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Comparative Phytochemical Profiling of Ethanol and Aqueous Extracts of Pisonia Grandis R.Br.

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ABSTRACT

Pisonia grandis R.Br. is a tropical plant species that has been used for generations in traditional medicine; nevertheless, little is known about its phytochemical makeup. Using phytochemical profiling, this study sought to investigate *P. grandis*'s therapeutic qualities, illuminating its potential as a source of new bioactive substances. Alkaloids, flavonoids, and terpenoids were among the phytochemicals that were identified through the use of spectroscopic and chromatographic methods. The results demonstrate that *P. grandis* is a useful resource for the creation of novel drugs and nutraceuticals, and that more study is necessary to fully understand its anti-inflammatory, anti-microbial, antioxidant, and anti-diabetic qualities as well as its potential medical uses.

Keywords: *Pisonia grandis*, phytochemical profiling, bioactive compounds, proximate composition, mineral assay.

1. INTRODUCTION

Traditional medical systems like Ayurveda, Unani, and Chinese medicine have their roots in plant-based therapies, which have been around for a very long time. Alkaloids, glycosides, and terpenes are just a few examples of the bioactive compounds found in medicinal plants, which are among the more than 400,000 species of plants known to science. These molecules have been used to make medicines that save lives. Many contemporary medications have their origins in plants, making medicinal plants an important resource for drug discovery in the modern day. In addition, people looking for alternative or complementary therapies may find plants appealing because they provide a natural, sustainable, and easily accessible method of healthcare (Poongothai et al., 2022).

Pisonia grandis R.Br., a member of the Nyctaginaceae family of flowering plants, is a popular name for plants in this group. It is said that the sticky seeds of several species in this genus can entice little birds, which is why these trees are called catch bird trees, bird catcher trees, or birdlime trees. According to Sangameswaran *et al.* (2023), some island species may have evolved these sticky seeds to help them stick to birds' beaks and be carried to other islands. The current investigation set out to catalog the bioactive components found in *P. grandis* foliage.

2. MATERIALS AND METHODS

2.1. Collection of plants

P. grandis leaves are sourced from the environs of Coimbatore in the Indian state of Tamil Nadu. Certification of the plant's identity was provided by BSI in Coimbatore, Tamil Nadu, India. Before being pulverized, the leaf samples were washed, shade-dried, and cleaned twice. Using analytical grade solvents, the extraction was carried out according to the normal practice. Aqueous and ethanol solvents were utilized for Soxhlet extraction using the coarse leaf powder, respectively. Analytical methods both qualitative and quantitative were applied to the final samples.

2.2. Qualitative phytochemical screening

Siddhuraju and Subramanian (2007) used conventional methods to evaluate the phytochemical screening of leaf extracts. To screen for important chemicals such as carbs, proteins, amino acids, alkaloids, saponins, phenols, flavonoids, glycosides, cardiac glycosides, phytosterols in aqueous leaf extracts, it was conducted using both methods.

2.3. Quantitative assays

2.3.1. Total phenolic contents:

Siddhuraju and Becker (2003) provided the methodology for determining the total phenolic content. We expressed the results as gallic acid equivalents after doing the analysis in triplicate.

2.3.2. Tannin contents:

After PVPP treatment, tannins were calculated from the same extracts. In a 100 × 12 mm Eppendorf tube, weigh 100 mg PVPP, add 1 mL distilled water, then add 1 mL sample extracts. The contents were vortexed and frozen at 4°C for 15 min. The Supernatant was recovered after centrifuging the sample at 4000 rpm for 10 min at room temperature. The tannins would have precipitated with the PVPP, leaving this supernatant with only simple phenolics. The Supernatant's non-tannin phenolic content was measured. Poongothai *et al*. (2018)

Total phenolics - Non-tannins = tannins

2.3.3. Flavonoid contents:

Using the methodology outlined in (Zhishen *et al*. 1999), the flavonoid concentrations of each extract were measured. To measure flavonoids, rutin was utilized as a reference. Every experiment was conducted three times, and the outcomes were reported as rutin equivalents (RE).

2.4. Proximate Composition

2.4.1. Total carbohydrates:

The methodology for total carb analysis was based on that of Krishnaveni *et al*. (1984). After consulting the work of Sadasivam and Manickam (2008), we were able to determine the total carbohydrate content of the leaf sample.

2.4.2. Total proteins:

The protein was measured using Bovine Serum Albumin (BSA) as a reference, following the methodology outlined in (Lowry *et al.* 1951).

2.4.3. Total free amino acids:

Thenmoli and Sadasivam (1987) developed a method to measure total free amino acids. A Spectrophotometer (Thermoscientific, Genesys) was used to measure the intensity of the purple color at 570 nm, in comparison to a reagent blank. By substituting 0.1 ml of 80% ethanol for the extract, we were able to create a reagent blank whose color remained constant for 1 hour.

2.4.4. Starch content:

According to the method outlined by Sadasivam and Manickam (2008), which used glucose as a reference, the starch content was estimated. After tallying up the amounts of total starch in each sample, the data was tabulated.

2.5. Moisture content:

The leaves were sliced one at a time, and their weight was recorded both before and after they were incubated for 24 hours at 80°C in a hot air oven before being cooled in a desiccator to determine their moisture content. (Arasaretnam *et al.,* 2017).

2.6. Ash value:

In a tared silica/platinum dish, weigh five grams of the sample. To remove carbon, carefully char the material on a burner, then move it to a muffle furnace and ash it at 550 ±10 0 C. For 30 minutes, reheat the dish at 550 ± 100 C. Weigh after desiccating. Weigh until the difference is less than 1 mg after heating once more for 30 minutes and chilling in a desiccator. Note the weight that is the lightest. Arasaretnam *et al.,* 2017.

2.7. Anti-nutritional composition assay

2.7.1. Trypsin inhibitor activity: Anti-nutrient content was estimated using the Sadasivam and Manickam (2008) trypsin inhibitor activity method. Samples were measured in TIU per mg protein for trypsin inhibitor activity. Sample aliquots must inhibit 50% of trypsin activity, one unit of inhibitor. The amount of trypsin inhibitor per mg protein that inhibits the enzyme 50% is one unit of activity.

2.8. Minerals profile: OES with inductively coupled plasma can detect and quantify elements in solution. The solution is nebulized and the aerosol is transferred to a high-frequency plasma that atomizes and partially ionizes the composition. Monochromators or polychromators distribute the emission lines of atoms and ions, and detectors record their intensity. Measure axially or radially. Axial measurement is more accurate. There are devices for both sorts of measurement. Due to the linear relationship between emission signal intensities and element concentration, the amounts are calibrated with suitable reference solutions. Rüdel *et al.* (2007).

3. RESULTS AND DISCUSSION

3.1. Qualitative Phytochemical Screening

Phytochemical analysis of extracts from P. grandis leaves identified a wide variety of chemicals. There were carbs, proteins, amino acids, alkaloids, saponins, phenolic compounds, flavonoids, glycosides, cardiac glycosides, phytosterols, and both ethanolic and water-based extracts included these substances. When comparing ethanol and aqueous extracts, it was found that phenolic chemicals and carbohydrate were more plentiful in the former, but cardiac glycoside was not present in either. Table 1 displays the results of the phytochemical analysis conducted on *P. grandis* ethanolic and water-based extracts.

The medicine and pharmaceutical industries have made considerable use of the secondary metabolites found in therapeutic plants, which include alkaloids, flavonoids, steroids, and related active metabolites. There has been a flurry of recent research on medicinal plants' phytochemistry, focusing on its vegetative components such as leaves, roots, stems, and fruits. (Dhanalakshmi and Krishnaveni, 2014.)

Table 1: Phytochemical screening of Ethanol & Aqueous Extracts of *P. grandis* R.Br.

Phytochemicals	**Ethanol**	**Aqueous**
Carbohydrates	+++	++
Proteins	++	++
Amino acids	+	++
Alkaloids	++	++
Saponins	++	++
Phenolic compounds	+++	++
Glycosides	++	+
Flavonoids	++	++
Cardiac glycosides	-	-
Phytosterols	++	++
Tannins	++	++

(+): Presence of chemical compound, (-): Absence of chemical compound

(+) < (++) < (+++): Based on the intensity of characteristic color

3.2. Quantification assays

3.2.1. Quantification of total Phenolic, Tannins and Flavonoid contents

The phytochemical screening results informed the analysis of the total phenol, tannin, and flavonoid content in both *P. grandis* extracts. The ethanol extract had a high concentration of tannin and phenol, while the water-based version had a high concentration of flavonoids. Comparing ethanolic extracts to aqueous ones, the quantitative quantification of tannin yields better results. As an added bonus, tannins have anti-inflammatory, antioxidant, anti-cancer, and antibacterial properties. An estimated 128.73 mg GAE/g ethanolic extracts of tannins were found in the leaf sample.

The ethanol extracts likewise had the highest phenolic concentration, measuring 141 mg GAE/g ethanolic extract. In addition to their antimicrobial and anti-carcinogenic capabilities, the phenolic components are thought to have a preventative role in the onset of chronic diseases such coronary heart problems. The aqueous extract has a greater flavonoid concentration than the ethanolic extracts, at 55.66 mg RE/g. One of flavonoid claimed benefits is the antioxidant power they possess. (Letchuman *et al.*, 2021) (Table 2).

Table 2: Total phenolic, Tannins and Flavonoids contents in the leaves of *P. grandis* R.Br.

Extracts	**Total Phenolics (mg GAE/g extract)**	**Tannins (mg GAE/g extract)**	**Flavonoid (mg RE/g extract)**
Ethanol	141 ± 1	128.73 ± 0.90	9.43 ± 0.36
Aqueous	64.66 ± 1.52	2.85 ± 0.07	55.66 ± 1.61

GAE – Gallic Acid Equivalents, RE – Rutin Equivalents

Values are means of triplicate determination (n=3) ± standard deviation.

3.3. Evaluation of composition

The analysis focused on the nutritional and anti-nutrient content of the leaves of *P. grandis* R.Br. Hydration and ash levels, as well as glucose, starch, protein, and amino acid present, were assessed. Along with trypsin inhibitor activity, the anti-nutrient investigation was conducted. The data indicate that amino acids are most abundant, followed by proteins. When evaluating the practical qualities of food, the ash content is often seen as an indicator of quality. It was found that the ash value in this case is 21.68%. Enzymes that do metabolic work become more active when there is a high concentration of moisture (Arasaretnam *et al*. 2017).

In Table 3,

Table 3. Proximate composition of *P. grandis*

Component	**Sample**
Total Carbohydrate (mg/ g)	0.16 ± 0.003
Total protein (mg/ g)	12.26 ± 0.23
Amino acid (mg/ g)	37.89 ± 1.67
Starch (mg/ g)	0.31 ± 0.009
Ash (%)	21.68 ± 0.26
Moisture (%)	10.34 ± 0.46
Trypsin inhibiting activity (TIU/mg protein)	Nil

Values are mean of triplicate determination (n=3) ± standard deviation

3.4. Minerals profile

To comprehend the medicinal uses of plants, one must research minerals. We have conducted a number of mineral analyses on the plants that were considered for this study. The results demonstrate that potassium is abundant in *P. grandis* leaves, with calcium and magnesium following closely after. Potassium has a crucial role in extracellular cation control, which in turn regulates plasma volume, neuronal conduction, and muscular contractions. In addition to being an essential component of teeth and bones, calcium also controls the activity of muscles and nerves. Anemia and cardiomyopathy are two conditions that magnesium has been shown to help avoid. In 2017, Arasaretnam et al. In addition, the outcomes are displayed in table 4.

Table 4 Minerals Profile of *P. grandis*

S. No	**Parameters**	**Result**
1	Magnesium (ppm)	122.34
2	Aluminium (ppm)	9.48
3	Sodium (ppm)	37.40
4	Barium (ppm)	2.12
5	Zinc (ppm)	0.64
6	Cobalt (ppm)	-
7	Manganese (ppm)	3.43

8	Potassium (ppm)	500.76
9	Copper (ppm)	0.39
10	Cesium (ppm)	6.69
11	Iron (ppm)	5.47
12	Calcium (ppm)	169.79
13	Lithium (ppm)	0.04

Pisonia grandis plant has various biologically active molecules which are promising sources of secondary metabolites. Further study aims to identify the antimicrobial, anti-inflammation, anti-diabetic and antioxidant compounds that may be exploited in herbal formulations.

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