INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA):IJPS00]
Journal Homepage: https://www.ijpsjournal.com

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Research Article

Physicochemical Standardization And Evaluation Of Immunomodulatory Activity By *In-Vitro* Method Of *Centella Asiatica Linn. Urban*

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ARTICLE INFO

Received: 07 Sep 2024
Accepted: 11 Sep 2024
Published: 22 Sep 2024
Keywords:
Centella Asiatica, Flavonoids, Immunostimulant, Phytochemicals, in-vitro study.
DOI:
10.5281/zenodo.13824768

ABSTRACT

Centella Asiatica is the most known medicinal herb in the Indian sub-continent. It is commonly known as Gotu, Kola, Mandukparni, and Bramhi. It has been used since ancient Ayurveda due to its being rich in phytochemicals like Madecassoside, Asiaticoside, Asiatic acid, Bramhic acid, D-mannitol, and centellose, etc. These triterpenoids and trisaccharides make the plant medicinal. About 124 chemical compounds are present in Centella Asiatica. These phytocompounds enhance the immunomodulatory potential. Bramhi is being used widely as a powerful antioxidant, helps during anxiety, boosts immunity, and improves cognitive functions. A recent study has proved that due to its phytochemicals like flavonoids present in it, it has very effective immunomodulatory activity. These studies are supported by some experiments carried out. So in our study, we have performed in-vitro studies which have shown some promising results by having cell proliferation and low cytotoxicity. We have performed an in-vitro study (MTT assay) by using Umbilical cord Mononuclear cells (UC-MNCs) which has critical components of the immunity system and they play a vital role in cell-mediated immunity. Elemental analysis was carried out to check the composition of elements present in it. As per the study, we have understood that it has stable immunomodulatory properties with low cytotoxicity and it would be the best alternative for immune disorders.

INTRODUCTION

Ayurveda, which means "Life" and "Knowledge of Science" in Sanskrit, is undoubtedly the "Science of Life." The Indian medicinal literature comprises an extensive inventory of plants that have demonstrated their remarkable ability to promote physical, mental, and defense mechanisms within the human body. The medicinal plants known as 'Rasayanas' which were also known as Vyadhivirodhak Chamatava have scientifically proven potent immunomodulatory

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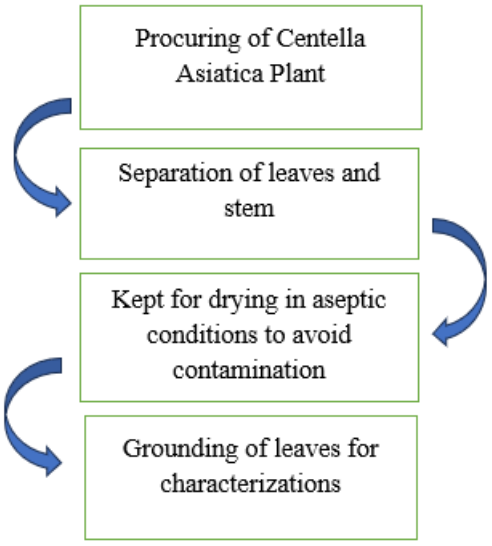
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

properties that stimulate both specific and non-specific immunity1. These herbal adaptogens have a significant impact on the immune system and possess remarkable immunomodulatory properties which could serve as a replacement for the chemical therapeutics for various diseases. This study was carried out for the evaluation of the immunomodulatory effect of Centella asiatica2,3. The longevity herb, Centella asiatica Linn. Urban, sometimes referred to as gotu kola (Family: Apiaceae), is widely utilized in traditional Ayurvedic treatment in India and Nepal2. Its name in Sanskrit is “Mandukaparni”" because its leaf appears from its rear like a standing frog. Its leaves and roots are utilized medicinally and offer significant health advantages for maintaining blood vessels and vein health, treating skin conditions, enhancing memory, and enhancing brain function4. The main ingredients of Centella Asiatica include terpenoids, saponins, and flavonoids, which are primarily thought to be in charge of the plant's numerous medicinal benefits5.

MATERIALS &METHODOLOGY:

Fig. no. 1



Flow chart. no. 1

The Centella Asiatica plants were grown and procured from Umargam, Gujrat. After the collection, the separation of leaves and stems was done followed by an airdrying process in a sterile environment to avoid contamination. Separated dried leaves were powdered by grinding process followed by ETO sterilization for decontamination.

Characterizations:

Proximate Analysis:

Proximate analysis quantifies the basic nutritional components of a substance such as moisture, ash, protein, fat, fiber, and carbohydrates6.

Phytochemical Analysis:

Phytochemical analysis helps to detect bioactive components, assures the purity and validity of herbal products. It confirms the plant's medical value, promotes drug research, assesses safety, and broadens scientific knowledge. This analysis increases the plant's commercial worth and encourages its use in medications, nutraceuticals, and cosmetics7.

Elemental Analysis:

The elemental analysis exposes the elemental composition and helps in the investigation of trace minerals such as calcium, magnesium, cadmium, and potassium. Understanding its elemental composition aids in determining its medicinal

capabilities and potential toxicity of the tested sample8.

In-vitro studies (MTT assay):

In-vitro experiments such as MTT assay help to assess biological processes and components in an aseptic lab-controlled environment, separate from living organisms. These studies are critical for understanding cellular functions, and metabolism, it helps to evaluate the drug efficacy, and also determines toxic effects, forming the base for further in-vivo research and other clinical aspects9.

RESULTS:

Proximate Analysis:

Physicochemical result of C. Asiatica leaves

Parameters	Mean Value (% w/w)
Total ash	14.98
Water insoluble ash	7.81
Acid insoluble ash	0.383
Water soluble extractive value	25.445
Alcohol soluble extractive value	11.835
Loss on Drying	

Physicochemical results of C. Asiatica Stem

Parameters	Mean Value (% w/w)
Total ash	10.89
Water insoluble ash	5.353
Acid insoluble ash	1.353
Water soluble extractive value	25.71
Alcohol soluble extractive value	13.405
Loss on Drying	

The ash value is a valuable tool for assessing the authenticity and purity of a sample, and it is also a crucial qualitative benchmark(6). The total ash value of Centella Asiatica leaf is 14.98% and the stem is 10.89%. Higher mineral concentration is indicated by a higher total ash value and a higher calorific value is indicated by a lower moisture content value(6). A lower value for acid insoluble ash in the leaf is 0.383% and, in the stem, it is 1.353%, which suggests that the plant is more easily digested when eaten. The leaves and stem have alkaloids, flavonoids, and saponins which have medicinal significance hence pharmacological screening of Centella asiatica leaves may be beneficial for identifying novel compounds.

Phytochemical Analysis:

Serial no	Name of Test	Solvent Extracts of Centella Asiatica Stem						
		H_2O	CH_3OH	EtOH	EtAc	$CHCl_3$	Hexane	Pet ether
a	Alkaloids							
	I}Dragendorff's Test	A	P	P	A	A	A	A
	II}Mayer's Test	P	P	A	A	A	A	A
	III) Hager's Test	P	P	P	A	A	A	A
b	Saponins	P	P	P	A	A	A	A
c	Flavonoids							
	I}Shinoda Test	P	P	P	P	A	A	A
	II}Slakaline reagent Test	P	P	P	P	A	A	A
d	Steroids							
	I}Salkowski Test	A	A	A	A	A	P	A
	II} Libermann burchard Test	A	A	A	A	P	P	A
e	Tannins							

	I}Lead acetate Test	A	A	A	A	A	A	A
	II}Ferric chloride Test	A	A	A	A	A	A	A
	III}Potassium dichromate Test	A	A	A	A	A	A	A
f	Phenols							
	Ferric chloride Test	P	P	P	A	A	A	A
g	Glycosides							
	Legal's Test	P	P	A	A	A	A	A

Using various solvent extracts, the phytochemical investigation of Centella asiatica leaves revealed the presence (P) and absence (A) of numerous chemicals. Alkaloids were tested using Dragendorff's test, which was negative in water, whereas Mayer's and Hager's tests revealed the existence of alkaloids. Saponins were detected in the water extract. The water extract included flavonoids, which were detected using the Alkaline Reagent NaOH and Lead Acetate tests. The Salkowski and Libermann-Burchard tests revealed no traces of steroids. Tannins were not discovered utilizing lead acetate, ferric chloride, or potassium dichromate test. Phenols were identified, as confirmed by the Ferric Chloride test. Glycosides were detected using Legal's assay10. These complex phytochemical findings emphasize the diverse phytochemical makeup of Centella asiatica leaves, suggesting their therapeutic potential. The phytochemical study of Centella asiatica leaves with several solvent extracts (water, methanol, ethanol, ethyl acetate, chloroform, hexane, and petroleum ether) confirmed the presence and lack of many bioactive chemicals. Alkaloids were found utilizing Dragendorff's (P in methanol and ethanol), Mayer's (P in water and methanol), and Hager's assays. Saponins were found in water, methanol, and ethanol extracts. Shinoda and Slakaline reagent tests were performed on water, methanol, ethanol, and ethyl acetate extracts to confirm flavonoids. Steroids were recognized using the Salkowski and Libermann-Burchard tests (P in hexane and chloroform, respectively). In all assays (lead acetate, ferric chloride, and potassium dichromate), tannins were not present. The Ferric chloride test was used to find phenols in extracts of water, methanol, and ethanol. Legal's test revealed that glycosides were found in the methanol and water extracts. The aforementioned findings underscore the varied phytochemical makeup of Centella asiatica leaves and their possible medical uses10.

Serial no	**Name of Test**	**Solvent Extracts of Centella Asiatica Stem**						
		H_2O	**CH_3OH**	**EtOH**	**EtAc**	**$CHCl_3$**	**Hexane**	**Pet ether**
a	**Alkaloids**							
	I}Dragendorff's Test	**A**	**P**	**A**	**A**	**A**	**A**	**A**
	II}Mayer's Test	**P**	**P**	**A**	**A**	**A**	**A**	**A**
	III) Hager's Test	**P**	**P**	**P**	**A**	**A**	**A**	**A**
b	**Saponins**	**P**	**P**	**P**	**A**	**A**	**A**	**A**
c	**Flavonoids**							

	I}Alkaline Reagent NaOH Test	**P**	**P**	**P**	**P**	**A**	**A**	**A**
	II}Lead acetate Test	**P**	**P**	**P**	**P**	**P**	**A**	**A**
d	**Steroids**							
	I}Salkowski Test	**A**	**A**	**A**	**A**	**A**	**P**	**A**
	Ii} Libermann burchard Test	**A**	**A**	**A**	**A**	**A**	**A**	**A**
e	**Tannins**							
	I}Lead acetate Test	**A**	**A**	**A**	**A**	**A**	**A**	**A**
	II}Ferric chloride Test	**A**	**A**	**A**	**A**	**A**	**A**	**A**
	III}Potassium dichromate Test	**A**	**A**	**A**	**A**	**A**	**A**	**A**
f	**Phenols**							
	Ferric chloride Test	**P**	**P**	**P**	**A**	**A**	**A**	**A**
g	**Glycosides**							
	Legal's Test	**P**	**A**	**A**	**A**	**A**	**A**	**A**

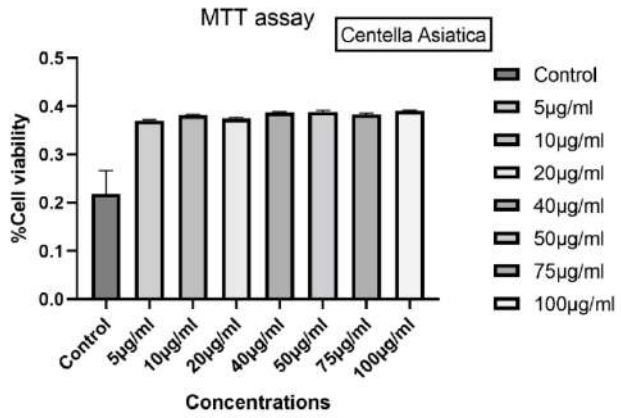
Alkaloids were identified in methanol, water, and ethanol extracts. According to the studies stated above, these solvents may be useful for extracting alkaloids with immunomodulatory properties. The aqueous, methanolic, and ethanolic extracts all contain saponins. They may also regulate immunological responses, making them suitable as immunomodulatory and/or therapeutic agents. The water extract, as well as the methanol, ethanol, and ethyl acetate extracts, contain flavonoids. These chemicals have been proven to have anti-oxidant properties and are also mediated immunomodulators, implying that these extracts may be effective in improving immunological function. Only hexane extracts demonstrate steroids (Salkowski Test)8. Due to the very low signal of steroids, they had no major impact on this extract though steroids are known to have an immunosuppressing action. Tannins are absent in all solvent extracts. Therefore, tannins do not contribute to the immunomodulatory potential of these extracts. Phenols are present in water, methanol, and ethanol extracts. Known for their antioxidant properties, phenols can modulate immune responses, suggesting these extracts have potential immunomodulatory activity. Glycosides are only detected in water extracts. These compounds can influence immune function, indicating potential immunomodulatory activity in the aqueous extract7.

Elemental Analysis:

Elements	Concentration in ppm
Chromium	13.152
Copper	34.194
Iron	393.592
Manganese	5.022
Cadmium	ND (less than 0.01ppm)

The presence of chromium concentration is 13.152ppm which shows immunological activity when present in a trace amount10.Concentration of copper is 34.194 ppm which helps in the differentiation of immune cells11. Iron is critical for macrophage differentiation which also helps the macrophage as a cofactor for the execution of the antimicrobial effector mechanism12. Manganese enhances antioxidant defenses and bone health at 5.022 ppm, however excess manganese can be harmful. When cadmium is found at less than 0.01 parts per million, it is classified as "ND" (Not Detected). These measures are essential for determining the sample's composition and any hazards, particularly in industrial or environmental contexts where the components may have an impact on ecosystem integrity and human health8,13.

MTT assay:



It is a colorimetric assay that helps in assessing the cell's metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present14.15. The assay was performed for the demonstration of the immunostimulatory effect of MTT assay15. It was performed by using cord blood MNC (Mononuclear cells) as they are critical components of the immune system and are involved in both humoral and cell-mediated immunity9. The absorbance is higher in the concentration of Centella asiatica as compared to the control group which clearly shows the spike in cell proliferation in the sample group. There is no decrease in cell proliferation seen in the treatment group though the concentration of the samples has been increased which shows it has Centella Asiatica could stimulate immunomodulatory activities and it could be developed as an immunostimulant.

CONCLUSION:

The prepared leaf powder of Centella Asiatica was assessed by using the Proximate analysis method which states that the leaf powder contains flavonoids that act as an immunostimulant and it was confirmed by the In-vitro method using MTT assay which showed the spike in the absorption in the sample group as compared to the control group and there was no decrease in the absorption seen in the multiple concentrations of Centella Asiatica leaves extract which states that the Centella Asiatica leaves powder could be used as immunostimulant as it supports the immunostimulatory activity.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

ACKNOWLEDGEMENTS:

We acknowledge profound gratitude to the Principal and the Head of the Department, Chemistry of Ramnarain Ruia College for

providing facilities and technical assistance for research work.

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HOW TO CITE: V. V. Pongade, M. S. Hate, Physicochemical Standardization And Evaluation Of Immunomodulatory Activity By *In-Vitro* Method Of *Centella Asiatica Linn. Urban* , Int. J. of Pharm. Sci., 2024, Vol 2, Issue 9, 1053-1059. https://doi.org/10.5281/zenodo.13824768