INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

INHIBITION OF PROTEIN DENATURATION BY EXTRACTS OF LEAVES, STEMS AND ROOTS OF *PISONIA GRANDIS* R.Br.

G.Poongothai, Shubashini K.Sripathi *

Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamilnadu, India

*Corresponding Author Email: adusks2@gmail.com

Article Received on: 03/05/18 Approved for publication: 18/05/18

DOI: 10.7897/2230-8407.09573

ABSTRACT

Pisonia grandis R.Br. is a rural folkloric medicinal plant of *Nyctaginaceae* family and has been extensively used as an anti-diabetic and anti-inflammatory agent.The present study is aimed to evaluate the *in-vitro* anti-arthritic activity of the solvent extracts and the column fractionates of this plant. Denaturation of tissue protein is a proven cause of inflammatory and arthritic diseases. Inhibition of protein denaturation is a measure of anti arthritic activity. The petroleum-ether and ethanol extract concentrates of leaves, stems and roots of *Pisonia grandis* exhibited inhibition of protein denaturation. Among the extract concentrates of *Pisonia grandis* the non-polar extract concentrate of leaves of *Pisonia grandis* exhibited a 90 % inhibition of protein denaturation at 50 µg/ml concentration, comparable with that of standard drug Diclofenac sodium. The commercially well-known anti-arthritic drug palmitic acid showed 76% inhibition only at 800µg/ml. Column chromatographic analysis of petroleum ether extract of *Pisonia grandis* led to the isolation of stigmasterol which showed 66% inhibition of protein denaturation at 800µg/ml.

Key words: *Pisonia grandis*, Diclofenac sodium, Palmitic acid, Anti-arthritic activity

INTRODUCTION

Rheumatoid arthritis (RA) is a form of arthritis that causes pain, swelling, stiffness and loss of function in the joints of the body. It is an autoimmune disease and is more common in women. The production of auto antigens in arthritis causes the denaturation of protein. Controlling the production of auto antigen and inhibiting the denaturation of protein and membrane lysis in rheumatic disease leads to anti-arthritic activity. Hence, inhibition of protein denaturation is taken as a measure of *in-vitro* anti-arthritic activity[1]. *Pisonia grandis* R.Br. is a folkloric medicinal plant that has been extensively used as an anti-diabetic and anti-inflammatory agent. Fresh leaves, moistened with Eau-de-Cologne, find use in reducing inflammation of a filarioid nature in the leg and other parts of the body[2]. South Indians cook and eat the young leaves as a salad and also use it for the treatment of arthritis[3]. There are scientific reports on anti-arthritic activity and of ethanol extract of leaves of the plant[4] and anti-inflammatory potential of the leaves and roots of *Pisonia grandis*[5-7].Despite much investigation on leaves, there are no scientific reports on anti-arthritic activity of stems and roots of *Pisonia grandis.* Hence the present investigation was taken up for documentation of the anti-arthritic activity the various extracts of leaves, stem and roots of *Pisonia grandis*. Inhibition of albumin denaturation was taken as a measure of *in-vitro* anti-arthritic activity[8].

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Leaves, stems and roots of *Pisonia grandis* were collected from the local areas of Coimbatore. The collected plant parts were cleaned, shade dried and pulverized into small pieces.

Authentication of Chosen Plant

The plant *Pisonia grandis* was authenticated at the Institute of Forest Genetics & Tree Breeding (IFGTB) Coimbatore. Herbarium voucher specimen number is F.No. 14932.

Preparation of Extracts

Shade dried and pulverized parts of the chosen plant material (leaves, stems and roots) were extracted with commercial petroleum ether and ethanol at reflux temperature for six hours. Each extract was concentrated in vacuum and the residue weighed and stored. The extract concentrates were designated PGLP (Petroleum ether extract of leaves of *Pisonia grandis*), PGSP (petroleum ether extract of stems of *Pisonia grandis*), PGRP (petroleum ether extract of roots of *Pisonia grandis*), PGLE (ethanol extract of leaves of *Pisonia grandis*), PGSE (ethanol extract of stems of *Pisonia grandis*) and PGRE (ethanol extract of roots of *Pisonia grandis*).

Assessment of *in-vitro* Anti-Arthritic Activity by Inhibition of Albumin Denaturation

Egg albumin was obtained from hen's egg. About 0.2 ml of egg albumin was mixed with 2.8 ml of phosphate buffer saline (PBS at pH =6.4) and 2 ml of varying concentrations of extract to obtain final concentrations of 50, 100, 200, 400 and 800 µg/ml. Double distilled water was used as control. Diclofenac sodium was used as standard. Reagent mixture without extract served as blank. The test solutions were incubated at 37°C in a BOD incubator for 15 minutes and then heated at 70 ◦C for 5 mins. After cooling the absorbance of the solution was measured at 660 nm.

Percentage Inhibition = 100 X [V_t/V_c -1]

V_t = absorbance of test sample; V_c = absorbance of control

Column Chromatographic Separation

The pet-ether extract of the leaves (PGLP) was subjected to column chromatographic separation over silica gel column built in petroleum-ether. The column was eluted using petroleum-ether and a gradient solvent system of petroleum-ether: ethyl acetate. Eluates of 200 ml were collected; concentrated and the homogeneity of the fractions was examined by TLC. Similar fractions were combined. The fractions are designated Frn I to Frn VIII. One compound designated as PGL1 was isolated from fraction III.

Spectral Measurement

The ^{13}C NMR spectrum was recorded in Burker Avance III 500 MHz Spectrophotometer with $CDCl_3$ as solvent

RESULTS AND DISCUSSION

Preliminary phytochemical screening results of the extracts of *Pisonia grandis* indicated the presence of alkaloids, flavonoids, steroids, phenols, tannins, carbohydrates and proteins. Bio active molecules pinitol and allantoin are found to be major constituents of the polar extracts of the leaves and stems of this plant [9-11] and C-methylated flavonoids are found to be constituents of roots[12].

Characterization of PGL-1

The compound PGL-1 was isolated from the 100 % petroleum ether eluate of the silica gel column of PGLP. Melting point 142-146°C. TLC R_f=0.56 (petroleum ether: ethyl acetate 8:2 v/v). Compound PGL-1 was inferred as steroid moiety from the Libermann-Burchard test. A comparison of the ^{13}C NMR spectra data with that reported in literature[13] revealed compound PGL1 as stigmasterol. The ^{13}C NMR spectrum of the isolated compound is illustrated by Figure 1.

Assessment of anti-arthritic potential was carried out by *in-vitro* model and percentage inhibition (PI) of protein denaturation was taken as a measure of *in-vitro* anti-arthritic activity. The extract concentrates of leaves, stems and roots of *Pisonia grandis* inhibited protein denaturation. The percentage inhibition of protein denaturation of extract concentrates of *Pisonia grandis* (PGLP, PGLE, PGSP, PGSE, PGRP and PGRE), standard diclofenac sodium and palmitic acid is listed in table 1. The comparison of the efficacy of the extracts and the column fractions is shown in figures 2 and 3. All the extract concentrates of *Pisonia grandis* inhibited protein denaturation with PGLP exhibiting maximum inhibition of 90.79**%** at 50µg/ml concentration comparable with that of standard diclofenac sodium. Hence the extract PGLP was selected for column chromatographic analysis.

Assessment of anti-arthritic potential of all column fractionates revealed that Fractions I, II, III, V, VII possessed dose-dependent response to anti-arthritic activity (Table 2). Fraction III expressed maximum inhibition 97.03% at 600 µg/ml concentrations and the isolated molecule PGL1 showed maximum inhibition 51.21% at 600 µg/ml concentrations. The commercially well-known anti-arthritic drug palmitic acid showed maximum inhibition 76% at 800µg/ml.

Palmitic acid (n-hexadecanoic acid) has been identified as a most prevailing phytoconstituent of the petroleum ether extracts of leaves, stems and roots of *Pisonia grandis*[14]. This molecule has been reported to possess anti-oxidant, anti-alopecic, anti-androgenic, anti-fibrinolytic, hypercholesterolemic, anti-inflammatory and anti-tumor activity[15-18] and it finds use in the Ayurvedic system of medicine as a component of medicated oils to treat rheumatism. The *in-vitro* anti-arthritic activity of the extracts and column fractionates was compared with that of palmitic acid and stigmasterol.

The results indicate that the leaf pet-ether extract PGLP and the leaf ethanol extract PGLE exhibit *in-vitro* anti-arthritic potential much higher than that of palmitic acid at the same concentration. This is the first report of *in-vitro* anti-arthritic potential of *Pisonia grandis*.

Table 1 Percentage inhibition of protein denaturation of various extracts of *Pisonia grandis*

Drug (µg/ml)	Percentage Inhibition							
	PGLP	PGLE	PGSP	PGSE	PGRP	PGRE	STD*	Palmitic acid
50	90.79±0.043	85.89±0.075	63.49±0.043	35.19±0.043	49.07±0.043	4.08±0.115	91.22±0.075	4.67± 0.104
100	90.75±0.048	88.07±0.083	61.70±0.048	39.82±0.048	43.52±0.048	19.88±0.083	90.64±0.083	10.55± 0.174
200	88.97±0.597	85.42±0.103	59.57±0.103	28.37±0.060	39.23±0.060	30.58±0.060	88.97±0.597	33.79± 0.076
400	87.39±0.068	86.29±0.118	51.26±0.068	7.60±0.068	40.46±0.651	30.14±0.118	87.27±0.068	57.90± 0.115
800	73.21±0.151	70.59±0.151	22.95±0.756	7.94±0.151	36.56±0.151	43.54±0.151	73.04±0.262	75.95±0.067

**Diclofenac sodium*

Table 2 Percentage inhibition of protein denaturation of column fractions and isolated compound PGL1

Drug µg/ml	Percentage Inhibition								
	Frn I	FrnII	Frn III	Frn IV	FrnV	FrnVI	Frn VII	Frn VIII	PGL1
100	35.27± 0.008	17.16± 0.231	- 37.04± 0.040	2.92± 0.209	12.54± 0.042	-30.13 ±0.057	10.33± 0.088	-24.85 ±0.143	-2.26± 0.034
200	44.15±0 .083	36.05± 0.137	-62.75 ±0.073	14.74± 0.067	26.22± 0.056	-13.65± 0.125	27.72± 0.014	36.11± 0.084	14.87± 0.135
400	55.58±0 .138	55.44± 0.150	49.09± 0.075	39.03± 0.142	39.48± 0.062	-25.84± 0.095	42.32± 0.071	13.27± 0.070	34.87± 0.120
600	60.98±0 .119	64.53± 0.127	97.03± 0.177	65.72± 0.132	71.11± 0.050	-13.11± 0.057	55.53± 0.112	21.44± 0.095	51.21± 0.046
800	85.11±0 .037	71.63± 0.092	83.76± 0.118	73.37± 0.015	77.21± 0.070	6.19± 0.077	64.86± 0.110	-35.08± 0.078	66.43± 0.025

Table 3 Comparison of ^{13}C NMR chemical shifts of PGL1 and Stigmasterol

Chemical Shift δ (ppm)					
Carbon number	**PGL-1**	**Stigmasterol[14]**	**Carbon number**	**PGL-1**	**Stigmasterol[14]**
C-1	37.2	37.0	C-16	28.5	**28.5**
C-2	31.5	31.6	C-17	55.9	**55.8**
C-3	71.1	71.7	C-18	12.1	**11.6**
C-4	40.8	41.5	C-19	19.0	**19.2**
C-5	139.7	140.2	C-20	40.3	**40.1**
C-6	117.5	121.6	C-21	21.1	**20.4**
C-7	31.4	31.7	C-22	138.2	**138**
C-8	31.9	31.9	C-23	129.5	**129.2**
C-9	49.9	50.1	C-24	51.2	**51.0**
C-10	37.1	36.7	C-25	31.9	**32.0**
C-11	21.5	20.8	C-26	21.1	**19.0**
C-12	39.5	39.5	C-27	21.4	**21.2**
C-13	43.2	42.5	C-28	25.4	**25.4**
C-14	55.1	56.6	C-29	12.1	**12.0**
C-15	**25.4**	**24.0**			

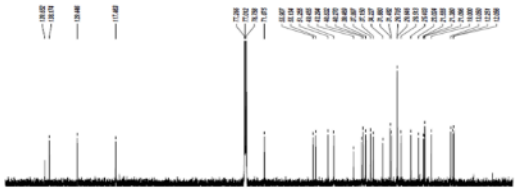


Figure 1 ^{13}C NMR spectrum of isolated compound PGL1

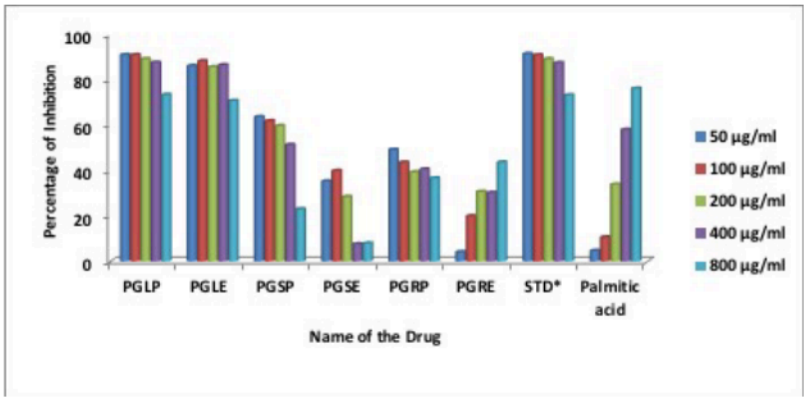


Figure 2 Percentage inhibition of protein denaturation of various extracts of *Pisonia grandis* compared with standard diclofenac sodium and palmitic acid

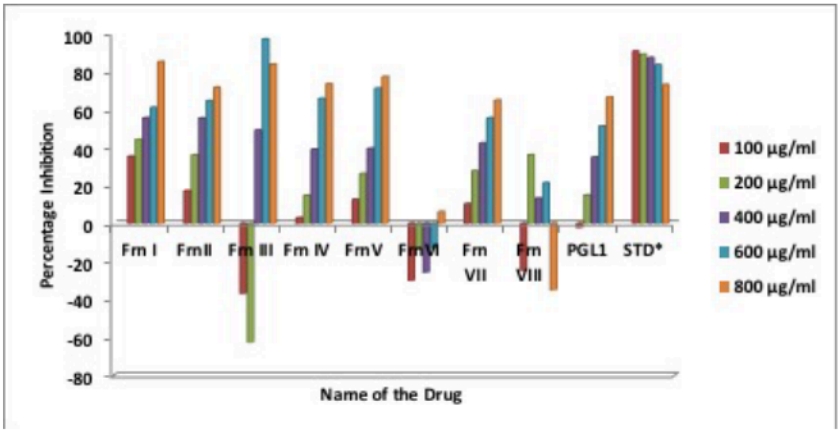


Figure 3 Percentage inhibition of protein denaturation of column fractions and isolated compound PGL1

CONCLUSION

The *in vivo* study[4] adopting Freund's adjuvant induced arthritis model[19] done to assess the anti arthritic potential of the defatted 90% ethanol extract of the leaves of *Pisonia grandis* on albino rats revealed that the percentage inhibition of paw volume with defatted ethanolic leaf extract was 68.32% at 300mg/kg body weight comparable to that observed with standard drug indomethacin at 10 mg/kg. In the present study the petroleum ether extract of the leaves of *Pisonia grandis* (PGLP) exhibited 90% inhibition of protein denaturation at 50 µg/ml concentration, comparable with that of standard drug diclofenac sodium. Hence the total ethanol extract of leaves of *Pisonia grandis* may be proposed as a prospective anti arthritic agent and this may be further validated by further *in vivo* studies.

ACKNOWLEDGMENT

The authors thank the authorities of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, India for providing necessary facilities. Authors acknowledge services of The KMCH College of Pharmacy, Coimbatore for the activity studies.

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Cite this article as:

G.Poongothai and Shubashini K.Sripathi. Inhibition of protein denaturation by extracts of leaves, stems and roots of *Pisonia grandis* R.Br. Int. Res. J. Pharm. 2018;9(5):53-56 http://dx.doi.org/10.7897/2230-8407.09573

Source of support: Nil, Conflict of interest: None Declared

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