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# 5 In-vitro Phytochemical Analysis and Antioxidants of the Ornamental Plant *Pandanus veitchii*

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## ABSTRACT

*Pandanus veitchii is an ornamental plant commonly known as screw pine that belongs to the family Pandanaceae. The objective of this work is to evaluate the phytochemical constituents and antioxidant activity through free radical scavenging assays using methanolic leaf extract of P. veitchii. Results showed, among the six compounds qualified, the maximum content was observed in flavonoids (77.4 ± 0.37 GAE/g). The IC50 values were 41.06 mg/ml and 67.5 µg/ ml in DPPH and ABTS assays respectively. The ferric reducing activity in different concentrations were recorded and the maximum scavenging was recorded at 200 µg/ml with 66.52 ±0.0058 $FeSO_4$ E mg/g DW.*

***Keywords:*** *Phytochemicals, Screw pine, DPPH, FRAP and ABTS*

## INTRODUCTION

Plants produce a wide range of chemical compounds called phytochemicals through primary and secondary metabolism. They generally have biological activity in the plant host and play a role in plant growth or defense against herbivores and other interspecies defenses. In humans, these are used as medicines to treat chronic as well as infectious diseases. The faster pace of life and the need for a rapid cure has led to the development of synthetic drugs. These synthetic drugs reveal side effects that result in complications. Therefore, treatment with herbal medicine is the alternative for numerous diseases. The World Health Organization (WHO) has emphasized the need to restore the indigenous systems of medicine based on locally available raw materials. The WHO reports that about 4 billion people depend on herbal medicine (Sultan *et al.* 2012).

Medicinal plants have been playing a vital role in the health and healing of man since the beginning of civilization. India is rich in all levels of biodiversity, namely species diversity, genetic diversity, and habitat diversity. Our mother nature has a remedy to heal every human ailment and the plants are utilized as herbal medications. The traditional use of medicinal plants can lead to the discovery of new natural drugs in the treatment of several diseases and also play a vital role in disease prevention. Plant-based compounds are still evidencing to be a valuable source of medicine for humans. And some of the plant-derived compounds, such as Atropine, Colchicine, Digoxin, Vinblastine, Taxol, Morphine, Reserpine is directly used as drugs (Fabricant and Farnsworth, 2001). Medicinal plants are sources of certain bioactive molecules that possess various pharmacological activities like anti-inflammatory, antiulcer, antibacterial, antioxidant, anticancer and antiurolithiatic, etc.

Antioxidants are substances that delay or inhibit oxidative damage of the substrate and have the property to stabilize free radicals leading to cytoprotection from the deleterious effects of free radicals. The potential to trap free radicals is an important characteristic of antioxidants. Antioxidant compounds like phenolic acids, polyphenols, and flavonoids scavenge free radicals such as peroxide, hydroperoxide, or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Wu *et al*. 2011) The imbalance between the formation and neutralization of pro-oxidants is the cause of many human diseases due to oxidative stress. The oxidation induced by reactive oxygen species (ROS) can result in cell membrane disintegration, the development of many

diseases, such as cancer, liver injury, and cardiovascular disorders (Deepa *et al*. 2014). Free radicals combined with the secondarily formed radicals are known to play an important role in the pathogenesis of many chronic conditions such as diabetes, arthritis, ischemia, and cancer. Hence, the study of antioxidant status during a free radical challenge can be used as an index of protection against the development of these degenerative processes in experimental conditions for therapeutic measures (Diwan, 2011).

*Pandanus veitchii* is an Ornamental plant and it is commonly known as Screw pine. It belongs to the family Pandanaceae and originated from Australia. In *P veitchii,* leaves are long that arch attractively outward from the center with bright yellow in the middle along with green margins and maintained at a minimum temperature of 13°C (55°F). Pandanus leaves are used for thatching, mats, hats, baskets, and fiber products. Mostly it is grown for ornamental purposes.

**Morphology of *Pandanus veitchii* (a)**

To our knowledge, the present scrutinization is the first report on the comprehensive study of antioxidant activity and phytochemical analysis of *P. veitchii* leaves. The results serves as a base for the drug discovery by isolation, identification, and categorization of active compounds. The objective of this work is to evaluate the phytochemical constituents and antioxidant activity of methanol leaf extract of *Pandanus veitchii* hort.

## MATERIALS AND METHODS

**Collection of plant material:**

The leaves of *P.veitchii* were collected from the campus of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India. A bunch of collected leaves were washed several times in sterile water to remove the traces of impurities and foreign matters. Healthy leaves were spread in plain paper and dried under shade for a few weeks and pulverized into a fine powder using an electric blender and stored in a sterile glass bottle at 30°C for further tests.

**Preparation of extraction:**

The powdered samples were weighed (10g) and extracted by methanol (100ml) using Soxhlet extraction. The solvent-extracted fractions (Methanol) were subjected to a rotary evaporator and lyophilized to remove the excess solvent. The dried leaf residues were weighed and stored at 4°C in an air-tight glass container for further tests.

**Quantitative Phytochemical Analysis:**

**Estimation of Total Phenolic Content (TPC):** (McDonald *et al.,* 2001)

The total phenolic content was expressed as Gallic acid equivalents (GAE g /100 g dry weight of extract) using the Folin-Ciocalteu method. To 1 ml of extract, 5 ml of Folin-Ciocalteu reagent and 4 ml of Sodium carbonate (75g/L) were added and vortexed. After 30mins of incubation at 20°C, the absorbance of the color developed was recorded using a UV-Vis spectrophotometer at 765 nm against the blank as water. From the standard curve, the concentrations of phenols in the sample were determined and expressed as GAE mg/g of material.

**Estimation of Total Flavonoid Content (TFC):** (Olajire *et al.,* 2011)

1 ml extract (each of 100 µg/ml concentrations) was added to 4 ml of $H_2O$ containing 300 µg of $AlCl_3$. The mixture was then incubated at room temperature for 5 min. After incubation, 2 ml of sodium hydroxide (1M) was added. Then the final volume was made up to 10 ml by further addition of distilled water. The absorbance of the sample and blank were determined at 510 nm by UV-VIS Spectrophotometer. The total flavonoid content was expressed in terms of mg Rutin equivalents RE/g of the sample.

**Antioxidant assay:**

**DPPH radical-scavenging activity (1, 1-diphenyl-2-picrylhydrazyl) assay:** (Riberio *et al*., 2008)

Reduction in the color from purple to yellow shows the ability to scavenge free radicals. Prepared the stock solution by dissolving 10mg of crude extract in 10ml of methanol. Different concentrations of a methanolic leaf extract from 20µl to 200µl were taken, to this 3ml of methanol and 1ml of DPPH (0.004%) was added, DPPH without plant extract serves as a negative control where methanol serves as a blank and ascorbic acid as standard. The test tubes were vortexed and incubated at 25 in the dark for about 30 mins and absorbed at 517 nm. The lower the absorbance of the reaction mixture, the higher is the free-radical scavenging activity.

DPPH % scavenging activity = [(Ab control – Ab sample) / Ab control] ×100

Where, Ab= Absorbance. The $IC_{50}$ value was calculated using a linear equation.

**FRAP Assay: Ferric Reducing Antioxidant Power Assay:** (Benzie and Strain, 1996)

The power of FRAP assay is determined by reduction of yellow ferric tripyridyl triazine complex (Fe(III)-TPTZ) to blue ferrous complex (Fe(II)-TPTZ). Different concentrations like 20, 40, 60, 80, 100, 200, 400, and 800µg of sample and standard solutions are made up of identical solvent. Added 2.5ml of phosphate buffer, 1% Potassium ferricyanide solution and kept at 50°C exceedingly in a water bath for 20 minutes. Cooled and added 2.5 ml of 10% Trichloroacetic acid and centrifuged at 3000 rpm for 10minutes. 2.5 ml of supernatant was mixed with 2.5ml of distilled water and 1ml of 0.1% ferric chloride was added and kept for 10 minutes. Control was prepared in the same manner excluding samples and measured at 700 nm.

FRAP value of Sample (µM) = Abs (sample) x FRAP value of Standard (µM) /Abs Standard.

**ABTS radical cation:** (Re *et al*., 1999)

ABTS radical cation (ABTS+) assay was performed by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate and the mixture was allowed to square within the dark at room temperature for 12-16 hours before use. The ABTS+ solution was diluted with 80% methanol to an absorbance of 0.700 ± 0.02 at 734 nm. After the addition of 100 µl of a sample and gallic acid standard to 3.9 ml of diluted ABTS+ solution, absorbance was measured at 734 nm exactly after six minutes. A gallic acid standard curve was plotted

and therefore the $IC_{50}$ values of plant extracts against ABTS+ solution was calculated. The procedure was performed in triplicates and results were expressed as gallic acid equivalent antioxidant capacity (GAE) and were calculated by using the subsequent equation:

ABTS radical cation activity = $(Ab_C - Ab_S) / Ab_C$ X 100

Where Abc = Absorbance of the control,

Abs = Absorbance of the test samples and blank.

Statistical Analysis were conducted in triplicates and evaluated with the one-way ANOVA and Turkey's Multiple Range Test, performed using SPSS statistical software. P ≤0.05 was observed to be statistically significant. Values were expressed in means ± SD.

## RESULT AND DISCUSSION

### Quantitative Phytochemical Analysis

Plants contain chemicals such as primary and secondary metabolites. The primary metabolites include proteins, amino acids, sugars, purines, and pyrimidines of nucleic acids, and chlorophylls and the secondary metabolites include alkaloids to terpenoids and acerogenins to different phenols. These are a diverse group of compounds with many functions widely used in human therapy, veterinary, agriculture, scientific research, and countless other areas.

**Table 1: Quantitative Estimation of Phytochemical Constituents of *P. veitchii* in methanolic leaf extract**

| Quantitative Analysis | Methanol leaf extract of *P. veitchii* | SED P<0.05 |
|---|---|---|
| **Phenol GAE(mg/g)** | 14.2±0.9 | 0.052<br>0.001 |
| **Flavonoid(RE / g)** | 77.4±0.37 | 0.213<br>0.001 |

* Data were expressed in mean values ± SD Significant level P<0.05

### Estimation of Total Phenolic Content (TPC):

The total phenolic contents of *P. veitchii* extract is 14.2±0.09 mg GAE/g in methanol extracts. Pure methanol produced extract with the highest levels of total phenolics. The content is significantly higher (P < 0.05). And results show that total phenolic content (TPC) was obtained from samples with high antioxidant activity. These values are shown in (Table 1).

The result of Afira and Shukor (2017) was satisfied with the total phenol content where the methanolic leaf extract of P. *amaryllifolius* showed 11.98±0.40mg QE/g in dry extract.

**Estimation of total Flavonoid content (TFC):**

The total flavonoid content of *P. veitchii* was recorded as 77.4 ± 0.37 GAE/g in the methanol extract. The methanol extract exhibited a total flavonoid content that is significantly higher ($P < 0.05$). And results show that total flavonoid content (TFC) was obtained from samples with high antioxidant activity. These values are shown in (Table 1).

Afira and Shukor(2017) reported the highest Flavonoid Content in methanolic leaf extract of *Pandanus amaryllifolius* 9.07±0.36mg QE/g in the dry extract which is similar to the results obtained.

***In vitro* Antioxidant Activity**

**Table 2. $IC_{50}$ values of DPPH, ABTS, and FRAP assay in *P. veitchii***

| Antioxidant assays | Standard | | Methanol leaf extract |
|---|---|---|---|
| | Ascorbic acid | Gallic acid | |
| DPPH% | 51.96±0.78 | - | 41.06 mg/ml |
| ABTS (µg/ml) | - | 53.8±0.109 | 67.5 µg/ ml |

**DPPH radical-scavenging activity (1, 1-diphenyl-2-picrylhydrazyl) assay:**

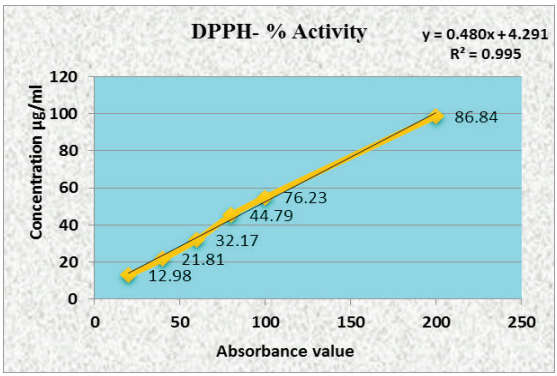


**Figure I: DPPH Free Radical Scavenging Activity of Leaf Extract of *P. veitchii***

The scavenging profile of the extract is shown in Figure 1, from which it can be observed that the extract possesses radical scavenging potential. The maximum radical scavenging of the methanolic leaf extract of *P. veitchii* was recorded at 200 µg/ml of 86.84(µg/ml**)** with an $IC_{50}$ value of 41.06 mg/ml and the minimum of 12.98 at 20 (µg/ml**)**. Regression analysis of the DPPH assays is $R^2$= 0.995.

These findings are similar to Nhu, (2015) reported the $IC_{50}$ value of DPPH radical-scavenging activity of ethyl acetate and butanol fractions from leaves as 0.280 ± 0.06 mg/ml of *P. tectorius*, when the results were compared with the $IC_{50}$ value of ascorbic acid (25.75 µg/mL). The extract of *P. odorus* showed an $IC_{50}$value of DPPH ranges from 1.66µg/ml and 4.69µg/ml and 26.21µg/ml in chloroform, petroleum ether, and ethyl acetate respectively which was done by Kaiser *et al., (*2010).

**The Ferric Reducing Antioxidant Power (FRAP) Assay:**

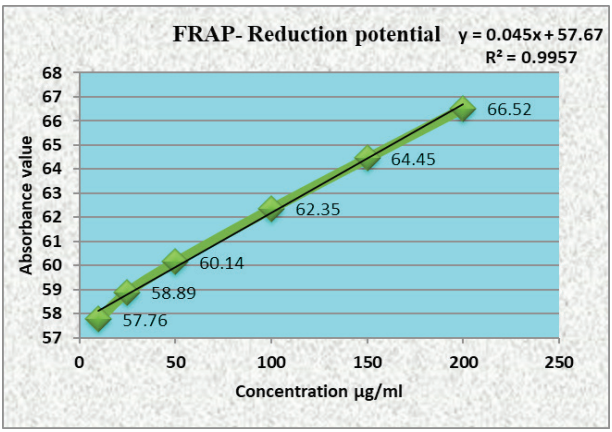


**Figure II: The Ferric Reducing Antioxidant Power (FRAP) Assay of Leaf Extract of *P. veitchii***

The color intensity is related to the amount of antioxidant reductants in the samples. The ferric reducing activity of methanol leaf extract of *P. veitchii* hort., were found to be 57.76 ± 0.003, 58.89 ± 0.0061,60.14 ± 0.0047, 62.35 ± 0.0029, 64.45 ± 0.0017, 66.52 ± 0.0058 $FeSO_4$ E mg/g dw in different concentrations of 10, 25, 50, 100, 150 and 200 µg/ml respectively when compared to the standard (53.8±0.109 µg/ml). Regression analysis of the FRAP assays is $R^2$= 0.995.

The results of ferric reducing antioxidant power (FRAP) were close to the methanolic leaf solvent of *P. amaryllifolius* showed 64.39 ± 2.79

Fe2+/g in dry extract conducted by Afira and Shukor (2017). The reducing antioxidant power assay in the methanolic leaf extract of *P. canaranus* Balamurugan *et al.,*(2020) which showed the potential activity of 17.74 ± 0.570 similar to *P. veitchii*

**ABTS radical cation:**

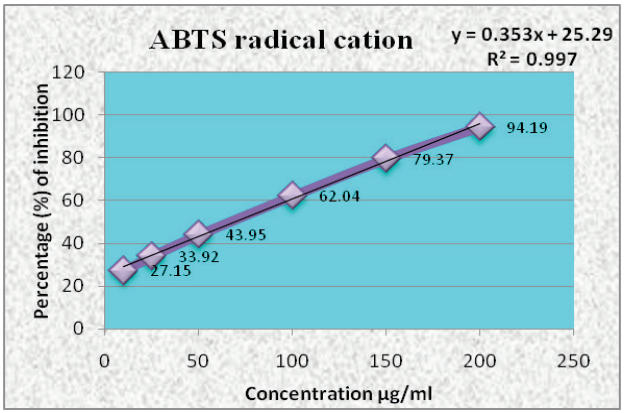


**Figure III: ABTS radical cation (ABTS) Assay of Leaf Extract of** *P. veitchii*

The leaf methanolic extract of *P. veitchii* showed *the* highest radical scavenging activity with an $IC_{50}$ value of 67.5 µg/ ml when compared to the standard gallic acid (48.3±0.255 GAE µg/ml).The amount of antioxidant reductants is related to the intensity of the color. The reducing activity of methanol leaf extract of *P. veitchii* in different concentrations were found to be 57.76 ± 0.003, 58.89 ± 0.0061,60.14 ± 0.0047, 62.35 ± 0.0029, 64.45 ± 0.0017, 66.52 ± 0.0058 $FeSO_4$ E mg/g dw in different concentrations of 10, 25, 50, 100, 150 and 200 µg/ ml respectively. It also inferred that the extract is efficient in scavenging more free radicals in contrast with the standard gallic acid. Regression analysis of the ABTS assays is $R^2$= 0.997.

The results were in matching with the ABTS free radical scavenging activity of aqueous and ethanol extract with *P.amaryllifolius* in which the $IC_{50}$ values are 204.99 µg/ml and 104.31µg/ml respectively in accordance with the Quyen *et al.,* (2020). Sang *etal.,*(2019) have conducted the ABTS assay in the ethanolic extract of *P. amaryllifolia* showed minimum value of 36.3 ± 2.9% when compared to *P. veitchii.*

## CONCLUSION

The present study reveals the presence of phytochemical components in the ornamental plant *P. veitchii.* They are commonly grown and

thus the availability and affordability are easy. As these investigation are limited, it will serve as base to access their chemical perspective in future.

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