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Invitro Assessment Of Anti Oxidant And Anti-Inflammatory Properties Of Centella Asiatica Aqueous Leaf Extract

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ABSTRACT

Centella asiatica (L) is one of the important medicinal plants in the International market of medicinal Plant Trade. Medicinal plants have been used all over the world as unique sources of medicines and may constitute the most common human use of biodiversity. A numbers of phytochemicals have been isolated from the various extracts of the plant. These include terpenoids, phenolic compounds, poly acetylenes, alkaloids, carbohydrates, vitamins, mineral and amino acid. The plant is attributed to possess a number of therapeutic uses such as antimicrobial, anti-inflammatory, astringent, bronchodilator, CNS-depressant, de-toxicant, diuretic, immune stimulant, anticancer and hepato-protective etc. The antioxidant activity of *Centella* plays a key role in acting against the reactive oxygen species in our body. Due to health benefits like antioxidant activity, the usage of *Centella* in food and beverages has been increased over these years. The use of *Centella* in food and beverages has increased over the years. The knowledge regarding ability of *Centella* as an alternative natural antioxidant especially of plant origin and its protection against age-related changes in brain antioxidant defense system, memory enhancing property has increased in recent years. In the present study, the antioxidant and Antiinflammatory property of fresh Aqueous Extract of *C. asiatica* was studied and the present research reported aqueous extract of *Centella asiatica* recognized minimum antioxidant and antitinflammatory property compared to the standards.

Keywords: *Centella asiatica (L), Antioxidant property, Antiinflammatory property, Aqueous Extracts*

1. INTRODUCTION

Centella asiatica Linn (Apiaceae) is native to Southeast Asian countries such as India, Sri Lanka, China, Indonesia, Malaysia as well as South Africa and Madagascar. *C. asiatica* is known to be consumed as leafy green vegetable in Sri Lanka and Philippines (Orhan, 2012). The leaves are used for preparing a drink and also eaten in raw form as salads or cold rolls in Vietnam, India, and Thailand due to its high amounts of medicinally important triterpenoids and beneficial carotenoids (Orhan *et al.,* 2013, Chandrika and Prasad Kumarab, 2015). *Centella asiatica* is mainly found in India, Pakistan, Sri Lanka, Madagascar, South Africa and Eastern Europe (*Brinkhaus et al.,* 2000). The most important constituents isolated from *C. asiatica* are primarily ursane- and oleanane-type pentacyclic triterpenoid saponins (up to 8%), also known as centelloids (*James J and Dubery I, 2011)*. These terpenoids include asiaticoside, centelloside, madecassoside, moside, thankuniside, sceffoleoside, centellose, as well as triterpenic acids such as asiatic, centellic, madecassic and terminolic acids (Hashim *et al.,* 2011). *Centella asiatica* is used in treatment of small wounds, hypertrophic wounds as well as burns, psoriasis and scleroderma (*Bylka et al., 2014).* The oxidative stress and inflammation have been implicated in neurodegenerative disorders like Alzheimer's disease (AD) and Parkinson's disease, among others (*Patten et al., 2010)*. The dried leaves of Indian penny wort (botanical name: *Centella asiatica*) is mixed with milk and consumed to improve memory (*Manyam BV, 1999)* and this is practiced traditionally in selected regions in India. (Nadkarni AK, 1954). There are studies on diverse effects

of *C. asiatica* such as acetylcholine-esterase inhibition, antioxidant, neuroprotection and amyloid load reduction (Ramesh *et al.,* 2010). The defense system of the human body against oxidative stress induced impairments includes antioxidant enzymes and non-enzymatic antioxidant proteins. These inbuilt defense system are reduced in AD (Uttara *et al.,* 2009). Keeping the medicinal property of *Centella asiatica* the anti-inflammatory and antioxidant property of fresh aqueous extract of *Centella asiatica* was studied.

2. MATERIALS AND METHODS

- **Chemicals and plant material**

1,1-Diphenyl-2-picrylhydrazyl (DPPH), adenosine triphosphate (ATP), dithiothreitol (DTT), arachidonic acid (AA), nordihydroguaiaretic acid (NDGA) from Sigma Chemical Co., MO, USA. All other chemicals and solvents used are of analytical grade. The fresh leaves of *C. asiatica* was harvested from the wet land of Thoothukudi District. The sample was cleaned and stored for further analysis.

***CENTELLA ASIATICA* Linn.**

- **Preparation of Aqueous Extracts of *C. asiatica***

The *C. asiatica* was harvested from the wet land of Thoothukudi District. The aqueous extract of leaves was prepared. A total of 40 g of leaf was washed in triple distilled water. The washed leaf was boiled in steam extractor (2 L of triple distilled water for ~ 3 h until the aqueous content become half). The extract was filtered through Whatman No. 42 filter paper to get the clear solution and lyophilized to powder (yield – 2.5%, w/w) (Ramesh *et al.,* 2010)

- **Antioxidant Assays**
  - ✓ **DPPH radical scavenging Assay**

DPPH is a stable free radical that accepts an electron or hydrogen atom to become a stable 1,1-diphenyl-2-picrylhydrazine molecules. The reduction of DPPH radical was determined by a decrease in the absorbance at 517 nm. The antioxidant activity of leaf aqueous extracts of *C. asiatica* and standard synthetic antioxidant ascorbic acid was measured in terms of hydrogen donating or radical scavenging ability. Briefly, 1 mL of 200 µM methanolic solution of DPPH was incubated with different concentrations of *C. asiatica* extracts and standard ascorbic acid for 20 min at room temperature. At the end of the incubation period, the absorbance was measured using an ultra violet-visible spectrophotometer at 517 nm. The percentage of scavenging or quenching of DPPH radicals (Q) by *C. asiatica* and ascorbic were calculated using the following formula.

$$Q = 100\ (Ao - Ac)/Ao$$

Where Ao is the absorbance of the control tube and Ac was the absorbance of the tube with 'c' concentration of the sample. All the experiments were performed in triplicates.

  - ✓ **Reducing potential: Potassium ferricyanide reducing method**

The reductive potential of *C. asiatica* extracts was determined according to the method of Oyaizu, 1986. Different concentrations of *C. asiatica* extracts in 0.5 mL of water were mixed with equal volumes of 0.2 M phosphate buffer, pH 6.6 and 1% potassium ferricyanide [K3Fe (CN) 6]. The mixture was incubated for 20 min at 50°C. At the end of incubation, an equal volume of 10% trichloroacetic acid was added to the mixture and centrifuged at 3200 g for 10 min. The supernatant was mixed with distilled water and 0.1% ferric chloride at 1:1:0.2 (v/v/v) and the absorbance were measured at 700 nm. An increase in the absorbance of the reaction mixture indicates the potential reducing power of the sample. Ascorbic acid was used as a standard for comparison.

- **AntiInflammatory Assay**

✓ **5-lipoxygenase Enzyme Assay**

5-lipoxygenase enzyme assay was performed using previously reported method. (Aharony D *et al.,* 1986). The enzyme reaction mixture contains 100 mM pH 7.4, 50 µM DTT, 200 µM ATP, 300 µM $CaCl_2$, 150 µM AA and 5 µg enzyme. The aqueous leaf extracts of *C. asiatica* were incubated with the enzyme for 2 min prior to the addition of AA. The enzymatic reactions were carried out at room temperature. 5-lipoxygenase activity was measured as 5-hydroxyeicosatetraenoic acid formed at 234 nm using spectrophotometer (Shimadzu).

3. RESULT AND DISCUSSION

1. DPPH radical scavenging assay of C. asiatica aqueous leaf extracts

To study the antioxidant properties of the leaf extract, the DPPH scavenging potential of *C. asiatica* leaf extracts were determined. We have used ascorbic acid as a positive control, due to its well-known antioxidant properties. Aqueous leaf extracts of *C. asiatica* scavenged DPPH radicals in a dose dependent manner. *C. asiatica* leaf extracts reported the IC50 value of 128.8 µg/mL) indicating that *C. asiatica* leaf extracts revealed a better DPPH radical scavenging activity. A study by Chintapanti *et al.* explored the antioxidant properties of *C. asiatica* in an animal model, demonstrating its ability to enhance antioxidant enzymes and decrease oxidative stress markers (Chintapanti *et al.,* 2018). Kandasamy et al., (2023) revealed the antioxidant property in shoot, callus, and cell suspension extracts of *C. asiatica,* in all three cultures, the antioxidant property was higher in callus culture with low IC 50 value. In the present study, the IC 50 value was higher for aqueous *C. asiatica* leaf extract compared to the standard representing less DPPH radical scavenging property.

Table. 1. DPPH Radical Scavenging Activity of Aqueous Leaf Extract of *C. asiatica*

SI NO	SAMPLE	IC50 VALUE (µg/mL)
1	Standard Ascorbic Acid	14.75±0.53
2	Aqueous Leaf Extract of *C. asiatica*	128.8±0.25

2. Reducing potential of C. asiatica aqueous leaf extracts

The reducing potentials of the extract indicate their ability to act as antioxidant. The reducing power of aqueous extracts of *C. asiatica* leaves were evaluated. The ascorbic acid was used as a standard. A dose dependent increase in reducing power of *C. asiatica* were observed. However, the standard ascorbic acid showed relatively higher reducing power compared to *C. asiatica* (Table.2). The iron reducing potential of a compound indicates its ability to act as antioxidant. In potassium ferricyanide reducing assay, the reductants in the extracts may reduce ferric to ferrous by donating electron. Our results showed that *C. asiatica* has a minimum reducing ability compared to the standard. The iron reducing potential of a compound indicates its ability to act as antioxidant. In potassium ferricyanide reducing assay, the reductants in the extracts may reduce ferric to ferrous by donating electron. The amount of ferrous can be monitored by measuring the complex formation with Perl's Prussian blue at 700 nm (Chung et al., 2002). Differences in their phenolic acids may be responsible for their differences in antioxidant properties. Renu Arora *et al.,* (2018) studied the *In vitro*, free radical scavenging assays (ABTS, DPPH, NO, NORAC, and ORAC) in three different extracts of *C. asiatica* and the result showed that methanolic extract recorded good antioxidant activity. In the present work, the aqueous leaf extract of *C. asiatica* demonstrated less potassium ferricyanide reducing property compared to the standard ascorbic acid.

Table. 2. Reducing Potential of Aqueous Extract of *C. asiatica* and Standard Ascorbic Acid

SI NO	Concentration	Absorbance	
		Aqueous Extract of *C. asiatica* (µg/mL)	Standard Ascorbic Acid (µg/mL)
1	50	0.14	0.41
2	100	0.19	0.54

3	150	0.23	0.66
4	200	0.32	0.75
5	250	0.39	0.86
6	300	0.45	0.96
7	350	0.52	1.02
8	400	0.64	1.12

2. Anti-inflammatory assay:

5-lipoxygenase assay of *C. asiatica aqueous* leaf extracts

The anti-inflammatory activities of aqueous leaf extract of *C. asiatica* was studied by inhibiting 5-lipoxygenase that was isolated from PMNLs assay. The results showed that *C. asiatica* with IC_{50} of 210 ± 1.81 µg/mL. Significant inhibition of 5-Lipoxygenase compared to the standard, NDGA had the lowest IC_{50} value of 9.6 ± 0.52 µg/mL (Table 3).

The 5-lipoxygense enzymes is a key enzyme in the biosynthesis of leukotriens. 5-lipoxygenase contains non-heme iron in the catalytic site. It catalyses incorporation of dioxygen into unsaturated fatty acid. It mainly converts AA to biologically active leukotriens. These leukotrienes are implicated in inflammatory and allergic reactions. The harmful effects can be prevented by inhibiting its production. Mairuae *et al.,* (2019) investigated the anti-inflammatory and anti-oxidative effects of *Centella asiatica* extract (CA) on lipopolysaccharide (LPS)-stimulated BV2 microglial cells. Saha *et al.,* (2013) showed Methanol extract of *C. asiatica* had significant anti-inflammatory effect against carrageenan-induced paw edema model, whereas chloroform extract had no such effect on rats. Carrageenan induced inflammation is a well-established method to detect orally active anti-inflammatory agents which shows biphasic response. In the present research, the fresh aqueous extract of *C. asiatica* represented less anti-inflammatory property compared to the standard NDGA.

Table. 3. Inhibition of 5-lipoxygenase activity by *C. asiatica* extract

SI NO	**SAMPLE**	**IC50 VALUE** (µg/mL)
1	Standard Nor Dihydro Guaiareic Acid (NDGA)	9.6 ± 0.52
2	Aqueous Leaf Extract of *C. asiatica*	210 ± 1.81

4. CONCLUSION

The declared studies highlights the antioxidant and anti-inflammatory property of fresh aqueous extract of *C. asiatica* and their pharmacological activities, offering insights into its potential therapeutic applications. However, further exploration is essential to illuminate the mechanisms of action and optimize the usage of *C. asiatica* in clinical practice. Empathetic the competence of *C. asiatica* in various medical applications could not only contribute to the development of new natural remedies but also help in the discovery of novel bioactive compounds for pharmaceutical applications. Hence, this research article aims to consolidate the present knowledge and encourage further investigations into the vast potential of *C. asiatica* as a valuable medicinal plant.

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