RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

A Path for Horizing Your Innovative Work

SCREENING FOR PHYTOCHEMICAL, ANTIBACTERIAL AND CYTOTOXIC ACTIVITY OF MEDICINAL PLANT OF TAMILNADU

[*]NAMEIRAKPAM NIRJANTA DEVI[1], P. JEEVA[1], W. FEMINA[2]

1. Department of Biotechnology, K.S.R. College of arts and science, Tiruchengode, Namakkal District, Tamilnadu, India.
2. Department Of Biotechnology, School Of Biotechnology and Health Sciences, Karunya University, Coimbatore, Tamilnadu, India

Corresponding Author Email: nnirjan@gmail.com

Accepted Date: 05/02/2012 **Publish Date: 27/02/2012**

Abstract: The present study was designed to evaluate the phytochemical, antibacterial activity, cytotoxic activity and GC-MS analysis of the medicinal plant, *Centella asiatica*. Methanol extract of this plant showed major phytochemicals and also showed good zone of inhibition for *Pseudomonas sp.* compare to other bacterial strains. The 47.6 µg/ml of plant extract showed more cytotoxicity activity for A431 cell line. Nine compounds also identified in the GC-MS analysis of this plant. All the results conclusively validate that phytochemicals treasures indulged in *centella asiatica* plant.

Keywords: *Centella asiatica*, phytochemicals, antibacterial, cytotoxicity, GC-MS.

INTRODUCTION

Centella asiatica (Linn.) urban belonging to the family Umbeliferae popularly known's as Brahmi. This plant is found in India, Sri Lanka, Madagascar and Africa up to an altitude of 650 m. In India, the plant is commonly known as Mandukaparni. *Centella asiatica* has reported for many pharmacological effects being used for wound healing, mental disorders, atherosclerosis, fungicidal, antibacterial, antiviral, antioxidant and anticancer purposes. *Centella asiatica* also reported to be useful in the treatment of venous hypertension, microangiopathy, inflammations, diarrhea, asthma, tuberculosis, various skin lesions and ailments like leprosy, lupus, psoriasis, and keloid[1, 2, 3, 4, 5, 6].

Many pathogenic microorganisms became resistance to the available drugs. In order to circumvent the problem of microbial resistance, there is always a need to search for new therapeutic agent[7, 8]. In this medicinal plants which plays a great role. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action[9].The objective of this work to evaluate the antibacterial and cytotoxicity activity of methanol cold extract of the *Centella asiatica* plant leaves.

MATERIALS AND METHODS

Plant material

The plant material of *Centella asiatica* was collected in and around Nammakal District Tamilnadu, India. The plant was taxonomically identified and authenticated by Botanical Survey of India, Tamilnadu Agricultural University. The voucher Specimen was deposited there with register number BSI/SRC/5/23/2011-12/Tech.-932. The plant leaves were washed with distilled water then air dried and then powdered for extraction in a Table model grinder.

Extraction

About 5grams of dried leaves powder were extracted with 50 ml of methanol for 24 h at room temperature by constant shaking and filtered twice through whatman no. 1 filter paper with the aid of a suction pump. Then the solvents were evaporated in water bath at 40°C and the residue were transferred to screw cap bottles and stored in refrigerator until use.

Qualitative evaluation of phytochemicals

The preliminary screening test were performed for the presence of the following secondary metabolites such as alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroids and phenols[10, 11, 12, 13].

Alkaloids test

The plant extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. Formation of turbidity or yellow precipitation showed the presence of alkaloid.

Glycosides

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycone) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.

Terpenoids and steroids

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids.

Flavonoids

Four milliliters of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

Saponins

0.5 gm of extracts was added to 5ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Phenols

The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of phenolic compounds.

Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for Gallic tannins and green black for catecholic tannins.

Preparation of microbial inoculums

Microorganisms grown in Nutrient broth at 37°C to a concentration of approximately 10^5–10^6 CFU/ml was used for the antimicrobial analysis.

Agar-well diffusion method

The agar-well diffusion assay was adopted for the present assay[14]. Each bacterial suspension was spread over the surface of Mueller-Hinton agar (Himedia, India) plates containing 4 wells of 6 mm diameter. The wells were filled with 25 µl each of the extracts. The plates were incubated at 37°C for 24 h. The results were expressed in terms of the diameter of the inhibition zone. The respective solvents were used as negative control, (DMSO).

Gas Chromatography Mass Spectrometry

For the GC-MS analysis a 30 m, 0.2 mm I. D. HP-5 fused silica capillary column; 25 µm film thickness, was used in Hewlett-Packard 5890 gas chromatograph with a HP 5972 MSD detector. The samples were introduced *via* an all-glass injector working in the split mode, with He as the carrier gas, linear velocity 32 cm/s. Temperature program: 80- 240 ^{o}C at 8 deg min1, 240-300 ^{o}C at 12 deg min 1 and a 20 min hold at 300 ^{o}C. The identification of components was accomplished using computer searches in commercial libraries.

Identification of Components

The database in the National Institute Standard and Technology has been used for the interpretation on GC-MS. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Then the structure, molecular weight, retention time of the compound were identified and tabulated.

Cytotoxicity assay

A431 Cell line was treated with plant methanolic extract. The plant extract was used as different concentration shows good activity against that cell line in 96 well plate. The viability was checked by the cell counting method.

RESULT AND DISCUSSION

The plant *Centella asiatica* methanol extracts pertaining potent phytochemicals (Table 1). Initial screening of antibacterial activity of methanolic extract assigned *in vitro* by the agar well diffusion method. The plant extract used in different concentration like 11.1µg/ml, 33.11µg/ml, 66.61µg/ml, 99.91µg/ml to check the antibacterial activity. In this 99.9 µg/ml of plant extract showed good inhibition of bacterial strains.

The extract showed activity against all tested pathogens. But its controlling more *Pseudomonas* sp. Next to this it's showing activity against *Proteus vulgaris, Bacillus sp. and Staphylococcus aureus* [Figure 1 & Table 2]

A431 Cell line was treated with plant methanolic extract. The plant extract was used as different concentration 2.4 µg/ml, 12 µg/ml, 23.6 µg/ml, 35.6 µg/ml, 47.6µg/ml shows good activity against that cell line in 96 well plates. The percentage of inhibition was more in 47.6µg/ml of plant extract [Figure 2 and Table 3]. The viability was checked by the cell counting method.

Percentage of viability = (Total no. of viable cells per ml aliquot *100) / Total no. of cells per ml aliquot.

The GC-MS analysis shown that 10 major peaks. In that 3 major peaks with nearest retention time were taken, 17.23, 14,45 and 15.37. In these peaks 3 compounds for each of the peak were identified and tabulated [Figure 3 and Table 4] with compound name, Molecular formula and Molecular weight.

CONCLUSION

For many years the adoptive significance of most plant secondary metabolites was unkown. The pharmacological value of secondary metabolites is increasing due to constant discovery of the potential roles in health care and lead chemicals for new drug development. The inference brown from our present study of *Centella asiatica* plant showed promising results for phytochemicals which indicate that this plant can use for treating diseases. The plant extracts revealed good antibacterial activity. In GC-MS also nine bioactive compounds were identified. Further study the separation and purification of bioactive compound and *in vivo* studies have to be performing to see

the activity of this plant extract in clinical trials.

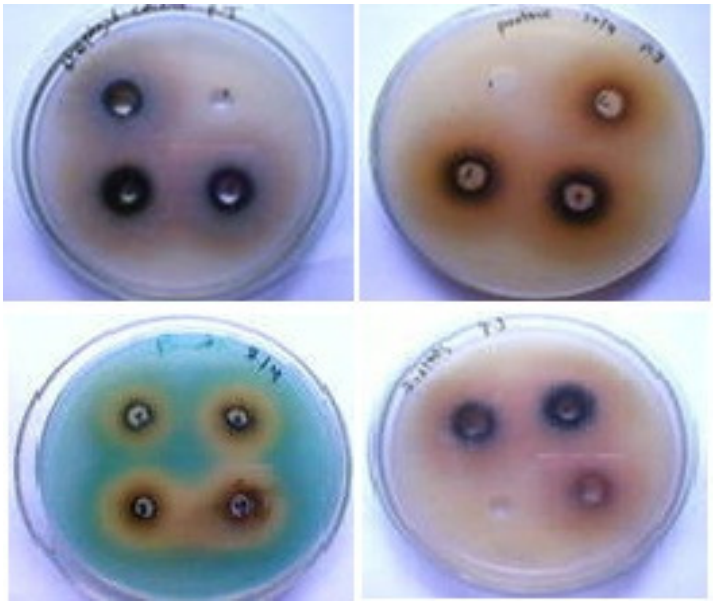

Figure No: 1 Plates showing antibacterial results

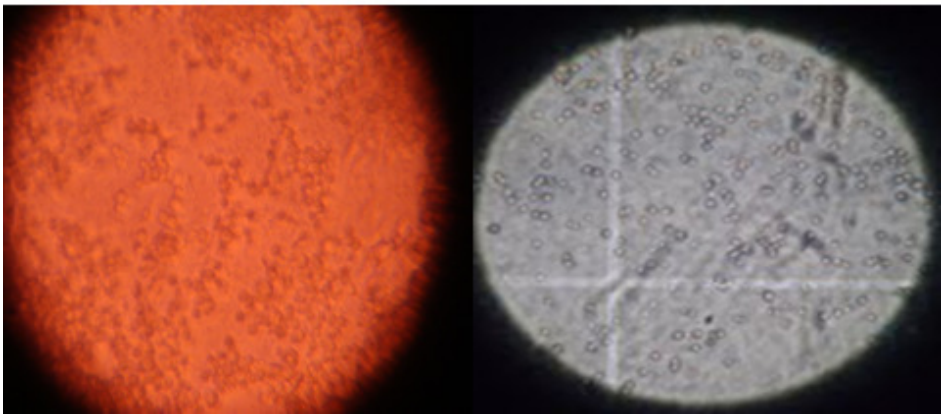

Figure No: 2 A431 cell line cytotoxicity result

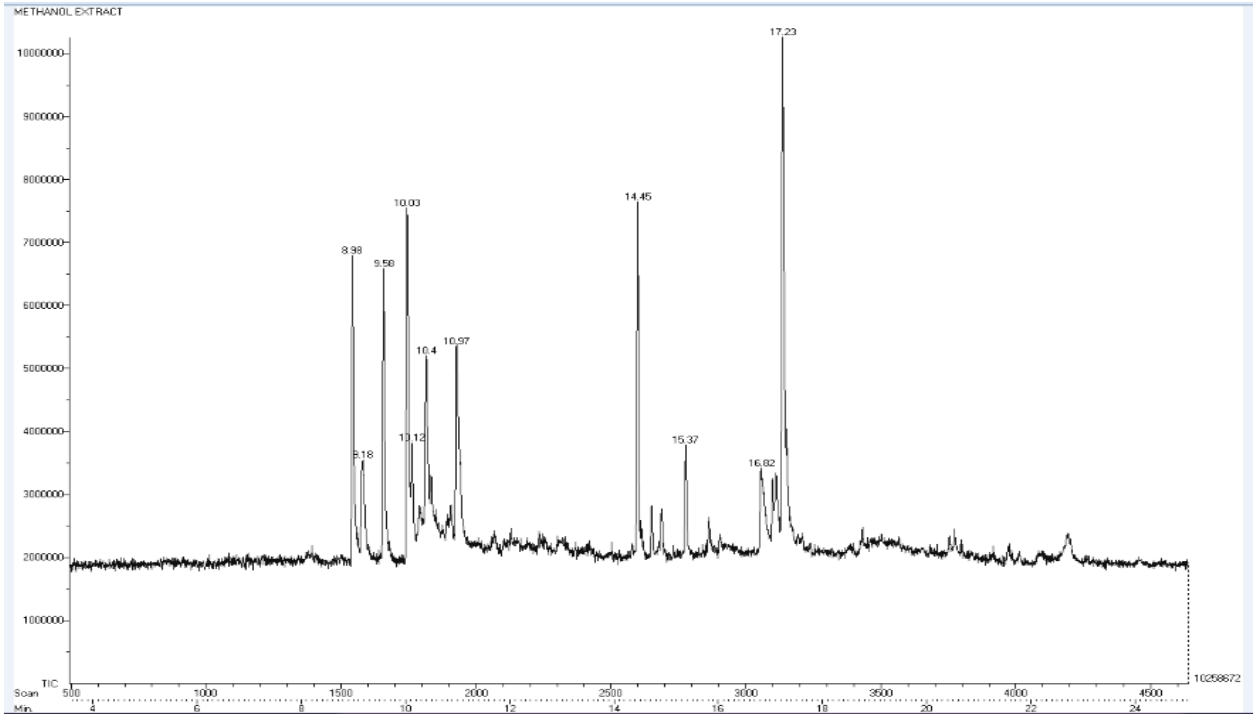

Figure No: 3 GC-MS results of *Centella asiatica*

Table 1

Phytochemical analysis of *centella asiatica*

Sr. No	Parameters	Results
1.	Flavonoids	+
2.	Alkaloids	+
3.	Tannins	+
4.	Phenol	+
5.	Steroids	+
6.	Saponins	_
7.	Glycosides	+
8.	**Terpenoid**	+

Table 2

Results of antibacterial tests

Sr. No	Organisms	Zone of inhibition in mm				
		Control	11.1 µg/ml	33.3 µg/ml	66.6 µg/ml	99.9 µg/ml
1.	*Staphylococcus aureus*	-	-	11	13	15
2.	*Bacillus* sp.	-	-	11	14	16
3.	*Proteus vulgaris*	-	-	10	13	16
4.	*Pseudomonas* sp.	3	11	13	17	20

Table 3

Results of cytotoxicity assay

Sr. No	Concentration(µg/ml)	Percentage %
1	2.4	62.20%
2	12	30.05%
3	23.6	54.86%
4	35.6	32.65%
5	47.6	29.54%

Table No: 4 Bioactive compounds present in *Centella asiatica* leaves extract

Sr. No	RT	Compound Name	Molecular formula	Molecular weight
1	14.45	2-Allyl-5-t-butylhydroquinone	C13H18O2	206
		4-Ethyl-2-(4-nitrophenyl)-4,5-dihydrooxazole	C11H12N2O3	220
		2,3-Dihydroxy-5-methoxy-2-methylchromen-4-one	C11H12O3	192
2	15.37	Undecane, 3-methyl	C12H26	170
		Dodecane, 2-methyl	C13H28	184
		1-Octadecanol	C18H38O	270
3	17.23	Pent-4-enal	C5H8O	84
		2-Octenal, (E)- (CAS)	C8H14O	126
		2-Nonenal, (E)- (CAS)	C9H16O	140

REFERENCES

1. Chengjian Z and Luping Q: Chemical components of *Centella asiatica* and their bioactivities. Journal o f Chinese Integrative Medicine 2007; 5(3): 348-351.

2. Joy PP, Thomas J, Mathew S and Skaria BP: Medicinal Plants. Tropical Horticulture (eds. Bose, TK, Kabir J, Das P. and Joy, PP.) Naya Prokash, Calcutta 2001; 2: 449-632.

3. College G and Naka K: Antimicrobial and Antifungal Activity of *Centella asiatica* (L) Urban Umbeliferae. Journal of Ethno pharmacology 2009; 2(2): 328-330.

4. Dash BK, Faruquee HM, Biswas SK, Alam MK, Sisir SM and Prodhan UK: Antibacterial and Antifungal Activities of Several Extracts of *Centella asiatica* L. against Some Human Pathogenic Microbes. Life Sciences 2011: 1-5.

5. Panthi MP and Chaudhary RP: Antibacterial activity of some selected folklore medicinal plants from West Nepal. Scientific World 2006; 4(4): 16-21.

6. Ullah MO: Antimicrobial, Cytotoxic and Antioxidant Activity of *Centella asiatica.* European Journal of Scientific Research 2009; 30(2): 260-264.

7. Cohen ML: Epidemiology of drug resistance: implications for a post antimicrobial era. Science 1992; 257: 1050-1055.

8. Gislene GF, Juliana L, Paulo C, Freitas and Giuliana LS: Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian Journal of Microbiology 2000; 31: 247-256.

9. Ellof JN: Which extract ant should be used for the screening and isolation of antimicrobial components from plants. Journal of Ethnopharmacol 1998; 60: 1-6.

10. Harborne JB: Phytochemical methods, London. Chapman and Hall, Ltd. 1973: 49-188.

11. Trease GE and Evans WC: Pharmacology, Saunders Publishers, London, 15th Edition (2002): 42-44, 221-229, 246-249, 303-306, 331-332, 391-393.

12. Mace ME: Histochemical localization of phenols in healthy and diseased tomato roots, Phytopathology 1963; 16: 915-925.

13. Kokate CK: Practical Pharmacognosy, Vallabh Prakashan, New Delhi, 4th Edition (1994): 107.

14. Bauer RW, Kirby MDK, Sherris JC and Turck M: Antibiotic susceptibility testing by single standard disc diffusion method, American Journal of Clinical Pathology 1966; 45: 493-496.