Journal of Pioneering Medical Sciences
Received June 14, 2023 Accept December 5, 2023 Publish December 23, 2023.
Volume 12, Issue 3, Pages 6-10

DOI https://doi.org/10.61091/jpms20231232

Antioxidant Activity and Cytotoxicity of Cyanthilium Cinereum and Cinnamon-in Vitro Study

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Abstract **Introduction:** Cyanthillium cinereum is a native of tropical Africa and Asia (India, Indochina, Indonesia) and is used as a smoking cessation drug and relief for cold. Leaves act as an abortifacient and blood purifier and have diuretic, antiviral, analgesic, antipyretic, and anti-inflammatory properties. Cinnamon is the inner bark of cinnamon cassia found on the west coast of India, Sri Lanka, and Indonesia. It is a forest evergreen tree that has antioxidant, anti-inflammatory, anti-diabetic, and anti-microbial properties, treats bronchitis, diabetes and has traces of Vitamin B & K. Hence, we aimed to study the antioxidant activity and cytotoxicity in the herbal synergy of cinnamon and cyanthillium cinereum. **Materials and Methods:** An in vitro study was conducted, and the polar aqueous extract of this combination of herbs cinnamon and cyanthilium cinereum was prepared and tested for DPPH assay H_2O_2 assay to study its antioxidant properties and the absorbance value was evaluated. Brine Shrimp Assay with viable nauplii cell lines was used for cytotoxicity assessment. **Results:** Antioxidant activity of the aqueous extract of Cyanthillium cinereum and Cinnamon revealed that there was a decrease in the antioxidant activity with an increase in the concentration of the aqueous extract. Cytotoxicity of the aqueous extract of Cyanthillium cinereum and Cinnamon reported an increase in the cytotoxic activity with a rise in the concentration of the aqueous extract. **Conclusion:** This research showed that aqueous extract of cinnamon and cyanthillium cinereum has good antioxidant activity, is less cytotoxic, and can be used as a therapeutic and preventive medicine.

Key Words Herbs, Nutrition, Well being, Health, Resources

1. Introduction

Ayurveda is the 5000-year-old ancient science of holistic healing. It emphasizes restoring the optimum balance in the body. The conventional healthcare system introduced numerous powerful herbal blends to support overall well-being [1]. Cyanthillium cinereum, or Little Ironweed, is an angiosperm belonging to the Asteraceae family. It has been reported to contain chemical compounds such as luteolin 7 mono beta-D-glucopyranoside and triterpene compounds like lupeol acetate and beta-amyrin acetate. Analysis of phyto-chemicals of Cyanthillium cinereum revealed the presence of steroids, alkaloids, flavonoids, phenols, cardiac glycosides, saponins, phlorotannins, and tannins. Notably, flavonoids and phenols, recognized for their potent antioxidant properties, play a significant role in healthcare. These constituents contribute to the plant's pharmacological properties and potential medicinal uses, have been utilized in treating diverse health conditions, and have demonstrated antioxidant capabilities. In Ayurvedic formulations, the entire plant addresses kidney disorders [2].

The plant is prepared as a decoction to alleviate swellings, stomach aches, and diarrhea. Additionally, it serves as a diuretic and is utilized for managing menstrual pains. Furthermore, it functions as a diuretic and aids in relieving menstrual pains. Additionally, the seeds serve as an anthelmintic agent. The seeds are employed as an anthelmintic agent, while the leaves treat diverse conditions, including analgesic, antimicrobial, antipyretic, and anti-inflammatory. Oxidative stress arises from an imbalance between the production of free radicals and the body's ability to counteract or eliminate their

harmful effects by neutralizing them with antioxidants [3]. Free radicals are reactive molecules that interact with other substances, causing damage to cells, tissues, or organs. These radicals result from reactive oxygen species encompassing highly reactive oxygen-containing molecules. Free radicals can interact with enzymes, membrane lipids, proteins, nucleic acids, and other small molecules. Antioxidants, whether synthesized within the body or obtained through the diet, are a natural defense mechanism against damage caused by free radicals [4], [5].

The plant is extensively found in tropical and subtropical regions globally. Its leaves, abundant in medicinal attributes, are employed for their analgesic, antipyretic, antibacterial, and antifungal properties [6]. Its considerable medicinal worth has led to its traditional application in Ayurveda for treating fevers. The plant's decoction or infusion is known to relieve spasms in the urinary bladder, and it also exhibits therapeutic potential against conditions like cholera, asthma, cancer, cough, colic pain, night blindness, diarrhea, dysentery, and impotence. Cyanthillium cinereum plant contains antioxidant compounds like flavonoids, catechins, and tannins, which shield (AAPH) oxidation in human red blood cells [7], [8]. Cinnamon, encompassing Cinnamomum zeylanicum and Cinnamon cassia, is a perennial tree in the Lauraceae family, renowned in tropical medicine. Cinnamaldehyde and trans-cinnamaldehyde (Cin), crucial components found in the essential oil, play a key role in cinnamon's various biological activities and aroma. Research on Cinnamomum zeylanicum demonstrated that the essential oil extracted from cinnamon leaves is rich in (E)-cinnamaldehyde, which exhibits antityrosinase activity. Cinnamon bark contains procyanidins and catechins, which specifically demonstrate antioxidant properties [9].

Antioxidant activity is vital for health as it combats oxidative stress caused by free radicals, preventing cellular damage and inflammation. The significance of antioxidant properties lies in their ability to counteract free radicals, mitigating oxidative stress and safeguarding cells from potential damage. Thereby, it has a crucial role in neutralizing harmful oxidative reactions, contributing to the body's overall well-being. By reducing oxidative damage, antioxidants support cardiovascular health, boost the immune system, and potentially mitigate the risk of chronic diseases like cancer. This is pivotal for maintaining overall health and exploring applications in diverse fields such as medicine and nutrition. Understanding the cytotoxicity of herbs is paramount for evaluating their safety and therapeutic potential. A cytotoxicity-based study investigates how herbal compounds affect living cells, providing insights into their impact on human health. This analysis is crucial in drug development, ensuring that herbal formulations do not harm normal cells while targeting diseased ones. By assessing cytotoxicity, researchers can identify potential risks and benefits, guiding the responsible use of herbs in medicine. This approach is fundamental in unveiling the nuanced interactions between herbs and cellular processes, fostering a safer and more effective integration of herbal remedies into healthcare practices. Hence, the present work aims to analyze the synergistic antioxidant and cytotoxic properties of Cyanthillium cinereum and cinnamon extracts.

2. Materials and Methods

Powdered plant samples were purchased, and biologically active components of the powdered sample were extracted using polar solvents like water. Aqueous extraction is more effective than ethanol extraction because water has high polarity and shorter chains. 10g of cyanthillium cinereum powder & 10g of cinnamon powder is taken & added with 100mL water. This mixture & was evaporated to dryness at 60-90 degrees C using a rotary evaporator until the solvent layer was completely volatilized. The concentrated aqueous extract is further used to screen for this herbal synergy's antioxidant activity and cytotoxicity. The aqueous extract has biologically active chemical components like saponins, flavonoids, terpenoids, glycosides, alkaloids, proteins, amino acids, carbohydrates, and phenols.

Antioxidant Activity

1) Diphenyl-1-Picrylhydrazyl (DPPH) Assay

Percentage of antioxidant activity is assessed by DPPH for radical assay in which samples reacted with stable DPPH radical in methanol solution. Reaction mixture contains 10μg/mL of aqueous extract in 1mL methanol, 20μg/mL, 30μg/mL, 40μg/mL, and 50μg/mL. When DPPH reacts with antioxidant compounds which can donate hydrogen, it is reduced. Absorbance (change in color) of deep violet to light yellow was read at 517 nm after 30 min reaction using UV-VIS spectrophotometer. The control solution prepared with methanol(1 ml) and DPPH radical solution (1 ml) acts as blank.

2) Hydrogen Peroxide (H_2O_2) Assay

Potassium permanganate solution of known strength is used to quantitatively oxidize hydrogen peroxide in a diluted portion of the sample through titration. This hydrogen peroxide turns $KMnO_4$ pink, as it is a strong oxidiser, but adding this prepared aqueous extract to this has reduced its oxidizing ability & the color change is observed to be slow and light showing that this solution inhibits oxidation proves that it is a good antioxidant. H_2O_2 is a key free radical within biological systems which induce cell membrane damage that decreases cell viability by causing oxidative damage in genomic DNA & mtDNA. Protective effect of aqueous extract against H_2O_2 is observed.

Cytotoxicity

3) Brine Shrimp Assay

The experiments used multiwell plates containing 5 ml of filtered (0.45μm pore diameter) and sterilized seawater. Each plate accommodated 10 viable nauplii, and aqueous extracts, prepared synergistically from herbs, were introduced

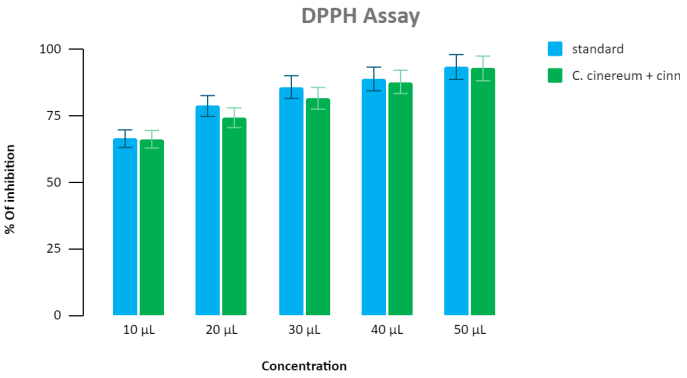

Figure 1: Antioxidant activity of Aqueous extract of Cyanthillium cinereum and Cinnamon (DPPH Assay)

Concentration	Absorbance	
	Ascorbic acid (Standard)	Aqueous extract of Cyanthillium cinereum and Cinnamon
10 μL	66.25	66.05
20 μL	78.52	74.12
30 μL	85.63	81.42
40 μL	88.68	87.58
50 μL	93.15	92.62

Table 1: Antioxidant activity of Aqueous extract of Cyanthillium cinereum and Cinnamon (DPPH Assay)

at varying concentrations (5μl, 10μl, 20μl, 40μl, and 80μl) along with a control. To facilitate optimal diffusion, 5 ml of seawater was gently shaken into each well. Ten replicates were executed for both treatment and control groups. The control involved the addition of the solvent used for extract dissolution, which allowed it to evaporate. Subsequently, the samples were left undisturbed for 24 hours to observe their effects.

3. Results

Antioxidant activity obtained by DPPH ASSAY (517 nm) showed that antioxidant activity of aqueous extract of Cyanthillium cinereum and Cinnamon is similar to the standard ascorbic acid value at 10 μl concentration, whereas in all other concentrations antioxidant values of aqueous extract of Cyanthilium cinereum and Cinnamon was lesser when compared with ascorbic acid. With the increase in the concentration, antioxidant values of the aqueous extract of Cyanthilium cinereum and Cinnamon raised (Table 1, Figure 1).

Antioxidant activity obtained by H_2O_2 ASSAY (517 nm) showed that the antioxidant activity of the aqueous extract of Cyanthillium cinereum and Cinnamon is lesser than the standard ascorbic acid value at all concentrations, whereas, with an increase in the concentration, antioxidant values of aqueous extract of Cyanthilium cinereum and Cinnamon increased (Table 2, Figure 2).

Cytotoxicity of aqueous extract of Cyanthilium Cinereum and Cinnamon increases with the increase in the concentration. 8 nauplii were viable at the concentration of 5 μl, and about 5 were viable at 80 μl (Table 3).

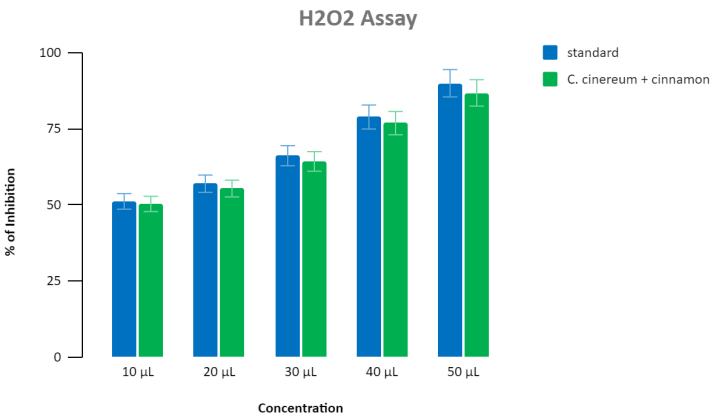

Figure 2: Antioxidant activity of Aqueous extract of Cyanthillium cinereum and Cinnamon (H_2O_2 Assay)

Concentration	Absorbance	
	Standard	(Cyanthillium cinereum+Cinnamon) aqueous extract
10 μL	51.1	50.27
20 μL	56.9	55.32
30 μL	66.1	64.22
40 μL	78.8	76.8
50 μL	89.9	86.72

Table 2: Antioxidant activity of Aqueous extract of Cyanthillium cinereum and Cinnamon (H2O2 Assay)

4. DISCUSSION

The therapeutic benefits of phytoconstituents are because they are chemical substances that elicit specific physiological effects on the human body. Substances such as glycosides, alkaloids, saponins, tannins, calcium, flavonoids, and phosphorus promote cell growth, facilitate tissue replacement, and contribute to body development [10]. Over the past two decades, the emergence of drug resistance and unwanted side effects from specific antibiotics has prompted the exploration of novel antimicrobial agents, primarily focusing on plant extracts. The objective is to identify new chemical structures with potential therapeutic benefits [11], [12].

Antioxidants play a pivotal role in the advancement and survival of humans, addressing free radicals and combating damage associated with metabolic diseases and age-related syndromes in humans and other animals [13], [14].

Lipid peroxidation results in increased oxidative stress within cells, causing damage to genomic and mitochondrial DNA. This damage can ultimately contribute to precarious cellular conditions, such as apoptosis or the formation of tumors. By disrupting the structural lipid bilayer, membrane

Aqueous extract of Cyanthilium cinereum and cinnamon (μl)	Viable nauplii (% of death)
5μl	8 (20%)
10μl	7 (30%)
20μl	7 (30%)
40μl	6 (40%)
80μl	5 (50%)

Table 3: Cytotoxic activity of aqueous extract of Cyanthilium cinereum and cinnamon

lipids' peroxidation can impact the durability of ligand-binding domains within the membrane. This, in turn, can derange membrane transport proteins and deactivate enzymes associated with the membrane, resulting in changes to downstream signal transduction cascades. Additionally, oxidative harm to the membrane alters its permeability and interferes with ionic channels, potentially triggering various diseases. The ability of antioxidant compounds to inhibit lipid peroxidation is crucial, as it enables them to mitigate the initiation and/or progression of diseases linked to oxidative stress. Thus, Cyanthillium cinereum could serve as a preventive remedy against oxidative stress, safeguarding cellular macromolecules and membranes from damage and thereby preserving the functional and structural integrity of the cells.

Hydrogen peroxide has effectively induced oxidative stress in animals and cell cultures. In the case of isolated frog hearts, this oxidative stress was induced, and it was noted that the ethanolic extract from the leaves of Cyanthillium cinereum demonstrated antioxidant activity against H2O2-induced oxidative stress in the isolated frog heart model. This activity was then compared with a standard antioxidant agent, namely Ascorbic acid [15], [16]. Several prior studies propose the medicinal values of Cyanthillium cinereum against various illness. Nevertheless, its effects on oxidative stress, cytotoxicity, and genotoxicity have not been investigated. This study employed various free-radical generating systems to examine the antioxidant properties of crude polar and non-polar extracts of Cyanthillium cinereum in scavenging free radicals. The assessment included evaluating total phenolic content, reducing power, and the protective effects of the extracts against DNA and cell damage. Additionally, the cytotoxicity of the extracts was tested on MDA-MB-435S cell lines [17].

A study reported that the antimicrobial efficacy of Cyanthillium cinereum leaves is notably robust against Staphylococcus aureus, Streptococcus mutans, and E. faecalis in comparison to the whole plant extract of Cyanthillium cinereum and superior anti-inflammatory activity when compared to established standard values [18]. Research reported the substantial antioxidant properties of diverse cinnamon extracts, including ether, aqueous, and methanolic variations [19]. In a rat study, the administration of 10% C. verum bark powder for 90 days demonstrated antioxidant effects, evident through the activity of hepatic and cardiac antioxidant enzymes, lipid-conjugated dienes, and glutathione. Another study by a research group suggested that cinnamon oil may possess superoxide-dismutase (SOD)-like activity, as reflected in its ability to inhibit the inhibiting capacity of pyrogallol autoxidation. Furthermore, different flavonoids extracted from Cinnamon demonstrate the properties of scavenging free radicals and exhibit antioxidant characteristics. An analysis of the inhibitory effects of cinnamaldehyde and other compounds from Cinnamon on nitric oxide production revealed that cinnamaldehyde demonstrates potential activity in suppressing both inducible nitric oxide expression and nitric oxide production [20]. The administration of cinnamon extract, whether orally (OA) or through intratumoral injection (IT), markedly decreased tumor cell proliferation rates in vivo by significantly enhancing the cytolytic activity of CD8+T cells [21].

Flavonoids are widely recognized for their robust antioxidant properties. Polyphenolic compounds and tannins are established as effective natural antioxidants. Flavonoids possess the capability to neutralize reactive oxygen species directly. Additionally, they can chelate free radicals through hydrogen atom donation or single-electron transfer. Flavonoids exhibit intracellular antioxidant activity by inhibiting enzymes that generate free radicals, such as xanthine oxidase, lipoxygenase, protein kinase, etc. Phenolic compounds, with their redox properties, serve as robust antioxidants. Therefore, the observed potent antioxidant potential of this herbal combination is deduced. Antioxidants choline, μ-Carotene, μ-Carotene, μ-cryptoxanthin, Lycopene, Lutein, and Zeanthin are found in Cinnamon. Flavonoid compounds (Gossypin) present in Cinnamon have anti-inflammatory properties [22]. Cyanthillium cinereum plant extract exerted dose-dependent cytotoxic and cell viability effects. Cyanthillium cinereum exhibited a significant reduction in cell viability compared to control in all MCF 7 breast cancer cells [17], [23].

While Cyanthillium cinereum is acknowledged for its demonstrated antioxidant properties, attributed to its ability to safeguard against oxidative harm to biological macromolecules such as lipids and DNA, it also exhibits anti-inflammatory effects. In this study, the aqueous extract of a combination of Cyanthillium cinereum and Cinnamon showed significant radical scavenging activity in both the in vitro models like DPPH scavenging and H_2O_2 assay, and cytotoxicity activity was studied in Brine Shrimp Assay. Surprisingly, 50% nauplii was observed to be dead, and cell viability was reduced with increased concentration like 5μl, 10μl, 20μl, 40μl and 50μl this synergistic herbal formulation is observed to exert more anti-inflammatory activity than individual extract.

Antioxidant and cytotoxicity studies on this herbal combination underscore their potential therapeutic benefits. These findings contribute valuable insights into the herbs' ability to mitigate inflammation and exhibit cytotoxic effects, suggesting their possible utility in pharmaceutical and medicinal applications. Further research is warranted to explore and harness the full spectrum of their therapeutic capabilities.

5. Conclusion

This in vitro study on this herbal combination underscores their potential therapeutic antioxidant and cytotoxicity effects. Cyathillium cinereum exhibits notable antioxidant properties, showcasing its potential in combating oxidative stress. Simultaneously, the cytotoxicity assessment sheds light on the impact of these substances on cell viability. These findings collectively contribute to understanding the therapeutic potential of Cyathillium cinereum and cinnamon, emphasizing the need for further exploration and clinical

investigations to validate their applications in health and medicine.

Acknowledgements

The authors would like to thank all data collectors and study participants involved in the study.

Conflict of Interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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