

Phytochemical Screening and In Vitro Antioxidant Analysis of *Colebrookea oppositifolia* Sm. Extract [†]

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Abstract

Dementia, a major cause of dependency, disability, and mortality, is characterized by a progressive cognitive decline. Alzheimer's disease, a major neurodegenerative dementia, primarily affects the elderly. This study aimed to investigate the antioxidant and neuro-protective potential of plant phytoconstituents for the treatment of Alzheimer's disease. Phytoconstituents of *Colebrookea oppositifolia* Sm. were investigated using aerial and root extracts. The antioxidant potential of the plant phytoconstituents was assessed using in vitro antioxidant assays such as 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and Ferric-Reducing Antioxidant Power (FRAP) assay. The plant extract (root) showed significant antioxidant potential. Additional studies are underway to comprehensively evaluate its potential applications.

Keywords: Alzheimer's disease; acteoside; antioxidant; dementia; plant phytoconstituents

1. Introduction

Dementia is becoming a more significant public health issue, and there are national and worldwide initiatives to speed up the diagnosis process. Even though the number of dementia sufferers is steadily rising, only 50% to 65% of cases are diagnosed in high-income nations; rates are significantly lower in low- and middle-income countries [1]. In addition to being the seventh most common cause of death worldwide, dementia is a significant contributor to reliance and impairment [2]. Numerous neurodegenerative and non-neurodegenerative illnesses can, and frequently do, cause impairment in patients with dementia. Alzheimer's disease (AD), which mostly affects the elderly population (5–6% of those 65 and over, and up to 30% of those 85 and older), is regarded as the major neurodegenerative dementia. Multiple hypotheses—amyloid- β , tau, inflammation, oxidative stress, and cholinergic neuron loss—have guided Alzheimer's disease drug development. Oxidative stress plays a significant role in Alzheimer's disease, as the brain's high oxygen consumption increases exposure to reactive oxygen species (ROS), leading to protein and lipid oxidation and A β accumulation; therefore, antioxidants have been explored to slow disease progression [3]. The burden of AD will be quite significant over the next ten years, affecting hundreds of millions of people, their families, and national health care systems due to aging patterns, particularly in industrialized nations. According to epidemiological data, one in three Americans over 85 will develop AD, and the number



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of Americans over 85 will treble by 2050. One of the top five causes of death in developed nations is AD [4]. In elderly adults, AD begins to manifest ten years or more before a diagnosis is made. The moderate cognitive impairment (MCI) phase, which can last for several years, is the initial stage that patients with AD experience. Additionally, MCI is divided into early- and late-stage features. Alternatives for AD diagnosis include brain imaging tests and equipment, such as various positron emission tomography (PET) scans and magnetic resonance imaging (MRI) machines. Furthermore, cerebrospinal fluid has been shown to include molecular indicators such as phosphorylated tau and amyloid-beta isoform 42 (A β -42) [4]. There is presently no known cure for Alzheimer's disease, despite a great deal of study, and the efficacy of current treatments has been questionable. To address the increasing burden of Alzheimer's disease, pharmaceutical approaches that reduce or stop the illness's incidence and progression are essential. A few drugs such as donepezil, rivastigmine, galantamine, and memantine are approved by the FDA for the treatment of AD [5]. Numerous molecules originating from Ayurvedic medicinal plants are now undergoing clinical trials, making them a viable route for drug discovery. The possible use of many Ayurvedic medicinal plants and their derivatives in the treatment of Alzheimer's disease has been investigated in scientific investigations [6]. Ashwagandha, Brahmi, Ginkgo biloba, Saffron, Turmeric, Cordia dichotoma, Acorus calamus and many more medicinal herbs hold great promise for both preventing and treating AD-related cognitive impairment. In order to treat AD symptoms including depression, memory loss, and reduced cognition, traditional medicinal practices prescribe a range of plants and their active constituents [7]. Plants contain a number of secondary metabolites, or phytochemicals, that can improve human health by preventing and treating diseases. Numerous phytochemicals, or plant metabolites, from the previous literature have undergone clinical testing to see if they can affect the immune system [8].

Compared to conventional drugs, plants are a cost-effective and safe source of traditional medicines that help treat a wide range of illnesses, including immune system disorders. The use of botanical supplements for immune defense reactions in the body can result in safe and efficient immunity responses, according to evidence reported in textual sources such as Traditional Chinese medicine and Indian Ayurvedic medicine. *Colebrookea oppositifolia* Smith is a significant traditional medicinal plant which belongs to the Lamiaceae family. It is a thick shrub that resembles wool and is mostly found in subtropical areas of several Asian nations, including China and India. It has been used extensively to treat conditions affecting the neurological system, such as epilepsy. Because polyphenols and flavonoids are the plant's primary chemical components, its active ingredients have demonstrated antioxidant, antimicrobial, and antifungal qualities [9]. Acteoside, a phytoconstituent present in a variety of plants, has drawn notice for its anti-inflammatory and neuroprotective qualities, indicating a possible role in treating AD through dietary treatments. Olives and a number of other plants, including Verbascum species, contain verbascoside, also known as acteoside, a phenylethanoid glycoside. Chronic neuroinflammation plays a major role in the development of AD. By reducing microglia activation, and reducing the production of pro-inflammatory cytokines, acteoside has been demonstrated to have anti-inflammatory properties [10]. The ethnopharmacological research on acteoside includes an analysis of its cultural and historical uses in traditional medicine. Table 1 lists some of the medicinal plants and their phytoconstituents that are used to treat AD, and the studies are documented in the scientific database.

Table 1. List of some plants and their phytoconstituents used in the treatment of Alzheimer's disease.

Plant Name	Reported Mechanism	Reference
Bacopa monnieri	Memory enhancer; antioxidant, anti-amyloid effects	[11]
Acorus calamus	Neuroprotective; memory enhancer	[7]
Cordia dichotoma	Cholinesterase inhibition; antioxidant effects	[7]
Withania somnifera	Neuroprotective; cholinesterase inhibition	[12]
Ginkgo biloba	Cognitive support; antioxidant and anti-inflammatory	[13]
Panax ginseng	Anti-amyloid, neurotrophic modulation	[12]
Polygala tenuifolia	Neuroprotective; cognitive function improvement	[14]
Crocus sativus	Anti-amyloid, antioxidant	[15]
Centella asiatica	Nootropic; antioxidant	[15]
Clitoria ternatea	Neuroprotective; antioxidant	[15]
Terminalia chebula	Antioxidant, possible anti-amyloid	[15]
Asparagus racemosus	Cognitive support in models	[15]
Salvia officinalis	Cognitive-enhancing effects	[16]
Melissa officinalis	Antioxidant & cholinergic modulation	[16]
Camellia sinensis	EGCG & polyphenols with neuroprotective roles	[16]
Tinospora cordifolia	Anti-inflammatory / neuroprotective	[17]
Convolvulus pluricaulis	Memory enhancer; neuroprotective effects	[17]

2. Material and Methods

2.1. Plant Collection and Extraction

Colebrookea oppositifolia Sm. was collected from the Nainital region of Uttarakhand, India. Extraction of the plant material was carried out using alcohol as the solvent with a Soxhlet apparatus to yield the final crude extract.

2.2. Chemical Used

Plant extract, distilled water, Mayer's reagent, sodium hydroxide, hydrochloric acid, sulfuric acid, chloroform, bromine water, glacial acetic acid, ferric chloride, potassium persulfate, ABTS, TPTZ, ascorbic acid, acetate buffer, ethanol, and methanol; all chemicals were purchased from OM Scientific Chemicals, India.

2.3. Phytochemical Screening of Secondary Metabolites Including Alkaloids Flavonoids, Steroidal Compounds, Tannins, Glycosides, Saponins and Terpenoids

The process of identifying the various classes of phytoconstituents found in different plant sections is known as phytochemical screening. The substances found naturally in plants are called phytochemicals. Among the many different types of chemical components found in plants are alkaloids, glycosides, phenol, flavonoids, terpenoids, and saponins. Phytochemical screening is useful for identifying bioactive substances that can be helpful for targeting the molecular mechanisms of diseases and determining which compounds predominate over the others [18]. The following standard techniques were used to evaluate the presence of phytochemicals in the plant extracts (aerial and root extracts).

A drop of Mayer's reagent was applied to the side of a test tube containing a few milliliters of the filtrates. The test is positive for alkaloids if a creamy or white precipitate forms [19].

Two milliliters of 2% NaOH were combined with an aqueous plant crude extract, resulting in a bright yellow color. However, when two drops of diluted acid were added, the mixture turned colorless. This outcome indicated that flavonoids were present [20].

Five milliliters of aqueous plant crude extract were mixed with two milliliters of chloroform and concentrated H₂SO₄. A red coloration emerged in the bottom chloroform layer, signifying the presence of steroids [20].

The 0.5 g aqueous extract was mixed with 10 ml of bromine water. The presence of tannins was indicated by the decolorization of bromine water [20].

Ten milliliters of aqueous plant extract and one milliliter of concentrated H_2SO_4 were combined with a solution of glacial acetic acid (4 milliliters) and one drop of 2% FeCl_3 . The presence of cardiac steroidal glycosides was observed as a brown ring formed between the layers [20].

In a test tube, 5 ml of distilled water and aqueous crude plant extract were well combined. When a few drops of olive oil were added and well stirred, the appearance of foam indicated the presence of saponins [20].

After adding 2.0 ml of chloroform to 5 ml of aqueous plant extract and letting it evaporate, 3 ml of concentrated H_2SO_4 was heated. Terpenoids were revealed by the appearance of a gray color [20].

2.4. Antioxidant Assay

2.4.1. 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic Acid) (ABTS Radical Cation Decolorization) Assay

The ABTS stock solution was made by reacting equal parts of ABTS aqueous solution (7 mM) and potassium persulfate aqueous solution (2.45 mM). The combination was then left to stand at room temperature in the dark for 12 to 16 h before being used. The stock solution was diluted with methanol to produce the working solution of ABTS. Next, 1 mL of the extracts at varying concentrations was combined with 2 mL of ABTS solution. After that, the mixture was incubated in the dark for precisely ten minutes at room temperature. Two milliliters of ABTS solution and one milliliter of double-distilled water were combined to create the control. A spectrophotometer was used to measure the absorbance at 734 nm in comparison to a blank. Three duplicates of each sample were produced and measured. The method was used to determine each extract's percentage of scavenging activity on ABTS as % inhibition [21].

2.4.2. Ferric-Reducing Antioxidant Power (FRAP) Assay

This technique relies on the sample's capacity to convert Fe^{3+} ions to Fe^{2+} ions. The ferric-tripyridyltriazine (Fe^{3+} -TPTZ) complex is reduced to the ferrous (Fe^{2+} -TPTZ) form at low pH in the presence of TPTZ, resulting in the development of a vivid blue color with an absorption maximum at 593 nm. 0.7 mL of the aqueous extracts at varying concentrations (0.5-5.0 mg/mL) was combined with 2.3 mL of the FRAP reagent. After that, the mixture was incubated in the dark for 30 min at 37 °C. Using a spectrophotometer, the absorbance was measured at 593 nm against a blank. An increase in the reaction mixture's absorbance indicates a greater capacity for reduction. Samples were measured three times. The standard was ascorbic acid. The regression formula was created from a similar approach that was used to prepare a standard curve of ascorbic acid solution. Ascorbic acid equivalents (AAE) in milligrams per milliliter of extract were used to express the results [21].

3. Result

The three independent determinations' mean $\pm$ standard deviation (SD) was used to express all experimental results ($n = 3$) (different days with a minimum interval of 24 h). Statistical software was used to determine the antioxidant parameters.

3.1. Percentage Yield of Plant Extract (Aerial and Root)

The yield percentage of the aerial plant extract was 13.80 percent (w/w), and for the root extract it was 14.10 percent (w/w) when the semi-solid residue was lyophilized to create a fine powder, as already reported in our previous findings [10].

3.2. Phytochemical Assay

Table 2 displays the findings of the phytochemical screening that was carried out utilizing chemical analysis for both the aerial and root plant extracts. The phytochemicals results were recorded as present (+) or absent (–) according to the results obtained from the respective chemical assays in the laboratory process.

Table 2. Phytochemical screening of secondary metabolites of plant extracts using aerial plant extract and root plant extract.

Phytochemicals	Aerial Extract	Root Extract
Alkaloids	+	+
Flavonoids	+	+
Steroidal compounds	+	–
Tannins	+	+
Cardiac glycosides	+	+
Saponins	+	+
Terpenoids	+	+

3.3. Antioxidant Assay

ABTS and FRAP tests were used to assess the antioxidant activity of the root and aerial extracts. The antioxidant effects of both extracts were concentration-dependent, although the activity of the root extract was much higher than that of the aerial extract. The root extract showed a higher percentage of radical scavenging activity in the ABTS assay, as shown in Table 3. Similar to this, the root extract showed noticeably better ferric-reducing capacity in the FRAP experiment, as evidenced by higher absorbance values at 593 nm and higher ascorbic acid equivalent (AAE) values, as shown in Table 4. Collectively, the results of the antioxidant parameters indicate that the root extract had higher in vitro antioxidant properties. The values are expressed as mean $\pm$ SD ($n = 3$).

Table 3. ABTS radical scavenging activity of plant extracts.

Concentration (mg/mL)	ABTS % Inhibition—Aerial	ABTS % Inhibition—Root	ABTS % Inhibition—Ascorbic Acid (Standard)
0.5	21.4 $\pm$ 1.2	32.6 $\pm$ 1.4	54.8 $\pm$ 1.6
1.0	29.8 $\pm$ 1.5	44.2 $\pm$ 1.7	66.3 $\pm$ 1.8
2.0	41.6 $\pm$ 1.8	58.9 $\pm$ 2.0	78.5 $\pm$ 2.1
3.0	52.3 $\pm$ 2.1	69.7 $\pm$ 2.3	86.9 $\pm$ 2.4
5.0	63.5 $\pm$ 2.4	81.2 $\pm$ 2.6	94.6 $\pm$ 2.8

Table 4. Ferric-Reducing Antioxidant Power (FRAP) activity of plant extracts.

Concentration (mg/mL)	FRAP—Aerial (mg AAE/mL)	FRAP—Root (mg AAE/mL)	FRAP—Ascorbic Acid (mg AAE/mL)
0.5	0.42 $\pm$ 0.03	0.68 $\pm$ 0.04	1.15 $\pm$ 0.05
1.0	0.63 $\pm$ 0.04	0.95 $\pm$ 0.05	1.82 $\pm$ 0.06
2.0	0.94 $\pm$ 0.05	1.34 $\pm$ 0.06	2.63 $\pm$ 0.08
3.0	1.21 $\pm$ 0.06	1.76 $\pm$ 0.07	3.28 $\pm$ 0.09
5.0	1.58 $\pm$ 0.08	2.21 $\pm$ 0.09	4.10 $\pm$ 0.12

4. Discussion

The results demonstrate potent antioxidant properties of plant extracts, as evidenced by their strong radical scavenging and reducing activities shown in Tables 3 and 4. These antioxidant effects are particularly relevant to the pathophysiology of Alzheimer's disease, where oxidative stress plays a central role in neuronal damage, protein oxidation, lipid peroxidation, and amyloid- β accumulation. By reducing oxidative stress, such antioxidants

may help protect neuronal cells and could contribute to therapeutic strategies aimed at slowing the progression of Alzheimer's disease.

Strong antioxidant activity, especially in the root extract, indicates that it may be able to lessen neuronal damage caused by oxidative stress. The presence of phenolic phytoconstituents, particularly acteoside (verbascoside), a well-researched glycoside with strong antioxidant, neuroprotective, and free-radical-scavenging qualities, may be primarily responsible for this effect. As a result, the antioxidant qualities shown in this ongoing research emphasize the medicinal potential of this plant extract in managing Alzheimer's disease via modulation of oxidative stress. In vivo and pathway-based research is needed to confirm the neuroprotective effectiveness of acteoside and clarify its molecular mechanisms and target profile.

5. Conclusions

In conclusion, this ongoing study supports continued investigations of the plant root extract, which shows greater pharmacological potential as a natural source of antioxidants for the treatment of Alzheimer's disease. In vitro results indicate potential antioxidant activity, notably associated with acteoside; further in vivo research is needed to evaluate efficacy and safety using suitable animal models. For better understanding and more robust results, a thorough investigation of molecular processes such as oxidative stress regulation, β -amyloid aggregation, neuroinflammatory pathways, and cholinergic signaling can be combined with biochemical, histological and hormonal assays.

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Abbreviations

The following abbreviations are used in this manuscript:

AD	Alzheimer's disease
MCI	Moderate cognitive impairment
PET	Positron emission tomography
MRI	Magnetic resonance imaging
A β	Amyloid-beta
ABTS	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
FRAP	Ferric-Reducing Antioxidant Power
Fe ³⁺ -TPTZ	Ferric-tripyridyltriazine
AAE	Ascorbic acid equivalent
SD	Standard deviation

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