Section A -Research paper

Antimicrobial activity and phytochemical analysis of extract of *Centella asiatica* against some human pathogenic microbes

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ABSTRACT

Plants are very useful source of various bioactive compounds which have direct and indirect use in the treatment of various human ailments. The plant named *Centella asiatica* belongs to the family Apiaceae showed Antimicrobial activity against various bacteria and fungi. The plant is mainly used for the treatment of wound healing , the herb is recommended for the treatment of various skin conditions such as leprosy ,lupus, ulcers. The most common bacteria that cause wound infections are *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus* and *Escherichia coli* causes diarrhea. The most commonly identified fungi in wounds are *Candida* sp, and *Aspergillus* sp.They cause infection usually in immunocompromised individuals. An experiment was carried out to study the antimicrobial activity of water ,ethanol, chloroform, extracts of *Centella asiatica* by agar well diffusion method and the phytochemical analysis was also performed for the plant extracts to detectthe presence of Alkaloids , Terpenoids , Saponin , Flavonoid. The result confirmed that the chloroform extract of *Centella asiatica* have higher antibacterial activity against *Escherichia coli* , *Staphylococcus* sp, *Bacillus* sp, *Pseudomonas* sp (Ranges 10-25mm).The antifungal activity of the chloroform extract *Centella asiatica* showed higher range (11-17mm) of activity against *Aspergillus sp.* and *Candida* sp. *Centella asiatica* (Gotukola) has been used to treat many conditions for thousand of years in India, china ,and Indonesia .It was used to heal wounds improve mental clarity and treat skin conditions .It can be consumed as a green leafy vegetable. *Centella asiatica* can be used in moisturizing cosmetic formulations and also to complement the treatment of dry and sensitive skin. Persons can take it by consuming the herb in capsules or as tea. The future scope of the study is to move on to the field of cosmetic microbiology, were the extracts can be used as skin care products against bacterial and fungal attacks in humans.

Keywords: Leprosy ,immunocompromised, *Centella asiatica* , agar well diffusion , phytochemical analysis, terpenoids ,saponin ,flavonoid.

INTRODUCTION

The use of plants and their parts as an ethno-Medicine for the treatment of various diseases is a common practice among the trial communities around the world ,Since time immemorial.

Despite tremendous progress in human medicines, infectious disease caused by the bacteria, fungi, virus and parasites are still a major threat to public disease caused by bacteria ,fungi and parasitesare still a major threat to public health. Their impact is particularly large in developing countries due to relative availability of Medicines and the emergence of widespread drug Resistance (Zampini *et al*., 2009) .The development of drug resistance in human pathogens against commonly used antibiotics has necessitated search of new antimicrobial substances from other sources including plants (Erdogrul, 2002).

Antimicrobial agents are chemical compounds that inhibit microbial growth or kill the microbes . These compounds are also use in food preparation as additives. Various antibiotics and antimicrobial medicine have been developed over the years to improve human quality of life. However unwise use of antibiotics makes the microbes resistant (Clardy *et al.,* 2006) and therefore required more powerful drugs to counteract the microbes which may cost more. The growing concern regarding the increase of bacterial resistance to antibiotics and increasing interest towards application of natural medicine have led to the search of new antimicrobial agents mainly from plant extract (Dash *et al*.,2011). Medical herbs are alternative treatment which is preferable for human and animal health.

Plants are the most important source of chemical compounds,and it has an antibacterial and antifungal property which are found in leaves, roots, stem, Bark, etc (*Baris et al*.,2006).Being situvated in tropics, Bangladesh has a rich in biodiversity that's why for a long time, a huge number of plants are being used in the medicinal history and also used as a control of fungal Diseases. Sustainable development has emerged as a new paradigm of development to maintain the human eco-system equilibrium from the last 40 years , and an example of sustainable development is *C.asiatica*(Esperienze Dermatol ,2018).

Scientific classification (morphological features)

Class : Dictyledenae
Sub class : Polypetalae
Order : Umbellales
Family : Umbelliferae (Apiaceae)
Genus : *Centella*
Species : *asiatica*

Botanical name

Family	Apiaceae
Latin name	*Centella asiatica*
Alternate name	*Hydrocotyle asiatica*
Tamil name	Vallarai

Vernacular name	Sanskrit - Mandukaparni Hindi - Brahmamanduki- Gotu kolas English - Indian pennywort Bengali - Thokuri Malayalam - Muttil Gujarati - Karbrahmi Japanese - Tsubokura Tibetan - Sin-mnar

Centella asiatica (L) urban belonging to the family Umbeliferae is a common perennial herbaceous creeper flourishing abundanthly in moist areas and distributed widely in tropical and sub tropical countries including Bangladesh. Various chemical constituents are reported in *Centella asiatica* like asiaticoside, madecassoide , madecassicacid, Asiatic acid, glucose, rhamnose, terpinoids, sitosterol, stigmasterol ,fattyoils ,consist of glyceriods of palmitic acid, stearic acid, linoleic acid, linolenic acid vitamins like ascorbic acid. It also contains calcium,iron,and phosphate (Trease and Evans,1996). *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 and Sultana ,2009).

Studies on microbial activity *C.asiatica* against microbial species such as Bacteria, fungi and yeast have been done (Arumurugam and Ayyanar , 2011) .In the type of solvents used for extraction which has been reported include aqueous, ethanol, Chloroform. Microbes used to test anti-microbial study of *C.asiatica* include bacteria from both Gram-positive and Gram -Negative groups ,Fungi ,molds and slightly yeast (Ahmad and paul,2015).Different studies published world wide have reported that up to seventy compounds have been extracted from *C. asiatica* (Alfara and omar , 2013) .The most abundant bioactive compounds found in *C.asiatica* are represented by asiaticoside, madecassoside, asiatica, and made cassic acid from the triterpene. Madecassoside can also stimulate the production of collagen type III (Monton *et al*.,2019). Triterpenes being the major components of *Centella* , they have regarded it as its biomarker components .Qualification of triterpenes of centella has been successfully established by several researchers using HPLC-UV (Inamdar *et al*.,1996).

In India , *centella asiatica* grow up to an altitude of 600-1800 meters above the level on moist, clayey or sandy soils forming a dense green carpet . *Centella asiatica* has a glabrous stem and long petiolated fleshy leaves rooting at nodes. It is a softly perfumed plant that attains height up to 15cm . stem is smooth and rooting occurs at the nodes. It grows extensively in damp,marshy and wet places and flowering occurs during April to June with white to purple or pink flowers. The whole plant is used for medicinial purposes and widely used as blood purifier as well as for the treating high Blood pressure , for memory enchancement and promoting longevity (Gohil*et al.*,2010).

In the present study the antimicrobial and phytochemical activity of *Centella asiatica*was determined against different species of Bacteria *Pseudomonas sp., Escherichia coli , Staphylococcus aureus* ,and *Bacillus subtilis* . against the fungi *Aspergillus sp ., Candida sp.*

MATERIALS AND METHODS

Collection of Sample.

The plant leaves of Vallarai (*Centella asiatica*) were selected for the study. The aerial part of plant of *Centella asiatica* was collected from the local area of choozhal, near Krishnapuram. The collected plants from the localities were identified taxonomically and authenticated. Different plant extract (Ethanol, water and Chloroform) were used for the further studies.

4.2 Preparation of the Plant Extracts.

The aerial part of *Centella asiatica* was cleaned with deionised water and dried in shade and pulverized into fine powdered substance by a grinding machine. Each one gram of powder of *Centella asiatica* was weighted with the electronic balance and transferred into three separate 100ml conical flasks. Then each 20ml of ethanol, 17 chloroform, and water was added in the flasks respectively. The conical flasks were closed by foil paper and placed on a shaker at 37 °C temperature for 24 hours. The crude extracts were then filtered by passing the extracts through Whatmann No. 1 filter paper and then concentrated under vacuum at 40 °C by using a rotary evaporator. The residual extracts were stored in refrigerator at 4 °C in small and sterile Screw Capped tubes**.**

Ethanol Extraction

1g of dried leaf powder was taken in separate conical flask. To this 20 ml of ethanol was added and then the conical flask was closed by the foil paper and placed on the shaker at 37°C for 24 hours. Then it was filtered with whatman no 1 paper and the filtrate was collected. The procedure was repeated three times. The collected filtrates were pooled and extracted samples were refrigerated at 4 °C for subsequent analysis.

Chloroform Extraction.

1g of dried leaf powder was taken in separate conical flask. To this of 20 ml of Chloroform was added and then the conical flask was closed by the foil paper and placed on the shaker at 37°C temperature for 24 hours. Then it was filtred with Whatmann no 1 paper and the filtrate was collected. The procedure was repeated three times. The collected filtrates were pooled and extracted samples were refrigerated at 4 °C for subsequent analysis.

Water Extraction.

1g of dried leaf powder was taken in separate conical flask. To this of 20 ml of distilled water was added and then the conical flask was closed by the foil paper and placed on the shaker at 37 °C temperature for 24 hours. Then it was filtred with whatman no 1 paper and the filtrate was collected. The procedure was repeated three times. The collected filtrates was pooled and extracted samples were refrigerated at 4 °C for future use.

Test Culture used in the Study

Antibacterial activity of *Centella asiatica* powder extracts was investigated against two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis)* and two gram-negative *(Escherichia coli and Proteus vulgaris*) bacterial isolates and two fungal isolates *(Aspergillus niger* and *Candida albicans*), which were Purchased from Nagercoil. The isolated bacteria were cultured on Nutrient Agar and the fungal isolates on Potato Dextrose Agar (PDA) at 37°C for 24 h. The cultures were sub cultured regularly and stored at 4°C.

Determination of Antibacterial Activity

Agar Disc Diffusion Method. The bacteria was first isolated and grown in a nutrient broth before use and standardize the culture to 108 CFU/ml. Nutrient agar was prepared and sterilized at 121 °C for 15 min. Sample loaded disc was placed on the surface of muller Hinton agar. Standarized cell culture and spread on nutrient agar. The plant powder extracts were introduced into the disc, allowed standing at room temperature. Then incubated at 37°C for 24 hours. After 24 hours of incubation, the plates were observed and inhibition zones were measured in millimeter.

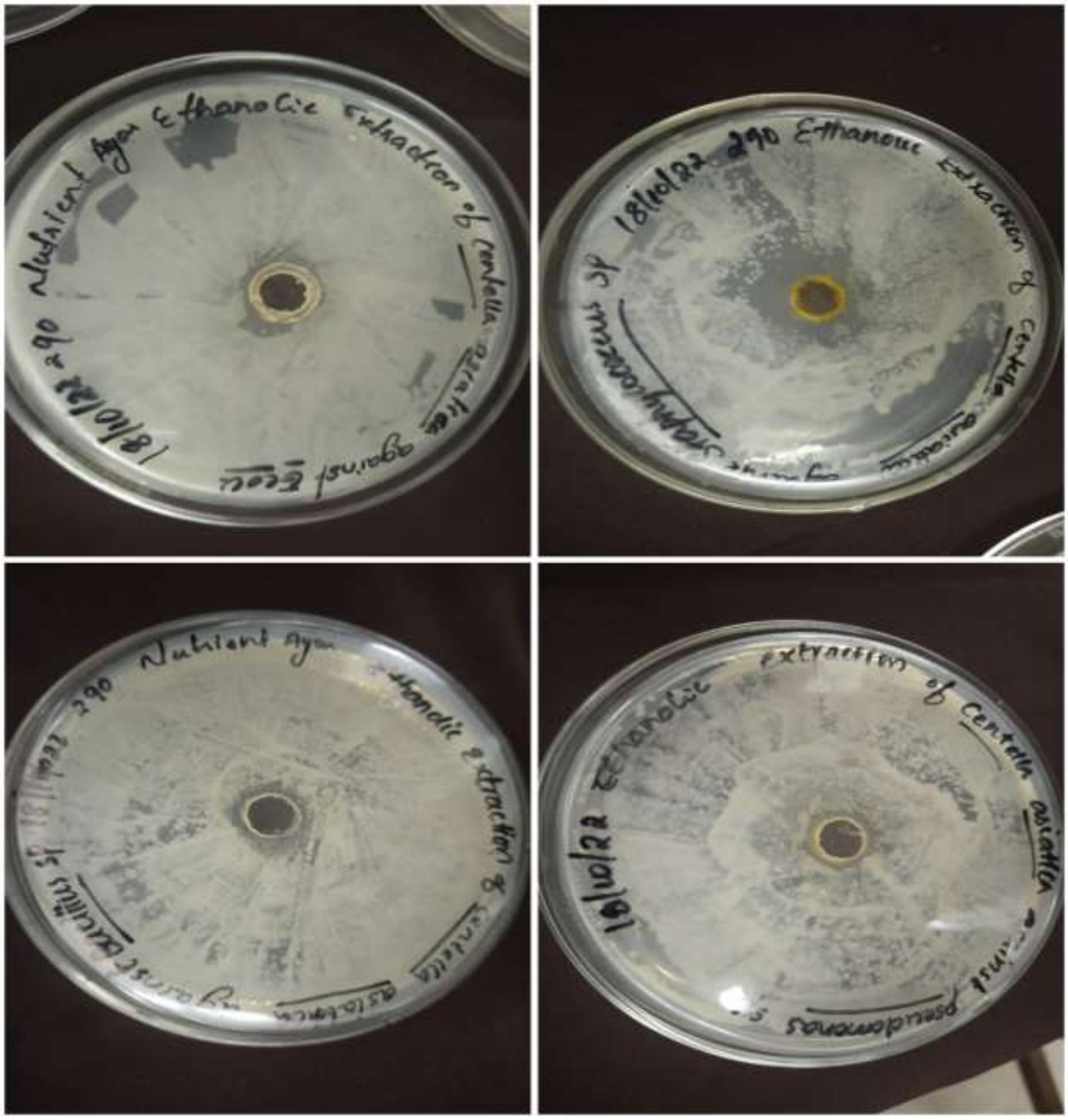
Determination Antifungal Activity(Agar Disc diffusion method)

The fungi was first isolated and grown in a nutrient broth before use and standardize the culture to 108 CFU/ml. Sabouraud Dextrose Agar was prepared and sterilized at 121°C for 15 mins at 15 lb/sq inches. Sample loaded disc was placed on the surface of Standarized cell culture spread on Sabouraud Dextrose Agar. The plant powder extracts were introduced into the disc, allowed to stand at room temperature. Then incubated at 20- 25 °C for 3-4 days. After 4 days of incubation, the plates were observed and inhibition zones were measured to the nearest millimeters.

RESULT

Antibacterial Activity of Aqueous Extract of *Centella asiatica* Against Human Pathogens.

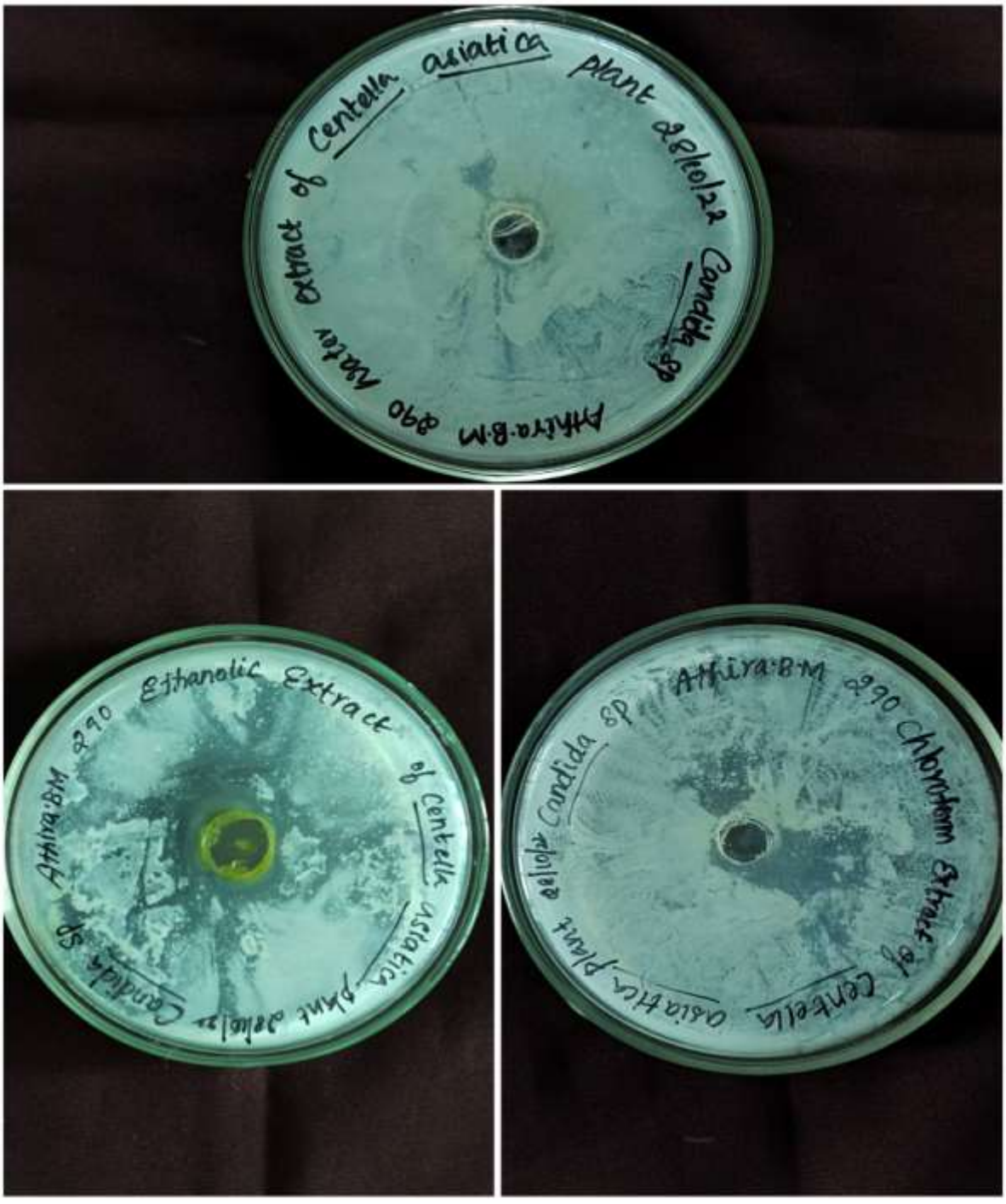
50µl of Aqueous *Centella asiatica* extract showed inhibitory activity against *pseudomonas sp (*9mm) and *staphylococcus sp* (10mm) and *E.coli* (8mm) and the *Bacillus sp* (14mm).



Antibacterial Activity of Ethanol Extraction of *Centella asiatica* Against Human Pathogens. 50µl of Ethanol *Centella asiatica* extract showed the inhibitory activity against *pseudomonas sp* (18mm) and *S.aureus(*23mm) followed by the *E.coli* (10mm), *Bacillus sp* (15mm).

Antibacterial Activity of Chloroform Extract of *Centella asiatica* Against Human Pathogens.

50µl of chloroform *Centella asiatica* extract showed the inhibitory activity against *pseudomonas sp* (25mm) and *staphylococcus sp* (10mm) followed by *E.coli* (14mm) and *Bacillus sp* (20mm).



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Antifungal Activity of *Centella asiatica* Plant Extract of Chloroform, Ethanol, Water against *Candida sp*

Candida sp showed resistant against Chloroform (13mm), water (10mm), Ethanol (17mm) from the extracts of *Centella asiatica* .

Antifungal Activity of *Centella asiatica* Plant Extract of Chloroform, Ethanol, Water against*Aspergillus sp*

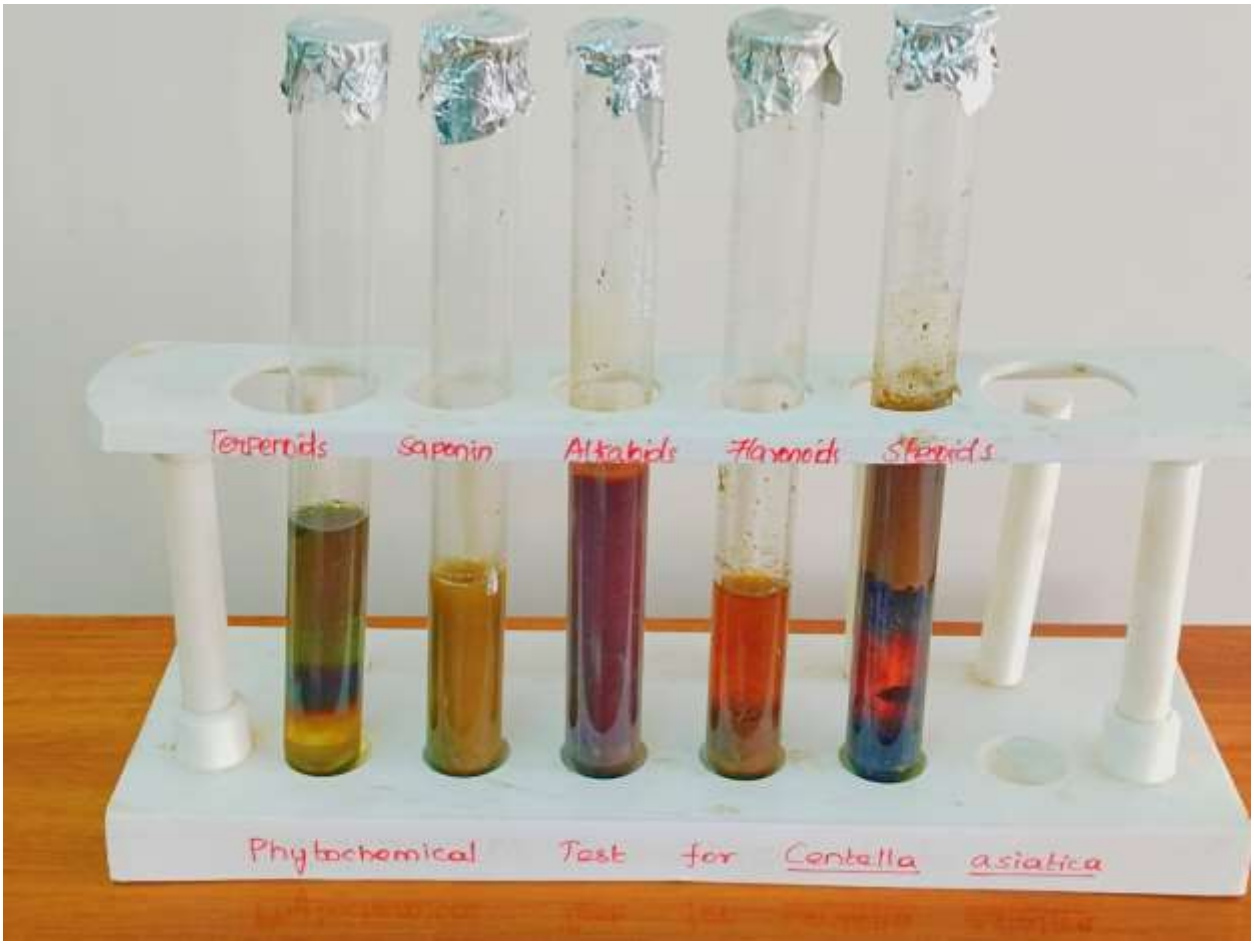
Aspergillus sp showed resistance against Chloroform (11mm), Water (10mm), Ethanol (17 mm) from the extracts of *Centella asiatica* .

Phytochemical Analysis of *Centella asiatica*

Phytochemicals	***Centella asiatica***
Terpenoids	+ve
Saponin	+ve
Alkaloids	+ve
Flavonoids	+ve
Steroids	-ve

Phytochemical Analysis.

The extracts showed the presence of Phytochemicals namely Terpenoids, flavonoids, alkaloids, steroids and saponins by colour change.



DISCUSSION.

Microorganisms are the concealed enemies to the mankind. There are a lot of antimicrobial drugs of which some are discovered or established and over 2,50,000 undiscovered flowering plants with medicinal properties exist worldwide (Madureira., 2008). Hence, the last decade witnessed an increase in the investigations on plants as a source of human disease management (Aiyelagable, 2001; Prashanth *et al.*, 2001; Mounishwamy *et.al*, 2002; Woldemicheal *et al*., 2003) and more natural antimicrobials have driven scientists to investigate the effectiveness of inhibitory compounds such as extracts from plants (Nasar – Abbas, Halkman, 2004). There are several reports of antibiotic resistance to human pathogens (Ganguly *et al*., 2001; Di Martino *et al.*, 2002).

In the present study, Effectiveness of antimicrobial agent is influenced by solubility, volatility and polarity of compounds in plants (Stratford and Eklund, 2003). Triterpenes in *C. asiatica* are polar compounds which on ionization of molecule combine with adsorption of

polyphenols to bacterial membranes leads to inhibition of bacterial growth by disrupting the bacterial membranes (Kalita and Saikia, 2012). *B. subtilis* which is a gram-positive bacterium was also found to be more susceptible towards *C. asiatica* extracts. This may be due to gram-positive bacteria was more sensitive than gram-negatives (Singh *et al.*, 2012).

Antifungal activity of *Centella asiatica* plant extract and extract at 100 % concentration against *A. niger* and *B. subtilis* showed little differences of inhibition zone as the 70 % concentration suggested the use of the extract at the less concentration but still giving significant inhibition of microbial growth. The results obtained in the present study indicated extracts of *C. asiatica* can be developed into broad spectrum of antibacterial and antifungal herbal formulations at the lowest cost. Essential oil from plants do have antimicrobial activity as proven by (Ferdes and Ungureanu., 2012) which have significant application against human pathogens, including those that cause enteric infections. They are reported to have curative properties against several pathogens and therefore could suggest their use in the treatment of various diseases (Hassan *et al.*, 2004)

This study deals with four pathogenic bacteria and two Fungi. In the present work, the antibiotic potential of three different extracts of*Centella asiatica* has been determined against different microorganisms such as *Pseudomonas* sp., *Bacillus* sp., *E.coli., Staphylococcus* spand the fungal microorganisms i.e., *Aspergillus* sp., *Candida* sp., In this study water, ethanol and chloroform extracts were used in which Chloroform and Ethanol were found to be very effective in inhibiting the growth of all the tested microorganisms ranging from 10-25mm zone of inhibition which are satisfactory comparing with water. In this study of *Staphylococcus* spexhibited the Zone of inhibition (10mm) for water extract of *Centella asiatica.* The Ethanolic extraction of *Centella asiatica* exhibited inhibition against various bacteria : *Staphylococcus* sp (23mm), *Pseudomonas* sp (18mm), *E.coli* (10mm), *Bacillus* sp (15mm). The Water extraction of *Centella asiatica* exhibited inhibition against the bacteria : *Staphylococcus* sp(10mm), *Pseudomonas* sp (9mm*), E.coli (*8mm), *Bacillus* sp (14mm). The Chloroform extraction of *Centella asiatica* exhibited inhibition against the bacteria : *Staphylococcus* sp(10mm), *Pseudomonas* sp (25mm), *Bacillus* sp (20mm), *E.coli* (14mm). In the present study the zone of Inhibition was observed for the fungi namely A*spergllus .niger* (8mm-15mm) and *Candida albicans* (10mm-17mm).

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