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Research Article

A STUDY ON PHYTOCHEMICAL SCREENING, ANTIOXIDANT, ANTIMICROBIAL AND α-AMYLASE INHIBITORY ACTIVITIES OF CRUDE EXTRACTS OF THE STEM BARK OF *PISONIA GRANDIS*, R.BR

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ABSTRACT

Objective: The aim of the present study is to determine the phytochemical screening, antioxidant activity and α-amylase inhibitory activity of the crude hexane, ethyl acetate and ethanolic stem bark extract of *Pisonia grandis*.

Methods: The evaluation of antioxidant and antimicrobial activity, total phenolic, and flavonoid content were assessed using 2,2-diphenyl-1-picrylhydrazyl, Folin–Ciocalteu's reagent, and aluminum chloride assay, respectively. The antidiabetic activity was assessed for porcine pancreatic α-amylase for the stem bark of *P. grandis.*

Results: Phytochemical screening confirmed the presence of phenolic, flavonoids, tannins, saponins, terpenoids, and steroids in all the three extracts. The antioxidant activity showed 148.2 µg/ml, total phenolic content (gallic acid equivalent), 0.0665±0.0002 mg/g, flavonoid content (quercetin equivalent), 0.6061±0.1817 mg/g, and inhibitory concentration 50% values were found to be 40.42 µg/ml and showed better in ethyl acetate extract. The antidiabetic activity exhibited mimic action with insulin due to the presence of pinnatol in the stem bark and leaves of *P. grandis.*

Conclusion: *P. grandis* stem bark crude ethyl acetate extract showed strong antioxidant activity, high phenolic, and flavonoid content. The antimicrobial activity was studied in both Gram-positive and Gram-negative strains against ampicillin and rifampicin as reference drugs. Antidiabetic activity shows effective result by α-amylase inhibitory activity.

Keywords: *Pisonia grandis*, Antioxidant, Antimicrobial, α-amylase, Total phenolic content, Total flavonoid content, Plant extract.

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INTRODUCTION

Pisonia is a genus of a flowering plants of family Nyctaginaceae. *P. brunoniana* of Australasia and Polynesia and *P. umbellifera*, which is widespread in the tropical Indo-Pacific region [1], belongs to the same species. *Pisonia grandis* has been extensively used in Indian traditional medicine as an antidiabetic, anti-inflammatory agent, and used in the treatment of analgesic, ulcer, dysentery, and snake bite.

As Ayurvedic literature reveals that *P. grandis* has tremendous traditional and medicinal uses including analgesic, anti-inflammatory, and diuretic activities [2], has a protective wound healing potential on Wistar rats excision wound and incision wound and antibacterial activity [3], possess antifungal activity against various microorganisms [4], antidiabetic activity [5], anxiolytic activity in mice [6] was studied, antioxidant activity [7,8], antiplasmodial activity [9], antipyretic activity [10], hepatoprotective [11], *anti-arthritic activity* [12], used in the treatment of analgesic, ulcer, dysentery and snake bite [13,14], anorexia, jaundice [15], *and various high performance thin layer chromatography fingerprinting analysis were performed and reported* [16,17].

In this study, we investigated the phytochemical and biological screening of *P. grandis* stem bark for the crude extracts. The novelty of the method includes the α-amylase inhibitory activity of pinnatol which helps in the inhibition of the α-amylase from the stem bark of *P. grandis* using pancreatic α-amylase enzyme [18,19]. Apart from this the antioxidant and the antimicrobial assay were also performed.

METHODS

Chemicals

The chemicals used in the present study were sodium nitroprusside, sulfanilic acid, phosphate buffer saline (pH 7.4), Naphthyl - N-ethylene diamine, phosphoric acid, dimethyl sulfoxide, ascorbic acid, α-amylase potato starch, sodium chloride, silver nitrate, sodium potassium tartrate, sodium hydroxide, 3, 5-dinitro salicylic acid, and acarose. All the chemicals utilized were of AR grade.

Collection and authentication of plant

The plant was collected in and around SRM University, Kattankulathur campus and authenticated by Dr. P Jayaraman, Director, Plant Anatomy Research Centre Medicinal Plants Research Unit, Tambaram, and Chennai - 45.

Chemical constituents

P. grandis has numerous bioactive compounds includes pinnatol, allantoin, β-sitosterol, α-spinasterol, β-sitosterol glucoside, octacosanol, dulcitol, flavonoids, and quercetin the major chemical constituents were shown in Fig. 1. These are the compounds which have been isolated from the leaves of the plant [20].

Preparation of plant extraction

Coarsely powdered the stem bark of *P. grandis* procured was extracted successively with various solvents ranging from nonpolar to polar, i.e., n-hexane, ethyl acetate, and ethanol using cold maceration method and the respective solvents were recovered under rotary evaporator. In each solvent, the plant material was soaked for 72 hrs at 30±2°C,

filtered and to the residue, the same solvent was added and repeated thrice to become colorless. All the filtrates were pooled, and solvent was removed in a rotary evaporator under vacuum.

Physiochemical evaluation

Ash values, extractive values and foaming index were determined according to the WHO guidelines and moisture content was determined by drying at 105°C until a constant weight was achieved.

The physiochemical parameters such as total ash, acid insoluble ash, and water soluble ash were performed as per the standard method [20]. The extractive value determines the actual percentage of active constituents extracted using solvents from a specified amount of plant extract and the alcohol extractive value, water soluble extractive, loss on drying, and foaming index were performed, and the results were tabulated in Table 1.

Preliminary phytochemical screening

Phytochemical analysis [21] was performed as per the standard methods for the presence of alkaloids, reducing sugars, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, resins, and starch.

Determination of phenolic content

The content of total phenolic compounds in plant extracts was determined by Folin–Ciocalteu's reagent using ultraviolet (UV) spectrophotometer (UV-1501PC) [22]. 0.5 ml of diluted plant extract and standard of different concentrations solution were taken in the test tube followed by adding 5 ml of Folin-Ciocalteu's (diluted 10-fold with water) and 4 ml of sodium carbonate (1 M), respectively. Solutions were then incubated for 15 minutes and protected from sunlight. The absorbance was measured at 765 nm. Calibration curve of gallic acid was set using a different concentration of 25, 50, 100, and 200 µg/ml. The extracts were calculated according to the following formula:

$T = (c \times V)/m$

Where, T is the total content of phenolic compounds in mg/g plant extract; c is the concentration of gallic acid established from the calibration curve in mg/ml; V is the volume of extract in ml, and m=the weight of extract in g. The value of total content of phenolic compounds is expressed as gallic acid equivalent (GAE) in mg/g extract [23].

Determination of flavonoid content

Total flavonoid in the crude extract was measured using the aluminum chloride colorimetric assay [24]. To 1 ml of plant extract or standard of different concentrations 3 ml methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml potassium acetate (1 M), and 5.6 ml of distilled water were added. Then, the solution was incubated for 30 minutes at room temperature. The absorbance was measured at 415 nm against a blank. Standard curve was prepared using quercetin by dissolving it in methanol followed by serial dilution to 25, 50, 100, and 200 µg/ml.

Biological activity

Antioxidant assay

Antioxidant activity [25] of the extracts was performed using method 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging [26,27]. 1 ml of 0.1 mM solution of DPPH in methanol was added to 3 ml of the solution of all the extracts in ethanol at different concentrations (5, 20, 40, 60, 80, and 100 µg/ml). The mixtures were shaken and were allowed to stand in dark room for ½ hr. Then, the absorbance was measured at 517 nm using UV spectrophotometer. Finally, scavenging capability of DPPH radical was determined by the formula:

Scavenging effect = $[(A_0 - c_1)/A_0] \times 100$

Where, A_0 is the absorbance of the control,
A_1 is the absorbance in the presence of all of the extract samples.

Nitric oxide scavenging method

The samples were treated with Griess reagent, and optical densities of the resultant chromophores were determined at 546 nm. Among the three extracts tested for stem bark of plant, ethyl acetate extract of *P. grandis* has shown high potent antioxidant activity with inhibitory concentration 50% (IC_{50}) value of 200.9 µg/ml.

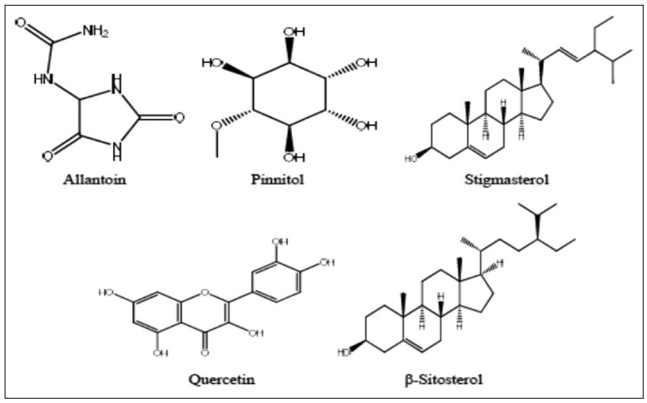

Fig. 1: Major chemical constituents of the leaves of plant

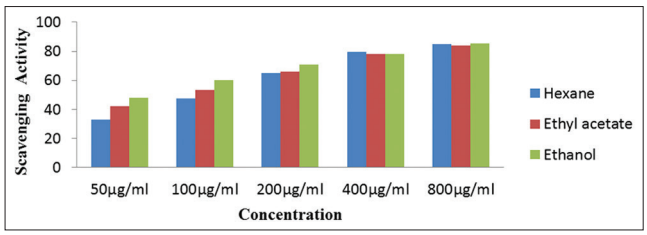

Fig. 2: Antioxidant activity of *Pisonia grandis* by 2,2-diphenyl-1-picrylhydrazyl radical scavenging method

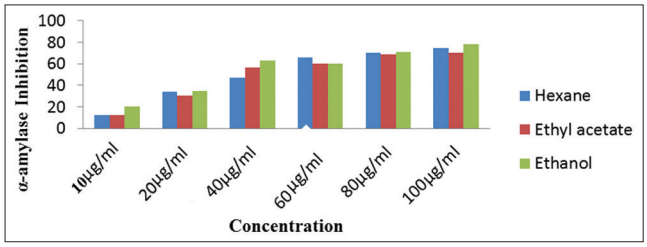

Fig. 3: Antidiabetic activity of *Pisonia grandis* by α-amylase inhibitory activity method

Table 1: Physicochemical parameters of *P. grandis*

Plant name	Ash value % w/w			Extractive value % w/w		Foaming index	Loss on drying %w/w
	Total ash	Acid insoluble ash	Water soluble ash	Water soluble	Alcohol soluble		
P. grandis	14.5	11.5	4.5	24.0	16.0	111.11	10.0

P. grandis: Pisonia grandis

Antimicrobial activity

As the leaves of the plant possess antimicrobial potency, the stem bark was screened for antimicrobial activity [25,28]. Four microbes, i.e., Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* while Gram-negative *Escherichia coli* and *Klebsiella pneumoniae* were used. Ampicillin (for Gram-positive) and tetracycline (for Gram-negative) were used as a standard.

α-amylase inhibitory activity

The plant extracts were screened for α-amylase inhibitory activity [29] at different concentrations using the chromogenic method from Sigma-Aldrich. Porcine pancreatic α-amylase (EC 3.2.1.1, Type VI, Sigma) was dissolved in ice-cold distilled water to give a concentration of 4 unit/ml solution. Potato starch (0.5, w/v) in 20 mM phosphate buffer (pH 6.9) containing 607 mM sodium chloride, was used as a substrate solution. α-amylase activity was determined by measuring the absorbance at 540 nm.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The extracts were subjected to preliminary phytochemical analysis, and the results were tabulated in Table 2.

Physicochemical properties

Physicochemical evaluation like ash values, i.e., total ash value, acid insoluble ash, water soluble ash, and extractive values such as alcohol extractive value, and water extractive value were performed foaming index and loss on drying were found to be 111.11% and 10.0% w/w, respectively.

Antioxidant assay

Estimation of total phenol content (TPC)

TPC of extracts of *P. grandis* was expressed in terms of GAE. Among the three extracts tested ethyl acetate extract has shown high TPC, 0.6061±0.1817 mg/g of gallic acid. The phenolic content in various solvents decreases in the order of EtOAc > hexane > ethanol, and the results were depicted in Table 3 and the DPPH scavenging activity was shown in Fig. 2.

Estimation of total flavonoid content (TFC)

TFC of extracts of *P. grandis* was expressed in terms of quercetin equivalents. Among the three extracts tested ethyl acetate extract has shown high TPC, 0.0665±0.0002 mg/g of gallic acid. The phenolic content in various solvents decreases in the order of EtOAc > hexane > ethanol, and the results were depicted in Table 3.

Antioxidant activity

In vitro, antioxidant activity was performed by DPPH and nitric oxide radical scavenging methods, and the results were tabulated in Table 4.

Antimicrobial activity

Ethanol extract of the stem bark of *P. grandis* showed a high level of inhibition against *E. coli* with a value of 94 µg/ml and the results were depicted in Table 5.

α-amylase inhibitory activity

The plant claimed to have antidiabetic activity as the leaves contain pinnatol which mimic the action of insulin. Among the three extracts tested ethyl acetate extract of *P. grandis* has shown high potent antidiabetic activity with an IC_{50} value of 40.42 µg/ml the results were depicted in Table 6 and the α-amylase inhibitory activity was represented in Fig. 3.

Table 2: Phytochemical analysis of stem bark of *P. grandis*

Phytoconstituents	Hexane	Ethyl acetate	Ethanol
Carbohydrates	-	+	+
Proteins	-	+	-
Starch	-	+	+
Amino-acids	-	-	+
Steroids	+	+	+
Cardiac glycosides	-	+	+
Anthraquinone glycosides	-	-	-
Alkaloids	-	+	+
Flavonoids	-	+	+
Tannins	-	-	-

P. grandis: Pisonia grandis

Table 3: Total flavonoid and phenolic contents of *Pisonia grandis*

Extracts	Total phenolic in µg of **GAE/mg of dried mass	Total flavonoid in µg of ⁺QE/mg of dried mass
Hexane	0.2926±0.0003	0.0359±0.0001
Ethyl acetate	0.6061±0.1817	0.0665±0.0002
Ethanol	0.1919±0.0003	0.0215±2.9463

**Quercetin equivalents, ⁺Gallic acid equivalents, each value listed in the table is expressed as mean±SD (n=3), *P. grandis: Pisonia grandis,* GAE: Gallic acid equivalents, QE: Quercetin equivalents

DISCUSSION

Phytochemical screening not only helps to reveal the constituents of the plant extracts and the one that predominates over the others but also is helpful in searching for bioactive agents those can be used in the synthesis of useful drugs. In this study, the hexane, ethyl acetate, and ethanolic extract of stem wood of *P. grandis* showed the dose-dependent antioxidant activity. Further, the significant antioxidant activity can be due to the presence of phenols, flavonoids, tannins, polyphenols, and reducing sugars. We reported the IC_{50} value of hexane and ethanolic extract to be lower than that of ethyl acetate extract. This showed that the radical scavenging property is higher in ethyl acetate extract of *P. grandis* and the IC_{50} value was found to be 148.2 µg/ml. The antimicrobial activity was studied against different bacterial strains against ampicillin and tetracycline as control, and the ethanolic extract of the stem bark showed greater inhibition. The minimum IC values were found to be 72 µg/ml for *S. aureus* and 83 µg/ml for *B. subtilis* against ampicillin as control at 20 µg/ml, whereas 94 µg/ml for *E. coli* and 63 µg/ml for *K. pneumonia* against tetracycline at 20 µg/ml as control, respectively.

The plant has pinnatol as the chemical marker in the leaves; a trial was performed with stem bark of *P. grandis* for the presence of pinnatol in the stem bark to mimic the action of insulin. The study confirms the significant antidiabetic activity on the porcine pancreatic α-amylase enzyme exhibiting the α-amylase inhibitory activity. Among all the three extracts the ethyl acetate stem bark extract of *P. grandis* showed potent antidiabetic activity, and the IC_{50} value was found to be 40.42 µg/ml.

This study may be proved to be an important step for the further study for identification, of compounds with the antioxidant, antimicrobial,

Table 4: *In vitro* antioxidant activity of *Pisonia grandis* by DPPH radical scavenging method

Plant extracts	% Scavenging activity					IC_{50} values
	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml	
Hexane	32.81	47.34	64.80	79.64	84.96	138.3 µg/ml
Ethyl acetate	42.25	53.27	66.03	78.23	83.91	148.2 µg/ml
Ethanol	47.79	60.22	70.65	78.03	85.22	55.27 µg/ml

P. grandis: Pisonia grandis, IC_{50}: Inhibitory concentration$_{50}$, DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 5: Antimicrobial activity of *Pisonia grandis* by MIC method

Strain	MIC value (µg/ml)			
	Hexane	Ethyl acetate	Ethanol	Ref. drugs
S. aureus	70	69	72	21[A]
B. subtilis	60	54	83	22[A]
E. coli	75	67	94	24[B]
K. pneumonia	65	72	63	23[B]

Ref. drugs=A-ampicillin 20 µg/mL, B-tetracycline 20 µg/mL, *S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia,* MIC: Minimum inhibitory concentration

Table 6: Antidiabetic activity of *Pisonia grandis* by α-amylase inhibitory activity

Plant extracts	% α-amylase inhibition						IC_{50} values (µg/ml)
	10 µg/ml	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
Hexane	12.38	33.92	47.10	65.80	70.41	74.61	39.46
Ethyl acetate	12.21	30.24	56.30	60.12	69.23	70.12	40.42
Ethanol	20.38	35.10	63.31	60.21	71.31	78.14	32.78

P. grandis: Pisonia grandis, IC_{50}: Inhibitory concentration$_{50}$

antidiabetic activities present in the stem bark of *P. grandis* for clinical use.

CONCLUSION

The results of the study revealed that different medically important phytochemicals were present in extracts of stem bark of *P. grandis*. This research has laid sufficient background for further study for identification, subsequent purification and isolation of compounds. The extracts possess antioxidant, antimicrobial, and α-amylase inhibitory activity. Thus, the study has helped in establishing scientific evidences in the rationality of traditional use of plants for curing different human diseases. Hence, these findings will be useful toward establishing pharmacognostic standards on identification, purity, quality, and classification of the plant, which is gaining relevance in plant drug research.

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