



IDENTIFICATION OF CHEMICAL CONSTITUENTS AND PHARMACOLOGICAL EVALUATION OF *PISONIA GRANDIS* R.BR USING CHROMATOGRAPHY AND IN-VITRO TECHNIQUES

Pankajaakshan P K

Lopaamudra Charitable Trust, Dhoni Road, Palakkad, Kerala, India

Hashim K M*

Uwin life sciences, Malappuram, kerala-india *Corresponding Author

Harsha Shailesh

Rvs college of arts and science, Sulur, coimbatore

ABSTRACT

Pisonia grandis R.Br is one such medicinal plant of the Nyctaginaceae family with a high medicinal potential and is freely available in India. It is commonly known as 'Leechaikottaikerai' in tamil and "Velati selat" in hindi. The leaves, stems and roots of this species are extensively used by the tribals in the preparation of several folk medicines. The plant is traditionally used as anti-rheumatic and antifungal. This plant is also pharmacologically studied for its anti-fungal, antioxidant, anti-microbial, anti-inflammatory, anti-diabetic, diuretic, analgesic and wound healing properties. The present scientific investigation deals with the GC-MS analysis of methanolic extract of the plant extract, F/P ratio and phytochemical preliminary analysis of the plant *Pisonia grandis* R. Br.

KEYWORDS :

INTRODUCTION

The plant *Pisonia grandis* R.Br belonging to the family Nyctaginaceae, is an evergreen glabrous garden tree with young shoots are minutely puberulous. It is a native of Hawaii Island also naturalized throughout India. In the alternative system of medicine, *pisonia grandis* leaves are used as analgesic, anti-inflammatory, diuretic and hypoglycemic agent [1]. Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently envogue in parts of the world[2]. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region[3]. The present study deals with the quantification of volatile compounds in leaves using GCMS analysis, F/P ratio and preliminary phytochemical analysis.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

Pisonia grandis plants were collected from around the areas of sulur and dried at room temperature while being protected from the direct sunlight. Then leaf were powdered and stored for further use.

PREPARATION OF THE EXTRACT

2.5g powder was weighed each and macerated in 25ml of ethanol, methanol, petroleum ether, aqueous, chloroform separately. The mixture was kept in room temperature for two days. Then these extracts were used for further analysis.

PRELIMINARY PHYTOCHEMICAL SCREENING

1. TEST FOR ALKALOIDS (Mayer's Test)

Extracts were dissolved individually in dilute Hydrochloric acid and filtered. Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

2. TEST FOR CARBOHYDRATES (Benedict's Test)

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used for the presence of carbohydrates. Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

3. TEST FOR GLYCOSIDES

To 2ml of the extract added 4 drops of chloroform and 2 drops of concentrated sulphuric acid is added along the side of the test tubes. Then development of a brownish ring at the interface of the two liquids and appearance of violet colour in the supernatant layer indicate the presence of glycosides.

4. TEST FOR SAPONINS (Foam Test)

0.5g of extract was shaken with 2ml of water. If foam produced persists for 10minutes it indicates the presence of saponins.

5. TEST FOR PHYTOSTEROLS (Salkowski's Test)

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpnes / phytosterols.

6. TEST FOR PHENOLS (Ferric Chloride Test)

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

7. TEST FOR TANNINS

About 1-2ml of the extracts was taken. A few drops of 5% ferric chloride was added and observed for brownish green or blue black coloration.

8. TEST FOR FLAVONOIDS (Lead acetate Test)

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

9. TEST FOR AMINO ACIDS (Ninhydrin Test)

To the extracts, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

10. TEST FOR DITERPENES (Copper Acetate Test)

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

ESTIMATION OF TOTAL PHENOLS:

The total phenolics were estimated by the Folin's phenol method. The calibration curve was obtained for standard gallic acid in the concentrations 0.10 to 5 mg/ml is presented in the standard graph. The quantity of the phenolics present in the methanolic extract of the was calculated as % mg Eq of Gallic acid.

ESTIMATION OF TOTAL FLAVONOIDS:

The total Flavonoids was estimated by the Aluminium chloride method. The calibration curve was obtained for standard quercetin in the concentrations 0.5 to 3 ug/ml is presented in the standard graph. The quantity of the flavonoids present in the sample was calculated as % mg Eq of Quercetin.

GCMS ANALYSIS

The GC-MS analysis of *Pisonia grandis* R. Br was carried out under the following conditions.

SPECIFICATIONS:

- **Detector:** Flame Ionization Detector
- **Fuel Gas:** Hydrogen
- **Carrier Gas:** Nitrogen
- **Flow Rate:** 2.5 ml/mn
- **Column:** HP-5 (5% Phenyl Methyl Siloxane) Capillary (30mX320umX0.25um)
- **Injection T:** 260°C
- **Detector T:** 240°C
- **Oven T:** 120 for 2 minutes
120 to 220 @4/min
220 to 260 @5/min
260 maintained for 5 minutes
- **Split Ratio:** 60:1
- **Split Flow:** 60 ml/mn
- **Total Run time:** 50 Minutes

SL NO	COMPOUND NAME	Content as per integrated peak area method
1	CAMPHENE	9.50
2	ALPHA HUMULENE	6.90
3	EUDESMOL	4.40
4	ERIOFLORIN METHACRYLATE	2.60
5	LINALOOL	2.45
6	ZERUMBONE	1.90
7	NEROLIDOL	1.00
8	BETA PINENE	0.98
9	KAEMPFEROL	0.20

CONCLUSION

From the present work we conclude that the leaves of *Pisonia grandis* have a variety of secondary metabolites. The biochemical parameters were also quantified and the F/P ratio was calculated. It also shows the biological efficacy of the leaves. The volatile constituents were identified using GCMS and significant compounds were found especially in the class of terpenoids.

RESULTS

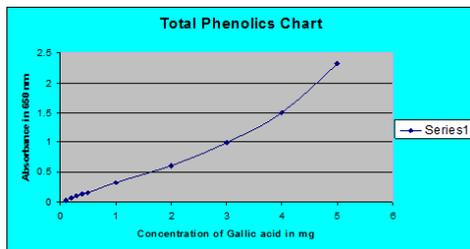
Table 1: Results of phytochemical screening

SI No	Compounds	Ethanol	Methanol	Petroleum Ether	Choloroform	Aqueous
1	Alkaloid	+	+	-	-	-
2	Flavonoids	+	+	+	+	+
3	Gycosides	-	-	-	-	-
4	Phenols	+	+	-	-	+
5	Saponins	+	+	-	-	-
6	Tannis	+	+	-	-	+
7	Polysterols	+	+	-	-	-
8	Aminoacid	+	+	-	-	-
9	Diterpens	+	+	-	-	+
10	Carbohydrates	-	-	+	-	+

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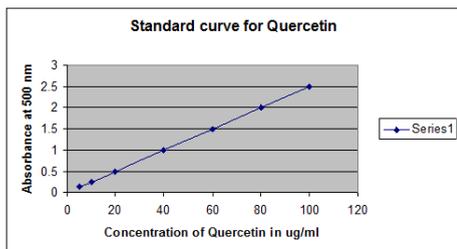
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Estimation of total phenol:



Total phenol content = 40.0 % mg Eq of Gallic acid

Estimation of total flavonoids content:



Total flavonoids content = 0.035 % mg Eq of Quercetin

F/P ratio = Total phenol / Total flavonoids = 1142.85

GCMS ANALYSIS

