Phytoconstituents and *In Vitro* Antimicrobial potentials leaf extract *Centella asiatica* and *Moringa oleifera* against selected bacterial and fungal strains

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Abstract

Plants are rich source of antimicrobial components. The present study was to find out the phytochemical and antimicrobial activity of ethanolic extracts of *Centella asiatica* and *Moringa oleifera* leaves. Both the plants are used in Ayurvedic and traditional medicines and are also wild edible plants. Preliminary Phytochemical analyses showed the presence of alkaloids, flavonoids, glycosides, carbohydrates, tannins, proteins and amino acids, phenols, saponins, diterpenes, terpenoids. Quantitative levels of alkaloids, flavonoids, saponins, phenols, tannins, crude fiber, ash content, and pectic substances are also reported. The antimicrobial activities of different concentrations (50 µl, 100 µl and 150 µl) of ethanolic extracts of *Centella asiatica* and *Moringa oleifera* were evaluated and determined by using disc diffusion method against the bacteriae *Escerichia coli, Staphylococcus. aureus* and fungi *Aspergillus niger, Aspergillus flavus*. The results showed that the etanolic leaf extracts of the plants *Centella asiatica* and *Moringa oleifera* have potent antimicrobial activity. Further detailed studies are required to determine the active components responsible for these effects and their mode of action.

Keywords : antimicrobial activity, *Centella asiatica*, disc diffusion, *Moringa oleifera,* Phytochemical screening.

INTRODUCTION

India is a varietal emporium of medicinal plants and is one of the richest countries in the world as regards to genetic resources of medicinal plants. A survey conducted by the All India Coordinated Research Project on Ethnobiology (AICRPE) during the last decade recorded over 8000 species of wild plants used by the tribals and other traditional communities in India for treating various health problems (Barmet, 1992). Medicinal plants and plant-derived medicines are widely used in different traditions all over the world and they are becoming increasingly popular in modern scientific communities as natural alternatives to synthetic chemicals (Karpagam, 2008). The trees and shrubs have wide range of benefits to human beings amongst other include medicine and foods. To prevent and cure different human diseases recently, considerable attention has been paid to eco-friendly and bio-friendly plants (Kokate *et al*., 2011).

World Health Organization has also identified the importance of herbal medicines. Approximately 60-70% patients living in rural areas are dependent on herbal medicine. In developing countries the practice of complementary and alternative medicine is now on the increase (Bauer *et al*., 1996). Several authors have reported favourable results with herbal drugs (mostly in the form of extracts) either in animal or in human studies (Deepti, 2013). Medicinal plants are the main source of pharmaceuticals and healthcare products and are a source of extraction and development of several drugs medicinal plants consist of many secondary metabolites such as alkaloids, phenolic compounds, etc which possesses antimicrobial properties (Raman, 2006). Several clinical and pre-clinical studies have provided the scientific basis for the efficacy of many plants used in folk medicine to cure diseases. There is a need for a search and development of new drugs from medicinal plant metabolites.

Centella asiatica is a popularly known as Centella, Pennywort or Indian pennywort, Brahmi or Gotu kola. It belongs to the family Apiaceae. It is used in Ayurvedic, traditional African and Chinese medicinal system. Root, stem, leaves, and aerial parts are used to the traditional drug formulas to decrease blood pressure, cure the fresh wound, heal bruised and diuretic (Ullah, *et al*., 2009).

Moringa oleifera Lam. (Family Moringaceae) is well – known for its various medicinal properties which are widely used for treating bacterial infection, fungal infection, antiinflammation, sexually-transmitted

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diseases, malnutrition and diarrhoea (Thakur and Sulekha Pathak, 2015). Moringa species have long been recognized by folk medicine practitioners as having value in the treatment of tumors (Rawat, 1997). Almost all the parts of this plant: root, bark, gum, leaf, pods, flowers, seeds and seeds oil have been used for the various ailments in the indigenous medicine. It is known to have antihelminthic, antimicrobial, detoxifying, immune boosting and anti –parasitic activities.

The present study evaluates the phytoconstituents of leaf extracts of *M. oleifera* and *C. asiatica* and their antibaterial and antifungal potentials.

MATERIALS AND METHODS

Collection, Identification and Authentication of plant materials

The plant species namely *Centella asiatica* and *Moringa oleifera* was collected by in and around Mannargudi, Thiruvarur District, Tamil Nadu, South India.

Preparation of plant powder

The plant leaves were air dried under shade for 10-15 days. Then the dried material were ground to fine powder using an electric grinder and stored in air tight bottles and was used for further analysis.

Preparation of the Ethanol extract

Ethanolic extracts were prepared according to the methodology of |Indian pharmacopoeia (Anonymous, 1996). The coarse powder materials were subjected to Soxhlet extraction separately and successively with 210ml ethanol and 90ml distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (40^0C – 50^0C). The paste form of the extracts was put in an air tight container and stored in a refrigerator.

Preliminary phytochemical analysis

The preliminary phytochemical investigations were carried out with the standard protocol (Dash *et al.*, 2016).

In vitro antimicrobial activity

The antimicrobial activity was performed by disc diffusion method of Beyar *et al*., 1966.

Antibacterial Activity

Disc Preparation

The 6mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C.After the sterilization the moisture discs were dried on hot air oven at 50°C.Then various solvent extract discs and control discs were prepared.

Assay of Antibacterial Activity

Antibacterial activity test was carried out following the modification of the method originally described by Bauer *et al.,* (1966). Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45°C. The cooled media was poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents extract prepared discs individually were placed on the each petriplates and also placed control and standard (Nitrofurantoin (300 µg) for Bacteria) discs. The plates were incubated at 37°C for 24 hrs.

Antifungal activity

Disc Preparation

The 6mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C.After the sterilization the moisture discs were dried on hot air oven at 50°C.Then various solvent extract discs and control discs were prepared.

Assay of Antifungal Activity

Antifungal activity test was carried out following the modification of the method originally described by Bauer *et al.,* (1966). Potato Dextrose Agar (PDA) was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45°C. The cooled media was added 10ml/L tartaric acid (10%) act as antibacterial agents and poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents extract prepared discs individually were placed on the each petriplates and also placed control and standard (Iitraconazole (10 µg)) discs. The plates were incubated at 28°C for 72 hrs.

Measurement of zone of inhibition

The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale.

RESULTS AND DISCUSSION

Phytochemical Analysis

Qualitative Phytochemical Analysis

Qualitative phytochemical analysis of ethanolic extract of *Centella asiatica* and *Moringa oleifera* were given in Table 1.

Phytochemical screening of ethanol extract of flowers in *Centella asiatica* and *Moringa oleifera*. The presence

Table 1. Phytochemical properties of ethanolic extract of leaves *Centella asiatica* and *Moringa oleifera*

S. No.	Name of the test	Ethanolic extract	
		Centella asiatica	*Moringa oleifera*
1	Alkaloids	+	+
2	Carbohydrates	-	-
3	Glycosides	+	+
4	Saponins	-	+
5	Phenols	+	-
6	Tannins	+	+
7	Flavonoids	+	+
8	Proteins and amino acids	+	+
9	Diterpenes	+	+
10	Terpenoids	+	+

(+) Indicates Presence (-) Indicates Absence

Table 2. Quantitative Phytochemical analysis of *Centella asiatica* and *Moringa oleifera*

S.No.	Phytochemical	Concentration in Percentage (%)	
		Centella asiatica	*Moringa oleifera*
1	Flavonoids	12.2	13.8
2	Alkaloids	5.8	5
3	Saponins	22.5	17
4	Phenols	10.6	14.2
5	Tannins	40.4	28.3
6	Crude fiber	6.5	4.6
7	Ash content	10.8	8.2
8	Pectic substances	35.8	28.9

Table 3. *In vitro* antibacterial activity (zone of inhibition in mm) of ethanolic extract of leaves of *Centella asiatica*

S. No.	Microorganisms	Treatments				
		Control	Nitrofurantoin (300µg)	50µl *	100µl*	150µl*
1	*Escherinchia coli*	-	16	20	21	24
2	*Staphylococcus aureus*	-	23	19	21	23

* Concentrations of leaf extract

Table 4. *In vitro* antifungal activity (zone of inhibion in mm) of ethanolic extract of leaves of *Centella asiatica*

S. No.	Microorganisms	Treatments				
		Control	Amphotericin (300µg)	50µl	100µl	150µl
1	*Aspergillus niger*	-	22	13	13	14
2	*Aspergillus flavus*	-	23	14	20	27

* Concentrations of leaf extract

Table 5. *In vitro* antibacterial activity (zone of inhibition in mm) of ethanolic extract of leaves of *Moringa oleifera*

S. No.	Microorganisms	Treatments				
		Control	Nitrofurantoin (300µg)	50µl	100µl	150µl
1	*Escherichia coli*	-	15	20	22	25
2	*Staphylococcus aureus*	-	21	20	22	23

* Concentrations of leaf extract

Table 6. *In vitro* antifungal activity (zone of inhibition in mm) of ethanolic extract of leaves of *Moringa oleifera*

S. No.	Microorganisms	Treatments				
		Control	Amphotericin (300µg)	50µl	100µl	150µl
1	*Aspergillus niger*	-	22	10	12	17
2	*Aspergillus flavus*	-	23	-	11	14

* Concentrations of leaf extract

of alkaloids, flavonoids, tannins, proteins and amino acids, diterpenes and terpenoids. Phytochemical constitutes such as tannins, flavonoids and several aromatic compounds or secondary metabolites of plants serves as defence mechanism against many microorganisms. The curative properties of medicinal plants might perhaps be due to the presence of various secondary metabolites such as alkaloids, flavonoids, tannins, phenolic compounds, saponins and phytosterols. The presence of alkaloids, saponins, flavonoids, phenolic compounds, tannins, phytosterol and terpenoids are used in analgesic and antiplasmodic and bacteriocidal activities ((Sahira Banu and Cathrine, 2015). Thus the extracts of the preliminary screening tof the present study might be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

Quantitative Phytochemical analysis

Quantitative phytochemical analyse of ethanolic extracts of *Centella asiatica* and *Moringa oleifera* revealed that Flavonoids (13.8%) and Phenols (14.2%) were higher in *M. oleifera,* while alkaloids (5.8%), saponins (22.5%), tannins (40.4 %), crude fiber (6.5 %), ash content (10.8 %) and pectic substances (35.8 %) were higher in *C.asiatica*. Tannins and phenols can synergize the antioxidant and anticancer potential of flavonoids.

Saponins in plants are responsible for antimicrobial, molluscidal and insecticidal activities. High fibre content indicates the nutritive value of the plants. Ash content roughly represents minerals and ash content adds to the nutritive value of plant as fodder. Pectic substances are complex polysaccharides, present in the plant cell wall and act as binder. Pharmacologically pectin helps in heavy metal excretion and act as anti-hypercholestrolemic.

Antimicrobial activity

The antimicrobial activities of aqueous and ethanolic extracts of *Centella asiatica* and *Moringa oleifera* were studied against two pathogenic bacterial strains (*Escherichia coli* and *Staphylococcus aureus*) and two fungal strains (*Aspergillus niger* and *Aspergillus flavus*). Standard antibiotics such as Nitrofarantoin and Amphotericin-B were also subjected to compare the antimicrobial potential plant extract.

Antibacterial and antifungal potential of different concentration (50,100,150µg/ml) ethanolic extracts were assessed in terms of zone of inhibition of bacterial and fungal growth and the results are presented in Tables 3,4, 5 and 6.

The antibiotics are sometimes associated with serious side effects such as hypersensitivity, immune suppression and allergic reactions. Therefore more interest is shown to develop alternative antimicrobial drugs such as herbal drugs without side effects for the treatment of infectious diseases.

The test organisms used in the study are associated with various forms of human infections. *E.coli* and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs, especially in debliate and the immunodeficient patients. Whole plant extract of *both Centella asiatica* and *Moringa oleifera* showed high inhibitory activity against *E.coli* (Tables 3 and 5) as the zone of inhibition of 23mm or more is considered as high antimicrobial activity (Veeramuthu *et al*., 2006).

Whole plant extract of *Centella asiatica* and *Moringa oleifera* showed high antifungal activity against *A.niger* with the zone of inhibition of 20mm (zone of inhibition of 18mm or more considered as high The antibiotics are sometimes associated with serious side effects such as hypersensitivity, immune suppression and allergic reactions. Therefore more interest is shown to develop alternative antimicrobial drugs such as herbal drugs without side effects for the treatment of infectious diseases.

The test organisms used in the study are associated with various forms of human infections. *E.coli* and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs, especially in debliate and the immunodeficient patients. Whole plant extract of *Centella asiatica* and *Moringa oleifera* also shows high activity against *E.coli* with zone of inhibition of *Moringa oleifera* but poor in *Centella asiatica* as the zone of inhibition 16mm or more considered as high antimicrobial activity and *Staphylococcus aureus* with zone of inhibition of *Centella asiatica* in ethanolic extract but poor in *Moringa oleifera* as the zone of inhibition 23mm more considered as high antimicrobial activity (Veeramuthu *et al*., 2006).

Leaf extracts of M.oleifera and C, asiatica showed high antifingal activity against A. niger with a zone of inhibition of 20 mm (zone of inhibition of 18 mm or more is considered to be indicative of high activity for this fubgal species) and against *A.flavus* with zone of inhibition of 23mm in ethanolic extract (zone of inhibion pf 15mm or more is considered to be indicative of high activity for this fungal species).

the present study, ethanolic extracts obtained from *Centella asiatica* and *Moringa oleifera* plant shows the significant activity against the tested bacterial and fungal strains. The present study supports the traditional usage of plant material *Centella asiatica* and *Moringa oleifera*. They possess compounds with antibacterial and antifungal potential that can be used as antimicrobial agents are new drugs for the therapy of infectious diseases caused by pathogens.

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that the ethanolic extracts of *Centella asiatica* and *Moringa oleifera* have antimicrobial activity. However, further detailed studies are required to determine the active components responsible for these effects and the mechanism of action.

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