

Full Length Research Article

# Phytochemical Screening, Antibacterial, Antifungal and Antioxidant Properties of Nachu Kottai Keerai (*Pisonia grandis*) Leaves Extract

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## Abstract

Plant plays an important role in discovering new therapeutic agents and have received significant focus because of their bioactive substance like antioxidants. The herbal products today are considered to be safer to human and environment. In different parts of India, several medicinal plants or their extracts are used for the treatment of various diseases. Hence the present study was carried out to determine phytochemical compounds qualitatively, to determine antibacterial, antifungal and antioxidant properties of leaves extract of *Pisonia grandis*. Plant sample was collected from Thiruvattiyur, Chennai, Tamilnadu, India and was authenticated by Herbal Plant Anatomy Research Centre, Chennai, Tamilnadu, India. Aqueous extract of leaves of *P. grandis* was prepared by following the standard method. The crude extract was subjected to preliminary screening to determine phytochemical compounds qualitatively. The results of the study revealed the presence of phytochemical components such as alkaloids, flavonoids, saponins, steroids and terpenoids. Further the study was extended to determine the antimicrobial activity of the leaves extract of *P. grandis*. The bacterial strains (*Klebsiella pneumoniae* and *Staphylococcus aureus*) and fungal strains (*Aspergillus niger* and *Candida albicans*) were used. The results of the antibacterial activity of leaves extract of *P. grandis* showed the maximum MIC value at 250 µg against *Klebsiella pneumoniae* and *Staphylococcus aureus*. The results of the antifungal activity of leaves extract of *P. grandis* showed the maximum MIC value at 250 µg against *Aspergillus niger* and 31.2µg for *Candida albicans*. Antioxidant activity of the leaves extract of *P. grandis* was also evaluated using DPPH radical scavenging assay. The highest percentage of inhibition were 41.5% and the lowest percentage of inhibition were 5.6% in the leaves extract of *P. grandis*.

**Key words:** *Pisonia grandis* leaves, Phytochemical screening, Antibacterial, Antifungal, Antioxidant properties

Plants are important source of drugs especially in traditional medicine [1]. Plants constitute various natural products that are important from medicinal point of view [2-3]. Medicinal plants are a natural gift for human beings' disease free and healthy life. The herbal products today are considered to be safer to human and environment. India has different types of several medicinal plants or their extracts that are used for the treatment of various diseases.

*Pisonia* is a genus of flowering plants in the 4 O'clock flower family, Nyctaginaceae. It is commonly referred to as "Leechai kottai keerai". *Pisonia grandis* is a large evergreen shrub. The leaves are edible. Young leaves are used as a vegetable. Leaves is a good cattle feed and are mostly used to treat rheumatism or arthritis. *Pisonia grandis* has high medicinal potential and reutilization, acting tradition and contemporary in the world [4]. Based upon literature survey, research related to *Pisonia grandis* is meagre. Hence the present study was carried out to determine phytochemical components

qualitatively and to assess the antibacterial, antifungal and antioxidant properties of *Pisonia grandis* leaves extract.

## MATERIALS AND METHODS

### Selection of plant

Fresh and healthy *Pisonia grandis* (Nachu Kottai keerai) leaves were collected from the plant from the locality in and around Chennai, Tamil Nadu, India. The fresh leaves of *Pisonia grandis* was authenticated by taxonomist (Certificate No. PARC/4809).

### Collection of bacterial isolates for antibacterial activity

Clinical isolates like *Staphylococcus aureus* and *Klebsiella pneumoniae* were collected from a tertiary hospital, Chennai. Samples were transported to the laboratory for further processing in an ice box.

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*Preparation of leaves extract of Pisonia grandis leaves: Hot method*

Preparation of aqueous decoctions (Hot method) of *Pisonia grandis* leaves were carried out by following the procedure of Senthilkumar and Sivakumar [5]. It was prepared by boiling 10gms of leaves in 100ml of sterile distilled water taken in conical flask for 30 minutes. The flask was then plugged and removed from heat and allowed to cool. After cooling the contents of flask was filtered and stored at 40 °C until use.

#### *Phytochemical screening of Pisonia grandis leaves extract*

The bioactive compounds present in Nachu Kottai (*Pisonia grandis*) leaves extract were analyzed by qualitatively. It was screened for alkaloids, flavonoids, saponins, steroids and terpenoids by following the procedure of Harborne [6].

#### *Antibacterial and antifungal activity*

Antibacterial activity of *Pisonia grandis* leaves extract against *Staphylococcus aureus* and *Klebsiella pneumoniae* and antifungal activity of *Pisonia grandis* leaves extract against *Aspergillus niger* and *Candida albicans* were determined by following the procedure of Sundararaj [7]. Minimum Inhibitory Concentration (MIC) was determined using Resazurin Microtitre Assay.

#### *Antioxidant activity*

Antioxidant activity of *Pisonia grandis* leaves extract was determined using DPPH Radical Scavenging Assay following the method of Tailor and Goyal [8].

## RESULTS AND DISCUSSION

Table 2 (a) Antibacterial activity of leaves extract of plant, *Pisonia grandis*

Micro-organisms	Growth of inhibition									DMSO
	1000 µg	500 µg	250 µg	125 µg	62.5 µg	31.2 µg	15.6 µg	7.8 µg	Standard streptomycin 10µg	
<i>Klebsiella pneumoniae</i>	-	-	-	+	+	+	+	+	-	+
<i>Staphylococcus aureus</i>	-	-	-	+	+	+	+	+	-	+

+ = Present, - = Negative

Table 2(b) Determination of Minimum Inhibitory Concentration (MIC) of leaves extract of plant, *Pisonia grandis* against *Klebsiella pneumoniae* and *Staphylococcus aureus*

Microorganisms	MIC Value (µg)
<i>Klebsiella pneumoniae</i>	250
<i>Staphylococcus aureus</i>	250

#### *Antifungal activity of leaves extract of plant, Pisonia grandis*

Table 3(a) Antifungal activity of leaves extract of plant, *Pisonia grandis*

Micro-organisms	Growth of inhibition									DMSO
	1000 µg	500 µg	250 µg	125 µg	62.5 µg	31.2 µg	15.6 µg	7.8 µg	Standard streptomycin 10µg	
<i>Aspergillus niger</i>	-	-	-	+	+	+	+	+	-	+
<i>Candida albicans</i>	-	-	-	-	-	-	+	+	-	+

+ = Present, - = Negative

#### *Antioxidant activity of leaves extract of plant, Pisonia grandis DPPH Assay*

The free radical scavenging assay is based on the reduction of 1,1 diphenyl-2-picrylhydrazyl (DPPH). The ability

#### *Results of phytochemical analysis of leaves extract of plant, Pisonia grandis*

The results of preliminary study of phytochemical screening of aqueous leaves extract of plant, *Pisonia grandis* were depicted in (Table 1). The results of the aqueous leaves extract of plant, *Pisonia grandis* revealed the formation of foaming appearance indicated the presence of saponins. The appearance of yellow colour indicated the presence of flavonoids. The appearance of red brown colour at the interface showed the presence of terpenoids. The formation of brown ring indicated the presence of steroids. The appearance of red brown colour showed the presence of alkaloids.

Table 1 Phytochemical screening of aqueous leaves extract of plant, *Pisonia grandis*

Contents	Aqueous extract
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids	+
Terpenoids	+

+ = Present

#### *Antibacterial activity of leaves extract of plant, Pisonia grandis*

The results of the antibacterial activity of leaves extract of plant, *Pisonia grandis* using Resazurin Microtitre plate Assay were presented in (Table 2a-b). The result of the antibacterial activity of leaves extract of plant, *Pisonia grandis* showed that Minimum Inhibitory Concentration (MIC) of leaves extract of plant, *Pisonia grandis* against *Klebsiella pneumoniae* and *Staphylococcus aureus* were recorded to be at 250µg of leaves extract of plant, *Pisonia grandis* thereby indicates that the leaves extract of plant, *Pisonia grandis* has a potent antibacterial activity.

The results of the anti-fungal activity of leaves extract of plant, *Pisonia grandis* using Resazurin Microtitre plate Assay were depicted in (Table 3a-b). The result of the antifungal activity of leaves extract of plant, *Pisonia grandis* showed that Minimum Inhibitory Concentration (MIC) of leaves extract of plant, *Pisonia grandis* against *Aspergillus niger* and *Candida albicans* were recorded to be at 250µg and 31.2 µg of leaves extract of plant, *Pisonia grandis* thereby indicates that the leaves extract of plant, *Pisonia grandis* has a potent antifungal activity.

of the sample and the standard ascorbic acid to scavenge the free radical at different concentrations from 100µg/ml to 500µg/ml of leaves extract of plant, *Pisonia grandis* was depicted in (Table 4a-b). The results of the antioxidant study

revealed that there was an increase in percentage of inhibition as the concentration of plant leaves extract increases. However, the highest percentage of inhibition was recorded as  $41.5 \pm 0.70$  in 500  $\mu\text{g/ml}$  of sample and lowest percentage of inhibition was recorded as  $5.6 \pm 0.08$  in 100  $\mu\text{g/ml}$  of sample. Thus, the study showed that leaves extract of plant, *Pisonia grandis* has good free radical scavenging ability. Plant plays an important role in discovering new therapeutic agents and have received significant focus because of their bioactive substance like antioxidant. Plants are the primary medicinal source used to

treat infectious diseases in the majority of developing nations in the world.

Table 3(b) Determination of Minimum Inhibitory Concentration (MIC) of leaves extract of plant, *Pisonia grandis* against *Aspergillus niger* and *Candida albicans*

Microorganisms	MIC Value ( $\mu\text{g}$ )
<i>Aspergillus niger</i>	250
<i>Candida albicans</i>	31.2

Table 4(a) Anti-oxidant activity of leaves extract of plant, *Pisonia grandis* using DPPH Assay BHT Concentration of the standard

BHT Concentration	100 $\mu\text{g}$	200 $\mu\text{g}$	300 $\mu\text{g}$	400 $\mu\text{g}$	500 $\mu\text{g}$
% Inhibition	$38.9 \pm 0.08$	$54.2 \pm 0.12$	$71.1 \pm 0.54$	$74 \pm 0.17$	$81.3 \pm 0.12$

$\pm$  = Standard deviation

Table 4(b) Antioxidant activity of leaves extract of plant, *Pisonia grandis*

Sample / concentration ( $\mu\text{g}$ )	100	200	300	400	500
% Inhibition	$5.6 \pm 0.08$	$11.3 \pm 0.12$	$22.6 \pm 0.16$	$32 \pm 0.34$	$41.5 \pm 0.70$

$\pm$  = Standard deviation

*Pisonia grandis* is the most common nest tree for the Seychelles warbler an endemic land bird brought back from near extinction by careful habitat management and translocation, thus showing that careful consideration of the entire island ecosystem is essential. There is evidence for therapeutic activity of *Pisonia grandis* due to the presence of numerous phytochemical substances. Hence an investigation was carried out to study the qualitative analysis of phytochemical, antibacterial, antifungal and antioxidant properties of the medicinal plant, *Pisonia grandis*. The result of the phytochemical screening of *Pisonia grandis* leaves extract showed the presence of bioactive components such as saponins, flavonoids, alkaloids, steroids and terpenoids.

Saponins reduce blood cholesterol levels, minimize the risk of cancer and slow down the response of blood sugar. Numerous alkaloids are effective pharmaceutical substances that can be used to treat a wide range of illnesses, including malaria, diabetes, cancer, heart failure, etc. Similar to this, the major cause of disorders connected to blood clotting is platelet aggregation that goes beyond the need for homeostasis [9]. The greatest antioxidant is flavonoid. They are biochemical transformers that alter the chemical pathways of the body in order to expose it to carcinogenic chemicals, viruses and allergens. Steroids are responsible for the cholesterol-lowering effects. They also aid in the regulation of the immune response [3]. Terpenoid molecules exhibit viable biological traits notably analgesic, anticonvulsant and anti-inflammatory activities [10].

The existence of these phytochemical compounds shows the therapeutic efficacy of *Pisonia grandis*. This is consistent with a large number of literature findings on their therapeutic uses [4]. The study was further extended to determine antibacterial efficacy of *Pisonia grandis* leaves extract. Antibacterial activity of *Pisonia grandis* leaves extract was determined against *Klebsiella pneumoniae* and *Staphylococcus aureus* using Standard Streptomycin. Colour change was observed. The result of antibacterial activity of leaves extract

shows that Minimum Inhibitory Concentration value is 250  $\mu\text{g}$ . Similar work on antimicrobial activity of *Pisonia grandis* was investigated in both Gram-positive and Gram-negative strains using ampicillin and rifampicin as reference drugs [11]. Antifungal screening of the leaves extract of *Pisonia grandis* was carried out against *Aspergillus niger* and *Candida albicans* using Streptomycin as standard. The result of antifungal activity of *Pisonia grandis* leaves extract showed the Minimum Inhibitory Concentration is 31.2  $\mu\text{g}$  [12].

Antioxidant activity of *Pisonia grandis* leaves extract was determined. The antioxidant potential of the leaves extract was assessed by employing DDPH. The result of antioxidant assay shows an important Scavenging activity for free radicals of DPPH (1,1-Diphenyl-2-picryl hydrazyl) widely used in pathogenesis of many diseases. The usage of synthetic antioxidant components may show many side effects like toxicity and mutagenic effects. The result of *Pisonia grandis* leaves extract showed the highest percentage of inhibition which was 41.5% and the lowest percentage of inhibition was 5.6% [13].

## CONCLUSION

Thus, it could be concluded from the results of the present study that *Pisonia grandis* contains various bioactive compounds. Further the leaves extract of *Pisonia grandis* has a potent antibacterial, antifungal and antioxidant properties. The presence of various bioactive compounds justified the use of the leaves for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. It can be used for the production of alternative new drugs in biomedical field. This encouraging result provides useful information for designing a much better anticancer compound using *Pisonia grandis* with minimum side effects.

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