



A Folk Medicine: *Passiflora incarnata* L. Phytochemical Profile with Antioxidant Potency

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ABSTRACT

Objectives: *Passiflora incarnata* L., commonly called folk medicine declaredly used for an enormous range of therapeutic purposes, one such is antioxidant potency. The study prioritized to determine the phytochemical analysis of total phenolics, flavonoids, alkaloids, and tannins contents as well as the antioxidant properties through 1,1-diphenyl-2-picrylhydrazyl (DPPH) quenching assay, 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation decolorization test, superoxide, and hydrogen peroxide radical scavenging assays of ethanol extract of *P. incarnata* leaves.

Materials and Methods: The organoleptic characteristics such as color, odor, appearance, taste, and other characters such as drying range and fiber contents were analyzed as preliminary data. Analytical parameters like total phenolic content, total tannins, total alkaloid content, and total flavonoid with multiple antiradical scavenging activity (DPPH, ABTS, superoxide and H₂O₂ scavenging assays) with IC₅₀ (µg/mL) in terms of inhibition percentage with various concentrations of the ethanolic extract studied.

Results: *P. incarnata* possessed a high radical scavenging activity with a phenolic content of 2.48 mg gallic acid equivalent/g of extract in leaves, whereas the total flavonoid content was 2.1, respectively.

Conclusion: High antioxidant activity was noticed in *P. incarnata* extract, in which might be of higher levels of flavonoids and phenols. Findings in the studies revealed that *P. incarnata* is a veritable source for antioxidant drug bioprospecting in scientific research and pharmaceutical industries.

Key words: *Passiflora incarnata*, phytochemical profile, antioxidant activity, DPPH, ABTS, radical scavenging activity

INTRODUCTION

Singlet oxygen is formed in the biological system in aerobic organisms for normal cell functions, if exceed, the reactive oxygen species (ROS) level in the living system causes oxidative stress and leads to oxidative damage. These ROS intermediate threaten various biomolecules including proteins, enzymes, lipids, and DNA^{1,2} and also cause physiological errors like blocks in arteries, strokes, cancer, and nervous disorders, which highly possess to increase in the study of compounds that protect against ROS and can prevent diseases.^{3,4} As a result, high priority to be enlightened to the purpose of antioxidants, particularly organic-based to protect from damage due to free radicals.⁵ Antioxidants, compounds that can delay or prevent the oxidation of biomolecules by blocking engagement of oxidative progress that can avoid or restore the damage by ROS.⁶ However, synthetic antioxidants, e.g. propylgallate, butylated

hydroxyanisole, butylated hydroxytoluene, and tertiary butylhydroquinone, are recognized as good oxidative costs, yet, they have limitations because of their carcinogenic effects on the lungs and liver parts.⁷ So, recently, several excessive efforts have been outworted to safeguard potent organic tagged antioxidants from ethnomedicinal sources.

Natural antioxidants, principally from plants with that sort of phenolic compounds, vitamin C, and carotenoids. Ethnomedicinal-based compounds have multiplex biological effects, including antioxidant potentials with its phytoconstituents including phenolics and flavonoids.⁸ Nutritional components from plants are the dynamic cause of various classes of polyphenolic components and some flavonoids.⁹ Some phytomedicines are traditional, among them, *Passiflora* sp. has been reported as folk medicine. *Passiflora* genus, Passifloraceae, includes about 520

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species,¹⁰ which are spotted mostly in tropical and subtropical regions of the world. *Passiflora incarnata* L., *P. alata* Curtis, *P. mucrinata* Lam., and *P. edulis* Sims reveal their potential biological activity by its various phytometabolites such as phenolic substances, alkaloids, and flavonoid contents, and it is known for its sedative properties as well as in the food and pharmaceutical industry.¹¹ In spite of extensive research on plant products, the efficacy of plant sources as novel drugs is still meagerly documented. Only a least fraction has been studied phytochemically and therefore, the fractions recommended for biological or pharmacological screening are even smaller. Hence, an attempt made to evaluate the leaves of *P. incarnata* to reveal its antioxidant potential.

MATERIALS AND METHODS

Collection of plant material

P. incarnata was collected from Keezanatham, Ariyalur (Dt.), Tamil Nadu, India (Figure 1). Identification of plant material was validated by Prof. Jegadeesan Head, Department of Environmental and Herbal Sciences, Tamil University, Thanjavur). Furthermore, it was confirmed with Herbarium sheets available in the Rabinat Herbarium, St. Joseph's College, Thiruchirappalli, Tamil Nadu, India. The specimen was kept in the Herbarium of Arignar Anna Government Arts College, Department of Botany (AAGAC/BOT-07). The fresh and fully-grown plant leaves were selected. The collected plant leaves were cleaned to remove mud and other adhering weed plants. Fresh leaf samples were desiccated at the room temperature and then shade-dried for 2-3 days and powdered mechanically, sieved using 80 meshes, and refrigerated.¹²

Preliminary phytochemical studies

Organoleptic characters

Color, odor, appearance, taste and other characters like drying range and fiber contents of the grounded sample were determined. The sample (2 g) was allowed to dry in a tarred dish and with the temperature of 100-105°C, it was then allowed to cool and weigh again.



Figure 1. *Passiflora incarnata* L.
Photo courtesy: P. Subramaniam

Analytical parameters

Total ash

5 g sample was exposed in silica crucible, which is ignited prior, allowed to cool and weighed. It was allowed to incinerate with slow progression of heat, up to 450°C, allow to cool and weigh again. The percentage of total ash was calculated using the reference range and repeated again until, a constant weight was noted.

Acid-insoluble ash

Total ash was allowed to boil with 25 mL (10%) of diluted HCl for about 5 min and filter it, then ignite it to obtain acid insoluble ash.

Water-soluble ash

To check the water-soluble ash, a portion of the total ash was allowed to boil in 25 mL of H₂O for 5 min and wash the filtered debris with hot water. The water-soluble ash was calculated.

Water soluble ash= Total ash value - Water-insoluble ash

Sulfated ash

A portion of the sample was ignited with 1 mL of H₂SO₄. It was then cooled, and percentage of sulfated ash was calculated.

Extractive values

The dried sample (5 g) was impregnated with moderately hot petroleum ether overnight. The extract was concentrated and weighed.¹³

Preparation of the extract

The shade-dried leaves were pulverized to get a coarse powder.¹⁴ The ground plant material (1 kg) was soaked separately in ethanol for 48 hrs. The aqueous extraction was gained through filtration method. The extract was then subjected to dryness in an evaporator under controlled pressure and temperature (40-50°C).

Chemicals

2,2-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1, Folin-Ciocalteu's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, Folin-Danis reagent, bromocresol green solution (BCG), dimethyl sulfoxide (DMSO), potassium persulfate, methanol, and sodium carbonate were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Estimation of total phenolic content

The extract was allowed to incubate for 5 minutes prior with 0.3 mL of Folin-Ciocalteu's reagent. Na₂CO₃ (7%, 10 mL) solution was mixed and incubated for 2 h absorbance was measured at 740 nm. Quantification was conducted using a gallic acid (GA) as the standard. The results are expressed as milligrams per gram dry weight.¹⁵

Estimation of tannins

Folin-Danis reagent (0.5 mL) was added to each tube containing different concentrations of the sample and kept for 3 minutes. Further, 2 mL of 20% Na₂CO₃ solution was added and gently vortex. The test tubes were kept in boiling

for 1 min and cooled down. The absorbance was measured at 650 nm.¹⁶

Estimation of total alkaloid content

A portion of extract residue was dissolved in 2N HCL for 20 minutes and then filtered. 1 mL solution was transferred to a separatory funnel and washed with 10 mL chloroform (thrice). The hydrogen ion range of the solution was adjusted to neutral. BCG with phosphate buffer (5 mL) was mixed with the mixture. The extract with chloroform was mixed by continuous shaking, then the extract was collected in a 10 mL flask, and diluted again with chloroform. The precipitate was collected and dried at 105°C to constant weight and weighed.

Estimation of total flavonoid content

The sample (10 g) was impregnated with 60 mL methanol and allowed to stand overnight. The residue was filtered and washed twice with 20 mL methanol. Filtrate was washed and concentrated to 10 mL. The concentrated solution was added drop wise continuously shaken into 100 mL ether. Mix vigorously for 10 minutes and allowed to stand for 10 minutes to settle. Filtrate was evaporated to dry and calculated.

In vitro antioxidant activity

DPPH radical scavenging activity

The plant extract at different concentrations was diluted with DMSO to get a sample solution. The sample (5 µL) was seeded in a 96-well plate followed by 195 µL DPPH working solution to each well. After 20 min reaction, the absorbance was measured at 515 nm. The free radical activity of the extract was determined by comparing its absorbance with blank.¹⁷ The scavenging ability by DPPH radical was expressed as a percentage of inhibition and was calculated.

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

where A_0 : Absorbance of the control, and A_1 : Absorbance of the sample.

ABTS radical scavenging activity

ABTS radical was formed with the addition of 5 mL of ABTS stock solution and 2.45 mM $K_2S_2O_8$ solutions respectively, and stored in the dark at room temperature for 16 hs. Before use, this solution was diluted and the absorbance was notably at 0.700 ± 0.020 at 734 nm and maintained at 30°C. The extract at various concentrations was diluted with DMSO, which counts for the sample solution. 5 µL of sample solution was mixed with 195 µL ABTS + solution, and incubated at room temperature for 6 min and the absorbance was recorded at 734 nm.¹⁸ ABTS scavenging activity was expressed as IC_{50} (µg/mL) and the inhibition percentage was calculated.

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

Hydrogen peroxide scavenging activity

Hydrogen peroxide was prepared with phosphate buffer (pH 7.4). Different concentrations of sample (200 µL) were mixed with 0.6 mL of H_2O_2 solution. A test tube containing 200 µL of phosphate buffer was processed as discussed above act as a control. Different concentrations of ascorbic acid were used as

the reference compound. Absorbance of H_2O_2 was determined against a blank.¹⁹

Superoxide scavenging activity

The sample (200 µL) of different concentrations was taken in test tubes. Superoxide radicals were produced by equal addition of 1 mL of Tris-HCl buffer (16 mM, pH-8), nitro blue tetrazolium (50 µM), nicotinamide adenine dinucleotide (78 µM) solution and phenazine methosulphate (10 µM) respectively. The mixtures were then incubated at 25°C for 5 min and measured the absorbance (560 nm).²⁰

Statistical analysis

The experimental results were expressed as \pm standard deviation. Data were analyzed with ANOVA and determined by Duncan's Multiple Range test using Graph Pad Prism software version 5.0 (San Diego, USA).

RESULTS

The organoleptic characteristics and analytical parameters of the leaf powder of *P. incarnata* were studied (Table 1). The ratio of active chemical components in unpolished drugs are mainly based on air-dried. Therefore, ranging the dryness of plant materials should be evaluated, particularly for the materials that imbibe moist easily. The residue remaining after incineration of the plant material is the ash content, which simply represents some inorganic salts and occurs naturally in unprocessed crude drug materials.

Phytochemical analysis

The total phenolic content in examined leaf extract was 2.8 mg GA equivalent/g and showed flavonoids (2.1 mg/g) but in merger range compared with phenolic contents (Table 2). Total tannin content of *P. incarnata* leaf extracts show 1.9 mg/g and alkaloids 0.031 mg/g. The high antioxidant activity was observed in *P. incarnata* extract, which evidenced the high level of flavonoids and phenolic presence in the plant (Table 3).

Table 1. Organoleptic characteristics of *Passiflora incarnata* leaves

| Appearance | Coarse powder |
|------------------|-------------------|
| Color | Green |
| Odor | No characteristic |
| Taste | Slightly bitter |
| % Loss on drying | 7.12 |
| Crude fiber | 9.4 |

Table 2. Analytical parameters

| Parameters | Value in w/w |
|--------------------|--------------|
| Total ash | 9.23 |
| Acid-insoluble ash | 10.3 |
| Water-soluble ash | 6.50 |
| Sulfated ash | 18.20 |

In vitro antioxidant activity

Inhibition% of DPPH by the extract at different concentrations (1, 2, 4, and 8 µg/mL) was observed to be 17.43 ± 0.31 , 30.12 ± 0.29 , 51.69 ± 0.43 , and 80.91 ± 0.37 , respectively, whereas the percentage inhibition of ascorbic acid was found to be 22.13 ± 0.28 , 39.87 ± 0.33 , 57.28 ± 0.25 , and 82.55 ± 0.41 . IC₅₀ values for DPPH scavenging activity of the leaf extract of *P. incarnata* and ascorbic acid were 4.30 µg/mL and 3.69 µg/mL, respectively (Figure 2, Table 4). The extract exhibited good ABTS radical scavenging activity and inhibition% (with various concentration 1, 2, 4, 8 µg/mL) was found to be 14.65 ± 0.17 , 32.16 ± 0.24 , 49.87 ± 0.30 , and 75.23 ± 0.33 , respectively, which were comparable to that of ascorbic acid 19.32 ± 0.21 , 40.28 ± 0.27 , 60.71 ± 0.31 , and 77.56 ± 0.29 . IC₅₀ values for scavenging ability on ABTS radical of the extract of *P. incarnata*, while IC₅₀ values by ascorbic acid were 4.60 µg/mL and 3.82 µg/mL, respectively (Figure 3, Table 5).

P. incarnata leaf extracts exhibited a strong scavenging effect on hydrogen peroxide were recorded as 12.43 ± 0.87 , 28.57 ± 0.67 , 41.32 ± 0.72 , and 62.84 ± 0.60 respectively (Figure 4, Table 6). Contrast, 18.21 ± 0.52 , 39.52 ± 0.41 , 50.43 ± 0.39 , and 66.16 ± 0.47 was noted in standard ascorbic acid. IC₅₀ values for H₂O₂ scavenging activity for ethanol extract of leaves of *P. incarnata* and ascorbic acid were 5.79 and 4.80 µg/mL. Superoxide radical scavenged by the plant extract at different concentrations were observed as 20.15 ± 0.45 , 29.42 ± 0.38 , 48.88 ± 0.35 , and 74.37 ± 0.51 for the leaf extract, while the reference was found to be 25.53 ± 0.29 , 37.19 ± 0.33 , 55.26 ± 0.34 , and 77.55 ± 0.39 respectively. IC₅₀ value of ascorbic acid was 3.90 µg/mL, while the leaf extract's was 4.63 µg/mL (Figure 5, Table 7). Values are

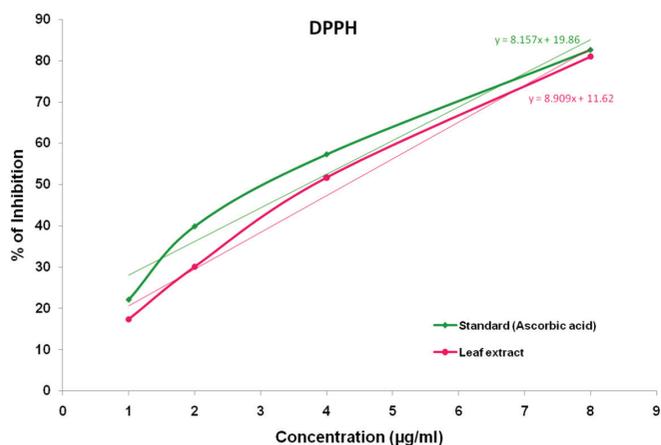


Figure 2. DPPH radical scavenging activity

Table 3. Polyphenol content of the ethanolic leaf extract of *Passiflora incarnata*

| Total phytochemicals | Leaf extract |
|-------------------------|--------------|
| Total phenolic content | 2.48 |
| Total tannins content | 1.9 |
| Total alkaloid content | 0.031 |
| Total flavonoid content | 2.1 |

the average of triplicate and represented as mean \pm standard deviation.

DISCUSSION

Uptrended studies have suggested the prevalence with increased levels of plant-derived compounds with low optional remedy rates of many diseases. These results suggest a connection between the protecting role of plant compounds in

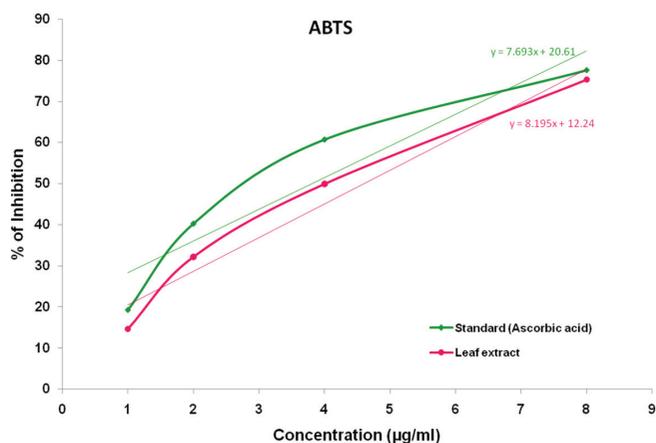


Figure 3. ABTS radical scavenging activity

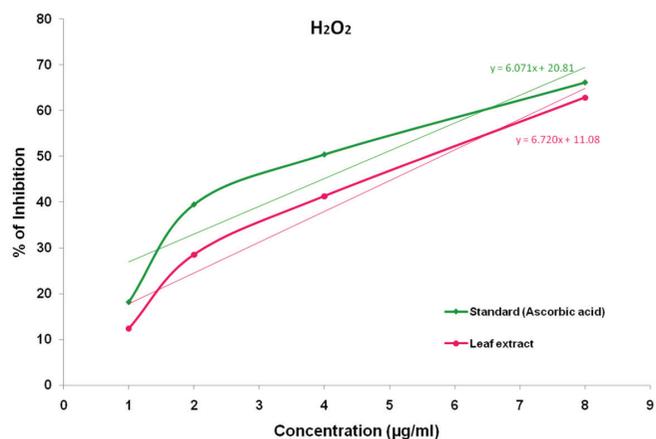


Figure 4. H₂O₂ scavenging activity

Table 4. DPPH

| Group | Concentration (µg/mL) | % of inhibition | IC ₅₀ value |
|--|-----------------------|------------------|------------------------|
| Ethanol extract of leaves of <i>Passiflora incarnata</i> | 1 | 17.43 ± 0.31 | 4.30 µg/mL |
| | 2 | 30.12 ± 0.29 | |
| | 4 | 51.69 ± 0.43 | |
| | 8 | 80.91 ± 0.37 | |
| Ascorbic acid | 1 | 22.13 ± 0.28 | 3.69 µg/mL |
| | 2 | 39.87 ± 0.33 | |
| | 4 | 57.28 ± 0.25 | |
| | 8 | 82.55 ± 0.41 | |

increasing the average life span of human health. Plant derived compounds have an enormous range of polyphenols, which play a prime role in minimizing the balance between free radicals and antioxidant potential. Substantially, it is needed to optimize these flavonoids to be acknowledged for their efficient action.²¹ Accumulated evidence has suggested that the ethanolic extracts of *P. incarnata* showed a prominent and potent *in vitro* antioxidant activity with high flavonoid contents (2.1 µg/mL). Flavonoids - a phenolic substance, labeled for its phytoconstituents of *P. incarnata*²² that can steadily repair the unpaired electrons located in its aromatic ring and can minimize the cause of free radical development. There are scientific reports showing that the number of phenolic compounds is directly proportional to antioxidant activity.²³ *In vitro* study has also evidenced a potential activity in aqueous and ethanolic extracts of *P. incarnata*.²⁴ A group of scientists from Italy studied the methanol extracts from five species of *Passiflora* obtained by zygotic embryo culture showed DPPH and ABTS radical scavenging activity.²⁵ Among the flavonoid constituents, in *P. incarnata*, vicenin, isovitexin, and orentin have major roles in the *in vitro* antiradical scavenging activity.²⁶ A number of flavonoid glycosides - isochoaftoside, schaftoside, isoorientin, orientin, vitexin, and isovitexin are considered standard markers to identify different *Passiflora*

species,²⁷ such as *P. edulis*, *P. incarnata*, *P. tripartita*. Isoorientin, major flavonoid credentials contributing in poly(2-methoxyethyl acrylate) fractions can be the most accountable for the antioxidant activity. Moreover, *in vivo* approaches, Wistar rats were gavaged with vitexin and isovitexin, noticing the increasing antioxidant capacity.²⁸ Most pharmacological studies have demonstrated effects on central nervous system, *e.g.* anxiolytic, sedative action, and anticonvulsant properties. About 294 volatile compounds have been identified in several passion fruit extracts.²⁹ From the results of this study, it is concluded that the ethanolic extract of *P. incarnata* with its remarkable phytochemical profile can ensure the promising antioxidant potential.

CONCLUSION

In green chemistry, the influence of basic phytochemical extraction methods was studied in the *P. incarnata* leaves in order to screen or to obtain its high phenolic compounds. This study investigated the leave extract act as an antioxidant agent in *in vitro* studies. The results revealed that the flavonoid contents present in *P. incarnata* (2.1 µg/mL) can be an optional to decrease or eradicate the ROS in active levels. Recently, the use of therapeutic ethnomedicinal products has been reliable with adverse effects. *P. incarnata* is a nutraceutical enriched with bioactive compounds, which are evident to possess it as

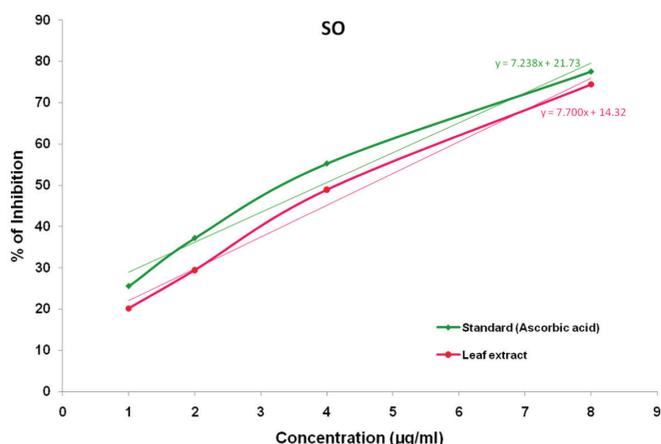


Figure 5. Superoxide scavenging activity

Table 5. ABTS

| Group | Concentration (µg/mL) | % of inhibition | IC ₅₀ value |
|--|-----------------------|-----------------|------------------------|
| Ethanolic extract of leaves of <i>Passiflora incarnata</i> | 1 | 14.65 ± 0.17 | 4.60 µg/mL |
| | 2 | 32.16 ± 0.24 | |
| | 4 | 49.87 ± 0.30 | |
| | 8 | 75.23 ± 0.33 | |
| Ascorbic acid | 1 | 19.32 ± 0.21 | 3.82 µg/mL |
| | 2 | 40.28 ± 0.27 | |
| | 4 | 60.71 ± 0.31 | |
| | 8 | 77.56 ± 0.29 | |

Table 6. H₂O₂

| Group | Concentration (µg/mL) | % of inhibition | IC ₅₀ value |
|--|-----------------------|-----------------|------------------------|
| Ethanolic extract of leaves of <i>Passiflora incarnata</i> | 1 | 12.43 ± 0.87 | 5.79 µg/mL |
| | 2 | 28.57 ± 0.67 | |
| | 4 | 41.32 ± 0.72 | |
| | 8 | 62.84 ± 0.60 | |
| Ascorbic acid | 1 | 18.21 ± 0.52 | 4.80 µg/mL |
| | 2 | 39.52 ± 0.41 | |
| | 4 | 50.43 ± 0.39 | |
| | 8 | 66.16 ± 0.47 | |

Table 7. SO

| Group | Concentration (µg/mL) | % of inhibition | IC ₅₀ value |
|--|-----------------------|-----------------|------------------------|
| Ethanolic extract of leaves of <i>Passiflora incarnata</i> | 1 | 20.15 ± 0.45 | 4.63 µg/mL |
| | 2 | 29.42 ± 0.38 | |
| | 4 | 48.88 ± 0.35 | |
| | 8 | 74.37 ± 0.51 | |
| Ascorbic acid | 1 | 25.53 ± 0.29 | 3.90 µg/mL |
| | 2 | 37.19 ± 0.33 | |
| | 4 | 55.26 ± 0.34 | |
| | 8 | 77.55 ± 0.39 | |

folk medicine for many years and possibly subsidizes to the prevention and cure of many disorders. Further investigations are required to determine the potential use of *P. incarnata* leaves in the pharmaceutical fields could be considered.

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Ethics

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Authorship Contributions

Concept: S.P., W.E.G., Design: N.B.M., Data Collection or Processing: H.S.R.M., W.E.G., Analysis or Interpretation: S.P., N.B.M., Literature Search: H.S.R.M., N.B.M., Writing: H.S.R.M., N.B.M.

Conflict of Interest: No conflict of interest was declared by the authors.

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