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Qualitative Phytochemical Investigation and Antioxidant Activity of Chlorophytum Borivilianum Santapau & R.R.Fern. Leaves

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Abstract

Background: The objective of this research was to determine how the phenolic profile and the corresponding biological activity were affected by various solvents—water, ethanol, and ethyl acetate. Methods: The 2,2"-azino-bis(3-ethylbenzothiazoline-6-sulphonic) acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were used to measure antioxidant activity. Results: Ethanol was found to be the most efficient solvent for polyphenol extraction, according to the findings. With IC50 values of 52.83 g/mL and 68.96 g/mL, respectively, the ethanol extract of C. borivilianum had the highest antioxidant potential for DPPH and ABTS radical scavengers, while the ethyl acetate and aqueous extracts had the lowest antioxidant potential. According to the Folin-Ciocalteu method, ethanol extract contained more phenolics (88.92 0.24 mg of equivalent gallic acid (GAE)/g) than aqueous extract (62.28 0.14 GAE/g). An aluminum chloride colorimetric method determined that the ethanol extract had the highest flavonoid content (67.24 0.14 mg quercetin equivalent (QE)/g), while the aqueous extract had the lowest (35.67 0.14 QE/g). Conclusion: According to the findings of this study, the ethanolic extract of C. borivilianum leaves may be a useful source of natural antioxidants for the formulation of functional foods due to its high antioxidant potential.

1. Introduction

Oxidative stress (ROS) is brought on by reactive oxygen species. ROS is a byproduct of oxygen reduction to singlet oxygen (O) in the atmosphere. The transfer of one or more electrons from O2 to a variety of different radicals, such as hydrogen peroxide (H2O2) and other radicals, is generally referred to as Numerous diseases, including atherosclerosis, cancer, ulcers, and degenerative diseases, can be exacerbated by these oxygen radicals [1]. Because they neutralize free radicals, antioxidants are important for preventing oxidative damage and helping to control chronic disease. On the other hand, synthetic antioxidants like butylate-d-hydroxytoluene (BHT) have been restricted because they have been linked to carcinogenesis and damage to the liver [2]. As a result, studies have been conducted on alternative antioxidants with fewer side effects that come from natural sources. “A number of diseases, such as cancer, cardiovascular and neurological disorders, hypertension, diabetes mellitus, and premature ageing, are associated with the pathophysiology of free radicals or ROS production” [3, 4]. “Reactive oxygen species (ROS), which promote the synthesis of aberrant proteins and cause antioxidant depletion in the immune system, are produced by radiation, chemicals, poisons, deep-fried and spicy foods, as well as physical stress” [5]. Glutathione peroxidase, catalase, and superoxide dismutase are examples of endogenous antioxidant enzymes that

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are capable of deactivating free radicals and preserving optimal cellular functions [6]. However, under conditions of increased oxidative stress, endogenous antioxidants may not be sufficient to maintain optimal cellular functions, so dietary antioxidants may be required [7].

Natural products play a crucial role in drug promotion and development [1,2]. Phyto-compounds produced from higher plants are present in over 25% of conventional medications. [3]. "The World Health Organization (WHO) estimates that plants provide primary healthcare for approximately 80% of the world's developing population" [8]. The antioxidant properties of medicinal herbs have been investigated [9]. According to the findings of a number of studies, the presence of phenolic compounds like flavonoids, phenolic acids, and carotenoids is primarily responsible for certain plants' antioxidant activity. The detrimental physiopathology brought on by oxidative stress, which is brought on by the overproduction of free radicals, can be greatly mitigated by these herbal antioxidants [12].

Chlorophytum borivilianum in the Ayurvedic system is referred to as "white gold" and is a highly prized medicinal plant in India [13, 14]. According to previous research, this had historically been used to treat high blood pressure. Chronic diarrhea, diabetes, arthritis, diarrhoea, dysentery, general weakness, and an enhanced immune system [15-17] This plant has been linked to a number of biological activities, such as antimicrobial, anti-inflammatory, antipyretic, hepatoprotective, antioxidant, hypolipidemic, and diabetes-fighting properties [18]. Male impotence has traditionally been treated with the root of *C. borivilianum*. 19] oligozoospermia and male infertility.

This plant's leaves have not been the subject of any research, despite the fact that there is information available regarding the antioxidant properties and phenolic content of its tubers. Consequently, there is a pressing need to investigate the leaves' antioxidant properties. In order to determine the phenolic and flavonoid content of C. borivilianum leaves' antioxidant activity, the current study used a range of in vitro models.

2. Materials and Methods

Plant Material

The *C. borivilianum* leaves were obtained from the vicinity of Kota, Rajasthan. The plant was identified and verified by Dr. Pankaj Sharma of the Himachal Pradesh State Biodiversity Board in Shimla, India.

Preparation of Extracts

The plant leaves were water cleaned to eliminate dirt and foreign debris before being shade dried. After being ground into a coarse powder from dried leaves, sieve No. 14. At 60-65 C for three to four hours, the dried powdered leaves of *C. borivilianum* (20 g) were extracted with a variety of solvents, including ethyl acetate, ethanol, and water (300 mL), in a thimble-shaped Soxhlet apparatus tube. The hot-filtered ethyl acetate (EAE), ethanol (EE), and aqueous (AE) extracts were dried using a rotary vacuum evaporator, and the final dried extract samples were stored in the refrigerator at a low temperature for further research.

Total Polyphenols and Flavonoid Contents

The previously described method [20] was used to determine each *C. borivilianum* leaf extract's total phenolic content (TPC) and flavonoid content (TFC). TFC was measured in milligrams of quercetin equivalents (QE) per 100 g of extract, while TPC was measured in milligrams of gallic acid equivalent (GAE) per 100 g of extract.

Antioxidant Activity

DPPH Radical-Scavenging Activity

As previously described [20], the capacity of each extract solution to scavenge free radicals on the DPPH radical was determined. Concentrations of EE, EAE, and AE solutions ranged from 20 to 100 g/ml. The standard ascorbic acid solution and the various plant extract concentrations were each supplemented with a 50 M DPPH radical solution. After being thoroughly shaken, the reaction mixtures were placed in darkness for thirty minutes. 2 mL of DPPH solution was mixed with 2 mL of ethanol to make the control solution. All

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reaction mixtures and the control solution's absorbance were measured at 517 nm. The percentage inhibition was determined using the formula below:

$$\% \text{ Inhibition} = \left[\left(\frac{AC517\,nm - AS517\,nm}{AC517\,nm} \right) \times 100 \right] \quad (1)$$

where AS is the absorbance of the Sample and AC is the absorbance of the Control.

Plotting a graph between the percentage of inhibition and various concentrations of plant extracts and ascorbic acid was used to determine the IC50 value.

ABTS Assay

As mentioned before, the ABTS test was used to determine the crude extracts' reducing power [21]. 0.2 mL of samples of various concentrations were combined with 1 mL of distilled dimethyl sulfoxide, and 0.16 mL of ABTS solution was added to make a volume of 1.36 mL for the EE, EAE, and AE solutions. Using a UV spectrophotometer, the absorbance was measured spectrophotometrically at 734 nm after 20 minutes. A sample was not provided to the control group. The percentage of inhibition was calculated as follows, and the scavenging capacity of ABTS was expressed as IC50 (g/mL):

$$\text{ABTS scavenging activity (\%)} = (A_0 - A_1)/A_0 \times 100 \quad (2)$$

where, A0: absorbance of the control, A1: absorbance of the sample.

Statistical Analysis

The mean standard error mean (SEM) was used to display the results. The Student's t-test was used to conduct a statistical analysis on the data, and a value of p 0.05 indicated that the findings were significant.

3. Results and Discussion

The technique used to assess the amount of phenolics in plant extracts is called Total Phenolic Content (TPC). Redox properties make these phenolic compounds potential antioxidants [22, 23]. The Folin-Ciocalteu reagent was used to measure each extract's phenolic content. Table 1 shows the calculated gallic acid equivalents (GAE) per gram of dry extract weight. The TPC of the EE was approximately 88.920.24 mg GAE/g, while that of the EAE was 75.830.19 mg GAE/g, and that of the AE was 62.280.14 mg GAE/g, according to the findings. The linear equation based on the gallic acid calibration curve (y = 0.0048x + 0.5699, R2 = 0.966) was used to calculate TPC.

Increased bioactivity, such as antioxidant and antimicrobial activity, may be indicated by the ME's higher phenolic level. The connection between antioxidant activity and phenolic content has been the subject of numerous studies. Velioglu and others [24, 25] discovered a strong connection between TPC and plant product antioxidant activity. Sengul and co. 26] also reported that phenolic compounds are used in plant defense mechanisms to prevent molecular damage and attacks from microorganisms, insects, and herbivores, as well as to prevent ROS formation and promote survival.

Table 1. Total phenolic and flavonoid content

Extracts	Phenolic Content (mg/g GAE)	Flavonoid (mg/g QE)
EE	88.92±0.24	67.24±0.14
EAE	75.83±0.19	46.73±0.19
AE	62.28±0.14	35.67±0.14

All values represent means ± SEM of three replicates. EE: Ethanolic extract; EAE: Ethyl acetate extract; AE: Aqueous extract. Statistical significance was determined at $p < 0.05$ and is indicated with different letters.

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Total Flavonoid Content (TFC)

The antioxidant activity of flavonoids, which are secondary metabolites, is determined by the number and position of free OH groups [27]. Using aluminum chloride in a colorimetric system as a foundation, the flavonoid content of selected plant extracts was quantitatively determined. Table 1 displays the results as quercetin equivalents (QE) per gram of dry extract weight. EE had a higher TFC than EAE and AE, with approximately 67.240.14 mg GAE/g, 46.730.19 mg QE/g, and 35.670.14 mg GAE/g, respectively, according to the findings. Based on the quercetin calibration curve, the linear equation (y = 0.004x + 0.511, R2 = 0.993) was used to calculate TFC.

"When extracting plant natural compounds, ethanol is frequently used; The widespread therapeutic use of the folklore botanical could be explained by the abundance of bioactive phytoconstituents found in *C. borivilianum* leaves ME" [28].

Plants have a lot of flavonoids, which are a type of phenolic compound. They significantly enhance the flavor and color of vegetables and fruits [29]. Flavonoids have the ability to scavenge free radicals because they contain hydroxyl groups. The same idea as TPC was used for TFC determination—a flavonoid-aluminum complex causing a change in color [30]. According to Table 1, the ME leaves of *C. borivilianum* had the highest values for TPC and TFC, indicating that EE is the most abundant in phenolic compounds of the examined extracts. This was supported by EE's antioxidant and cytotoxic properties. Therefore, *C. borivilianum* can be considered a promising source of these compounds in comparison to other natural sources.

Antioxidant Activity

DPPH Radical Scavenging Activity

The radical scavenging action of DPPH is based on one-electron reduction, which is the free radical lowering activity of antioxidants. As a positive control, ascorbic corrosive (AA) was utilized. At various concentrations, the crude extracts and standard of *C. borivilianum* were examined to determine the percentage of DPPH radical inhibition. The IC50 value of ascorbic acid was 16.51 g/mL, while the IC50 values of EE, EAE, and AE were 52.83, 71.97, and 92.22 g/mL, respectively. Table 2). TPC and EE's high antioxidant activity were positively correlated. Antioxidant capacity has been linked to both TFC and TPC in previous studies [31, 32].

Table 2. IC50 values of *C. borivilianum* extracts in DPPH and ABTS antioxidant assay.

Crude Extracts	DPPH Assay (µg/mL)	ABTS Assay (µg/mL)
Ascorbic acid	16.51±0.24[a]	24.63±0.25[a]
ME	53.83±0.17[b]	68.96±0.19[b]
EAE	71.97±0.15[c]	74.90±0.18[c]
AE	92,22±0.21[d]	90.54±0.21[d]

Statistical significance was determined at $p < 0.05$ and is indicated with different letters.

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assays

Dose-dependent ABTS radical scavenging potential was found in *C. borivilianum* EE. With IC50 values of 68.96, 74.90, and 90.54 g/mL, respectively, EE had the lowest IC50 (Table 2). With an IC50 of 24.63 g/mL, ascorbic acid outperformed EAE and AE in free radical scavenging performance because lower IC50 values (concentration required for 50% inhibition) indicate greater antioxidant activity.

Cell reinforcements are fundamental in forestalling oxidative harm. They manage oxidative stress-related illness by neutralising and dismantling free

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radical chains. However, synthetic antioxidants like BHT are controlled substances because they are thought to harm the liver and cause cancer [33]. Natural sources should be thoroughly investigated to find safer and more effective alternatives to antioxidants in order to lessen the negative effects [34]. Over the past ten years, it has been demonstrated that a number of natural compounds derived from plants, such as the lignan secoisolariciresinol [35], are viable alternatives to these synthetic antioxidants, which have the potential to be harmful.

"Due to its great antioxidant potential and preventive actions against mutagens, carcinomas, and inflammatory pathological processes (including free radical scavenging)" [27,28], a number of phenolic compounds found in plants, including simple phenolics, phenolic acids, anthocyanins, and flavonoids, have piqued the interest of researchers. Due to their redox properties, phenolic compounds can be effective metal chelators, hydrogen givers, reducing agents, and singlet oxygen inhibitors [29].

The ABTS assay revealed a very high ABTS+ radical scavenging capacity in comparison to EE. "Plant extract concentration increased DPPH radical scavenging activity in the current study, suggesting a greater capacity to transfer a hydrogen atom and produce a lighter solution proportional to the number of electrons obtained" [30]. Due to its high hydrogen atom transferability, it is possible that *C. borivilianum* reduces the radical to the corresponding hydrazine and plays a DPPH scavenging role. However, extracts were found to have a much lower ABTS+ scavenging role than DPPH radical activity.

It has been extensively discussed how phenolic chemicals affect antioxidant activity; Yet by using these two different radical scavenging tests in relation to the unique phytochemical makeup of each extract, we offer the most thorough phytochemical investigation of this important folk medicine plant to date. "In addition, it is widely acknowledged that antioxidant activity can be significantly impacted by a number of factors, such as phytochemical composition, which is closely linked to the extraction method" [33]. To determine how the phytochemical composition and antioxidant activity of *C. borivilianum* extract were affected by various extraction solvents, we conducted tests. In this instance, the phytochemical diversity and antioxidant potential of *C. borivilianum* extract are best achieved through ethanol extraction. For instance, EE contains more phenolics, flavonoids, and arylheptanoid antioxidant substances such p-coumaric acid, catechin, quercetin, and myricanone in comparison to the other extracts. "For these compounds, the effect of the extraction solvent on the antioxidant activity of the corresponding extracts has already been described" [34]. Additionally, a distinct difference in the composition of the extracts was observed, with EE having a higher concentration of phenolics and arylheptanoids, which are known to have the potential to fight microorganisms [35].

4. Conclusions

Due to their high concentration of phytochemicals like phenolics and flavonoids, this study found that *C. borivilianum* leaf extracts have intriguing antioxidant properties. For each of the C. borivilianum extracts (EE, EAE, and AE), TPC and TFC quantification revealed that the extraction solvent had a significant impact on the quantity and composition of bioactive compounds. The strain C. borivilianum EE contained the most TPC and TFC. This study also provided new information regarding the potential antioxidant mechanisms of *C. borivilianum*, specifically its capacity to eliminate free radicals. The level of phenolic acids and flavonoids in the various *C. borivilianum* extracts appears to be strongly correlated with their biological activity. Given the wonderful cancer prevention agent impacts of *C. borivilianum* leaf extricates, its utilization ought to be extended, as this plant might assume a significant part in the avoidance of a few wellbeing problems related with free extreme overproduction, like carcinoma, cardiovascular sickness, and untimely maturing. However, more study on the isolation and individual characterisation of bioactive chemicals is needed in order to create potential food and/or cosmetic preservatives.

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