RESEARCH ARTICLE

Phytochemical Investigations and *In-vitro* Antioxidant Analysis of *Passiflora incarnata* L.

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ABSTRACT

The present study aims to investigate the presence of the phytochemical and *in-vitro* antioxidant study of hydroalcoholic (HA) and ethyl acetate (EA) preparations of *Passiflora incarnata* L. leaves. The hydroalcoholic (HA) and ethyl acetate (EA) extracts showed strong antioxidant activity when examined in a laboratory setting using DPPH, H2O2, NO, reducing power, total phenol, and TOC assays. Also compounds like saponin, alkaloids, flavonoids, and phenolics were all identified through phytochemical testing. In addition to expanding our understanding of the pharmacological characteristics of *P. incarnata* L., the results of this study have the potential to change the way medicinal botanicals are viewed forever. Nature's treasure trove, concealed within the leaves of *P. incarnata* L., offers a pharmacological narrative that transcends traditional boundaries, suggesting new dimensions for antioxidant-based therapeutic strategies for the development of novel botanical interventions in preventive healthcare and disease management.

Keywords: Antioxidants, Medicinal plants, *Passiflora incarnata* L.

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INTRODUCTION

The investigation of botanical sources is becoming an ever more pressing necessity in the search for novel therapeutic agents that can be utilized in a variety of different pharmacological contexts.[1-6] Analgesia, spasticity, asthma, worms, hysteria, and neurasthenia are some of the conditions treated with *Passiflora incarnata* L. in Poland. In the US, it's prescribed for a variety of medical conditions, including headaches, neuralgia, burns, hemorrhoids, epilepsy, neurosis, insomnia, and burns. People who suffer from opiate addiction can find relief through its usage in India.[7-11] This research article embarks on a journey of phytochemical investigations and *in-vitro* antioxidant analysis, seeking to unravel the molecular intricacies that underlie its pharmacological prowess. Among the many kinds of secondary metabolites discovered in *P. incarnata* L. are phenolic chemicals, alkaloids, diterpenoids, and flavonoids.[11-17] The metabolic actions of these many secondary metabolites are distinct from one another. To find out what these chemicals are and how much of them there is in *P. incarnata* L. we wish to use state-of-the-art analytical techniques to carefully examine the plant's phytochemical composition.[13,18-22] This will set the stage for understanding the plant's possible medical applications. *P. incarnata* L.'s potential for using its antioxidant properties is a major selling point for the plant. In order to protect oneself from oxidative stress, which has been associated with numerous degenerative diseases, this quality is crucial. The byproducts of cellular metabolism known as reactive oxygen species (ROS) are a persistent danger to the integrity of cells and constitute a hazard to cellular health.[23-27] The *in-vitro* antioxidant study of *P. incarnata* L. is of the utmost importance in determining whether or not it is able to deactivate these harmful ROS, hence reducing the amount of oxidative damage caused.[10,11,15] Our research intends to measure and qualify the antioxidant potential of *P. incarnata* L. by conducting a number of rigorous studies, such as radical scavenging assays, determination of total antioxidant capacity, and assessment of enzymatic antioxidant activities.[28-30] Our goal is to shed light on the therapeutic implications of this botanical entity in the prevention and management of illnesses that are associated to oxidative stress by clarifying the various mechanisms via which it combats oxidative stress.[22,31] As we get started on this scientific journey, it becomes clear that combining phytochemical research with antioxidant testing is a potentially fruitful path to go in the direction of deciphering the medicinal mysteries that are concealed inside *P. incarnata* L.[27,32,33] The results of this research have the potential to not only further our understanding of the pharmacological properties of the plant but also to open the way for the creation of revolutionary therapeutic approaches based on antioxidants.

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MATERIAL AND METHODS

Ingredients for Preparation of Herbal Extract

The plant material of *P. incarnata* L. was collected and authenticated. The first step in extracting the leaves is washing them under running water to remove any dirt or other particles that may have adhered to them. The next step is drying them in an oven at a temperature of 35 to 40°C. The local collection of *P. incarnata* L. leaves was followed by shade drying and grinding into a coarse powder. Using the Soxhlet apparatus, a mixture of hydroalcohol in a 60:40 ratio and 100% ethyl acetate was used to extract 250 g of dried plant leaves. Then they were kept at 4°C until they were required.[24-29]

Method

Phytochemical analysis

Each consecutive extract was made using a 1% (W/V) stock concentration of ethyl acetate and hydro-alcohol using the matching solvents. Using conventional procedures, the presence of active phytochemicals such as saponin, alkaloid, terpenoid, steroid, glycoside, flavonoid, tannin, protein, carbohydrate, amino acid, and fixed oil was qualitatively assessed in these extracts and those used as controls.[30,31]

In-vitro antioxidant activity

- *Assay of reducing power*

Using ascorbic acid as a reference, the inhibitory concentration (IC_{50}) value was computed by using standard method.[32]

- *Assay of H_2O_2 scavenging activity*

An IC_{50} value is the concentration of a substance at which it inhibited H2O2 by a magnitude equal to half was computed by using standard method.[33]

Nitric oxide radical scavenging activity

In order to determine the nitric oxide scavenging activity, the Griess reagent was employed and further assays was performed using standard methods.[34,35]

Table 1: Phytochemical analysis

S. No	*Phytoconstituent*	*P. incarnata L.*	
		EA (Ethyl acetate)	*HA (Hydroalcohol)*
1	Saponin	+	+
2	Alkaloid	-	+
3	Terpenoid	-	-
4	Steroid	-	-
5	Glycosides	-	+
6	Flavonoids	+	+
7	Tannins	+	+
8	Proteins	-	-
9	Carbohydrates	+	+
10	Amino acids	+	-
11	Fixed oils	-	-

TAOC

After the absorbance at 695 nm was measured, a 0.3 mL volume of plant sample with 1 mg/mL of phosphomolybdate was heated to 95℃ for 10 minutes. Then, following a range of 10 to 320 g/mL for ascorbic acid in the calibration curve, the total absorbance concentration (TAC) was calculated [36].

Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical scavenging activity [37]

The assay for DPPH was carried out by using standard procedure.[37]

Estimation of total phenolic compounds

The Folin-Ciocalteau reagent was used to estimate gallic acid, a standard phenolic compound and the estimation of total phenolic content was measured using standard protocol.[38]

RESULTS

Phytochemical Analysis

The study of plants and their bioactive components is known as phytochemical analysis, and it is an important branch of science. Phytochemicals encompass an extensive array of secondary plant metabolites, including but not limited to alkaloids, flavonoids, terpenoids, and phenolic compounds. To put traditional applications of medicinal plants into context, establish a scientific basis for them, and pave the path for the development of novel pharmaceuticals or alternative treatments, phytochemical study is essential (Table 1).

In-vitro Antioxidants

DPPH free radical scavenging activity

The IC_{50} values for EA (197.34 µg/mL) and HA (172.85 µg/mL) and ascorbic acid were 119.85 µg/mL. The results show that both plant extracts are just as effective as the gold standard, ascorbic acid, for scavenging DPPH (Figure 1).

Reducing Power Activity

The reducing power of both extracts was observed to increase with concentration, reaching its peak at 320 µg/mL. EC50 values of 27.27 µg/mL for EA and 28.77 µg/mL for HA were recorded. Ascorbic acid standards were 19.32 µg/mL (Figure 2).

Hydrogen Peroxide-scavenging Activity

The highest degree of hydroxyl radical inhibition that was detected in the extract of EA and HA was determined to

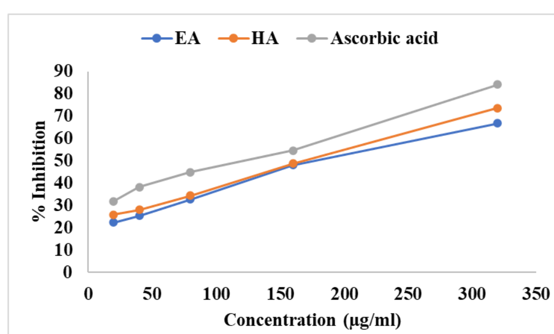

Figure 1: DPPH free radical scavenging activity

be 62.37 and 68.531%, respectively, when the maximum concentration was 320 µg/mL. Ascorbic acid, on the other hand, demonstrated an inhibition of 79.41%. It was demonstrated that the extracts' radical scavenging capabilities were concentration-dependent. Extracts of EA and HA were determined to have an IC50 value of 220.44, 193.11 µg/mL and ascorbic acid was 145.92 µg/mL (Figure 3).

Total Antioxidant Capacity (TAC)

The antioxidant potential of EA was 80.73 mg/AAE, while that of HA was 139.26 mg/AAE (Ascorbic acid equivalent) (Figure 4).

Nitric Oxide Radical Scavenging Activity

In NO scavenging activity, IC_{50} value of EA 216.17, HA 196.45 µg/mL while that of ascorbic acid was found to be 154.44 µg/mL (Figure 5).

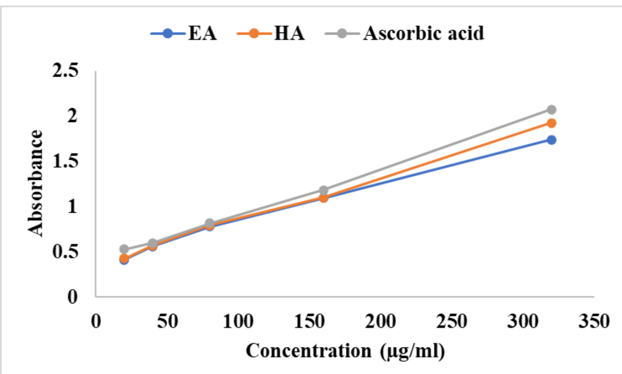

Figure 2: Reducing power activity

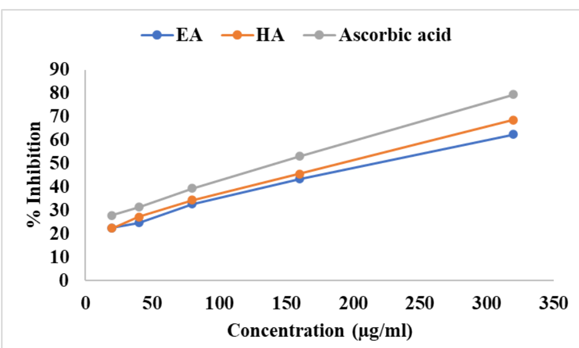

Figure 3: Hydrogen peroxide-scavenging activity

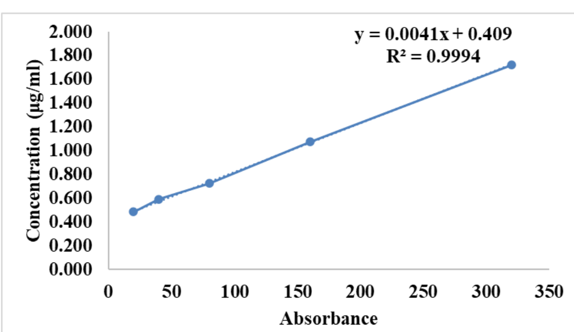

Figure 4: Total antioxidant capacity (TAC)

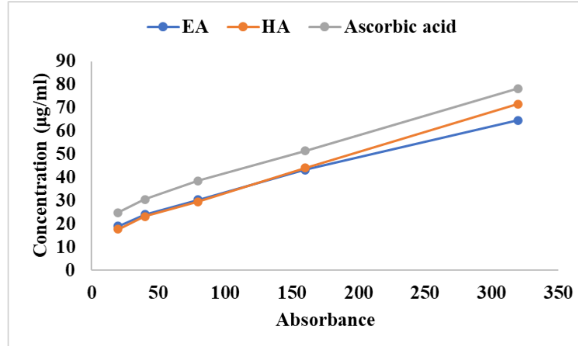

Figure 5: Nitric oxide radical scavenging activity

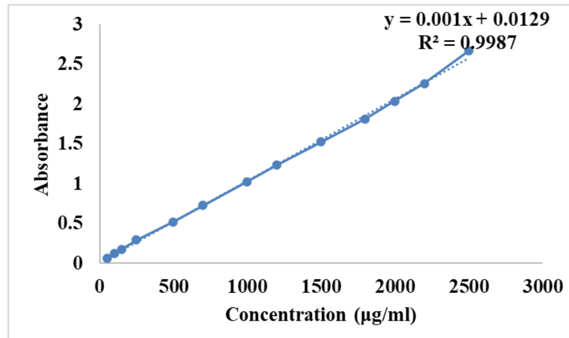

Figure 6: Total phenol content

Total Phenol Content

The total phenol concentration of the EA extract was 499.1 mg/ GAE, whereas the HA extract had a level of 507.1 mg/ GAE (Figure 6).

CONCLUSION

In conclusion, our comprehensive exploration into the phytochemical composition and *in-vitro* antioxidant potential of *P. incarnata* L. has illuminated the multifaceted pharmacological prowess of this botanical treasure. The richness of secondary metabolites, including flavonoids, alkaloids, and phenolic compounds, underscores the complexity and potential therapeutic value inherent in this succulent plant. The *in-vitro* antioxidant analyses have unequivocally demonstrated *P. incarnata* L. capacity to neutralize reactive oxygen species (ROS) and mitigate oxidative stress, positioning it as a promising candidate for addressing conditions associated with oxidative damage. *P. incarnata* L. has strong antioxidant potential, according to the results of radical scavenging assays, total antioxidant capacity evaluations, and enzymatic antioxidant activity evaluations. Botanical therapies for disease prevention and management have new potential thanks to the synergy between the detected phytochemicals and antioxidant properties. *P. incarnata* L. is not only a pretty flower, but it may also contain medicinally useful chemicals when we look at the bigger picture of our research. The results of this study lay the groundwork for future studies and possible translational applications in botanical medicine, which is a complex field with many moving parts. Harnessing the phytochemical richness of *P. incarnata* L. may offer innovative solutions to combat oxidative stress-related disorders and contribute to the expanding repertoire of natural therapeutic interventions. In the pursuit of sustainable healthcare solutions, the integration of *P. incarnata* L. into the pharmacological arsenal represents a paradigm shift, emphasizing the importance of exploring nature's reservoirs for novel bioactive compounds. As we conclude this exploration, the *P. incarnata* L. attention not only for its aesthetic appeal but for its potential to redefine our approach to health and wellness through the harnessing of phytochemicals and antioxidants.

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