutrient agar plates swabbed with test bacterial strains. Controls were also maintained by incorporating the respective solvents only, on sterile discs. Then the plates were incubated at 37 ± 0.5°C for 12 to 14 hours. The zone of inhibition was observed and recorded in terms of diameter of zone as millimeters.

8. **Antibiotic Sensitivity Test**:

Determination of antimicrobial effectiveness is essential to show which agents are more effective against specific pathogens and give estimate of proper therapeutic dose. Here, all the test bacterial strains were checked for antibiotic sensitivity test by employing antibiotics such as streptomycin and ampicilin. Streptomycin (10units/disc) and ampicilin (10 units/disc) were employed for the evaluation of its spectrum of activity against the bacterial.

The antibiotic discs were impregnated on nutrient agar plate which were pre-seeded with test organisms, and incubated at 37 ± 0.5°C for 12 to 14 hours. The antibiotic sensitivity pattern of the test pathogen was deduced from the standard interpretation chart.

Methods:

Preliminary phytochemical analysis – The extracts using different solvents were screened for the qualitative identity of different classes of natural compounds, using the methodology of modified Sofowora and Kepm (2007). The major pharmaceutically valuable phytochemical compounds investigated in the present study were:

a. Alkaloids
b. Carboxylic acids
c. Coumarins
d. Flavanoids
e. Phenols
f. Proteins and free amino acids
g. Quinones
h. Resins
i. Saponins
j. Sterols, phytosterols and triterpinoidal sapogenins
k. Tannins
l. Xanthoproteins
m. Sugars.
n. Volatail Oils
o. Cardiolipins

Detection of alkaloids:

A few drops of dilute HCl was separately treated with 1ml each of various extracts. Then it was filtered and the filtrates were treated with 1ml of Dragendoff's reagent. Formation of reddish orange precipitation indicated the presence of alkaloids.

Detection of carboxylic acids:

1ml each of various extracts was separately treated with a few ml of saturated solutions of sodium bicarbonate. Observation of effervescence (due to liberation of CO_2) indicated the presence of carboxylic acids.

Detection of coumarins:

1ml each of alcoholic extracts was treated with alcoholic NaOH solution. Production of dark yellow colour indicated the presence of coumarins.

Detection of flavanoids:

5ml each of the various extracts were separately dissolved 1 ml each of alcohol (stock solution) and then subjected to the following tests.

Ferric chloride test: 1 ml each of stock alcoholic solution was added with a few drops of neutral $FeCl_3$ solution. Formation of blackish red colour indicated the presence of flavanoids.

Shinoda's test:

With 1 ml each of alcoholic solution a small piece of Mg ribbon or Mg foil was added followed by the addition of a few drops of concentrated HCl. Change in colour (red to pink) showed the presence of flavanoids.

Detection of phenols:

1ml of the various extracts dissolved in 5ml of alcohol was treated separately with a few drops of neutral $FeCl_3$ solution. Any change in colour indicated the presence of phenolic compounds.

Detection of protein and free amino acids:

5 ml each of various extracts were dissolved in 5ml of water separately and were subjected to the following tests.

Biuret test: 1 ml each of the various extracts was warmed gently with 10% NaOH solution and a drop of diluted $CuSO_4$ solution. Formation of reddish violet colour indicated the presence of proteins and free amino acids.

Ninhydrin test:

1 ml each of the various extracts was separately treated with a few drops of Ninhydrin solution. Change in colour showed the presence of proteins and free amino acids.

Detection of quinines:

1 ml of the various extracts was separately treated with alcoholic KOH solution. Quinones give colorations ranging from red to blue.

Detection of resins:

1 ml each of various extracts were subjected to treat with a few drops of concentrated HNO_3 and a few drops of acetic anhydride solution followed by 1 ml of concentrated H_2SO_4. Resins give colouration ranging from orange to yellow.

Detection of Saponins:

1 ml each of the various extracts were separately mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Foam formation indicated the presence of saponins.

Detection of steroids / Phytosterols /Triterpenoidal sapogenins:

5 ml each of various extracts were dissolved in 5 ml each of chloroform separately (stock solution) and was subjected to the following tests.

Salkowski test: 1 ml each of concentrated H_2SO_4 was added to the stock solution and allowed to stand for 5 minutes after shaking. Turning of golden yellow colour in the lower layer indicated the presence of steroids, phytosterols and triterpenoidal sapogenins.

Liebermann- Burchard test:

To 1 ml each of the stock solution, a few drops of acetic anhydride and 1 ml of concentrated H_2SO_4 were added from the sides of the test tubes and allowed to stand for 5 minutes. Formation of brown ring at the junction of the two layers and the upper layer turned green indicated the presence of steroids, phytosterols and triterpenoidal sapogenins.

Detection of tannins:

5 ml each of the various extracts was dissolved in minimum amount of water separately, filtered and the filtrated were then subjected to the following tests.

Ferric chloride test:

To the above filtrate a few drops of ferric chloride solution were added. The colour change indicates the presence of tannins.

Basic lead acetate test: To the filtrate, a few drops of aqueous basic lead acetate solution were added. Formation of reddish brown precipitate indicated the presence of tannins.

Detection of xanthoproteins:

One ml of various extracts was treated separately with a few drops of concentrated HNO_3 and NH_3 solution. Formation of reddish orange precipitation indicated the presence of xanthoprotein.

Detection of sugars:

5 ml each of the various extracts was dissolved separately in distilled water filtered and then subjected to the following tests.

Molisch's test:

To the filtrate a few drops of alcoholic α-naphthol and 2 ml of concentrated H_2SO_4 were added slowly through the sides of the test tube. Formation of reddish brown precipitate indicated the presence of sugars.

Fehling's test:

A small portion of various filtrates were treated with 1 ml of Fehling's solution 1 and 2 and then heated gently. Change in colour indicated the presence of sugars.

Anthrone test:

1 ml each of the various extracts in a watch glass were separately taken and mixed thoroughly using a glass rod with an equal quantity of anthrone reagent and a few drops of concentrated H_2SO_4 and heated on a water bath. Formation of dark green colour indicated the presence of sugars.

Test for volatile oils:

2ml of various extract was treated with 0.1ml NaOH solution and dilute HCl .Observed for the presence of white precipitate.

Antibacterial assays:

The antibacterial activity was assessed using the disc diffusion assay (Kirby et al., 1966). 10ml of sterile nutrient broth were aseptically inoculated with test cultures and incubated at 37 ± 0.5°C for 18 hours. After incubation, the test bacterial cultures were spread on air-dried nutrient agar plates using a sterile cotton swab. Using a sterile forceps, sterile disc (Hi-Media) of different concentration (25,50,100) loaded with plant extract and was placed on the surface of Nutrient agar plates swabbed with test bacterial strains. Controls were also maintained by incorporating the respective solvents only, on sterile discs. Then the plates were incubated at 37 ± 0.5°C for 12 to 14 hours. The zone of inhibition was observed and recorded in terms of diameter of zone as millimeters

Antibiotic Sensitivity Test:

Determination of antimicrobial effectiveness is essential to show which agents are more effective against specific pathogens and give estimate of proper therapeutic dose. Here, all the test bacterial strains were checked for antibiotic sensitivity test by employing antibiotics such as Streptomycin and ampicilin (10 units/disc) and ampicilin were employed for the evaluation of its spectrum of activity against the bacterial. The antibiotic discs were impregnated on nutrient agar plate which were pre-seeded with test organisms, and incubated at 37 ± 0.5°C for 12 to 14 hours. The antibiotic sensitivity pattern of the test pathogen was deduced from the standard interpretation chart.

3. Results & Discussion

Phytochemical Analysis

Table 1 shows the phytochemical analysis of the leaf extract of Centella asiatica using solvent methanol and petroleum ether. The results showed the presence of secondary metabolites in the sample. Most of the phytochemicals were present on the methanol leaf extract. The petroleum ether leaf extract showed the presence of volatile oils. The phytochemicals such as saponin, carboxylic acid, flavanoids, tannin, were present in both leaf and root extract. The flavanoids presence were found on methanol while it was absent on petroleum ether leaf. Secondary metabolites such as sterol, quinines and cardiolipin were absent on both the leaf extract. The methanol leaf extract showed the presence of additional phytochemical coumarins.

Table 2 shows the phytochemical analysis of root extract of Centella asiatica using methanol and petroleum ether. Most of the phytochemicals such as alkaloids, tannin, carbohydrate, saponin, coumarins, and carboxylic acid were present on the root extract. Presence of phenol was indicated only on the methanol root extract. Both of the extract does not indicated the presence of flavanoids, sterols, quinine and Xanthoprotein. On the same time methanol root extract does not shows the presence of volatile oils.

Table 1: Phytochemical screening of *C.asiatica* leaf on methanol and petroleum ether extract

Phytochemicals	Methanol root	P.ether root
Alkaloids	+	+
Tannin	+	+
Amino acids and proteins	+	+
Flavanoids	-	-
Sterol	+	-
Carbohybrate	+	+
Saponin	+	+
Quinines	-	-
Coumarins	+	+
Carboxylic acid	+	+
Volatile oils	-	+
Cardiolipin	-	+
Sugar	+	+
Xanthoprotein	-	-
Phenol	+	+

Presence +, Absence -

Table 2: Phytochemical screening of *C.asiatica* root on methanol and root extract

phytochemical	*Centella* leaf (methanol)	*Centella* leaf (petroleum)
Alkaloids	-	+
Tannin	+	+
Aminoacids and proteins	-	+
Flavanoids	+	-
Sterol	-	-
Carbohybrate	+	+
Saponin	+	+
Quinines	+	-
Coumarins	+	-
Carboxylic acid	+	+
Volatile oils	-	+
Cardiolipin	-	-
Sugar	+	+
Xanthoprotein	+	+
Phenol	+	+

Prescence +, Absence –

Antibacterial Activity Study

In this study the bacterial strains such as *Pseudomonas aeroginosa*, *Klebisiella pneumonia*, and *Staphylococcus aureus* used for the antibacterial activity of *Centella asiatica*, ampicilin and streptomycin is used as a control. Methanol and petroleum ether were used as the solvent. Leaf and root of the plant is used to check the antibacterial activity.

Antibacterial activity of *S.aureus* on methanol leaf and root extraction

Table 3showed the antibacterial activity of methanol leaf and root on *S.aureus.* From the data its clear that the methanol leaf extract showed maximum inhibition against S.aureus (16mm) and also methanol root have less activity on the test organism. The 50% and 75% methanol leaf also showed inhibition zone (10mm) against the organism. Methanol leaf and Petroleum ether root extract showing the zone of inhibition against *S.aureus*

Methanol root and petroleum ether root extract showing the zone of inhibition against *P.aeroginosa*

Methanol root and Petroleum ether leaf showing the zone of inhibition against *K.pneumonia*

Antibacterial activity of *S.aureus* on petroleum ether leaf extract and root extract

Table 4 showed the antibacterial activity of leaf and root on S.aureus. The result indicated that the crude extract of petroleum ether showed maximum zone of inhibition (14mm) on test organism. Among the extract 75% petroleum ether root showed inhibition zone (13mm).

Antibacterial activity of *P.aerogienosa* on methanol leaf and root extract

Table 5 showed the antibacterial activity on methanol leaf and root on P.aeroginosa. The result indicated that methanol root extract showed maximum zone of inhibition (16mm) and the 50% methanol root also shows zone of inhibition.

Antibacterial activity of *P.aeroginosa* on petroleum ether leaf extract and root extract

Table6 showed the antibacterial activity on petroleum ether leaf and root on P.aeroginosa. The result indicated that both the leaf and root extract of C.asiatica is less active towards the test organism. The crude extract of petroleum ether root indicated a maximum zone (10mm) on P.aeroginosa.

Antibacterial activity of *K.pneumonia* on methanol leaf extract and root extract

Table 7 showed the antibacterial activity on methanol leaf and root on K.pneumonia. The control shows maximum zone of inhibition against the test organism (13mm). The leaf and root extract showed less effect towards the organism.

Antibacterial activity of *K.pneumonia* on petroleum ether leaf and root extract

Table 8 showed the antibacterial activity on petroleum ether leaf and root on *K.pneumonia.* The petroleum ether extract of leaf and root is less active against the organism used. The crude extract also showed poor activity.

Antibacterial Activity

Table 3: Antibacterial activity of methanol leaf extract and root extract on *S.aureus*

S. no	Plant name	Extract and conc.	Inhibition Zone (dia.in mm) of *S.aureus*
1	Centella asiatica (methanol leaf)	Crude	16
		Solvent	4
		25%	8
		50%	10
		75%	11
2	Centella Asiatic (methanol root)	Crude	2
		Solvent	4
		25%	3
		50%	2
		75%	6

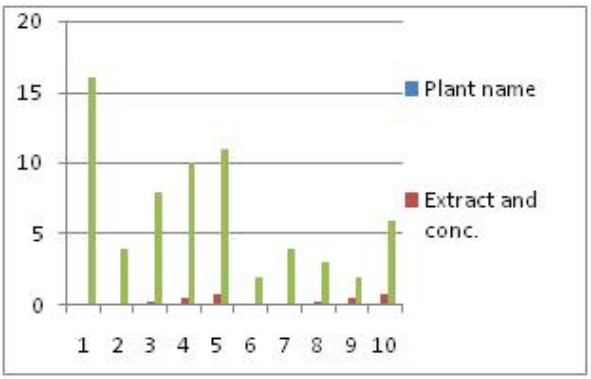

Figure 1:. Antibacterial activity of methanol leaf extract and root extract on *S.aureus*

Table 4: Antibacterial activity of petroleum ether leaf extractand root extract on *S.aureus*

S. no	Plant name	Extract and conc.	Inhibition Zone (dia.in mm) of *S.aureus*
1	Centella asiatica (petroleum leaf)	Crude	4
		Solvent	3
		25%	9
		50%	5
		75%	11
2	Centella asiatica (petroleum root)	Crude	14
		Solvent	5
		25%	6
		50%	8
		75%	13

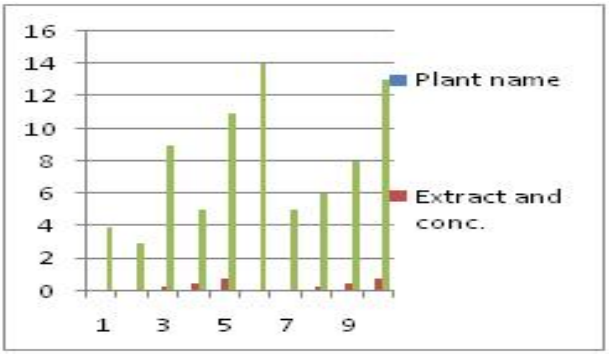

Figure 2: Antibacterial activity of petroleum ether leaf extract and root extract on *S.aureus*

Table 5: Antibacterial activity of methanol leaf extract and root extract on *P.aerugenosa*

Sl no	Plant name	Extract and conc.	Inhibition Zone (dia.in mm) of *P.aerugenosa*
1	Centella asiatica (methanol leaf)	Crude	7
		Solvent	3
		25%	4
		50%	6
		75%	7
2	Centella asiatica (methanol root)	Crude	16
		Solvent	5
		25%	4
		50%	13
		75%	8

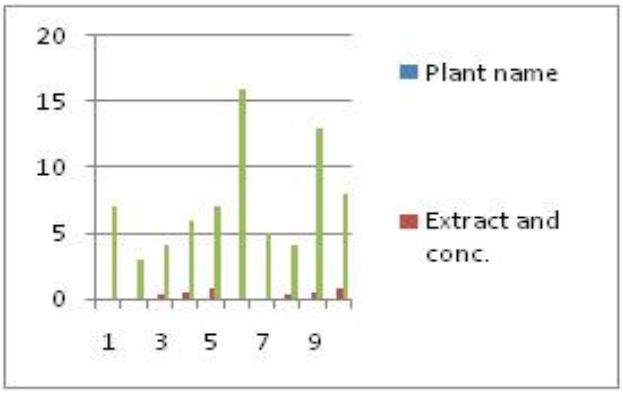

Figure 3: Antibacterial activity of methanol leaf extract and root extract on *P.aerugenosa*

Table 6: Antibacterial activity of petroleum ether leaf extract and root extract on *P.aerugenosa*

S. no	Plant name	Extract and conc.	Inhibition Zone (dia.in mm) of *P.aerugenosa*
1	Centella asiatica (petroleum leaf)	Crude	-
		Solvent	2
		25%	6
		50%	3
		75%	5
2	Centella asiatica (petroleum root)	Crude	10
		Solvent	4
		25%	9
		50%	11
		75%	8

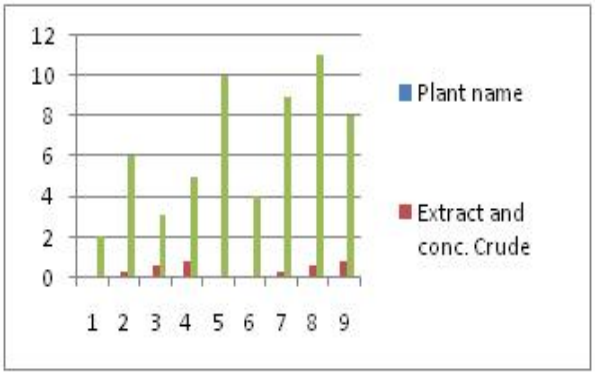

Figure 4: Antibacterial activity of petroleum ether leaf extract and root extract on *P.aerugenosa*

Table 7: Antibacterial activity of methanol leaf extract and root extract on *K.pneumonia*

S. no	Plant name	Extract and conc.	Inhibition Zone (dia.in mm) of *K.pneumonia*
1	Centella asiatica (methanol leaf)	Control	17
		Crude	7
		Solvent	3
		25%	3
		50%	4
		75%	3
2	Centella asiatica (methanol root)	Control	18
		Crude	10
		25%	9
		50%	10
		75%	7

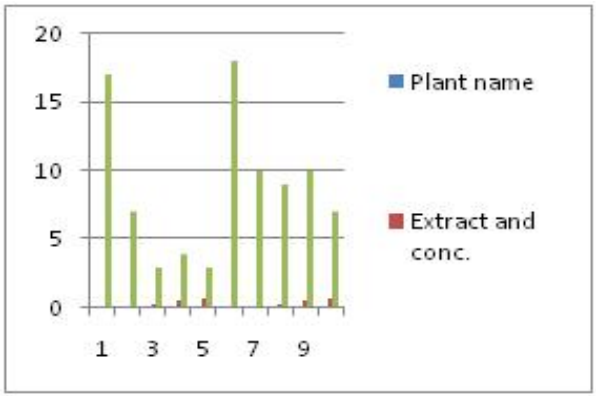

Figure 5: Antibacterial activity of methanol leaf extract and root extract on *K.pneumonia*

Table 8: Antibacterial activity of petroleum ether leaf and root on *K.pneumonia*

Sl no	Plant name	Extract and conc.	Inhibition Zone (dia.in mm) of *K.pneumonia*
1	Centella asiatica (petroleum leaf)	Crude	8
		Solvent	4
		25%	4
		50%	10
		75%	8
2	Centella asiatica (petroleum root)	Crude	5
		Solvent	2
		25%	6
		50%	7
		75%	4

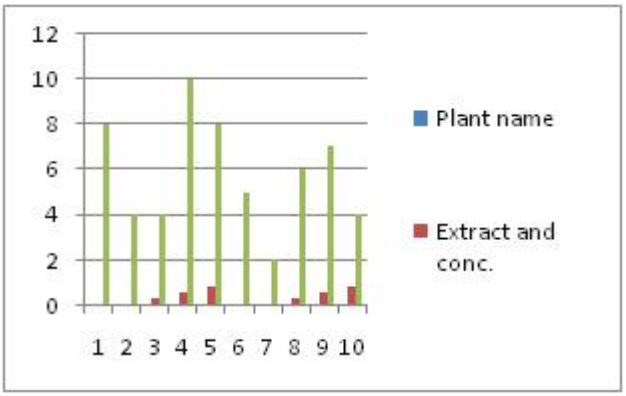

Figure 6: Antibacterial activity of petroleum ether leaf and root on *K.pneumonia*

Discussion

The plant part contains most of the important secondary metabolites like carboxylic acid, flavanoids, saponin, steroids, resins, Xanthoprotein, coumarins etc. The medicinal properties of the plant are mainly resided on these phytochemicals.During the antibacterial study the methanol leaf extract showed maximum inhibition against *S.aureus* (16mm) and also methanol root have less activity on the test organism. The crude extract of petroleum ether showed maximum zone of inhibition (14mm) on test organism**.** The methanol leaf and root extract showed maximum zone of inhibition (16mm) against *P.aeroginosa*. Among them the methanol root showed maximum zone of inhibition. The petroleum ether root also showed (10mm) zone of inhibition against *P.aeroginosa.* Most of the extract was less active on *K.pneumonia*. The leaf and root extract showed less effect towards the organism. The petroleum ether extract of leaf and root is less active against the organism used. The crude extract also showed poor activity**.** Among the plant part studied methanol leaf and petroleum ether root showed high antibacterial activity than. The different concentration 25%, 50%, 75% also showed zone of inhibition, the solvent such as methanol and petroleum ether also showed zone of inhibition.

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