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Exploration of Ethnopharmacological Potential of Antimicrobial, Antioxidant, Anthelmintic and Phytochemical Analysis of Medicinally Important Plant *Centella asiatica* (L.) Urban in Mart. and Eichl.

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Abstract

As there is a huge pressure on the cultivated medicinal plants and due to this pressure a large number of plants are being eradicated yearly. So to reduce this pressure on the cultivated plants an effort is being done to use the wild plants as a good medicinal agent and a cheaper source as well. The present study was undertaken to find out the Antimicrobial activity, Antioxidant activity and Pharmacological Analysis of *Centella asiatica*. It is a wild plant and mostly found on the damp places of plains and foothills. It was collected, dried and extracted by maceration method in different polar and non-polar solvents *i.e.* petroleum ether, chloroform, methanol and distilled water. These extracts were further used to find out the antimicrobial, antioxidant and anthelmintic activities. *Centella asiatica* showed remarkable values comparable with the standard antimicrobial and antioxidant agents. Well defined zones of inhibition were recorded indicating that the plants were potent against pathogenic microbes, such as *i.e.* Bcteria (*Staphylococcus aureus*, *Staphyllococcus saprophyticus*, *E. coli*) and fungi (*Pseudomonas aeruginosa*, *Aspergillus parasiticus* and *Rhizopus oryzae*). The antioxidant activity of all the plant extracts was studied by DPPH Assay, Total Antioxidant Assay and Total phenolic Assay and the remarkable values comparable with the standard antioxidants were recorded. For pharmacological analysis different secondary metabolites indicated positive results whereas some others gave negative results.

Keywords

Antimicrobial Activity, Antioxidant Activity, Phyochemical, Anthelmintic

1. Introduction

Plants are oldest source of pharmacologically active compounds and have been very useful for human kind with reference to medically important compounds from centuries. Today it is estimated that more than two third of the world's population relies on plant derived drugs. 7000 medicinal compounds used in the Pharmacopoeia are derived from plants. Previously the plant *C. asiatica* was also known as *Hydrocotyl asiatica* and commonly as Brahmi Booti. It belongs to family Apiaceae. It is mostly found on the damp places of plains and foothills. The stems are slender, creeping stolons, green to reddish green in color, interconnecting one plant to another. It has long-stalked, green, reniform leaves with rounded apices which have smooth texture with palmately netted veins. The leaves are borne on pericardial petioles, around 2 cm. The rootstock consists of rhizomes, growing vertically down. They are creamish in color and covered with root hairs. The flowering period ranges from April to September [1]. In previous literature, a variety of chemical compounds from *C. asiatica* have been documented as: alkaloids, hydrocotyle, pectic acid, essential oils and Asiatic Acid. Medicinally the plant is considered very important being tonic, diuretic and local stimulant of skin diseases and as well memory sharper [2].

In Africa, chewing sticks are the most common means of maintaining oral hygiene, and roots, stems and twigs of numerous plants are employed for this purpose. Chewing sticks are recommended for oral hygiene by the World Health Organization, and some of them, or their extracts, are also used in the ethnomedical treatment of oral infections. Primary screens have demonstrated that extracts from many chewing sticks have antimicrobial activity against a broad spectrum of microorganisms, including those commonly implicated in orofacial infections. Some chewing stick extracts have additional biological activities. Preparation, extraction and antimicrobial screening methodologies are largely unstandardized and bioactivity-guided fractionation has only been conducted on a few chewing stick extracts. It is therefore highly likely that many chewing sticks contain secondary metabolites with as yet unreported antimicrobial activity. Antimicrobial principles that have been identified include novel flavenoid compounds and alkaloids. Chewing sticks offer considerable and underexploited potential as sources of new antimicrobial backbones [3]. Jeewan *et al.*, [4] observed the ethnopharmacological and antimicrobial properties of certain medicinal plants used by Adivasi tribes of the Eastern Ghats of Andhra Pradesh, India. They used 23 crude drug samples for various skin diseases and assayed for antimicrobial activity against four bacterial and one fungal human pathogen.

2. Materials and Methods

The plan of work was designed to qualitatively analyze the plant for its phytochemicals and the quantitative analysis of pharmacological aspects as antimicrobial, antioxidant and anthelmintic activities. The plant was collected from the GCU Botanic garden during the month of February. The plant specimen was authenticated and submitted to GCU herbarium. The collected specimen was dried at room temperature and then obtained the finely grind powder. Later on the extraction was done through maceration method in a series of non-polar and polar solvents *i.e.* petroleum ether, chloroform, methanol and distilled water.

2.1. Phytochemical Analysis

Qualitative phytochemical analysis of the crude extracts of the *Centella asiatica* was carried out by using standard procedures to identify the constituents as described by Edeoga *et al.* [5].

2.2. Antimicrobial Activity

Antimicrobial activity of the extracts was done according to Ortega *et al.* [6] and Ferreira *et al.*, (1996) by agar well diffusion method. The fungi were cultured on potato dextrose agar medium, which was prepared according to Johansen [7]. The antimicrobial activity was done against four bacterial (*Staphylococcus aureus*, *Staphylococcus saprophyticus*, *E coli* and *Pseudomonas aeruginosa*) and two fungal (*Aspergillus parasiticus* and *Rhizopus oryzae*) strains. The standard antimicrobial discs were used in comparison as:

- Ampicillin disc (10 ug) against *Staphylococcus aureus*
- Ampicillin disc (10 ug) against *Staphylococcus saprophyticus*
- Amikacin disc (30 ug) against *Pseudomonas*
- Sulphomethoxazole disc (23.75 ug) against *E. coli*
- Fucanozole medicine in the form of dilution as 250 mg/625 ml against *Aspergillus parasiticus* and *Rhizopus oryzae*

The whole process was carried out in the aseptic conditions. The zone of inhibition became prominent after the incubation time, *i.e.* 24 hours for bacteria and 48 hours for the fungi.

2.3. Antioxidant Activity

For antioxidant evaluation of the plant extracts different assays were run as DPPH (Diphenyl Picryl Hydrazyl Radical) assay, Total antioxidant assay and Total Phenolic assay.

DPPH Assay was done according to Erasto *et al.* [8]. The total antioxidant capacity of all the extracts was assayed according to the method of Prieto *et al.* [9] whereas Total Phenolic Assay was done by following the methodology of Makkar *et al.* [10].

2.4. Anthelmintic Activity

Haemonchus contortus, intestinal parasite of sheep were used by following the methodology of "Sharma *et al.* (1971), Lal *et al.* (1976) and Singh *et al.* (1985)" with a little modifications in the methodology of (Fasiuddin and Campbell, 2000).

3. Results and Discussion

Different polar and non-polar solvents were used for the extraction and thus their antimicrobial aspect and different values of inhibitory action against the microbes in mm were observed. In most of the cases the fungal species were strongly effected. Moreover, the water extracts in majority of the cases showed the maximum inhibitory values against the micro-organisms used.

The plant showed positive results for Alkaloids, Saponins, Tannins, Phlobatannins, Cardiac glycosides and Flavonoids by producing characteristic precipitates, froth or ring formation. Whereas in some cases it showed negative results that are for Terpenoids, Coumarins and Anthraquinones (**Figures 1-9**).

Centella asiatica petroleum ether leaf extract produced the maximum value of zone of inhibition, *i.e.* 68 ± 1.64^a against *S. aureus* among all the bacteria and the minimum values for inhibitory zone were observed by rhizome methanol and chloroform extracts against *S. saprophyticus* 3 ± 1.84^b and 3 ± 1.69^b respectively. Whereas for fungal strains the maximum inhibition value was given by rhizome methanol extract (52 ± 0.76^a) against *R. oryzae*, and the minimum by leaf petroleum ether extract (4 ± 0.76^a) against *A. parasiticus*.

The absorption values at 517 nm and %age DPPH values of all the extracts were recorded and later on compared with the standard antioxidant chemicals, BHT (Butyl HydroxyToluine) and α-Tocopherol. Among all the extracts of *Centella asiatica*, leaf chloroform extract and rhizome chloroform extracts showed DPPH activity that was closer to that of BHT, with the absorption values 0.13 ± 0.007 and 0.13 ± 0.01 respectively.

For total antioxidant assay, the absorption values were recorded at 695 nm and the rhizome extracts of *Centella asiatica* showed more closeness with α-Tocopherol with the values (0.54 ± 0.03), (0.57 ± 0.01) and (0.57 ± 0.01) respectively recorded.

As well as the total phenolic assay is concerned the results in this case were noted down by comparing them with the Gallic acid equivalent and the resultant values were recorded in the form of μg/g of Gallic acid. As the value increases, it means the quantity of phenol is increasing. The whole procedure was carried out on three sample replicates and the mean of these values were being recorded in association with the standard deviation among the replicates, the maximum value was shown by rhizome water extract, 422 ± 8.62 while rhizome petroleum ether extract showed the lowest value.

The results indicated that the plant is antimicrobial in nature. Some of the extracts showed very highly antimicrobial potential against bacteria and fungi, while some of them were comparatively less antimicrobial in nature. A variety of standard antimicrobial discs were run to compare the zones of inhibition against bacteria like *S. aureus*, *S. saprophyticus*, *E. coli* & *P. aeruginosa*, and fungi like *A. parasiticus* & *R. oryzae*. *Centella asiatica* rhizome chloroform extract showed the

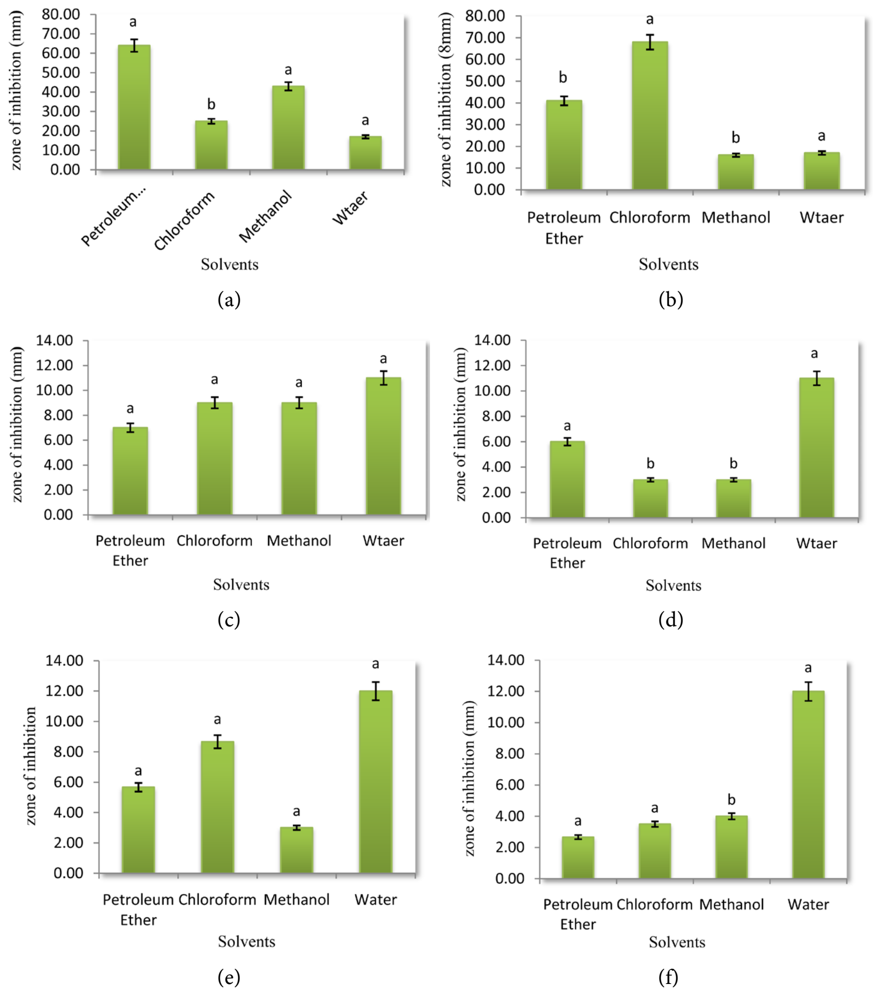
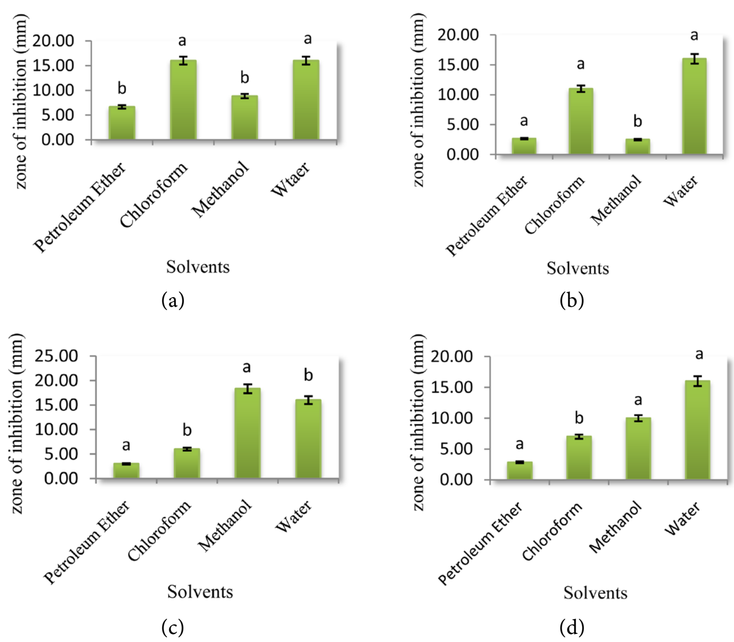


Figure 1. (a)-(f) Zone of inhibition (mm) produced by various extracts of *Centella asiatica* leaf extracts against *S. aureus*, *S. saprophyticus*, *E. coli*, *P. aeruginosa*, *A. parasiticus* and *R. oryzae.*



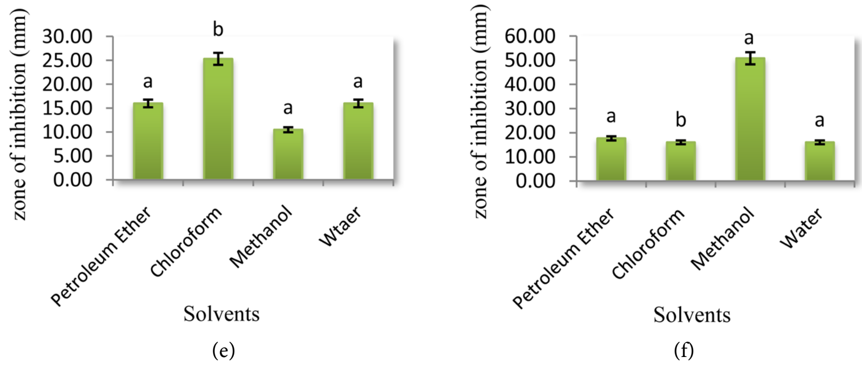


Figure 2. (a)-(f) Zone of inhibition (mm) produced by various extracts of *Centella asiatica* rhizome extracts against *S. aureus*, *S. saprophyticus*, *E. coli*, *P. aeruginosa*, *A. parasiticus* and *R. oryzae*.

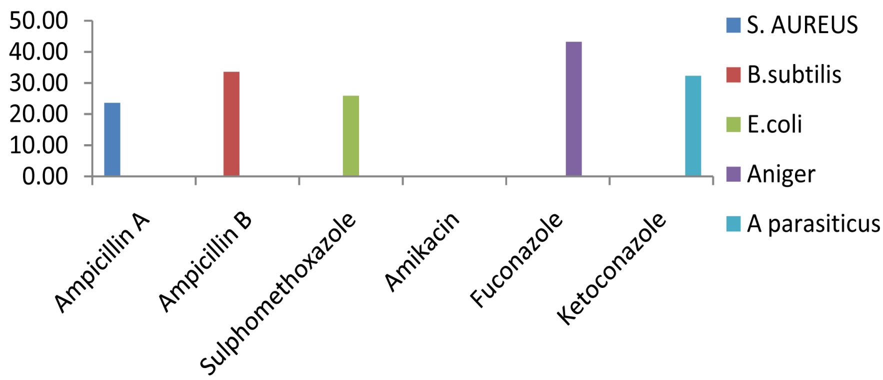


Figure 3. Zone of inhibition produced by standard discs against microbes.

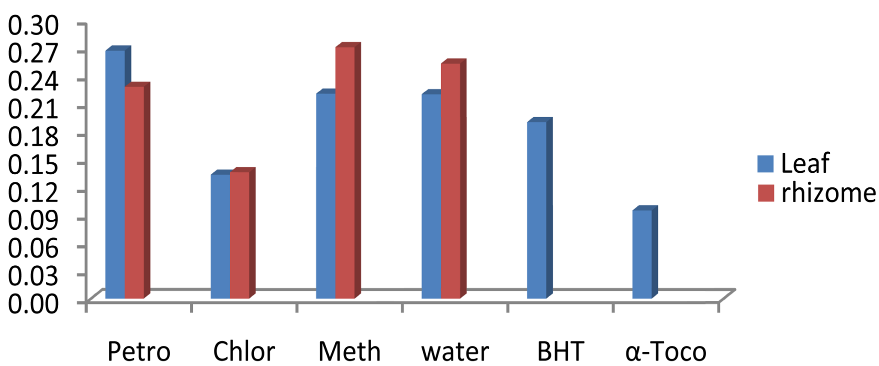


Figure 4. Antioxidant activity of various extracts of *Centella asiatica* in different solvents through DPPH assay.

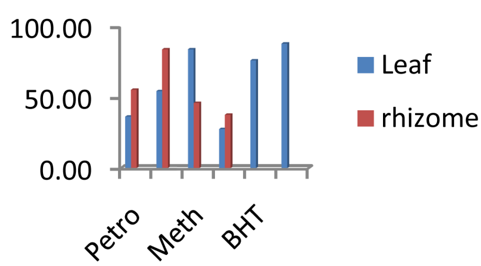


Figure 5. Percentage DPPH value.

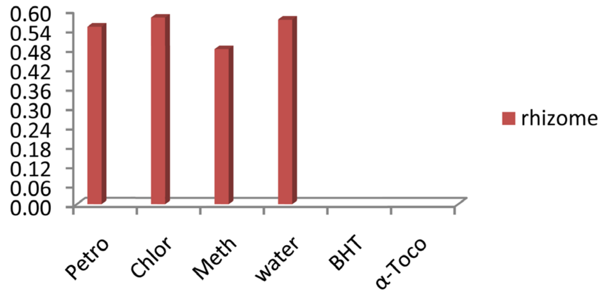


Figure 6. Antioxidant activity of various extracts of *Centella asiatica* indifferent solvents through total antioxidant assay.

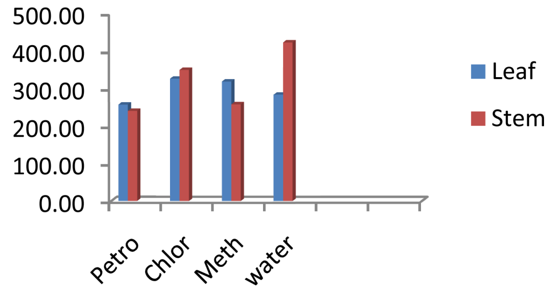


Figure 7. Antioxidant activity of various extracts of *C. asiatica* through total phenolic assay.

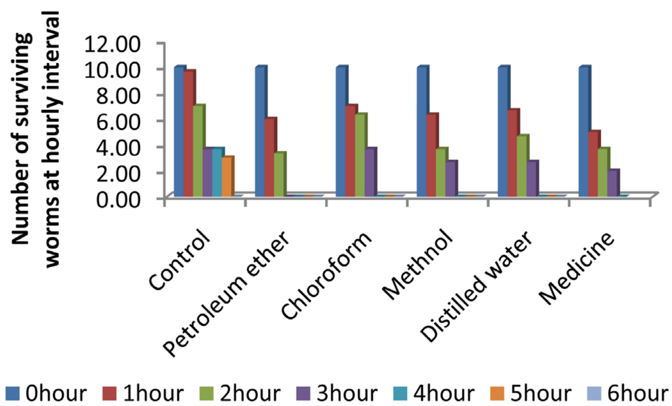


Figure 8. Anthelmintic activity of *Centella asiatica* leaf extracts.

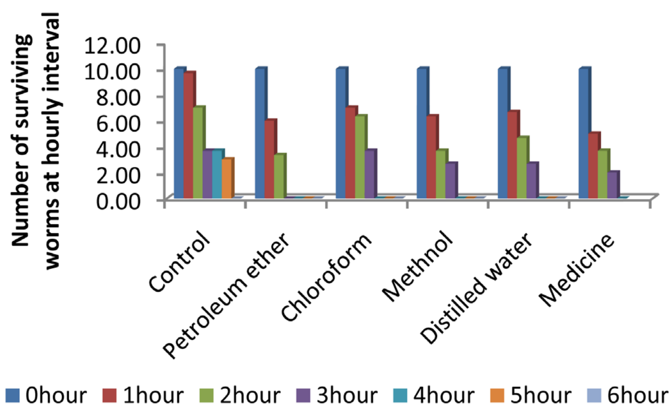


Figure 9. Anthelmintic activity of *Centella asiatica* rhizome extracts.

maximum value for zone of inhibition 68 ± 1.64[a] against *S. aureus* among all the bacteria, this very high antimicrobial value may be due to the compounds having strong antibiotic potential present in the extract. The maximum antifungal value was observed in rhizome methanol extract (52 ± 0.76[a]) against *R. oryzae*. Generally the secondary metabolites like alkaloids, terpenoids and tannins, etc. in the plants are readily extracted in the methanol like solvents showing higher polarity. Therefore the high antifungal value of the extracts may be due to the presence of such compounds in the extracts. While the lower value of antimicrobial activity may be due to the less concentration or absence of such compounds Rios and Recio [11] carried out somewhat related work on antimicrobial activity and recorded the results related to the results obtained in the present work.

In the same way Ibrahim *et al.* [12] observed the leaf extracts of two Nigerian edible vegetables by agar well diffusion method on selected food borne pathogens of medical importance for their antimicrobial activity. Both aqueous and ethanolic extracts of these plants were tested against *E. coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Shigella dysentriae and Salmonella typhimurium* in which the later one showed better and significant anitibacterial activity among all tested samples.

As well as the antioxidant activity is concerned, the results revealed that the plant had significant free radical scavenging activity. The free radical scavenging activity might be one of the mechanisms by which the plant extracts exhibited high antioxidant activity. Hence the present study provided a strong evidence for their use in food industry and medicine. *Centella asiatica* leaf chloroform extract and rhizome chloroform extracts showed the values closer to the BHT with the absorption values of 0.13 ± 0.007 and 0.13 ± 0.01 respectively. As these values were very close to the standard samples, it means that the plant is highly antioxidant. The same work done by Irina *et al.* (2001) recorded somewhat similar findings, in which they utilized the same methodology for testing the antioxidant activity of different extracts.

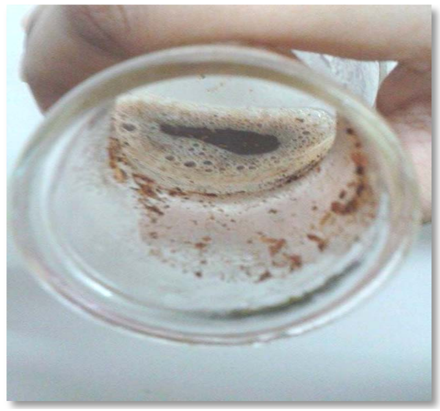
Just like the present work Seneviratne and Kotuwegedara [13] compared the antioxidant activities of the phenolic extracts of seed oils and seed hulls of five plant species with those of butylated hydroxyl toluene (BHT) solutions at comparable phenolic concentrations in order to understand the phenolic dependence of the antioxidant activity and to evaluate the potentials of these phenolic extracts as alternatives for synthetic antioxidants. Antioxidant activities of the phenolic extracts from different plants varied even at equal total phenol concentrations. o-Diphenol contents showed better correlations with the antioxidant activities than total phenol contents.

In *Centella asiatica*, the petroleum ether leaf extracts was the strongest one as the worms survived in this extract only for the duration of 2 hours while the leaf distilled water extract was mild one as the worms survived for 4 hours. When compared with the standard medicine (Levamisole), in which the living duration of worms was 4 hours, and then the water leaf extract was almost equal in strength with the standard medicine whereas the petroleum ether leaf extract was proved to be much stronger than the standard medicine

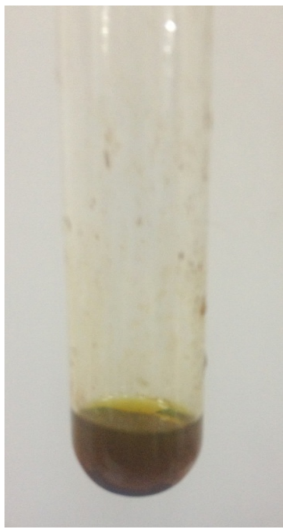
Among the extracts of rhizome, all of them were showing equal mortality rate *i.e.* 3 hours living duration, hence they are all weaker than standard medicine.

PLATES

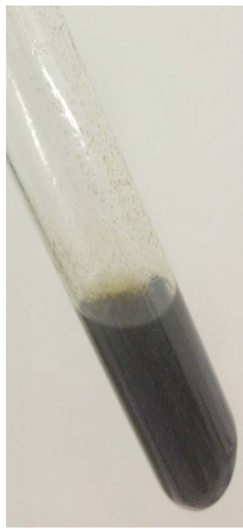
C. asiatica plant



Saponin frothing

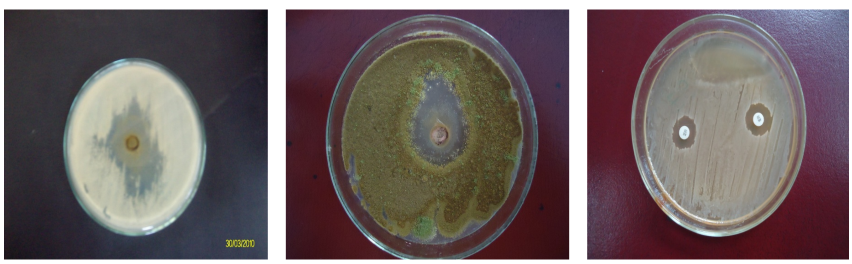


Alkaloids



Terpenoids

Glycosides



Zones by plant extracts against bacteria, fungi and standard discs

4. Conclusion

In the present study an effort has been done to find out the different ethno-pharmacological effects of *Centella asiatica*. The plant fractions showed very potent antimicrobial results and thus it can be concluded that the plants can be used as the better sources for diminishing the microbes. As the antioxidant activity is concerned, many of the extracts showed strong antioxidant activity and as well a very high number of the phenolic agents; thus these can be considered as good anti-aging agents. The anthelmintic activity of the plant also showed good results against the worms. If we consider all these aspects collectively we can conclude that the plant is very active medical agents and can be used as an authentic source for reducing the pressure on the pharmaceutical industry as they are all the natural resources thus without any side effects.

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