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Phytochemical screening and In-vitro antioxidant activity of *Centella asiatica* extracts

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Abstract

Centella asiatica also known as *mandukparni* or Indian pennywort or *jalbrahmi*, which has been used as a medicine in the Ayurveda from ancient times and mentioned in many classical texts of Ayurveda. *Centella asiatica* has long been used to improve memory and cognitive function. The study aimed to identify the phytochemicals present in different solvent extracts of *Centella asiatica* (i.e. PECA- Petroleum ether extract of *C. asiatica,* CCA- Chloroform extract of *C. asiatica,* EACA- Ethyl acetate extract of *C. asiatica,* ECA- Ethanolic extract of *C. asiatica,* HACA- Hydro-alcoholic extract of *C. asiatica*) and evaluate the respective in-vitro antioxidant potentials. The phytochemical screening of extracts was done with standardized procedures and the antioxidant potential of different solvent extracts of *Centella asiatica* was assessed by its free radical scavenging activity 2, 2-diphenyl -1- picrylhydrazyl (DPPH) as well as hydrogen peroxide scavenging assay respectively for reducing capability. In all different solvent extracts of *C. asiatica* revealed excellent free radical scavenging activity as revealed by 2-2- diphenyl-1-picryl-hydrazyl (DPPH) assay with EC_{50} values for ECA=128.752±1.85 µg/ml, HACA=274.884±1.21 µg/ml and hydrogen peroxide assay against the standard (Butylated hydroxytoluene) BHT, with the EC_{50} values ECA=429.69±0.92 µg/ml HACA=458.08±0.58 µg/ml while rest solvent extracts shown very less antioxidant activity. The present study indicates that the *Centella asiatica* extracts have good antioxidant activity which can be used in stress and anxiety and also a good source to be used as natural drugs.

Keywords: (Butylated hydroxytoluene) BHT, *Centella asiatica*, 2-2- diphenyl-1-picryl-hydrazyl (DPPH), Phytochemicals.

Introduction

Neurodegeneration, a slow and progressive dysfunction in which loss of neurons and axons in the central nervous system is the primary pathological feature of acute and chronic neurodegenerative conditions. During aging some neuronal changes can result in impaired cognitive ability [1, 2]. Amyloid-β (Aβ) peptide accumulates in the brain leading to the formation of plaques that are the pathological hallmark of the Alzheimer's disease (AD).

Centella asiatica L. has been used as a medicinal herb for thousands of years in India, China, Sri Lanka, Nepal and Madagascar [3]. *Centella asiatica* is one of the chief herbs for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells. *C. asiatica* reported to possess various pharmacological activities: antimicrobial activity, anticancer activity, wound healing activity, neuroprotective activity, immunomodulatory activity and anti-inflammatory activity. *C. asiatica* is also rich in flavonoids and terpenoids compounds among them asiatic acid, asiaticoside, madecassoside is well characterized for its pharmacological value. *Centella asiatica* (L.) Urban (Apiaceae), also known as Gotu Kola, is used in traditional Chinese and Ayurvedic medicine to improve cognitive function and reverse cognitive impairments. The neuroprotective and cognitive enhancing effects of *C. asiatica* have been well-documented. *C. asiatica* extracts have reported its potential as an antimicrobial agent, agent of collagen synthesis and even as a wound healer [4, 5].

Materials and methods

Plant material

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Whole plant material of *Centella asiatica* was collected from village Ramnapur, Varanasi, Uttar Pradesh, India in October 2015 and authentication was done by Department of Botany, Banaras Hindu University, India and also herbarium of *C. asiatica* (voucher specimen no. Apia/02/2015) of plant was deposited in the Department of Botany, Banaras Hindu University, India.

Chemicals and Reagents

Plant extraction

The extraction of both plants was done with soxhlet method in petroleum ether, chloroform, ethyl acetate, and ethanol and hydro-alcoholic solvents at 72-100°C for 72 hours. The Soxhlet extraction has widely been used for extracting valuable bioactive compounds from various natural sources. It is used as a model for the comparison of new extraction alternatives. Generally, a small amount of dry sample is placed in a thimble. The thimble is then placed in distillation flask which contains the solvent of particular interest. After reaching to an overflow level, the solution of the thimble-holder is aspirated by a siphon. Siphon unloads the solution back into the distillation flask. This solution carries extracted solutes into the bulk liquid. Solute is remained in the distillation flask and solvent passes back to the solid bed of plant. The process runs repeatedly until the extraction is completed.

Phytochemical Screening

For preliminary phytochemical analysis the freshly prepared crude ethanolic extracts of leaves were tested for the presence or absence of phytoconstituents such as alkaloids, tannins, Flavonoids, Saponins by using standard phytochemical procedures [6].

Table-1. Phytochemical examination results of different solvent extracts of *C. asiatica.* (PECA- Petroleum ether extract of *C. asiatica,* CCA- Chloroform extract of *C. asiatica,* EACA- Ethyl acetate extract of *C. asiatica,* EECA- Ethanolic extract of *C. asiatica,* HACA- Hydro-alcoholic extract of *C. asiatica).*

S. No.	Phytochemical	Test	PECA	CCA	EACA	ECA	HACA
1	Alkaloids	Wagner's reagent	+	-	+	+	+
2	Glycosides	Keller Kelliani's test	-	-	-	+	+
3	Flavonoids	Alkaline reagent Test	-	-	-	+	+
4	Phenols	Ferric chloride test	-	-	-	+	+
5	Saponins	Foam test	-	-	+	+	+
6	Tannins	Braymer's test	-	-	-	+	+
7	Terpenoids	Salkowki's test	+	+	-	+	-
8	Quinones	Acid test	+	+	+	-	-

Antioxidant assays

DPPH scavenging activity

Free radicals are extremely reactive species and are known to damage proteins, cause breakdown of DNA strands, initiate the peroxidation of various compounds and thus leads to many health problems and degenerative diseases such as cancer, inflammation, atherosclerosis, accelerated aging, etc. In plant tissues many compounds are potential antioxidants and they may act as free radicals scavengers. Therefore, these phytochemicals play a significant role in health promotion. The free radical scavenging activity was measured by the DPPH method [7].

0.5 ml different solvent extracts of *Centella asiatica* with different concentration i.e. 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml. were added to 1.5 ml of 0.3 mM methanolic solution of DPPH. Incubated for 20 min in dark and absorbance was measured at 517 nm against blank sample (DPPH solution only) and L-ascorbic acid (1.0 mg/ml) used as standard. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_b - A_s}{A_b} \times 100$$

Where,

Ab = Absorbance of Blank

As = Absorbance of Test sample

Hydrogen peroxide activity

Hydrogen peroxide assay

40 mM solution of H_2O_2 prepared in phosphate buffer (pH= 7.4). Plant extract (0.1 – 1.0 mg/ml) added to 0.6 ml of 40 mM solution of H_2O_2. (Butylated hydroxytoluene) BHT of 1.0 mg/ml concentration used as standard. Absorbance taken at 230 nm against Blank solution (Phosphate buffer without H_2O_2) [8]. H_2O_2 Scavenging activity was measured by the following equation:

$$H_2O_2 \text{ Scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 = Absorbance of Control
A_1= Absorbance of Sample

Result and Discussion

DPPH scavenging activity

DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers. It was found that the radical scavenging activities of extracts increased with increasing concentration. In all different solvent extracts, ethanolic extract showed more percent inhibition than hydro-alcoholic extract of *C. asiatica* against the standard ascorbic acid. EC_{50} for ECA=128.752±1.85 µg/ml and for HACA=274.884±1.21 µg/ml.

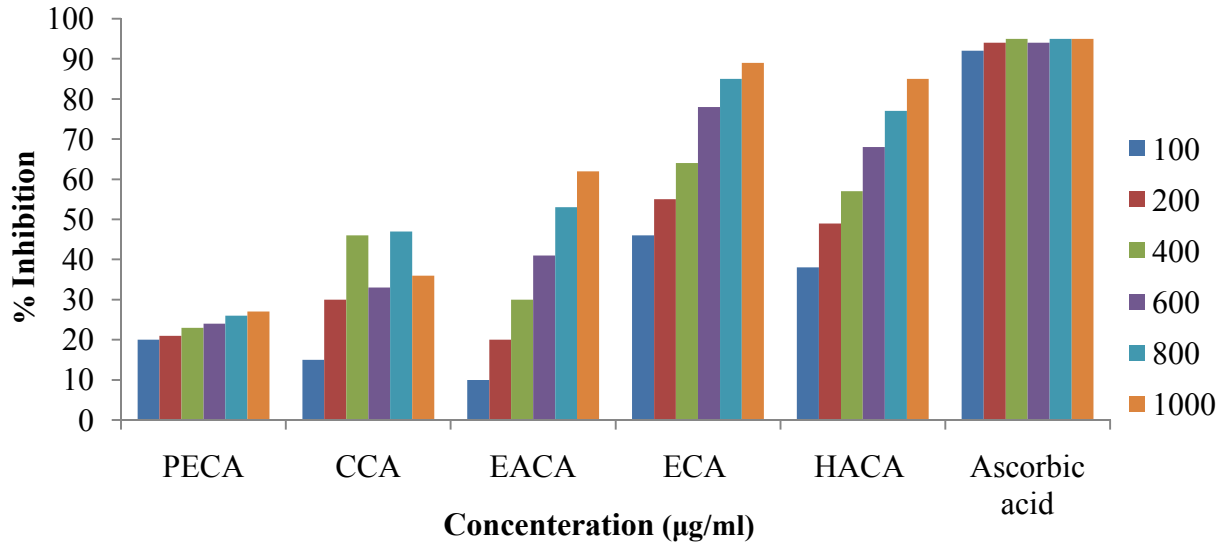
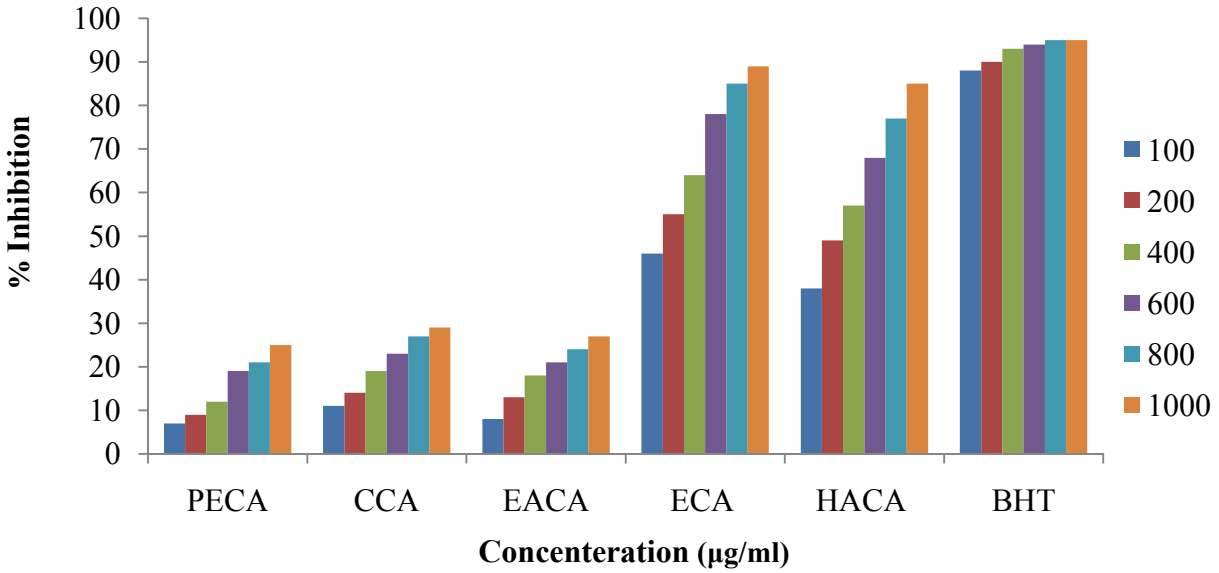


Figure 1. DPPH scavenging activity of different solvents of *Centella asiatica.*(i.e. PECA- Petroleum ether extract of *C. asiatica,* CCA- Chloroform extract of *C. asiatica,* EACA- Ethyl acetate extract of *C. asiatica,* ECA- Ethanolic extract of *C. asiatica,* HACA- Hydro-alcoholic extract of *C.asiatica*)

H_2O_2 scavenging activity

Hydrogen peroxide activity

Scavenging of H_2O_2 by ethanolic and hydro-alcoholic extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The differences in H_2O_2 scavenging capacities between the two extracts may be attributed to the structural features of their active components, which determine their electron donating abilities. In all different solvents the ethanolic extract showed more percent inhibition than hydro-alcoholic extract of *C. asiatica* against the standard Butylated hydroxytoluene. The EC_{50} for ECA=429.69±0.92 µg/ml HACA=458.08±0.58 µg/ml. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems.



Conclusion

This study shows that in the different solvent extracts of *C. asiatica,* only ethanolic and hydro-alcoholic extracts have effective antioxidant activity in in-vitro DPPH radical scavenging assay and hydrogen peroxide scavenging assay when compared to standard Antioxidant compounds L-ascorbic acid and BHT respectively and also found that ethanolic extract is more effective than hydro-alcoholic extract. As a result, *C. asiatica* seem to be good sources of natural antioxidants.

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