

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

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ABSTRACT

Objectives: *Passiflora incarnata* commonly called folk medicine declaredly used for enormous range of therapeutic purposes, one such are 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 DPPH quenching assay, (1, 1-diphenyl-2-picrylhydrazyl) ABTS cation decolorization test, Superoxide and Hydrogen Peroxide radical scavenging assay of ethanol extract of leaf *Passiflora incarnata*.

Methods: The organoleptic characters such as color, odour, appearance, taste and other characters like drying range and fibre contents were analysed as preliminary data. Analytical parameters like Total phenolic content, Total tannins, Total alkaloid content and Total flavonoid contents were analysed with multiple anti radical scavenging activity (DPPH, ABTS, Superoxide and H₂O₂ scavenging assay) with IC₅₀ in terms of inhibition percentage with various concentrations of ethanolic extracts (µg/ml) were studied.

Results: *Passiflora incarnata* possessing a high radical scavenging activity with phenolic content as 2.48 mg gallic acid equivalent (GAE)/g of extract in leaves whereas the total flavonoid content was 2.1 respectively.

Conclusion: The high antioxidant activity was noticed in *Passiflora incarnata* extract which might be of increased level of flavonoids and phenols in the plant extracts. Findings in the studies revealed that the *Passiflora incarnata* projects the veritable source for antioxidant drug bioprospecting in scientific research and pharmaceutical industries.

Keywords: *Passiflora incarnata*, Phytochemical profile, Antioxidant activity, DPPH quench, ABTS

INTRODUCTION

Singleton O₂ is formed in the biological system in aerobic organisms for normal cell functions, if exceed, the ROS (Reactive Oxygen Species) level in the living system cause oxidative stress and lead to oxidative damage. These ROS intermediates threaten to various biomolecules including proteins, enzymes, lipids and DNA^{1,2} also cause physiological errors like blocks in arteries, strokes, cancer and nervous disorders which highly possess to increase in the study of compounds which protect against ROS and possibly can prevent diseases.^{3,4} As a result, high priority to be enlighten to the purpose of antioxidants, particularly organic-based to protect from damage due to free radicals.⁵ Antioxidants, compound that can delayed or prevent the oxidation of biomolecules by blocking the engagement of oxidative progress which can avoid or restore the damage by ROS.⁶ However, synthetic antioxidant like propylgallate, butylated hydroxyanisole, butylated hydroxytoluene and tertiary butylhydroquinone are recognized as good oxidative costs yet they are having limitations because of the carcinogenic effects to the lungs and liver parts.⁷ So, recently, several excessive efforts has been outworthed to safeguard with 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 includes phenolics and flavonoids.⁸ Nutritional components from plants are the dynamic cause of various classes of polyphenolic components and also some flavonoids⁹ Some phytomedicine are traditional, among them, *Passiflora* sp have been reported as folk medicine. *Passiflora* genus, Passifloraceae, includes about 520 species¹⁰ which are spotted mostly in tropical and subtropical regions of the world. *Passiflora incarnata*, *Passiflora alata*, *Passiflora mucrinata* and *Passiflora edulis* revealed its potential biological activity by its various phytometabolites like phenolic substances, alkaloids and flavonoid contents, as familiar for its sedative properties and shown its interests in food and pharmaceutical industry.¹¹ In spite of extensive research on plants 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 to biological or pharmacological screening is even smaller. Hence an attempt made to evaluate the leaves of *Passiflora incarnata* to reveal its antioxidant potentials.

MATERIALS AND METHODS

Collection of Plant material

Passiflora incarnata was collected from Keezanatham, Ariyalur (Dt.), Tamil Nadu, India (Fig 1). The 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, Tamilnadu, India. A specimen was kept in Herbarium of Arignar Anna Government Arts College, Department of Botany (AAGAC/BOT-07). The fresh fully-grown plant leaves were selected. Collected plant leaves were cleaned to remove mud and other adhering weed plants. Fresh leaves sample 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, odour, appearance, taste and other characters like drying range and fibre contents of grounded sample were determined. 2 g of sample 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.



Fig. 1.
incarnata

Passiflora

Analytical parameters

Total ash

5 g of sample was exposed in silica crucible which is ignited prior, allow to cool and weighed. It was allowed to incinerate with slow progressing of heat, up to 450°C, allow to cool and weigh again. The percentage of total ash was calculated with reference range and repeat 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

In order to check the water-soluble ash, a portion of 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.

$$\text{Water soluble ash} = \text{Total ash value} - \text{Water insoluble ash}$$

Sulphated ash

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

Extractive values

5 grams of the dried sample was impregnated with moderately hot PE (Petroleum Ether) for overnight. The extract was concentrated and the dehydrated extracts was weighed.¹³

Preparation of extract

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

Chemicals

2, 20 – azinobis – 3 – ethylbenzothiazoline - 6-sulfonic acid, 1, Folin–Ciocalteus's

phenol reagent, 1- Diphenyl -2 - picrylhydrazyl, ascorbic acid, Folin-Danis reagent, Bromocresol green solution (BCG), DMSO, Potassium persulfate, Methanol, 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 reagent. 10 mL of 7% Na₂CO₃ solution was mixed and incubate for 2 hrs. The absorbance was measured at 740 nm. The quantification was conducted using a gallic acid as standard. The results expressed as milligram per gram dry weight¹⁵.

Estimation of tannins

0.5 mL Folin-Danis reagent was added to each tube containing different concentration of samples 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 nm¹⁶.

Estimation of total alkaloid content

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

Estimation of total flavonoid content

10 grams of sample was impregnated with 60 mL of methanol and allowed to stand for 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 100ml of ether. Mix vigorously for 10 minutes and allowed to stand for 10 minutes to settle. Filtrate was evaporated to dry and calculate.

***in vitro* Antioxidant activity**

DPPH radical scavenging activity

The plant extract at different concentrations was diluted with DMSO to get sample solution. 5 µL of the sample was seeded in a 96-well plate following with 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 extracts was determined by comparing its absorbance with blank.¹⁷ The scavenging ability by DPPH radical was expressed as percentage inhibition and calculated.

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

where A₀ - absorbance of the control, and A₁ - 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 K₂S₂O₈ solutions respectively, and stored in the dark at room temperature for 16 hrs. 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 extracts at various concentrations were diluted with DMSO which counts for 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 nm¹⁸. ABTS scavenging activity was expressed as IC₅₀ (µg/ml) and the inhibition percentage were calculated.

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

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide was prepared with phosphate buffer (pH 7.4). Different concentrations of sample (200 μ L) were mixed with 0.6 mL of H₂O₂ solution. A test tube containing 200 μ L of phosphate buffer are processed as discussed above act as control. Different concentration of ascorbic acid was used as reference compound. Absorbance of H₂O₂ was determined against a blank.¹⁹

Superoxide radical scavenging activity

200 μ L of test sample of different concentrations was taken in test tubes. Superoxide radicals produced by equal addition of 1 mL of Tris-HCl buffer (16 mM, pH-8), NBT (50 μ M), NADH (78 μ M) solution and PMS (10 μ M) respectively. The mixtures 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 error of mean (SEM). Data were analysed with ANOVA and determined by Duncan's Multiple Range test using Graph Pad Prism software version 5.0 (San Diego, USA).

RESULTS

Organoleptic characters and analytical parameters of the leaf powder of *Passiflora 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 remains after incineration of plant material is the ash content, which simply represents some inorganic salts, occurs naturally in unprocessed crude drug materials.

Table 1. Organoleptic characters of *Passiflora incarnata* leaves

Appearance	Coarse powder
Color	Green
Odour	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
Sulphated ash	18.20

Phytochemical analysis

Total phenolic contents in examined leaf extracts were 2.8 mg GAE/g and also showed the presence of flavonoids (2.1 mg/g) but in merger range compare to phenolic contents (Table 2). Total Tannin Content of *P. incarnata* leaf extracts show the 1.9 mg/g and alkaloids – 0.031 mg/g. The high antioxidant activity was observed in *Passiflora incarnata* extract which evidenced the high level of flavonoids and phenolic presence in the plant (Table 3).

Table 3. Polyphenol contents of the ethanolic leaf extracts of *P. incarnata*

Total phytochemicals	Leaf extracts
TPC	2.48

TTC	1.9
TAC	0.031
TFC	2.1

***in vitro* Antioxidant activity**

The percentage inhibition of DPPH by plant extract in different concentrations like 1, 2, 4, 8 $\mu\text{g/mL}$ were observed as 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 . The IC_{50} values for DPPH scavenging activity of leaves of *Passiflora incarnata* and ascorbic acid were $4.30 \mu\text{g/mL}$ and $3.69 \mu\text{g/mL}$ respectively. (Fig. 2). The extracts of *P. incarnata* exhibited food ABTS radical scavenging activity and the percentage of inhibition (with various concentration 1, 2, 4, 8 $\mu\text{g/ml}$) was found to be 14.65 ± 0.17 , 32.16 ± 0.24 , 49.87 ± 0.30 and 75.23 ± 0.33 respectively and comparable with standard ascorbic acid 19.32 ± 0.21 , 40.28 ± 0.27 , 60.71 ± 0.31 and 77.56 ± 0.29 . IC_{50} values in scavenging abilities on ABTS radicals of the extract *Passiflora incarnata* and ascorbic acid were $4.60 \mu\text{g/mL}$ and $3.82 \mu\text{g/mL}$ respectively (Fig. 3).

P. incarnata leaf extracts exhibited a strong scavenging effect against hydrogen peroxide were recorded as 12.43 ± 0.87 , 28.57 ± 0.67 , 41.32 ± 0.72 and 62.84 ± 0.60 respectively (Fig. 4). Whereas, 18.21 ± 0.52 , 39.52 ± 0.41 , 50.43 ± 0.39 and 66.16 ± 0.47 was noted in standard ascorbic acid. IC_{50} values for H_2O_2 scavenging activity for ethanol extract of leaves of *Passiflora incarnata* and ascorbic acid were $5.79 \mu\text{g/mL}$ and $4.80 \mu\text{g/mL}$. Superoxide free radicals scavenged by plant extract in 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 standard was found to be 25.53 ± 0.29 , 37.19 ± 0.33 , 55.26 ± 0.34 and 77.55 ± 0.39 respectively. The IC_{50} values of ascorbic acid was $3.90 \mu\text{g/ml}$ while leaf extracts were $4.63 \mu\text{g/mL}$ respectively (Fig. 5). Values are the average of triplicate and represented as mean \pm standard deviation.

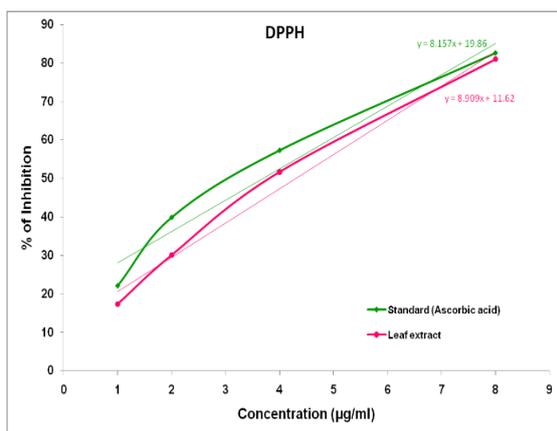


Figure 2. DPPH

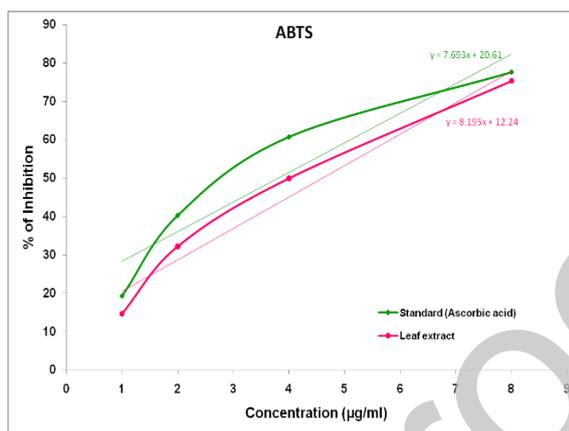


Figure 3. ABTS

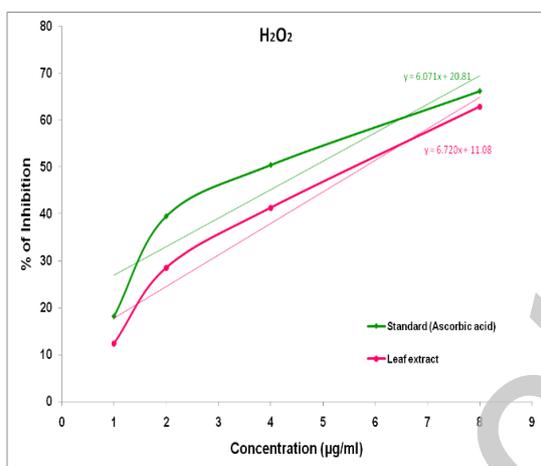


Figure 4. H₂O₂ scavenging activity

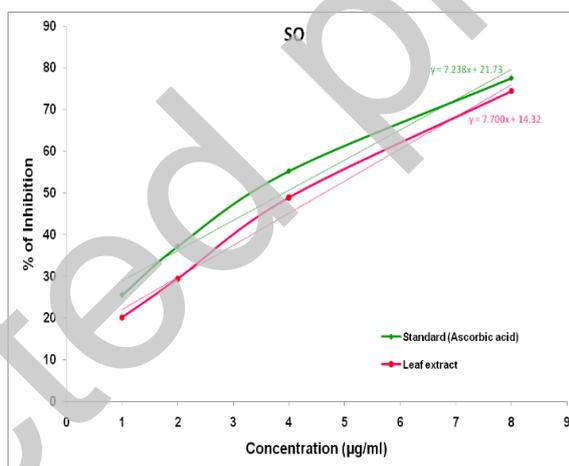


Figure 5. Superoxide scavenging activity

DISCUSSION

Up trended studies have suggested the prevalence with increased levels of plant derived compounds with low optional remedy rates of many diseases. These results suggesting the connections between the protecting role of plant compounds in increasing average life span of human health. Plant derived compounds have enormous range of polyphenols, which are the prime role in minimizing the balance between free radicals and antioxidant potential. Substantially, it is in need to optimize these flavonoids to be acknowledged for its efficient action.²¹

Accumulated evidence has suggested that the ethanolic extracts of *P. incarnata* showed prominent and potent *in vitro* antioxidant activity with the high flavonoid contents (2.1 µg/mL). Flavonoids – a phenolic substance, labelled for its phytoconstituents of *P. incarnata*²² that can steadily repair the unpaired electron located in its aromatic ring and can minimize the cause of free radical development. There are scientific reports showing the amount 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 The group of scientists from Italy studied the methanol extracts from five

species of *Passiflora* obtained by zygotic embryo culture showed the DPPH quench and ABTS radicals.²⁵ Among the flavonoids constituents, in *P. incarnata*, vicenin, isovitexin and orentin have major role in the *in vitro* antiradical scavenging activity.²⁶ A number of flavonoid glycosides - Isochaftoside, schaftoside, isoorientin, orientin, vitexin and isovitexin are considered as standard markers to identify different *Passiflora* species²⁷, such as *Passiflora edulis*, *P. incanata*, *P. tripartite*. Isoorientin, major flavonoid credentials contribute in PMEAF fractions can possibly be the most accountable for the antioxidant activity. Moreover, *in vivo* approaches, wistar rats gavaged with vitexin and isovitexin, noticing the increasing capacity of antioxidant.²⁸ Most of pharmacological studies are demonstrated within the central systema nervosum effects, like 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 ethanol extract of *P. incarnata* with its remarkable phytochemicals profile can ensuring the promising antioxidant potentials.

CONCLUSION

In green chemistry, the influence of basic phytochemical extraction methods was studied in the *Passiflora incarnata* leaves in order to screen or to obtain its high phenolic compounds. The present study investigated the leaves extract acts as an antioxidant agent in *in vitro* studies. The results revealed that flavonoid contents present in *Passiflora incarnata* (2.1 µg/mL) can be an optional to decrease or eradicate the ROS in active levels. In recent years, the use of therapeutic ethnomedicinal products have been reliable with adverse effects. *Passiflora incarnata* is a nutraceutical enriched with bioactive compounds which are evident to possess it as folk medicine for many years and possibly subsidize to the prevention and cure of many disorders. Further investigation required to determine the potential use of *Passiflora incarnata* leaves in the pharmaceutical fields could be considered.

Conflicts of interest: No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper

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