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A COMPREHENSIVE REVELATION ON *PISONIA GRANDIS* R. BR.

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ABSTRACT: Medicinal plants are a huge treasure for mankind and a source of remedy for all illnesses as they are storehouses of bio-actives. The therapeutic efficiency of formulations and medicines depends on using standardized plant extracts, as The World Health Organization emphasized. The present documentation on the medicinal plant *Pisonia grandis* covers its pharmacological, phytochemical, biological standardization studies and the quantification protocols for the bioactive pinitol and bioactive allantoin earlier reported from this plant. This documentation sheet will aid as a reference for formulating *Pisonia grandis*-based herbal medicinal products.

INTRODUCTION: The plant *Pisonia grandis* is scientifically well-documented for its medicinal potential. Folkloric data on this plant indicates that leaves dipped with Eau-de-Cologne reduced inflammation due to filarioid in leg. Sugar levels reduce on chewing two leaves of the plant. An interesting observation is that the sticky seeds of the plant trap small birds; therefore, it is commonly termed bird-catcher tree [1]. Sea-birds that shelter in this tree are sources of guano that farmers use to grow plants. It is a symbiotic host for many mycobionts and demonstrates ectomycorrhizal fungus association in autotrophic plants [2]. Due to this nature, the plant serves as a good source of nitrogen and is further proved by another report on symbiotic association [3]. The plant is reported to possess anti-diabetic, wound healing, anti-oxidant, anti-microbial, anti-cancer, anti-inflammatory, anxiolytic, anti-pyretic, and hepato-protective potential. Two medicinally valuable molecules pinitol and allantoin have been isolated and characterized from the plant leaves [4, 5]. This revelation validates the anti-diabetic and wound-healing potential expressed by the leaf extracts.

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FIG. 1 *PISONIA GRANDIS* [4]

nitrogen and is further proved by another report on All parameters about standardization reported to date on this plant and its extracts have been documented in this review and comprise of data on pharmacognostic and phytochemical aspects, bio marker quantification and biological activity. All reports of earlier work, including figures are duly cited. **Fig. 1** represents the picture of the plant *Pisonia grandis.*

Rationale: There is paucity in standardization data on medicinal plants and quantification of bioactive present in them. This remains an impediment to the large-scale production of herbal medicines and consequently leads to their unsung popularity in the drug market. To overthrow these hindrances, documentation of potential medicinal plants is a prerequisite.

TABLE 1: PROVIDES A FACT SHEET ON THE PLANT *PISONIA GRANDIS*

Scientific Name	***Pisonia grandis* R. Br**
Taxonomic Identity Number	504419
Subkingdom	*Viridiplantae*
Class	*Magnolopsida*
Order	*Caryophyllales*
Family	*Nyctaginaceae*
Genus	*Pisonia*
Synonyms	*Pisonia alba Span; Pisonia morindifolia R.Br. Pisonia sylvestris Teijsm & Binn*
Vernacular names	Hindi: Chinaisalit, Tamil: Lechaikottai, Maruval, Chandu, Telugu: Lanchamundaku, Kannada: Sulesoppu, Gujarati: Velatisalet
Common names	Lettuce tree; Cabbage tree; Bird eating tree
Special name	The grand devil's-claw
Morphological Characteristics	A tall attractive an evergreen tree, 9-12 m high; Large Leaves 12-15 cm. pale greenish or yellowish; smooth bark, brittle and soft nature; buttress-like roots; produces flowers rarely; fruits are narrow club-shaped
Distribution	Seychelles; Indo-pacific islands; India, Sri Lanka, Malaysia, Australia, Indonesia, Madagascar
Adaptation	Salt and drought resistant plant. Its fibrous nature helps with water storage and allows for year-round flowering. (www.greenpatio.com)
Propagation	Natural Propagation takes place through birds. The sticky substance in the seeds of the plant makes it feasible to get attached to the belly of birds thus aiding propagation. Propagation in nursery takes place by planting the grafts
Chemical Constituents	Pinitol [4], allantoin[5] ,β-sitosterol[6], α-spinosterol, β-sitosterol glucoside, dulcitol, quercetin [7] from leaves C-Methylated glycosides[8] from roots
Biomarkers	Pinitol, Allantoin, Quercetin
Therapeutic Uses	Used for the cure of arthritis, diabetes, fever and topical wounds
Folk Use	Decoction of leaves is consumed to cure diabetes; Paste of leaves is applied externally to alleviate rheumatoid arthritis pain in the joints
Medicinal Part	Leaves, Stem-bark
Pharmacological Potential	Analgesic, anti-pyretic, diuretic, wound healing, anti-diabetic, free radical scavenging, anti-inflammatory, anti-arthritic, anti-microbial, hepatoprotective, anxiolytic, anti-plasmodial
Dosage and Safety	Leaf ethanol extracts - safe and non-toxic up to 2000mg/kg body weight [8, 9, 10, 11, 12]
Mention in Databases	Napralerttm database, National Resource Conservation Service –Plants Database of United States Department of Agriculture, Flora of British India' Hooker *et al.*, 1973, Flora of China' XianGuoTengShu, 2003
History	Discovered by Robert Brown from islands in the Gulf of Carpentaria (Flinders, 1814)

This review is a thorough revelation of the medicinal potential of *Pisonia grandis*. Standardization of plants and plant extracts involves the assessment and documentation of data on the following aspects.

Pharmacognostic and Phytochemical Analysis: Details of plant authentication, morphological characteristics, physicochemical characteristics, proximate parameters and qualitative phytochemical tests on plant material are necessary requisites for the standardization of plant and plant extracts

Fingerprinting of Extracts: Plant extracts' chromatographic and spectral fingerprint aid in selecting authentic plant material for herbal formulation.

Chemical Standardization: Quantifying phytochemical content and quantifying major bioactive markers in a plant extract is significant in enhancing the market value of herbal formulations.

Toxicity Studies: Toxicity studies on herbal extracts are mandatory for preparing safe herbal formulations. This review paper comprehensively documents all available data on *Pisonia grandis* for the ease of reference of herbal researchers and manufacturers. Standard methods and optimized protocols adopted in acquiring data on this plant are also mentioned in this document and are duly cited.

Plant Authentication: Authentication of *Pisonia grandis* was done at the Institute of Forest Genetics and Tree Breeding IFGTB, Coimbatore. Voucher specimens have been deposited in the herbarium of the Institute for further reference [F.No. 14932].

Extraction of Plant Material: The powdered plant material was sequentially extracted. The total ethanol extract was prepared by refluxing the plant material with ethanol for 6 hours. The sample codes of *Pisonia grandis* are presented in **Table 2A** and **2 B.**

TABLE 2A: DESIGNATION OF EXTRACTS OF *PISONIA GRANDIS*

Extract code	Extract
PGLP	Pet ether extract of leaves
PGSP	Pet ether extract of stems
PGRP	Pet ether extract of roots
PGLE	Ethanol extract of leaves
PGSE	Ethanol extract of stems
PGRE	Ethanol extract of roots
DPGLE	Dewaxed ethanol extract of leaves
DPGSE	Dewaxed ethanol extract of stems
DPGRE	Dewaxed ethanol extract of roots
PGLW	Aqueous extract of leaves
PGSW	Aqueous extract of stems
PGRW	Aqueous extract of roots

TABLE 2B: DESIGNATION OF PLANT MATERIAL OF *PISONIA GRANDIS*

Plant material code	Extract
PGL	Powdered leaves
PGS	Powdered stems
PGR	Powdered roots

Physicochemical Analysis: The data regarding the various physicochemical parameters from our earlier paper [14] is reproduced here in **Tables 3** to **7** for the sake of complete documentation in this data sheet on *Pisonia grandis.*

TABLE 3: ORGANOLEPTIC CHARACTERISTICS OF POWDERED LEAVES, STEM AND ROOTS OF *PISONIA GRANDIS*

Organoleptic Characteristic	PGL	PGS	PGR
Colour	Green	Pale yellowish green	Pale orangish yellow
Odour	Medicine like	Tree odour	Odourless
Taste	Tasteless	Bitter	Tasteless

*Data reproduced from our earlier paper [14].

TABLE 4: PROXIMATE ANALYSES

Proximate Parameters	% Proximate Content		
	PGL	PGS	PGR
Surface Moisture	10.0	8.0	9.0
Inherent Moisture	6.0	6.3	5.8
Ash	14.0	6.9	4.5
Alcohol extractive	16.20	7.20	9.80
Water extractive	34.70	13.80	16.40
Crude fiber	11.67	8.09	12.31
Volatile matter	74.20	77.00	76.60

*Data reproduced from our earlier paper [14]

TABLE 5: EXTRACTIVE VALUES

Extract	% Yield
PGLE	5.9
PGSE	5
PGRE	6
PGLW	29
PGSW	10
PGRW	14.5

*Data reproduced from our earlier paper [14]

The gross calorific value for leaves, stems and roots of *Pisonia grandis* is 3848.00, 3648.00 and 4026.00 respectively. The high volatile matter content indicates that the plant is host to essential oils and fatty material, which may contribute to its wound healing potential.

TABLE 6: ELEMENTAL CONTENT OF *PISONIA GRANDIS* (ICP-AES METHOD)

Element	% Element in Sample		
	PGL	PGS	PGR
Carbon	39.36	38.01	38.52
Nitrogen	3.69	1.06	2.08
Hydrogen	6.99	6.31	7.26
Sulphur	0.47	0.29	0.23
Calcium	2.64	1.36	1.29
Potassium	4.11	2.67	2.02
Magnesium	0.35	0.31	0.29
Sodium	0.88	0.55	0.48
Zinc (ppm)	31.58	24.56	19.26

*Data reproduced from our earlier paper [14]

TABLE 7: TOXIC METAL CONTENT OF *PISONIA GRANDIS* (ICP-AES METHOD)

Toxic Metal	Toxic Metal Content(ppm)		
	PGL	PGS	PGR
Lead	3.3	0.42	BDL
Cadmium	ND	0.01	0.01
Arsenic	ND	0.09	0.09

BDL= Below detection limit; ND = not detected

*Data reproduced from our earlier paper [14]

EDX Analysis: Elemental composition of the extract of leaves, stems, and roots of *Pisonia grandis* was done by energy Dispersive X-ray analysis (EDX) analysis. The respective EDX spectra are represented in **Fig. 2, 3** and **4**. The EDX analysis ascertains the absence of heavy metals and toxic elements in the extracts.

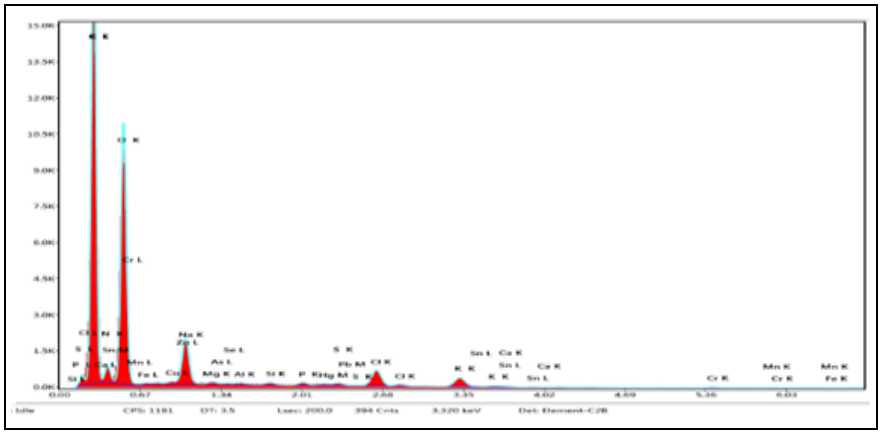

FIG. 2: EDX SPECTRUM OF PGLE

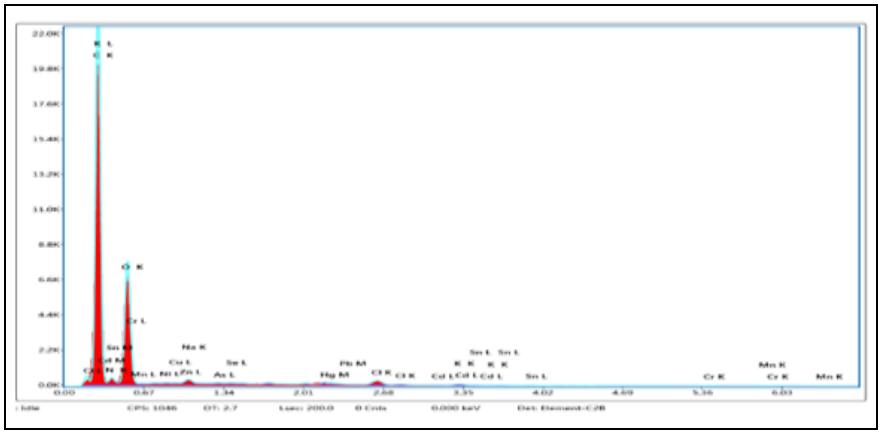

FIG. 3: EDX SPECTRUM OF PGSE

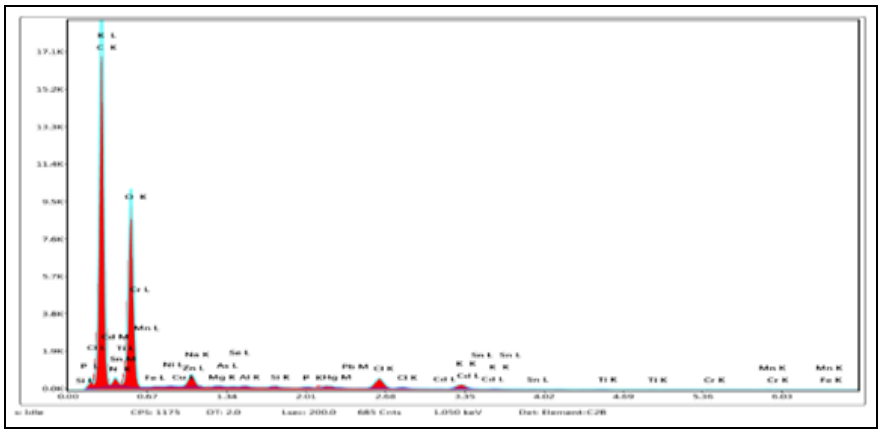

FIG. 4: EDX SPECTRUM OF PGRE

Fluorescence Analysis [14]: The fluorescence displayed by dry powdered leaves, stems and roots of *Pisonia grandis* when treated with chemical reagents **Fig. 5** shows the presence of secondary metabolites in the plant.

FIG. 5: FLUORESCENCE ANALYSIS

Chemical Standardization of Extracts:

Qualitative Analysis: Qualitative color test results are presented in **Table 8**.

TABLE 8: QUALITATIVE PHYTOCHEMICAL TESTS

Class of Compound	PGLP	DPGLE	PGLW	PGSP	DPGSE	PGSW	PGRP	DPGRE	PGRW
Alkaloids	-	-	-	-	+	+	-	+	+
Flavonoids	-	-	+	-	+	+	-	+	+
Phenols	-	+	+	-	+	+	-	+	-
Steroids	+	+	+	+	+	-	+	-	-
Triterpenoids	-	-	-	-	-	-	-	+	-
Tanins	-	+	+	-	+	+	-	+	-
Carbohydrate	-	+	+	-	+	+	-	+	+
Saponins	-	+	+	-	+	+	+	-	+

Spectral Finger Printing of Extracts of *Pisonia Grandis:* Spectral fingerprinting of medicinal plant extracts serves as a valuable authentication tool and is a recent trend adopted in plant standardization.

UV-Visible Spectral Fingerprinting: UV-Visible fingerprints of the ethanol extract of leaf, stem, and roots of *Pisonia grandis* were recorded in a double beam UV spectrophotometer (Shimadzu, 1601) as represented in **Fig. 6.**

All the fingerprints indicate the presence of polar secondary metabolites in the extracts.

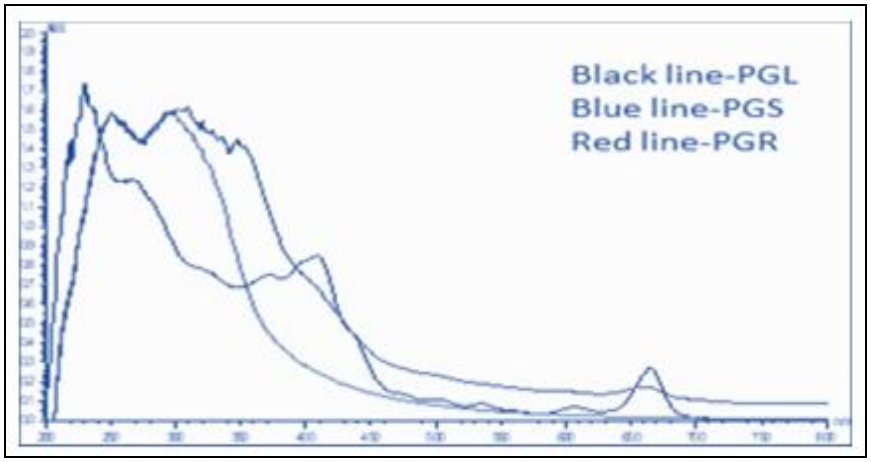

FIG. 6: OVERLAY OF UV VISIBLE FINGERPRINTS OF PGLE, PGSE AND PGRE

FT-IR Spectral Fingerprinting: The FT-IR spectral fingerprints were recorded in FTIR spectrometer Shimadzu, IR Affinity 1. The fingerprints **Fig. 7, 8 and 9** may be considered specific for the respective parts of this plant. While the IR spectrum of the leaf extract indicates a moderate absorption, the stem and root extracts reveal intense absorptions due to glycosides. All three fingerprints indicate comparably similar absorptions in the range 3400 to 2800 cm^{-1}.

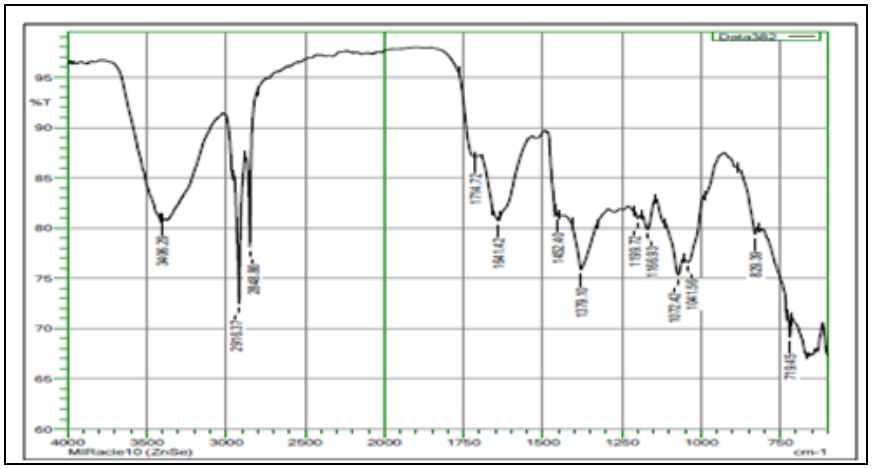

FIG. 7: IR FINGERPRINT OF PGLE

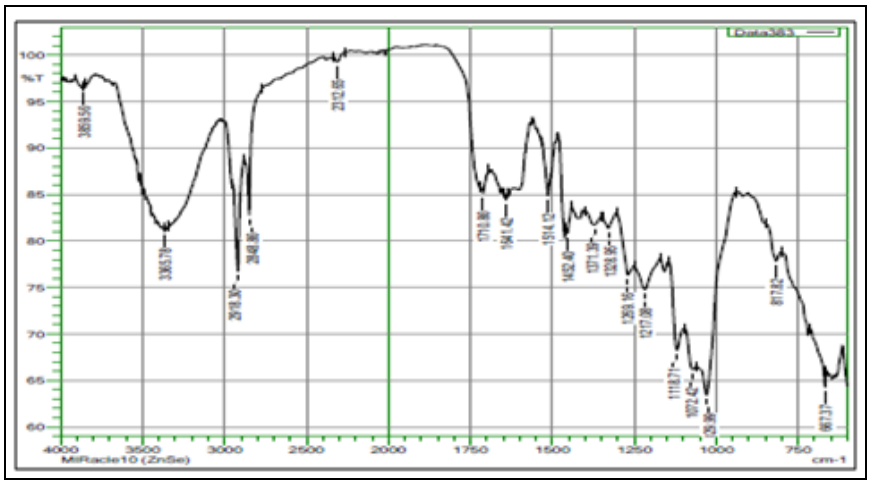

FIG. 8: IR FINGERPRINT OF PGSE

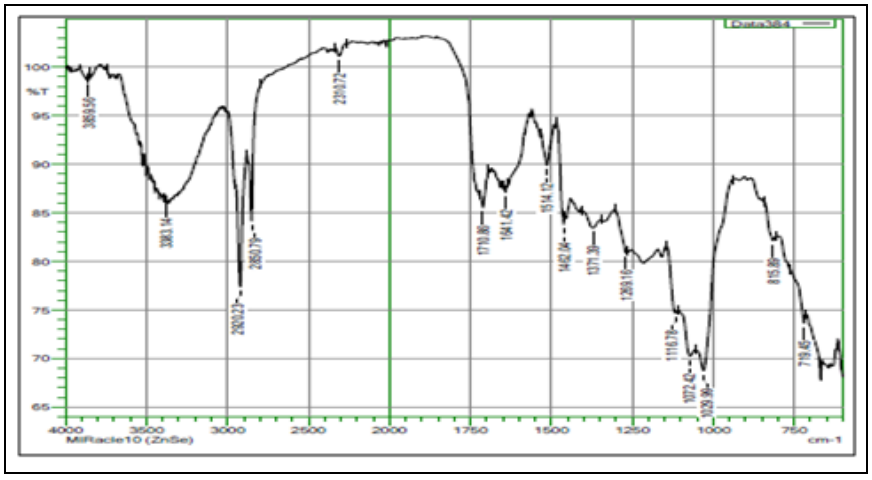

FIG. 9: IR FINGERPRINT OF PGRE

NMR Spectral Fingerprinting: ^{1}H NMR spectral fingerprints of pet-ether and ethanol extracts of leaves, stems and roots of *Pisonia grandis* were recorded in Bruker Avance III 500 MHz spectrometer with DMSO/$CDCl_3$ solvent as the case may be. The ^{1}H NMR spectral fingerprints of the pet-ether, ethanol and water extract of leaves, stems and roots, of *Pisonia grandis* are represented in **Fig. 10** to **15.** The ^{1}H NMR spectral fingerprints of the polar ethanol extracts indicate peaks characteristic of the biomarker allantoin (expected at 5.24 ppm as doublet, 5.83ppm as singlet, 6.96 ppm as doublet, 8.06ppm as singlet [4, 5] and of biomarker pinitol in the range 3.0-4.0 ppm [4, 5].

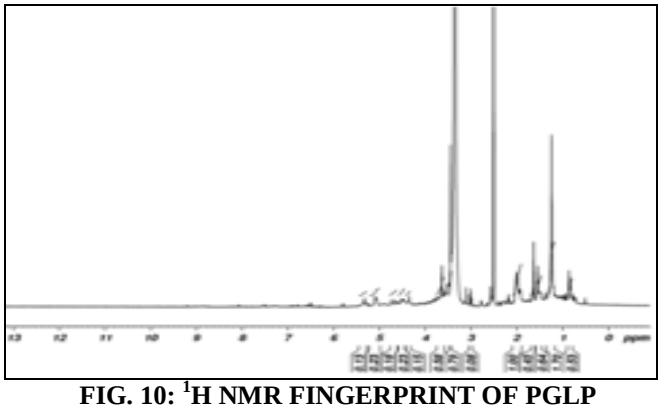

FIG. 10: ^{1}H NMR FINGERPRINT OF PGLP

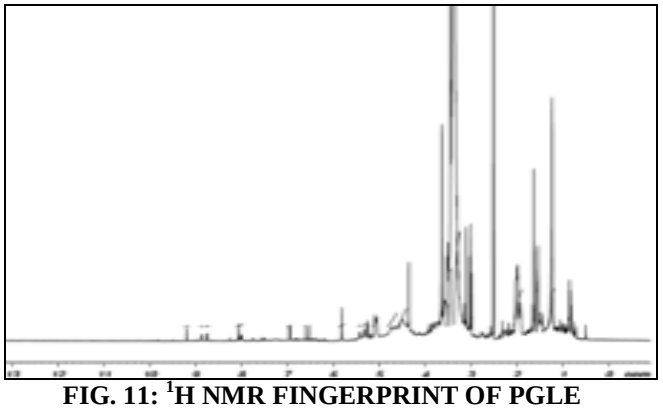

FIG. 11: ^{1}H NMR FINGERPRINT OF PGLE

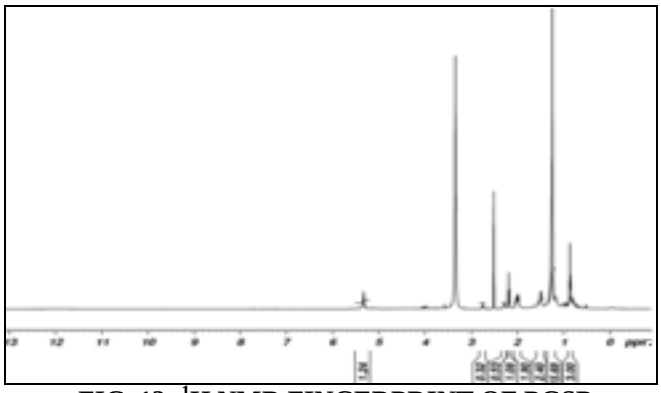

FIG. 12: ^{1}H NMR FINGERPRINT OF PGSP

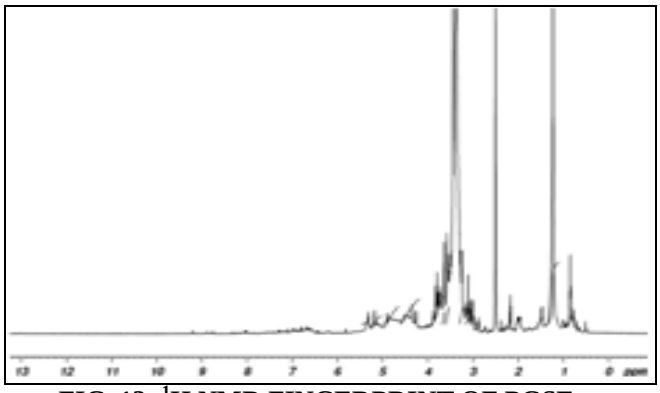

FIG. 13: ^{1}H NMR FINGERPRINT OF PGSE

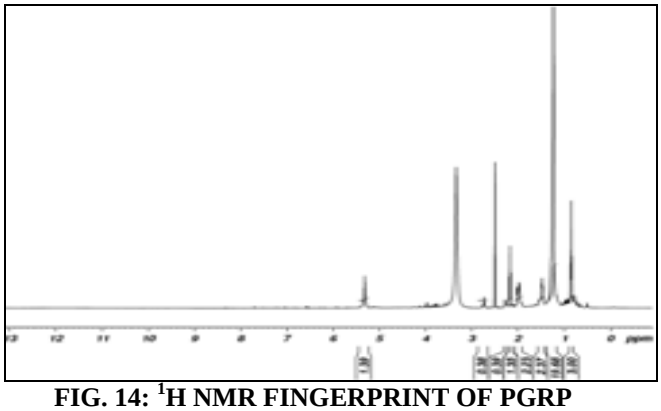

FIG. 14: ^{1}H NMR FINGERPRINT OF PGRP

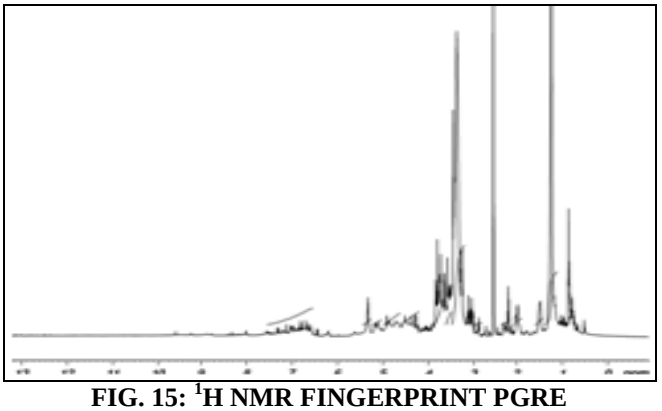

FIG. 15: ^{1}H NMR FINGERPRINT PGRE

^{13}CNMR Finger Printing: ^{13} C NMR spectral finger prints of the pet-ether and ethanol extract of leaves, stems and roots of *Pisonia grandis* were recorded in Bruker Avance III 500 MHz spectrometer. The respective ^{13}C NMR spectral finger prints are represented by **Fig. 16** to **21.**

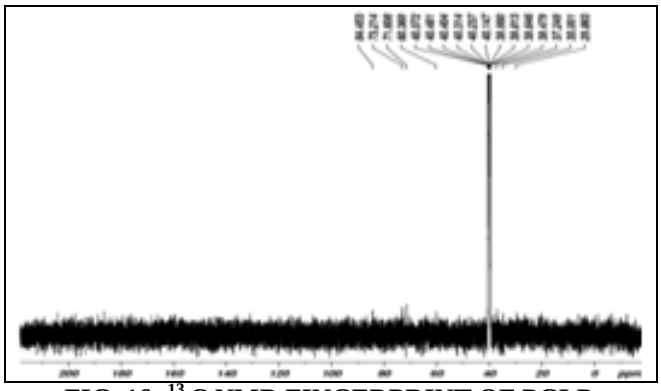

FIG. 16: ^{13} C NMR FINGERPRINT OF PGLP

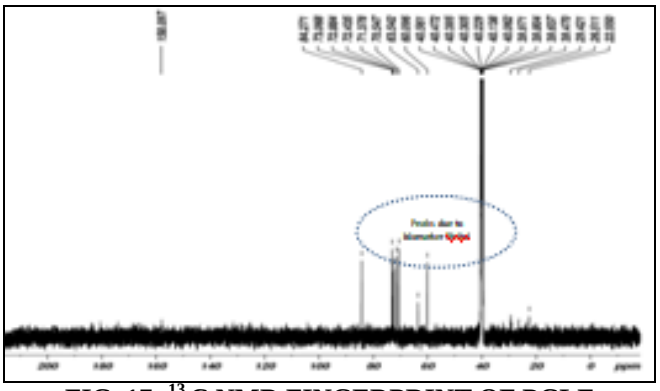

FIG. 17: ^{13} C NMR FINGERPRINT OF PGLE

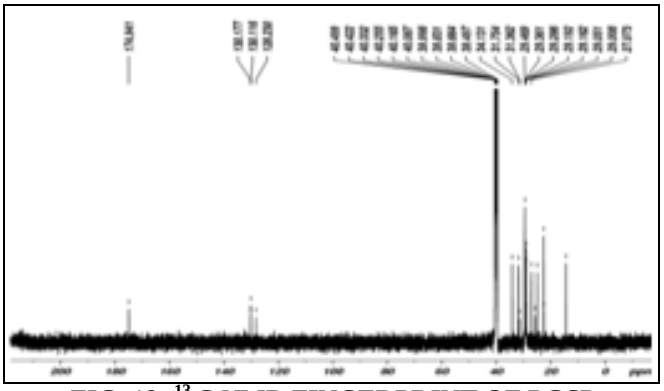

FIG. 18: ^{13}C NMR FINGERPRINT OF PGSP

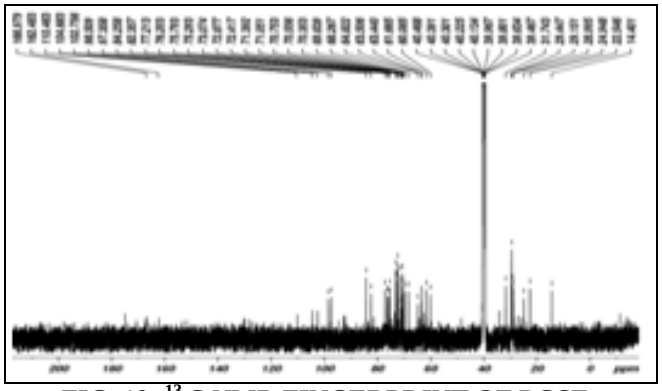

FIG. 19: ^{13}C NMR FINGERPRINT OF PGSE

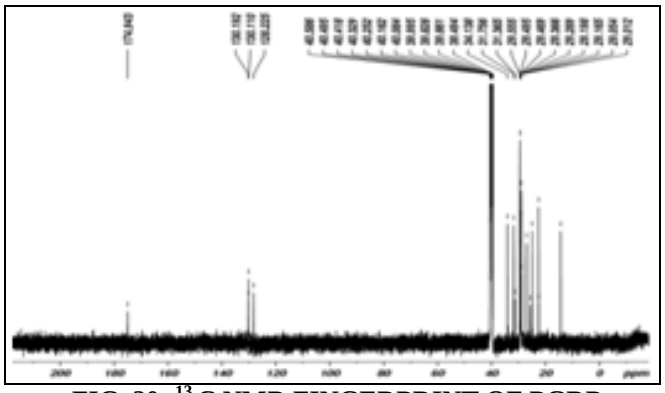

FIG. 20: ^{13}C NMR FINGERPRINT OF PGRP

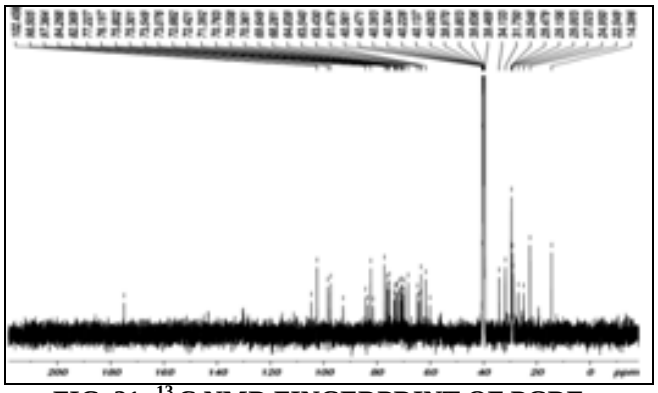

FIG. 21: ^{13}C NMR FINGERPRINT OF PGRE

The ^{13}C NMR spectral fingerprints of the polar ethanol extract of leaves clearly indicate peaks characteristic of the biomarkers allantoin (at 63.5 and 158.9 ppm) and pinitol (at 60.0 ppm due to the lone methoxyl carbon and at 84.3 ppm due to the ipso carbon to which the methoxyl group is attached in pinitol [4, 5]. The stem and root ethanol extracts additionally indicate the presence of glycosides.

Chromatographic Finger Printing:

Thin Layer Chromatographic Analysis: The optimized solvent systems for TLC analysis of pet-ether, dew axed ethanol, aqueous extracts of *Pisonia grandis* are listed in **Table 9**. Thin layer chromatograms for the standard and isolated bioactive are represented in **Fig. 22** and **23.** The relative factor of pinitol is 0.5 and that of allantoin is 0.62 in the respective developing solvents indicated below.

TABLE 9: OPTIMIZED SOLVENT SYSTEMS FOR TLC

Sample	Solvent System (V/V)
Pet-ether extracts	Pet-ether-ethyl acetate mixture (9:1)
Dewaxed ethanol extracts	Chloroform- methanol mixture (7:3)
Aqueous extracts	n-Butanol-acetic acid-water (5:0.5:4.5)
Pinitol	Chloroform-methanol-water (6:3.5:0.5)
Allantoin	Chloroform-methanol-water (6:4:3drops)

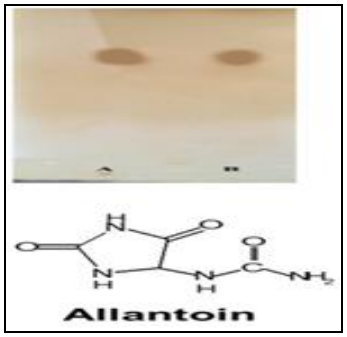

FIG. 22: TLC CHROMATOGRAM OF ISOLATED ISOLATED ALLANTOIN WITH STANDARD

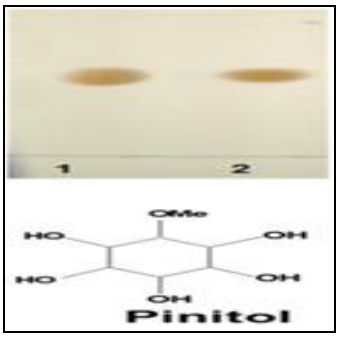

FIG. 23: TLC CHROMATOGRAM OF PINITOL WITH STANDARD

HPTLC Fingerprinting: HPTLC analysis was performed in Camag HPTLC system (Camag Linomat V semi automatic spotting device and WinCATS 4 software version 4.05).

The protocols adopted for HPTLC analysis are given below. HPTLC densitograms of the ethanol extracts of leaves, stems and roots of *Pisonia grandis* and the respective dewaxed extracts are represented in **Fig. 24** to **29.**

HPTLC Protocol for Pinitol	HPTLC Protocol for Allantoin
Stationary Phase: Pre-coated silica gel 60 F254 for TLC Mobile phase: Chloroform: Methanol: Water (6:3.8:0.2) Developing Agent: Iodine Detection Wavelength : Visible, UV 254nm and 366 nm	Stationary Phase: Pre-coated silica gel 60 F254 for TLC Mobile phase: Chloroform: Methanol: Water (6:3.5:0.5) Developing Agent: Ammoniacal Silver Nitrate Detection Wavelength: UV 254nm and 366 nm

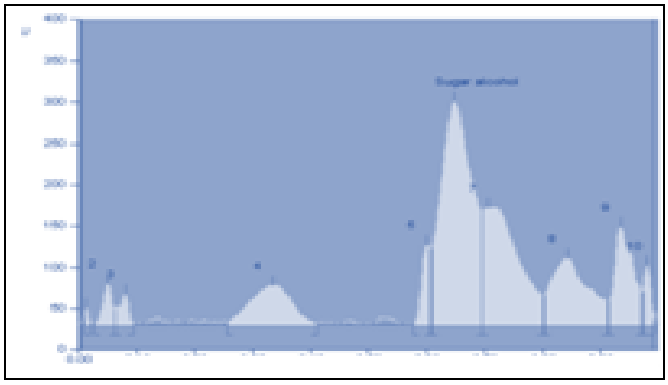

FIG. 24: HPTLC DENSITOGRAM OF PGLE

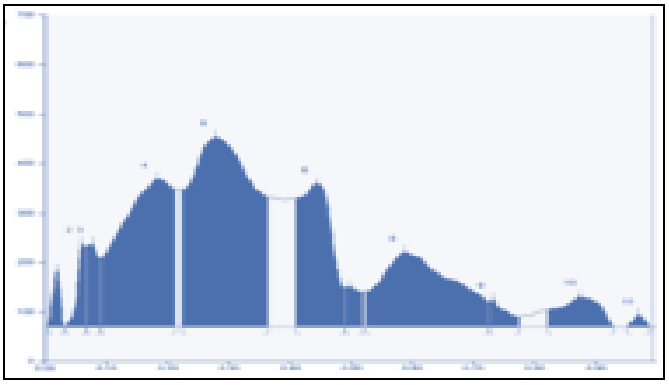

FIG. 25: HPTLC DENSITOGRAM OF PGSE

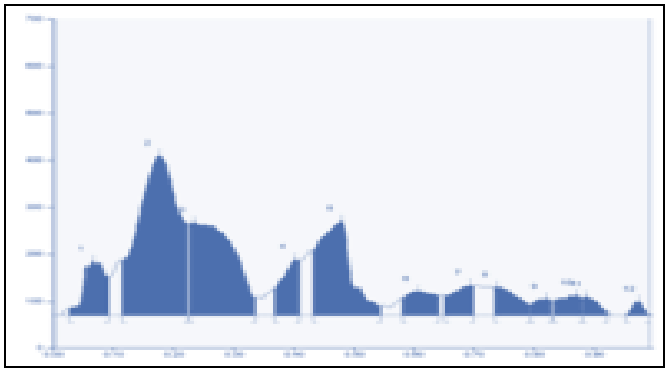

FIG. 26: HPTLC DENSITOGRAM OF PGRE

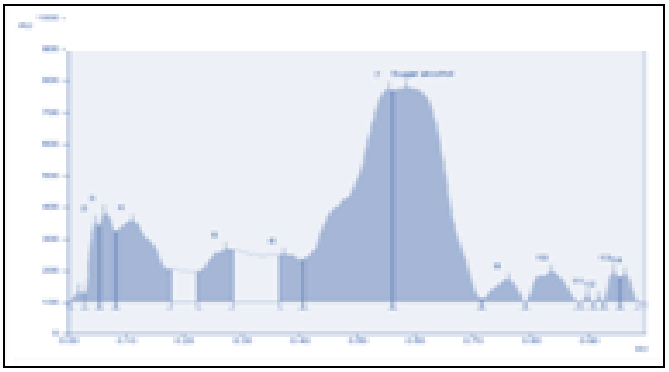

FIG. 27: HPTLC DENSITOGRAM OF DPGLE

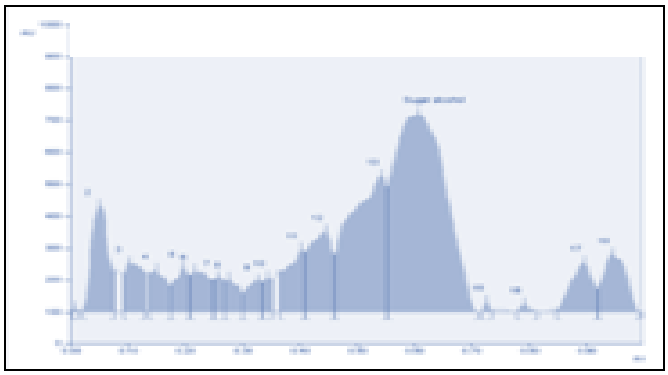

FIG. 28: HPTLC DENSITOGRAM OF DPGSE

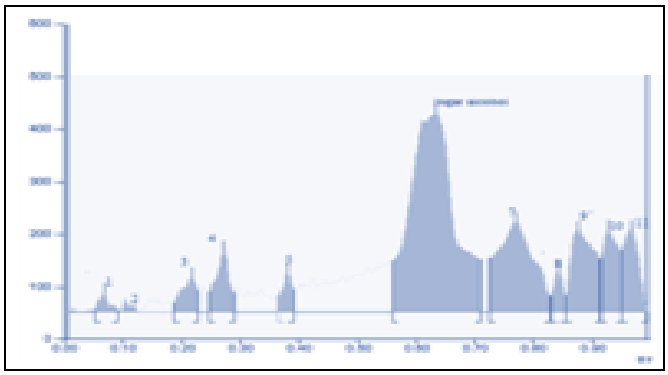

FIG. 29: HPTLC DENSITOGRAM OF DPGRE

High-Performance Liquid Chromatographic Fingerprinting: High-Performance Liquid Chromatographic (HPLC) analysis was done to assess the phytochemical profile of the plant *Pisonis grandis* (Schimadzu Class VP V6.14SP2 HPLC instrument). Overlay of HPLC Chromatograms of ethanol extracts of *Pisonia grandis* (PGLE, PGSE and PGRE) with standard markers pinitol and allantoin are presented in **Fig. 30 and 31**. The HPLC protocols adopted are presented below:

HPLC Protocol for Pinitol	HPLC Protocol for Allantoin
Colum: Amine Column	Colum: C 18 Column-Reverse Phase
Mobile phase: Acetonitrile: Water (70:30)	Mobile phase: Acetonitrile:Buffer (20:80)
Detector: RI	Detector: PDA (Photo diode array detector)
Standard: Pinitol	Standard: Allantoin
Concentration of Standard: 10mg in 1ml of water	Concentration of Standard: 1mg in 2ml of H_2O
Injected volume: 20µl.	Injected volume: 20µl

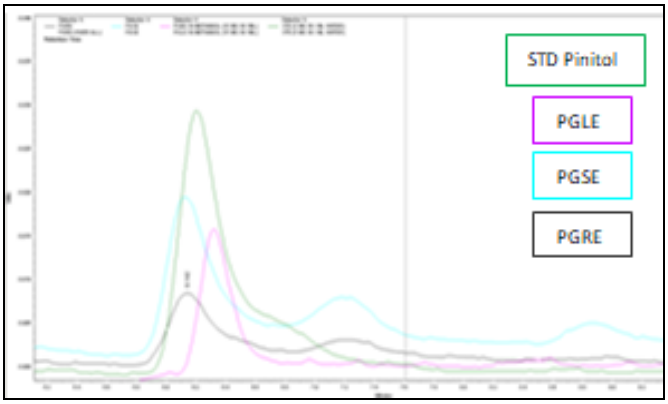

FIG. 30: OVERLAY OF HPLC CHROMATO-GRAMS OF PGLE, PGSE AND PGRE AND STANDARD MARKER PINITOL

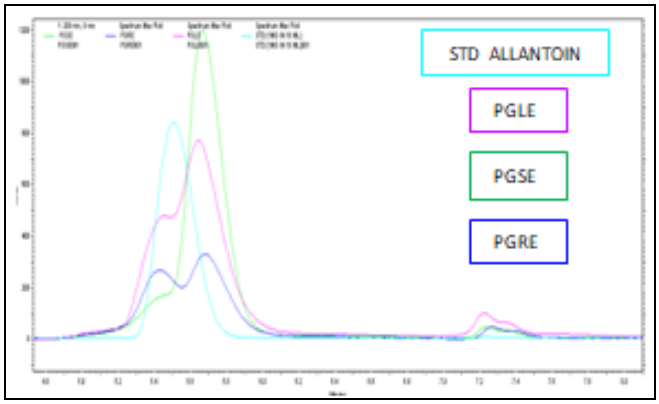

FIG. 31: OVERLAY OF HPLC CHROMATOGRAMS OF PGLE, PGSE AND PGRE ANDSTANDARD MARKER ALLANTOIN

GC-MS Fingerprinting: GC-MS fingerprints of the extracts were recorded in Hewlett–Packard 6890 gas chromatograph (Agilent Technologies, CA) coupled to an HP5973 mass selective detector. The protocol adopted is given below.

GC MS Protocol:

Column used: Agilent Ultra 2 fused silica capillary column (12 m length, 0.2 mm internal diameter).

Carrier Gas: Helium.

Flow Rate: 1 ml/min.

Sample Injection: Splitless mode.

Initial Temperature: 100°C.

Final Temperature: 400 °C.

Concentration of the Sample: 1 ppm.

Run Time: 36 min.

The total ion chromatograms **Fig. 32** to **37** indicate that the non-polar extracts possess major constituents with retention time ranging from 22 to 26 minutes. The polar extracts express a broad peak in common and other major peaks beyond 30 minutes retention time. As predicted by the NIST database, the major phyto-constituents in the non-polar extracts have been reported as n-hexadecanoic acid (palmitic acid), 6-octadecenoic acid, 9-octadecenoic acid and 9-octadecenoic acid 1,2,3-propanetriyl ester [45].

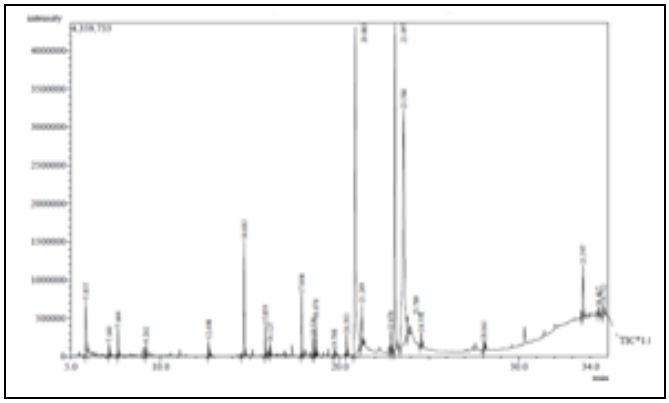

FIG. 32: GC FINGERPRINT OF PGLP

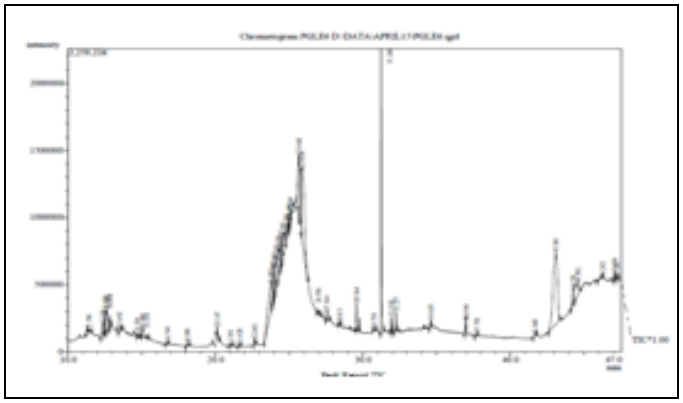

FIG. 33: GC FINGERPRINT OF PGLE

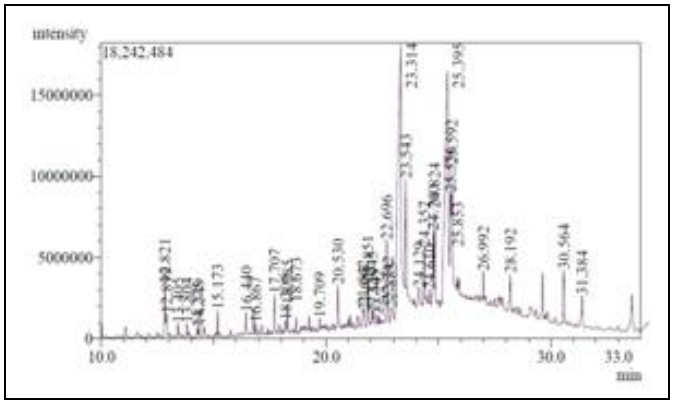

FIG. 34: GC FINGERPRINT OF PGSP

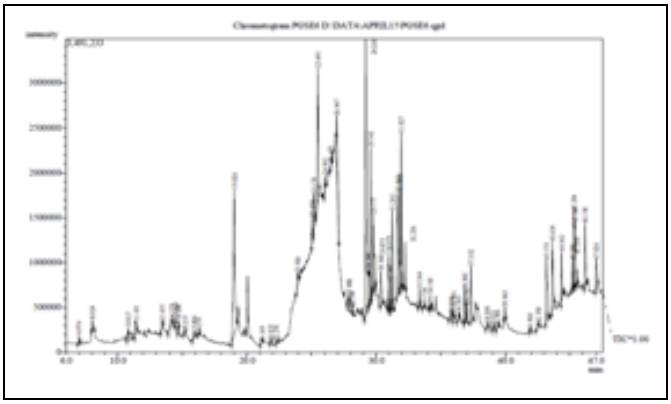

FIG. 35: GC FINGERPRINT OF PGSE

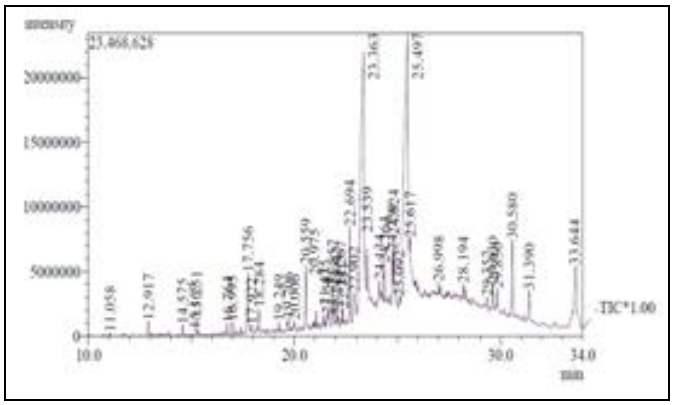

FIG. 36: GC FINGERPRINT OF PGRP

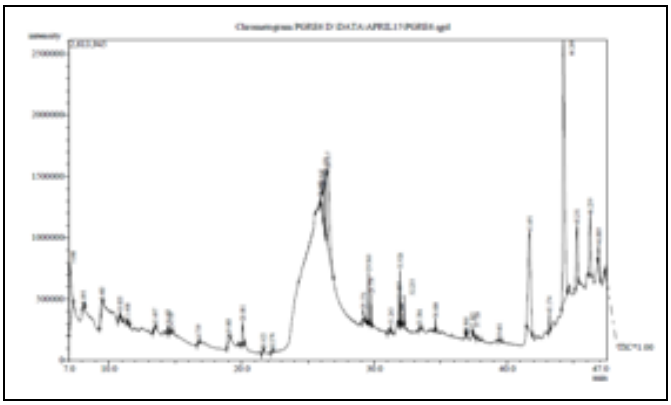

FIG. 37: GC FINGERPRINT OF PGRE

Quantitative Analysis:

Quantification of Phytochemicals: The phenolic and flavonoid content of the leaves and stem bark was estimated by Folin-Ciocalteu and aluminium chloride colorimetric assay, respectively. The phytochemical content reported for crude extracts of *Pisonia grandis* is presented in **Table 10**.

TABLE 10: QUANTIFICATION OF PHYTOCHEMICALS

Plant Part Analysed	Extract	Phytochemical Content mg/g			
		Phenolic content	Flavonoid content	Tannin content	Literature
Leaves	Pet ether	74.45	83.22	71.47	47
	Methanol	58.84	54.14	117.04	
	Ethyl acetate	89.82	84.05	65.72	
Stem bark	Hexane	0.2926±0.0003	0.0359±0.0001	-	
	Ethyl acetate	0.6061±0.1817	0.0665±0.0002	-	15
	Ethanol	0.1919±0.0003	0.0215±2.9463	-	

*For leaf extract phenolic, flavonoid and tannin contents are expressed as catechol, quercetin, and catechin equivalents per gram extract. Stem bark extracts are expressed as gallic acid and quercetin equivalents for phenolic and flavonoid contents, respectively as Mean±S.D (n=3).

Quantification of Nutritive Content: The fiber, protein and soluble carbohydrate (g/100g) content of leaves was determined by acid-base digestion, Kjeldahl method and difference method respectively [16]. The leaves are a source of fiber also.

TABLE 11: NUTRITIVE CONTENT OF *PISONIA GRANDIS* [16]

Plant Part	Nutritive Content (%)			
	Fat	Fibre	Protein	Carbohydrate
Leaves	4.09±0.32	10.87±0.23	1.67±0.04	2.57±0.06

Quantification of Biomarkers: The biomarkers allantoin and pinitol present in the leaves of this plant were quantified by HPLC (Shimadzu Class VP V6.14SP2) [46]. The protocols adopted are presented below, and the quantification details are tabulated in **Table 12**. The respective

chromatograms are represented in **Fig. 38** and **39.** These biomarkers were first reported from leaves of this plant [4, 5].

HPLC Protocol for Allantoin:
Column: C18 column

Mobile Phase: acetonitrile: phosphate buffer (20:80)

Detector: Photo Diode Array detector (PDA)

Standard: Allantoin Sigma brand

Concentration of Standard: 10mg in 1ml of water

Injected Volume: 20µl

HPLC Protocol for Pinitol:
Column: Amine column

Mobile Phase: acetonitrile: water (70:30)

Detector: Refractive Index detector (RI)

Standard: Pinitol Sigma brand

Concentration of Standard: 10mg in 1ml of water

Injected Volume: 20µl

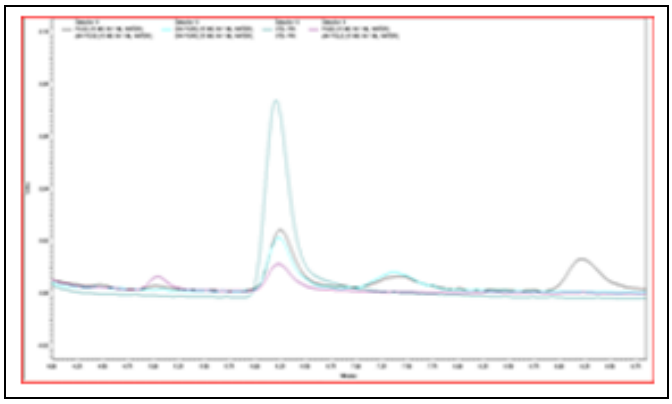

FIG. 38: HPLC OF ALLANTOIN

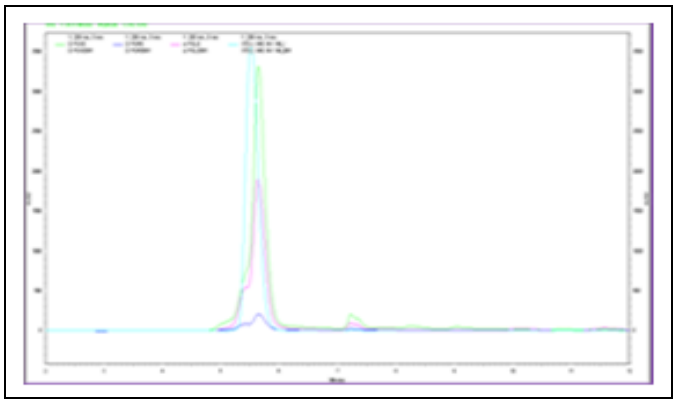

FIG. 39: HPLC OF PINITOL

TABLE 12: QUANTIFICATION OF BIOMARKERS

Extract	Biomarker Quantity (µg/100 µg extract)	
	Pinitol	Allantoin
PGSE	16	18
PGLE	14	16
PGRE	10	6

Biological Standardization: The plant is known for its anti-diabetic, anti-arthritic and wound healing potential and there are numerous reports on the medicinal potential of leaves in particular. The bioactive molecules allantoin and pinitol have been isolated and reported from this plant and since then there is a surge of scientific reports on the medicinal potential of *Pisonia grandis.* Available reports on the biological potential of the plant have been reviewed in this section and the results of the studies compared and presented in **Tables 13-15**. The references are duly cited.

Antidiabetic Activity: The first report on *in-vivo* anti-diabetic effect of the ethanol extract of the plant, revealed the α-glucosidase inhibitory activity in a dose dependant manner and the IC_{50} value was 416.7 µg/ml [13]. The other reports are *in-vitro* studies on the anti-diabetic efficacy of the leaf ethanol extract revealing high inhibition of α-amylase [17]. The stem bark hexane extract of the plant was also found to exhibit potent α-amylase inhibitory activity [15].

Antioxidant Activity: The first report of antioxidant activity of the plant was reported by Subhasree *et al.* in 2009. The methanol extract of leaves was evaluated for its capacity to scavenge free radicals by *in-vitro* method at concentrations ranging from 50 to 250µg/ml. The IC_{50} values of the methanol extract in DPPH radical scavenging assay, ABTS radical cation-scavenging assay, and inhibition of lipid peroxidation assay was 175µg/ml, 80µg/ml and 505µg/ml, respectively. This study highlights the plant's tremendous nutritional potential and its importance in the prevention of diseases caused by free radicals [18]. A similar report of the antioxidant activity of the leaf methanol extract (maceration with methanol for 15 minutes) was reported by Jagadeesan *et al.,*[19]. The crude ethyl acetate extract of stem bark exhibits strong antioxidant activity with IC_{50} value of 148.2µg/ml [15]. A biherbal extract containing *Pisonia grandis* and *Cardiospermum helicacabum*

also showed high antioxidant activity [20]. Antiquorum sensing effect of the methanol extract of leaves was also assessed and the extract shows 88.25±0.82 % biofilm inhibition against *Pseudomonas aeruginosa* [21]. The electrochemical response of the extracts of *Pisonia grandis* by cyclic voltammetry was reported by Shubashini *et al.,* [22]. **Table 13** gives the results of antioxidant assays on various parts of *Pisonia grandis* carried out in our laboratory [45]. **Table 14** provides data on antioxidant activity for the free radical-scavenging assays reported earlier.

TABLE 13: *IN-VITRO* ANTIOXIDANT ACTIVITY (BY DPPH RADICAL SCAVENGING)

Percentage Inhibition of DPPH radical by Extracts of *Pisonia grandis*						
Concentration (µg/mL)	**PGLE**	**PGSE**	**PGRE**	**PGLP**	**PGSP**	**PGRP**
10	1.46	3.14	1.24	2.18	4.02	3.14
30	9.42	7.46	4.68	7.46	16.01	12.43
50	19.19	17.43	7.02	10.43	24.01	26.38
70	25.92	24.23	10.23	15.42	32.98	42.32
90	34.89	31.96	15.42	21.09	42.67	61.27

TABLE 14: FREE RADICAL-SCAVENGING ASSAY

Plant Part	Extract Analysed	MIC (µg/ml)				
		DPPH Radicals Scavenging	ABTS Cation Radicals	Inhibition of Lipid Peroxidation	Nitric Oxide Assay	References
Leaves	Ethyl acetate	50	-	-	76.40	23
	Ethanol	45.5	-	-	52.3	23
	Methanol	175	80	505	110	18,23
	Aqueous	137.11	67.24			24
Stem bark	Hexane	138.3				15
	Ethyl acetate	148.2				
	Ethanol	55.27				

Antimicrobial Activity: There are five reports till date on antimicrobial potential of the leaves of this plant and one report on antimicrobial potential of the stembark of the plant. The antimicrobial potential of the extracts of *Pisonia grandis* as reported till date is documented in **Table 15**.

TABLE 15: ANTIMICROBIAL ACTIVITY OF SOLVENT EXTRACTS OF *PISONIA GRANDIS*

Extract	Strain	Leaf	Stem bark	Reference
		Disc Diffusion Assay	MIC Method	
		Zone of Inhibition(mm)	MIC	
Hexane Extract	*S. aureus*	-	70	15
	B. subtilis		60	
	E. coli		75	
	K. pneumonia		65	
Ethyl acetate Extract	*S. aureus*	17	69	26,15
	B. subtilis		54	15
	E. coli	15	67	26,15
	K. pneumonia	13	72	26,15
	C. albicans	14		26
Ethanol Extract	*S. aureus*	10	72	27,15
	B. subtilis	8.6	83	27,15
	E. coli	8.6	94	27,15
	K. pneumonia	7.3	63	27,15
	C. albicans	8	-	27
	S. paratyphi	9	-	27
	Monascus purpureus	25	-	25
	A. niger	7.3	-	27
	M. luteus	8	-	27

Anti-Inflammatory Activity: Leaves have been well investigated for their acute and chronic anti-inflammatory potential. Anti-inflammatory activity was first reported in 2002 for the chloroform extract of leaves of the plant. The study revealed that the chloroform extract exhibited chronic anti-

inflammatory activity at a dosage of 500mg/kg, equivalent to diclofenac at 50mg/kg [28]. A similar report wherein the leaf ethanol and aqueous extracts of *Pisonia grandis* (cold maceration for 3-7 days) was tested against carrageenan-induced paw edema indicated that both extracts exhibited substantial anti-inflammatory effects against infusion of carrageenan injection [8]. The methanolic extract and flavonoid rich ethyl acetate extract of leaves of *Pisonia grandis* were found to significantly decrease acute and chronic phase inflammation [23]. Compared to aqueous and alcoholic extracts of roots of the plant, the alcoholic extract reduced paw edema significantly [29]. A topical formulation prepared with chloroform extract of leaves showed a considerable reduction in carrageenan induced paw edema [30].

Anti-Arthritic Activity: Anti-arthritic activity of the plant was first reported in leaves by Elumalai *et al.,* in 2012. The ethanol extract of leaves [Soxhlet extraction] was assessed by Freund's adjuvant induced arthritis model [31]. *In-vitro* assessment of anti-arthritic potential of pet-ether and ethanol extract of leaves, stems and root was carried out by Poongothai *et al.* [32] The study revealed that the pet-ether and ethanol extracts of leaves exhibited significant anti-arthiritic potential equivalent to that of palmitic acid at the same concentration. In yet another assay of a biherbal extract composed of ethanolic extract of *Pisonia grandis* and *cardiospermum helicacabum*, indicated a greater inhibition of protein denaturation compared to the individual extracts [33].

Wound Healing Potential: The leaf methanol extract (1% and 2% w/w concentration) in an ointment formulation, demonstrated considerable wound healing activity in incision and excision wound models [34]. The potential of the plant to cure injuries has also been validated by chick chorioallantoic membrane (CAM) *in-vitro* assay by Poongothai *et. al* in 2019.

In addition to significant anti-diabetic, anti-microbial, anti-oxidant, anti-inflammatory, anti-arthritic and wound healing potential, the plant has also been tested for its effectiveness as an analgesic [28], diuretic [28], anxiolytic [35], anti-ulcer [36] and hepatoprotective [29, 37] agent. A polyherbal formulation consisting of leaves of *Pisonia grandis* is reported with anti-pyretic activity comparable with the standard drug paracetamol at the dose levels of 150mg/kg and 300mg/kg [9]. The hepatoprotective and anti-inflammatory action of roots was reported by Majumdar *et al.,* in 2012 [29]. An *in-vitro* anti-quorum sensing effect of the methanol extract of the leaves of the plant with 88.25 ± 0.82% inhibition has been reported recently [21]. This report opens up avenues for the discovery of anti-quorum sensing agents from *Pisonia grandis*. The findings may be significant in the light of increased multi-drug resistance by pathogenic bacteria.

In summary, with regard to the medicinal potential of *Pisonia grandis,* significant research has been done on the leaves, whereas roots and stem bark are found to be less investigated. Revelation on the presence of two potentially medicinal molecules allantoin and pinitol in the extracts of this plant provides the intellectual input to formulating wound healing ointments and anti-diabetic formulations. Both molecules have been thoroughly validated scientifically.

Toxicity Studies: Acute oral toxicity studies have been reported on leaf ethanol extract of *Pisonia grandis*. As reported [8, 12, 35, 36], no toxic symptoms were observed up to a dosage of 2000mg/kg body weight. *Pisonia grandis* leaf ethanol extract is considered safe for oral administration. This fact was reaffirmed by the toxicity study of a polyherbal formulation [9] of which *Pisonia grandis* constituted one-fifth of the concentration of the formulation that showed no mortality even at the dosage of 1000mg/kg body weight. Yet another biherbal formulation [33] containing *Pisonia grandis* ethanolic extract as one of its constituents revealed no toxic symptoms upto 2000mg/kg body weight. Still, a higher dose level of 5000mg/kg body weight brought adverse symptoms like dyspnea. Hence the recommended therapeutic dose level of leaf extract of *Pisonia grandis* is 2000mg/kg body weight. All studies on toxicity assessment are reported to have been carried out according to OECD 423 guidelines.

***Pisonia Grandis* Mediated Nanoparticle Synthesis:** Silver nanoparticles (AgNPs) synthesized from *Pisonia grandis* [38] exhibited significant antifungal activity [39] and found to be

useful in biomedical imaging [40]. The zinc oxide (ZnO) and magnesium oxide (MgO) nanoparticles from leaf extract of *Pisonia grandis* were found to exhibit high anti-bacterial activity against *M. luteus* and *K. pneumonia* and a higher anti-fungal activity against *C. albicans* strain. Thus, *Pisonia grandis* may find prospective applications in the biomedical field also.

Medicinal Products Formulated with Leaf Extract of *Pisonia Grandis:* Herbal food supplements in the form of tablets comprising of the *Pisonia grandis* leaf extract have been formulated to treat diabetes mellitus [41]. The allantoin-rich leaf extract of the plant was incorporated in skin whitening, moisturizing creams, and sunscreen lotions [42, 43]. Fabric coated with leaf extracts of *Pisonia grandis* has been validated as an anti-microbial herbal bandaging [44]. A topical wound healing ointment with extracts of *Pisonia grandis* formulated in our laboratory has received a patent grant [48].

CONCLUSION: All available reports up to date on the plant *Pisonia grandis* are documented herein as a ready record, which will aid herbalists in preparing standardized formulations of the plant that will fetch global market value. Literature reports on the medicinal plant *Pisonia grandis* since the last two decades are a scientific validation of its medicinal potential, in particular its leaf extracts. Safe and efficacious medicinal preparations such as skin creams and wound healing ointments based on allantoin and pinitol containing leaf extracts of *Pisonia grandis* will be prospective for communities that largely rely on folkloric and traditional medicines. This comprehensive revelation of the medicinal potential of *Pisonia grandis* provides substantial ground for formulating safe and efficacious herbal products by the local people from fresh leaves of *Pisonia grandis* from their garden. Since the leaves are host to anti-diabetic, insulin-mimetic pinitol and wound-healing allantoin, the leaf extract may be potentially effective in curing diabetic foot ulcers. The validation of this efficacy will be taken up in our laboratory.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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