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COMPARATIVE STUDY ON THE CRUDE DRUG OF TWO IMPORTANT TRADITIONAL MEDICINAL PLANTS, *CENTELLA ASIATICA* AND *BACOPA MONNERIA*

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ABSTRACT

Medicinal plants have provided a source of inspiration for novel drug compounds. As plant derived medicines have made large contributions to human health and wellbeing. In traditional system of medicines, the plants are reported to possess a lot of beneficial effects. Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic values. In recent times, focus on medicinal plant research has increased all over the world. The medicinal plants Centella asiatica and Bacopa monneria are the most useful traditional medicinal plants in India. The plants are commonly known as "Brhami" after Bramha, the creator God of the Hindu pantheon. Both these herbs are important in Indian system of medicine and are commonly used for memory enhancement activity due to the presence of some important bioactive components. The whole parts of the plants are used to treat various human ailments. These plants have different active principles, but they possess nerve stimulation, antibacterial, and antioxidant properties etc. In the present study FT-IR spectroscopic method was carried out for the determination of bio active constituents present in Centella asiatica and Bacopa monneria. In addition to that the antioxidant activity and the antibacterial activity were carried out to find out the systematic authentication for the efficacy of the crude drugs.

KEY WORDS

Centella asiatica, Bacopa monneria, FT-IR, Antioxidant property, Antibacterial activity, Memory enhancement.

INTRODUCTION

India is one among the top 25 biological hotspots of the world and it harbors various species of rare and endangered fauna and flora. India has about more than 20000 species of medicinal plants with high production potential. Plants have been a valuable source of natural products for maintaining human health. There are numerous traditional medicines are complementary and alternative medicines of herbal origin used all over India. Medicinal plants contain substances that can be used for therapeutic purpose or precursors for the synthesis of useful drugs[1]. Natural products such as herbs, fruits and vegetables become popular in recent years due to public awareness and increasing interest among consumers and scientific community[2]. Ethano pharmacological evidences has been provided that constituents in natural products show many biological and pharmacological activities including anti-oxidant and anti-bacterial activities. Various plants are used traditionally by ethnic societies from remote place in the forms of extracts of different plant parts such as fruits, leaves, stem-bark and roots [3]. Both the plants

Centella asiatica and Bacopa monneria are described as Brahmi It is used in Indian medicine as tonic, churana and tablets. It has naturally occurring nitric compounds that have positive effects in brain activity [4]. The antibacterial activity was carried out by using standard disc method for augment the scientific validation of the crude drug of *Centella asiatica* and *Becopa monneria*. The helative properties of the plant are due to the presence of antioxidant in the green plants. In order to find out this property the antioxidant activity of the plant were analyzed by using the Hydrogen Peroxide scavenging activity and $FeCl_3$ Reducing Power Assay followed by the FT-IR spectroscopic method for identification of bioactive phytpochemical constituents. *Centella asiatica*, commonly known as Centella and Gotu kola, is a small, herbaceous, frost-tender perennial plant of the family Apiaceae is native to wetlands in Asia[5]. It is used as a medicinal herb in Ayurvedic medicine, traditional African medicine, and traditional Chinese medicine. It is also known as the Asiatic pennywort or Indian pennywort in English, among various other names in other languages. Vallari in Tamil, Mandukparni in Sanscrit, Tholkui in Bengali, Brahmi or Brahmhmanduki in Marati. Centella is commonly grows in tropical swampy areas[6]. The stems are slender, creeping stolons, green to reddish-green in color. It has long-stalked, green, rounded apices which have smooth texture with palmately netted veins. The leaves are kidney shaped and borne on pericladial petioles. The rootstock consists of rhizomes growing vertically down. *Centella asiatica* is indigenous to the Indian subcontinent, Southeast Asia, and wetland regions of the Southeastern US. Because the plant is aquatic, it is especially sensitive to biological and chemical pollutants in the water, which may be absorbed into the plant. Centella is most often used to treat varicose veins and chronic venous insufficiency and in ointments to treat psoriasis and help to heal minor wounds, due to its triterpenoids contents. Studies have also shown positive effects on anxiety and scleroderma[7]. The chemical constituents of *Centella asiatica* have Asiaticoside, Medacassoside, Brahmoside, Alkaloids- Hydrocotylin, Vellarine, New triterpene glycoside- Thankuniside, Triterpene acid- Thankunic acid, Anthrone, Asiaticoside, Asiatic acid, Madegascaric or madecassic acid, Isothankuniside, Brahmic acid, Centelloside and Centic acid[8].The drug produced from Centella *asiatica* has significant intellectual improvement in mentally retarded children. Clinical trials conducted on normal adults showed that the drug increased the mean level of RBC, blood sugar, serum cholesterol, vital capacity and total protein. The increase in the hemoglobin percentage was quite high. The drug also decreased the mean blood urea level[9].

***Centella asiatica* (Habit)**

Bacopa monneria is a perennial, creeping herb belongs to the family Scrophulariaceae, native to the wetlands of Southern India, Australia, Europe, Africa, Asia, and North and South America[10] Bacopa is a medicinal herb used in Indian system of medicine, the common name of the plant Nirbrahmi in Tamil, Thyme-leaved gratiola in English, Barahmbhi in Sanskrit, Brahmi in Bengali and Marati[11]. The leaves of this plant are succulent, oblong and 4–6 mm (0.16–0.24 in) thick. Leaves are oblanceolate and are arranged oppositely on the stem. The flowers are small, white with five petals. Its ability to grow in water makes it a popular aquarium plant. It can even grow in slightly brackish conditions. Propagation is often achieved through cuttings[12]. It is commonly grow throughout India, Nepal, Sri Lanka, China, Pakistan, Taiwan, and Vietnam. It is also found in Florida, Hawaii and other southern states of the United States where it can be grown in damp conditions in a pond or bog garden[13]. This plant can be grown hydroponically. Bacopa has been used in traditional Ayurvedic treatment for nervous stimulation and epilepsy. It is also used for ulcers, tumors, ascites, enlarged spleen, inflammations, leprosy, anemia, and gastroenteritis[14]. The chemical constituents of *Bacopa monneria* have Alkaloids, Brahmine, Nicotine, Herpestine, D-mannitol, Apigenin, Hersaponin, Monneriasides 1-3, Cucurbitacin, Plantainoside and Bacoside A. It is a cardiac and nervine tonic, leaves and stalks are diuretic and aperients. The alcoholic extract of the plant showed a sedative effect[15].

***Bacopa monneria* (Habit)**

METHODOLOGY

Collection of plant material

The plant samples were collected from the Kani settlement of Agasthiyamalai Biosphere Reserve forest of Pechiparai, Kanyakumati, District, Tamil Nadu, India.

Processing of the plant material

The collected plant samples were washed thoroughly for 2-3 times with running water and rinse with sterile distilled water for removal of dust and soil particle then cut in to pieces. Then allowed to air dried and homogenized to a fine powder and stored in air tight containers.

Solvent Used

The organic solvents such as Chloroform, Methanol, Ethanol and distilled water were used for the extraction process.

Extraction of Bioactive Compounds

Dried powder form of plant sample was used to extract bioactive principles. Five grams of plant sample was weighed and ground in to a part by using Chloroform, methanol, ethanol and water separately. The grounded material was made up to 50 ml using the respective solvent and it was maintained in refrigerator for 24 hours. Then it was centrifuged at 5000 rpm for 20 minutes. The supernatant extract was used for the analysis of sensitivity assay.

Culture Used

Six bacterial strains (3 Gram Positive and 3 Gram Negative Human pathogenic bacteria) were used for testing the antibacterial activity of the *Centella asiatica* and *Becopa monneria*

Maintenance of Bacterial Strains

Bacterial strains to be tested were streaked in selective agar plates to get pure cultures and stored at 40˚C to keep the bacterial strains viable.

Preparation of Inoculum

Ten ml of Nutrient broth was prepared in test tubes. These tubes were cotton plugged and autoclaved. The test tubes were labelled according to the type of the bacterial cultures to be inoculated. Then the broth were inoculated with the known bacterial strains under aseptic conditions and incubated at 37˚C for 24 hours.

FTIR Spectroscopic Analysis

Samples were placed directly on the germanium piece of the Bruker Alpha T Infrared spectrometer with constant pressure applied and the data of infrared absorbance collected over the wave number ranged from 4000 cm–1 to 5000 cm–1 and computerized for analyses by using the IRPAL. The reference spectra were acquired from the cleaned blank crystal prior to the presentation of each sample replicate. All spectra were collected with a resolution of 4-1 cm and to improve the signal-to-noise ratio, 256 scans were co-added and averaged. Samples were run in triplicate and all of them were undertaken within a day period.

***In-vitro* Antibacterial Assay**

Standard Disc method was followed to determine the antimicrobial activity, Muller Hinton Agar (MHA) plates were prepared and swabbed (sterile cotton swabs) with 24 hours old broth culture of respective bacteria. Discs were made from Whatman No.1 filter paper. Stock solution of each plant extract was prepared using methanol, chloroform, ethanol and water as solvent. About 100µl and 200µl of different concentrations of plant solvent extracts was added using micropipette into the paper discs allowed to dry at room temperature. The discs were placed in the Muller Hinton Agar medium plates. The plates were incubated at 37˚C for 18-24 hours. At the end of incubation, inhibition zones formed around the disc were measured with the transparent ruler in millimeter. These studies were performed in triplicate and the values were recorded.

Antioxidant Activity

$FeCl_3$ Reducing Power Assay

An about 100µl of extract was prepared in ethanol, it was mixed with 500 µl of 1% potassium ferric cynade. The mixture was incubated 50^0C for 20 minutes. After incubation, 500 µl of 10% trichloro acetic acid was added to the mixture, which was then centrifuged at 3000rpm for 10minutes. The upper layer of the mixture 500 µl was mixed with an equal volume of distilled water and 100 µl of 0.1% $FeCl_3$ solution. The absorbance was measured at 700nm. Blank was prepared by adding 200 µl ethanol instead of the extract. The reducing power of each sample were calculated.

Hydrogen Peroxide scavenging activity

A solution of 2mM H_2O_2 (Hydrogen peroxide) was prepared in 50mM phosphate buffer (pH 7.4). An aliquot of 100 µl of the sample was transferred to the test tube to have final concentration in a reaction mixture of 200 µl and the volume was made up to 400 µl with 50mM phosphate buffer (pH 7.4). After addition of 600 µl of H_2O_2 solution, tubes were vortexed and absorbance of H_2O_2 at 230nm was determined after 10 minutes, against a blank. Ascorbic acid was used as positive control. (Ruch et al., 1989). The percentage H_2O_2 scavenging ability of samples (extraction/fraction) was then calculated by using the following equation:

$$\text{Hydrogen peroxide scavenging activity} = [1\text{-As/Ac}] \times 100$$

As = Absorbance of sample

Ac=Absorbance of control was expressed as ascorbic acid equivalent

RESULT AND DISCUSSION

The FT-IR analysis of absorption spectrum and functional groups of *Centella asiatica* and *Becopa monneria* were given in the figures 1&2 and tables 1&2 respectively. *Centella asiatica* there are ten functional groups are present.

The FT-IR analysis showed that the presence of bio active constituent group present in the plants *Centella asiatica* and *Bacopa monneria*. In Centella there are 10 functional groups but in Becopa there are 6 functional groups. This 6 functional groups present in the Becopa are also present in the Centella. But in Centella there are 4 additional functional groups than Becopa. The presence of 6 common functional groups like alkyl halides, carboxylic acids, ethers, alkenes etc in both the plants, and it indicates that the plants possess same active principles which is useful for memory enhancement activity. So, both the plants can be used to induced nervous stimulation and other related human ailments because of the presence of 6 common functional group constituents. Among the two plants the *Centella asiatica* is having more bioactive constituents when compared to *Becopa monneria.*

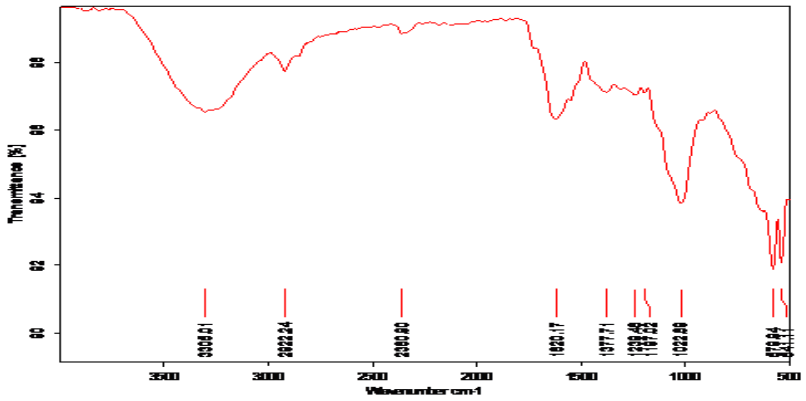


Fig 1. FTIR Spectrum analysis of *Centella asiatica*

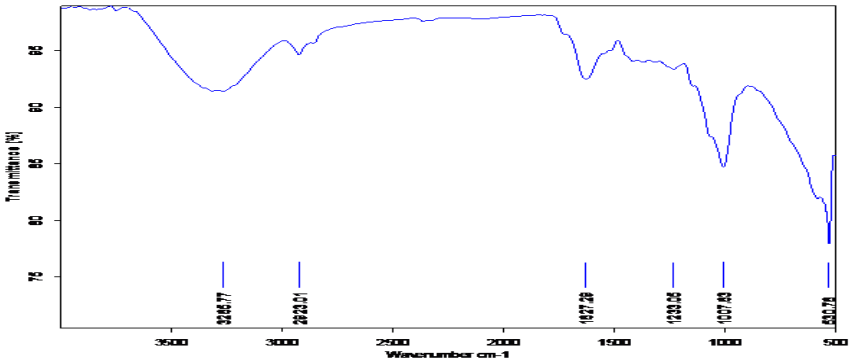


Fig 2: FTIR Spectrum analysis of *Bacopa monneria*

Table 1: FTIR Functional groups of *Centella asiatica*

No.	Frequency	Functional Groups
1.	541.11	Alkyl halides
2.	579.94	Alkyl halides
3.	1022.89	Carboxylic acids
4.	1197.02	Amines
5.	1239.46	Ethers
6.	1377.71	Alkanes
7.	1620.17	Alkenes
8.	2360.90	Miscillenious
9.	2922.24	Carboxylic acids
10.	3305.01	Phenols

Table 2: FTIR functional groups of *Bacopa monneria*

No.	Frequency	Functional Groups
1.	530.76	Alkyl halides
2.	1007.63	Carboxylic acids
3.	1233.05	Ethers
4.	1627.29	Alkenes
5.	2923.01	Carboxylic acid
6.	3265.77	Phenols

Antimicrobial Activity

The leaf extract was taken in different solvents such as Chloroform, Methanol, Ethanol, and Aqueous and tested against the human pathogenic bacteria of three Gram negative and three positive organisms. The results were given in Tables 3,4 and Figs 1,2. On comparison the two plants showed good antibacterial activity and the *Centella asiatica* is having higher antibacterial activity than *Becopa monneria.* The standard was in Kanamycin.

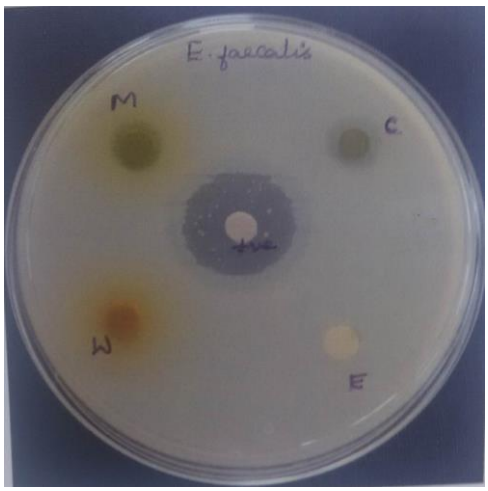
Table 3: Antibacterial activity of *Centella asiatica* in different Solvents

Organisms	Zone of Inhibition of *Centella asiatica* – Leaf extract in mm				
	Chloroform	Methanol	Ethanol	Water	Control
Bacillus subtilis	7	8	-	-	22
Escherichia Coli	8	11	-	-	25
Enterococcus faecalis	8	10	-	-	20
Pseudomonas aueroginosa	13	12	-	-	14
Staphylococcus auerus	9	8	-	-	16
Vibrio cholerae	7	10	-	-	27

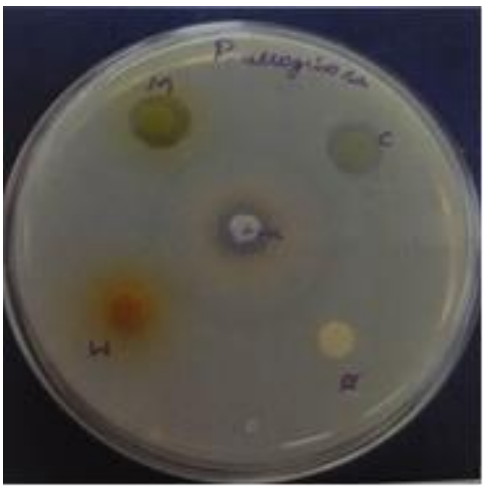
Table 4: Antibacterial activity of *Becopa monneria* in different Solvents

Organisms	Zone of Inhibition of Becopa monneria- Leaf extract in mm				
	Chloroform	Methanol	Ethanol	Water	Positive control
Bacillus subtilis	6	5	-	-	22
Escherichia Coli	10	12	-	-	23
Enterococcus faecalis	5	6	-	-	19
Pseudomonas aueroginosa	12	9	-	-	11
Staphylococcus auerus	9	7	-	-	25
Vibrio cholerae	7	10	-	-	27

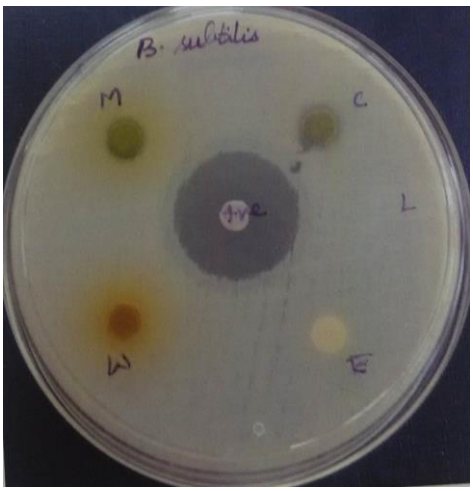
Fig:1: Antibacterial Activity of *Centella asiatica* Leaf Extract in Different Solvents



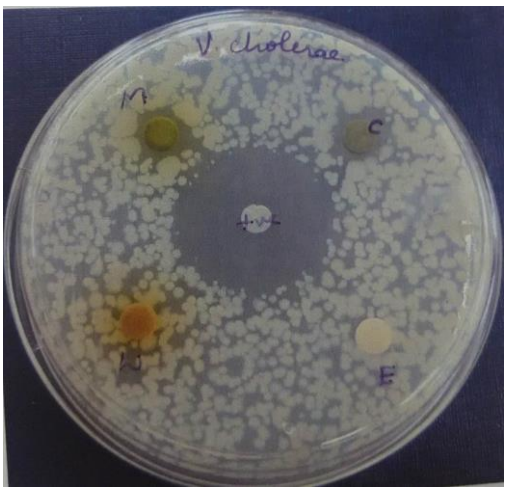
E.Faecalis



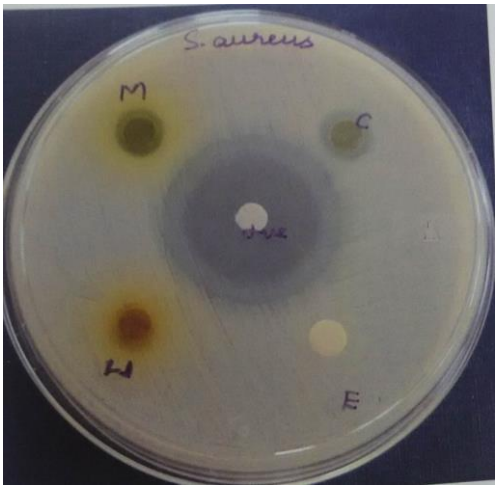
P.aueroginosa



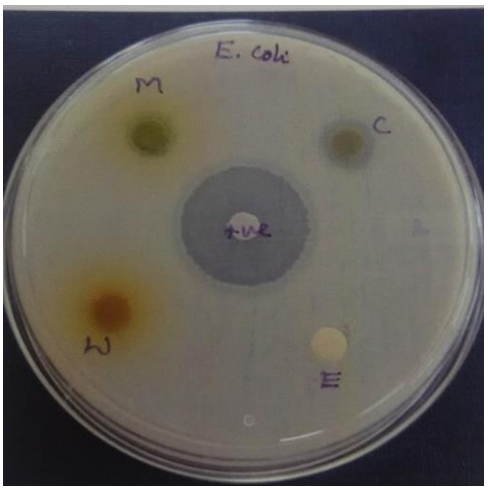
B.subtilis



V.colerae

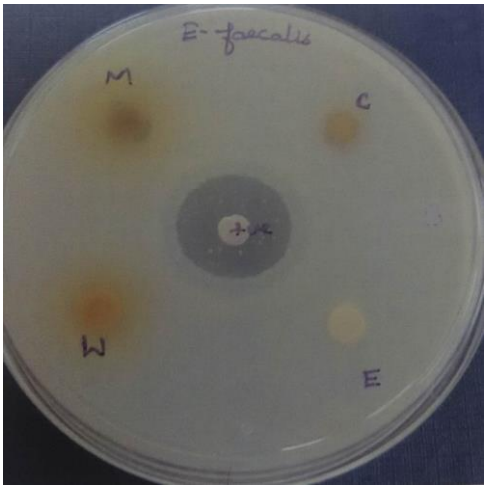


S.auerus

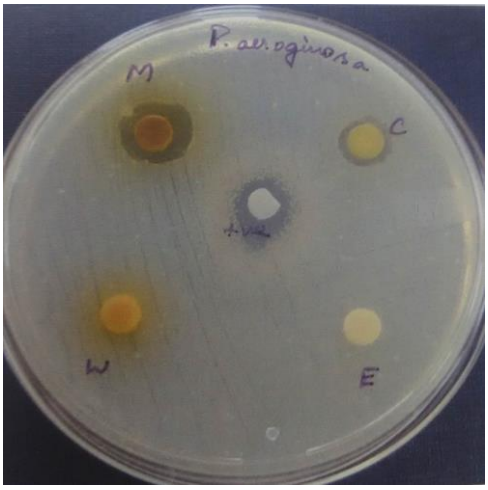


E.coli

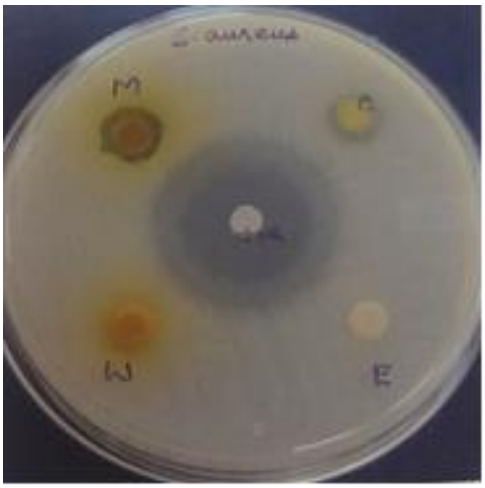
Fig:2: Antibacterial Activity of *Becopa Monneria* Leaf Extract In Different Solvents



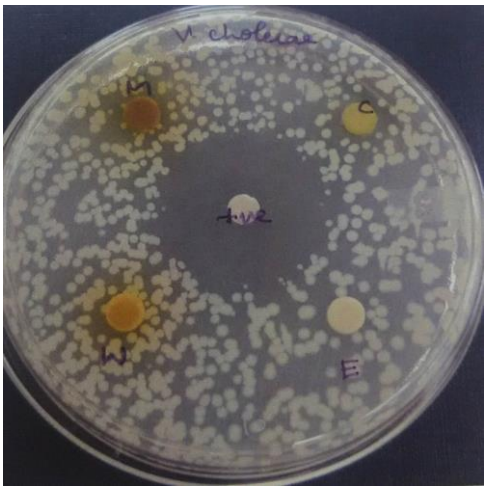
E.Faecalis



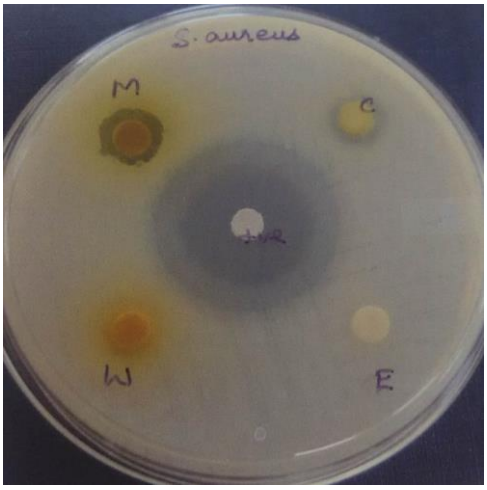
P.aueroginosa



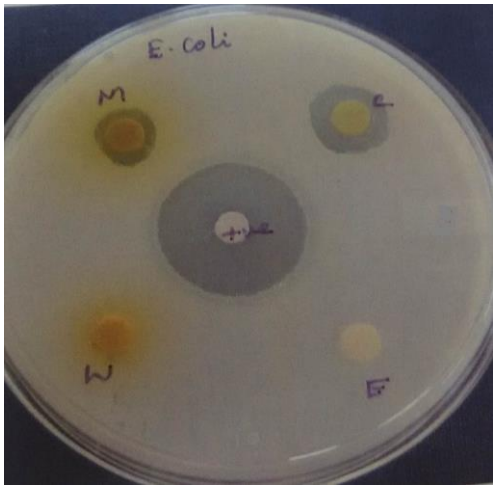
B.subtilis



V.colerae



S.auerus



E.coli

Antioxidant Activity of *Centella asiatica* & *Bacopa monneria*

The results of the antioxidant activity of *Centella asiatica* and *Becopa monneria* were given in the table 5&6. In this the $FeCl_3$ Reducing Power Assay and Hydrogen Peroxidase Scavenging assay were analyzed and tabulated. The *Centella asiatica* have more than 50% of activity and *Becopa moneria* showed 40% antioxidant activity. The reducing power and scavenging activity of Centella is higher than *Becopa monneria*

Table: 5: $FeCl_3$ Reducing Power Assay of *Centell asiatica* and *Becopa monneria*

Sl. No	Sample	Reducing Power Assay (100 µg)	Reducing Power Assay (200µg)
1	***Centella asiatica***	**41.8**	**66.4**
2.	***Becopa monera***	**35.6**	**61.2**
3.	**Standard**	**84.25**	**91.55**

Table: 6: Hydrogen Peroxidase Scavenging Activity of *Centell asiatica* and *Becopa monneria*

Sl. No	Sample	H_2O_2 Scavenging Activity (100 µg)	H_2O_2 Scavenging Activity (200 µg)
1	***Centella asiatica***	**40.08**	**62.08**
2.	***Becopa moneria***	**33.50**	**52.05**
3.	**Standard**	**84.25**	**91.55**

CONCLUSION

Both the plants *Centella asiatica* and *Bacopa monneria* have nervous stimulation properties in addition to other medicinal uses. The present study is to determine the active principles and the medicinal property of the *Centella asiatica* and *Bacopa monneria*. In the traditional medicinal preparation of Ayurvedic & Siddha, both the plants are used as an alternative to each other based on the availability. The present investigation also confirms that both the plants are having the bioactive constituents of the same functional groups. Even though they are having common functional groups in both the plants. *Centella asiatica* is having more functional group than *Bacopa monneria*. For our medicinal preparation it is better to use *Centella asiatica* than *Bacopa monneria.* In south the medicinal practitioners are using *Centell asiatica* and in North India the Becopa *monneria* is used based on the availability of the plant in abundance. It can be can used as Immunomodulatory drug in the Indian system

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