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Research Article

Phytochemical Constituents, Antioxidant Activity and FT-IR Analysis of *Pisonia grandis* Leaf Extracts

Murugesh S, Vino P*

Department of Botany, School of Life Sciences, Periyar University, Salem, Tamil Nadu-636 011

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ABSTRACT

Pisonia grandis is a traditional medicinal plant. It is commonly known as Leeachai kottai keerai. The present investigation focus on screening of phytochemical constituents, antioxidant activity and FT-IR analysis of *Pisonia grandis* leaf extracts. The leaf extracts were prepared using three different solvents (Aqueous, Methanol, Ethanol). The antioxidant potential of the extracts was assessed by employing different assays such as DDPH and ABTS. The screening tests also were performed for the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, phenols, steroids, saponins and tannins in the extracts. The results of FT-IR analysis confirmed the presence of alcohols, alkenes, Phenols, aromatic carboxylic acid, esters, aliphatic amines, and primary secondary amines in the ethanolic extract. It is concluded that the ethanolic extract of leaves of *Pisonia grandis* has potential bioactive compounds. The results of the present study suggest that the leaves of *Pisonia grandis* may be used as a herbal alternative to cure various diseases after various scientific validation studies.

Keywords: *Pisonia grandis*, Phytochemical, DDPH, ABTS and FT-IR.

INTRODUCTION

Plants, especially those with ethnopharmacological uses, have been the primary sources of medicine for early drug discovery[4].Medicinal plants are the nature's gift to human beings to make disease free healthy life[24]. Plants possess miraculous and perilous power, which could alleviate pain and cure illness. Plants have been used from the early civilization onwards as a source of medicine for all types of diseases[19]. In spite of recent development in the synthetic drug discoveries, plants still occupy an important place in the modern and traditional systems of medicine all over the world[3]. Modern medicines are primarily from synthetic origin and they may have toxic side effects while, the phyto medicines have less toxicity and their importance being realized in both developed and developing countries. It is expected that, in near future the plant products will play a major role in the healthcare programmes of all countries. Plant biodiversity is an outward manifestation of chemical diversity, often with very attractive bioactivities[13]. They are the traditional source of many chemicals used as pharmaceuticals, biochemicals, fragrances, food colours and flavours[5]. The therapeutic effects of medicinal plants are associated with their chemical peculiarities. A medicinal herb is considered to be a chemical factory as it contains a number of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins sesquiterpenes, lactones and oils (essential and fixed)[21]. Secondary metabolites are compounds that are biosynthetically derived from the primary metabolites and their distribution in the plant kingdom is restricted. These compounds are detected in lower volume compared to the primary metabolites and also possess significant biological activities[14]. The secondary metabolites are indeed chemically complex and may contain one or more structurally related active compounds that produce a synergistic effect. Therefore, medicinal plants are perhaps the most valuable source of new bioactive chemical entities to benefit mankind against various ailments[6]. Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables. They are also available as dietary supplements[2]. Vegetables and fruits are rich sources of antioxidants[8]. There is good evidence that eating a diet with lot of vegetables and fruits is healthy and lowers risks of certain diseases. But it isn't clear whether this is because of the antioxidants, something else in the foods, or other factors[10]. Natural antioxidants present in plants have the ability to scavenge harmful free radicals generated in our body. Therefore, protection against free radicals can be improved by sufficient intake of dietary antioxidants. It is also suggested that intake of antioxidant rich foods play a vital and beneficial role in preventing or postponing the onset of degenerative diseases[1]. At present, the chromatography is the main tool used to identify the adulterants from the medicinal materials and extract products based on the chemical profile. Therefore, an effective and inexpensive analysis method to entirely monitor the whole constituents of the medicinal materials and their corresponding extract a product is required[9]. FT-IR has played a vital role in pharmaceutical analysis in recent years[22]. The FT-IR method measures predominantly

*Author for Correspondence: pvino726@gmail.com

Table 1: The analyses of phytochemicals in different extracts of *Pisonia grandis* leaves.

S.no	TEST	Aqueous	Methanol	Ethanol
1.	Alkaloids	++	+	++
2.	Saponins	-	+	+
3.	Steroids	+	+	++
4.	Terpenoids	+	+	++
5.	Tannins	+	++	++
6.	Phenols	++	+	++
7.	Flavonoids	++	+	++

(++) Copiously present, (+) Moderatly present, (-) Absence.

Table 2: Total Phenolic content and Total Flavonoid content of *Pisonia grandis* leaf extracts.

S.no	Extract	Total phenolic content(mg of GAE/g)	Total Flavonoid content (mg of RE/g)
1.	PGAE	21.26 ± 0.84	10.06 ± 0.42
2.	PGME	19.21 ± 0.41	09.16 ± 0.14
3.	PGEE	24.03 ± 0.17	11.21 ± 0.07

PGAE - *P. grandis R.Br.* Aqueous extract, PGME- *P. grandis R.Br.* Methanolic extract.
PGEE - *P. grandis R.Br.* Ethanolic extract.

Table 3: DDPH activity of different extracts of *Pisonia grandis* Leaves.

S.no	Extracts	IC50 values (µg/ml)
1	BHT	39.41
2	PGAE	137.11
3	PGME	79.12
4	PGEE	51.03

Table 4: ABTS activity of different extracts of *Pisonia grandis* Leaves.

S.no	Extracts	IC50 values (µg/ml)
1	BHT	45.27
2	PGAE	67.25
3	PGME	64.21
4	PGEE	42.43

Figure 1*: Pisonia grandis* R.Br.

the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic "fingerprint" of the sample[12]. In the present study demonstrate that *Pisonia grandis R.Br.* posses *in vitro* antioxidant activity and the bioactive compounds present in the *Pisonia grandis* leaves extracts were identified with the aid of FT-IR technique, which may provide an insight in its use as traditional medicine.

MATERIALS AND METHODS

Collection of plant material

The Fresh leaves of *Pisonia grandis* R.Br. (Nyctaginaceae) was collected from Omalur, Salem district, Tamilnadu, India (during 2016-2017). The collected plant material was identified and their authenticity was confirmed by comparing the voucher specimen at the herbarium of Botanical Survey of India, Southern circle Coimbatore, Tamil Nadu. Freshly collected plant material was cleaned to remove adhering dust and then dried under shade. The dried samples were powdered and used for further studies.

Solvent extraction

The air dried, powdered plant material was extracted in soxhlet apparatus successively with aqueous, methanol and ethanol solvent (1:5 ratio (w/v). The extract was then evaporated to dryness by rotary flash evaporator (Buchi type Rotavapor, Mumbai, India) and the resultant crude methanol and ethanol extract was used for further analysis. The leaves were separated and washed with double distilled water then frozen in liquid nitrogen, lyophilized using a vacuum freeze dryer (Christ alpha 1-2/LD plus, Germany) and the powder was stored at --20 degree Celsius until use. Then the lyophilized powder was resuspended with distilled water to produce the desired concentrations for further analysis.

Phytochemical Analysis

The Phytochemical screening of the *Pisonia grandis R.Br.* leaf extract was assessed by standard method[23,7]. Phytochemical screening was carried out on leaf extracts using different solvents (aqueous, methanol, ethanol) to identify the major natural chemical groups such as Alkaloids, Saponins, Steroids, Terpenoids, Tannins, Phenols, Flavonoids.

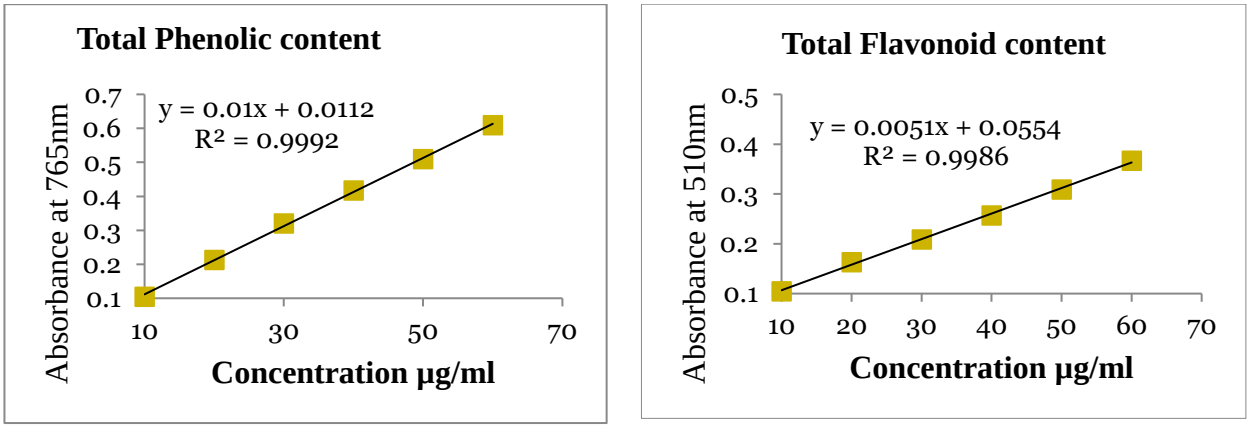
Quantitative Analysis

Determination of total phenolic content (TP)

Total phenolic content was determined by using Folin-Ciocalteu method[17]. Briefly, 93 an aliquot of the crude extract was made up to 3 ml with distilled water and allowed to react with 0.5 ml of Folin-ciocalteu reagent. After 3 minutes of reaction, 2 ml of 20% sodium carbonate was added to the reaction mixture and incubated for 30 minutes at room temperature. The absorbance was read at 765 nm using UV-Visible spectrophotometer (Shimadzu UV-1601, Columbia, MD, USA). The total phenolic content was expressed as mg of Gallic acid equivalents (GAE) / gram of the extract. The estimation of total phenolics in the extract was carried out in triplicate and the results were averaged.

Determination of total flavonoid content (TF)

A modified aluminum chloride method was used for determining the total flavonoids content in the plant



A B

Figure 2: (**A**) Gallic acid standard curve for total phenolic content (**B**) Rutin standard curve for total flavonoids content.

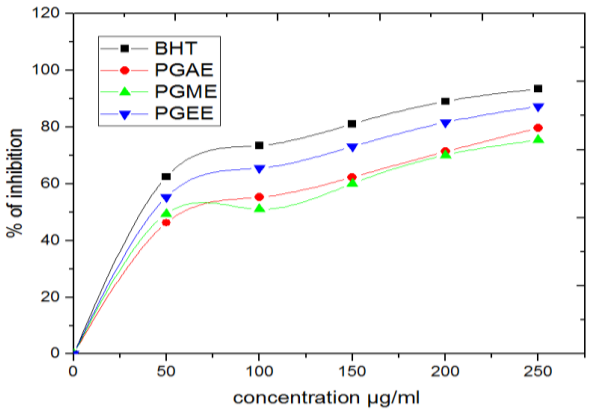


Figure 3: Graphical representation of DDPH activity of different extracts of *Pisonia grandis* Leaves.

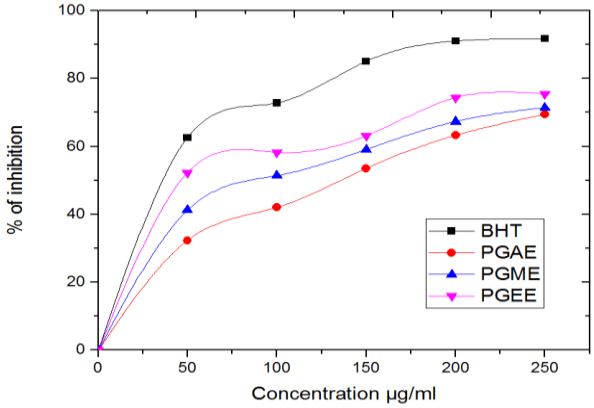


Figure 4: Graphical representation of ABTS activity of different extracts of *Pisonia grandis* Leaves.

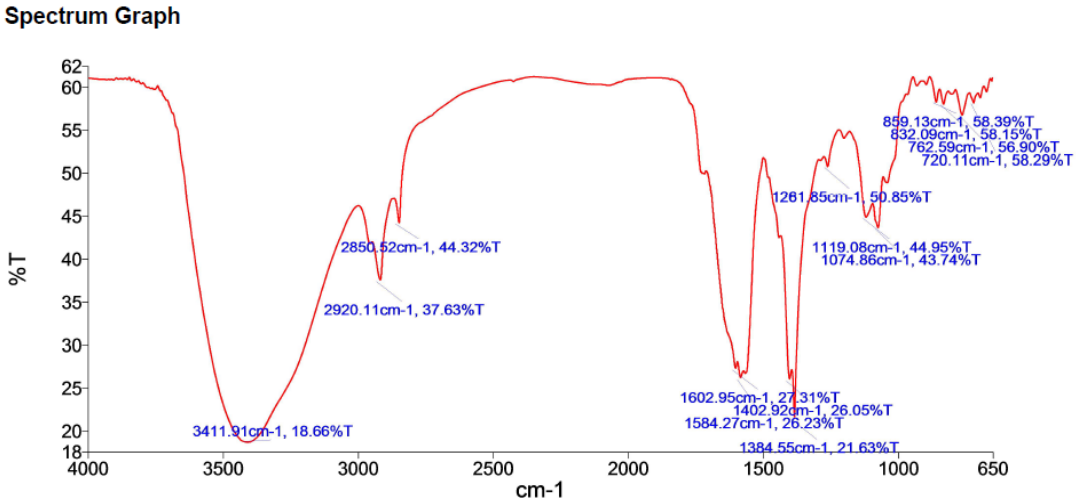


Figure: 5 FT-IR analysis of ethanolic extract of leaves of *Pisonia grandis.*

extract. A total of 100 µl of sample (1 mg/ml) was mixed with 600 µl of methanol, 40 µl of 10% aluminum chloride, 40 µl of 1 M potassium acetate and made up to 2 ml with distilled water. The tubes were kept at room temperature for 30 min and the absorbance was measured at 420 nm using UV–visible spectrophotometer. The total flavonoids content was obtained from extrapolation of the calibration curve, which was made by preparing various concentrations of a Quercetin solution in methanol. The total flavonoids content was expressed as mg of Quercetin equivalents (QE) / gram of the extract.

In vitro antioxidant activity

The methods followed to determine the antioxidant activity of ethanol extract of the leaves were DPPH radical

Table 5: FT-IR peak values of ethanolic extract of *Pisonia grandis* Leaves.

S. No	Peak	Frequency range	Functional groups
1	3411.91	3500-3200 (s,b)	O-H stretching vibration presence of alcohols, phenols
2	2920.52	3000-2850(m)	C-H stretching vibration presence of alkenes
3	2850.52	3300-2500(m)	O-H stretching vibration presence of carboxylic acids
4	1602.95	1680-1640(m)	-C=C- stretching vibration presence of alkenes
6	1402.92	1500-1400(m)	C-C stretching vibration presence of aromatics
7	1261.85	1320-1000(s)	C-O s stretching vibration presence of alcohols, carboxylic acids, esters, ethers
8	1119.08	1250-1020(m)	C-N s stretching vibration presence of aliphatic amines
9	1074.86	1250-1020(m)	C-N stretching vibration presence of aliphatic amines
10	859.13, 832.09	910-665(s,b)	N-H wag stretching vibration presence of primary secondary amines

scavenging assay and scavenging of ABTS radical cation activity.

Determination of 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity(DDPH)

DDPH radical scavenging activity was determined using the method described previously[20] with minor changes. An appropriate dilutions of the extract (1 mg/ mL) were mixed with 1 mL of 0.4 mM methanolic solution of DPPH radical. Absorbance was measured at 516 nm after 30 min of reaction. The inhibition percentage was calculated using the following formula:

% Radical scavenging activity

= [(Abs con–Abs sample)] / (Abs con)] × 100

Where, Abs con is the absorbance of control; Abs sample is the absorbance of test sample extract/standard. Butyl hydroxyl toluene (BHT) was taken as a reference standard.

Determination of 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid ABTS test

ABTS decolorization activity was determined by the method described previously[16].One ml of ABTS·+ solution was reacted to varying concentrations of the extract for 6 min, and the absorbance was immediately measured at 734nm after 7min using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of BHT. Results are expressed as percent of radical scavenging activity. The inhibition percentage was calculated using the following formula:

% Radical scavenging activity

= [(Abs con–Abs sample)] / (Abs con)] × 100

Where, Abs con is the absorbance of control; Abs sample is the absorbance of test sample extract/standard.

Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of different solvent extracts of each plant materials were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm $^{-}$1 with a resolution of 4cm^{-1}.

RESULTS AND DISCUSSION

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. The interest of medicinal plants exploration as a source of pharmacologically active compounds has increased worldwide. In most developing countries of the world, plants are the main medicinal sources used in treating infectious diseases. The various phytochemical compounds detected are known to exhibit medicinal activity. In the present study preliminary phytochemical analysis of methanol and ethanol extracts of *Pisonia grandis* showed the presence of alkaloids, flavonoids, phenols, saponins, steroids, terpenoids and tannins. The aqueous extract of *Pisonia grandis* showed the presence of alkaloids, flavonoids, phenols tannins, steroids and terpenoids except saponins **(**Tab:1). The Antioxidant shows an important Scavenging activity for free radicals of DPPH (1,1-Diphenyl-2-picryl hydrazyl) and ABTS (2, 2′-azino-bis 3-ethylbenzothiazoline- 6-sulfonic acid action decolorization) is widely used in pathogenesis of many diseases. The usage of synthetic antioxidant components may shows many side effects like toxicity and mutagenic effects, it made an alternative search of naturally occurring antioxidants [19]. Different accessions of *Pisonia grandis* leaf samples were used for antioxidant studies. The free radical scavenging activity of the methanol, ethanol and aqueous extracts of leaves of *Pisonia grandis* by the DPPH method exhibited potent scavenging effect against DDPH with inhibition of IC 50 values ranged from 42.43µg/ml, 64.21 µg/ml and 67.25 µg/ml compared with that of standard BHT 45.27 µg/ml(Tab:3, Fig:3). The ABTS scavenging activity with inhibition of IC 50 values ranged from 51.03 µg/ml, 79.12 µg/ml and 137.11 µg/ml compared with that of standard BHT 39.41 µg/ml.(Tab:4, Fig:4). Also, the antioxidant effect of PGEE, PGME, PGAE was due to various mechanism like hydrogen donating. Phenolics are the most widespread secondary metabolite in plant kingdom. These

diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as radical scavengers. Phenolic compounds are a class of antioxidant agents which act as free radical terminators [15]. In our study, total phenol content (TPC) of *Pisonia grandis* leaf extract was estimated by using Folin-Ciocalteau colorimetric method and represented in terms of gallic acid equivalent (GAE). The result of the present study showed that the phenol contents of the PGEE, PGAE, PGME are 24.03 ± 0.17 mg GAE/g, 21.26 ± 0.84mg GAE/g, 19.21 ± 0.41 mg GAE/g.(Fig:2A) The flavonoid shows an important role in antioxidant activity and their effects in human nutrition. The mechanisms of action of flavonoids are through scavenging or chelating process[11]. The result of the present study showed that the flavonoid contents of the PGEE, PGAE, PGME are 11.21 ± 0.07mg RE/g, 10.06 ± 0.42 mg RE/g, 09.16 ± 0.14 mg RE/g. (Fig:2B). The TFC was determined using spectrophotometry method with aluminum chloride and is expressed as rutin equivalent .The FT-IR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FT-IR, the functional groups of the components were separated based on its peaks ratio. The results of FT-IR analysis confirmed the presence of alcohols, alkenes, Phenols, aromatic carboxylic acid, esters, aliphatic amines, and primary secondary amines.

CONCLUSION

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 could be concluded that *Pisonia grandis* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance. The presence of significant amount of respective bio-active compounds in these plants under study and further use in the pharmaceutical and nutraceutical sector.

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