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ANTIMICROBIAL ACTIVITY GUIDED PHYTOCHEMICAL CONSTITUENTS PROFILING OF *PONGAMIA PINNATA* SEED EXTRACT

Meenu Verma [1, 2], Shiwa Chaubey [1], Vaishali Mishra [1], Mohd. Ashraf [3], Shefali Singh [4], Swati Sharma [4], Mahesh Pal [1] and Meenakshi Singh [*2]

Phytochemistry Division [1], CSIR- National Botanical Research Institute, Lucknow - 226001, Uttar Pradesh, India.
Department of Chemistry [2], Isabella Thoburn College, Lucknow - 226007, Uttar Pradesh, India.
Department of CIF - National Botanical Research Institute [3], Lucknow - 226001, Uttar Pradesh, India.
Department of Biosciences [4], Faculty of Science, Integral University, Lucknow - 226026, Uttar Pradesh, India.

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Correspondence to Author:
Meenakshi Singh

Professor,
Department of Chemistry,
Isabella Thoburn College, Lucknow - 226007, Uttar Pradesh, India.

E-mail: meenu16266@gmail.com

ABSTRACT: The current study investigates the antimicrobial activity of different extracts and fractions of *P. pinnata* using a chemical profiling approach to identify bioactive compounds responsible for its antimicrobial potential. Various fractions (hexane, chloroform, butanol and methanolic) were prepared from the seeds of the plant and tested against two different bacterial strains *Salmonella abony* (gram positive) and *Klebsiella pneumoniae* (gram-negative). The antimicrobial activity was assessed using standard disc diffusion method to determine zone of inhibition. In parallel, chemical profiling of the active extract and fractions was conducted through chromatographic technique (HPLC) and spectroscopic analysis GC-MS/MS to identify bioactive constituents. The results revealed significant antimicrobial activity, with methanolic extract and hexane fraction from seeds exhibiting the strongest inhibition against *S. abony*, on the other hand it was found less potent against *K. pneumonia*. Several bioactive compounds, including flavonoids, alkaloids, and phenolics were identified, suggesting their role in the antimicrobial activity. The *in-silico* ADMET and acute rat toxicity prediction suggested that the major bioactive compounds Pongamol and Lanceolatin B as the most effective drug-like and pharmacologically favorable candidates, whereas Rutin is least suitable due to poor pharmacokinetics and safety concerns.

INTRODUCTION: Global healthcare is facing a major challenge in the development of a novel, more effective and cost effective affordable drugs for the treatment of various microbial infections particularly in the developing countries [1].

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Having a strong immune system cannot always inhibit the spreading and multiplication of the bacteria. There may be many symptoms when one faces bacterial infections but the most common being diarrhea, fever and fatigue [2, 3].

Salmonella abony (gram positive) and *Klebsiella pneumonia* (gram negative) are two common pathogenic bacteria which are responsible for causing a wide range of infections in humans. The harmfull effects of *S. abony* may lead to gastroenteritis involving inflammation of the

gastrointestinal tract, sepsis leading to shock and may also result in organ failure, reactive arthritis in which the joints become inflammed [4, 5, 6]. *Klebsiella pneumonia* responsible for causing *Klebsiella* infection lives normally and harmlessly in the intestine, but may lead to serious infections specially in hospitals responsible for pneumonia, UTI, blood stream infections in immune-compromised patients [7]. They are responsible for 83% of hospital acquired pneumonia and are the main cause of ventilator associated pneumonia. The growing resistance of bacteria to several antibiotics such as cephalosporins, fluoroquinolones and carbapenems is one of the main cause of concern as infections caused by carbapenem resistant *K. pneumoniae* are challenging to treat and are responsible for maximum death rate. Various plants since ancient times have been used in traditional system of medicine by the folklore for treatment of different diseases. *Pongamia pinnata* also known as karanjais an indigenous Indian medicinal plant found in Indian subcontinent and South East Asia. The plant has been used in traditional system of medicine for the treatment of diabetes, antimicrobial, antifungal, anti-inflammatory, antioxidant besides this it also has been found to inhibit proliferation of certain cancer cell lines [11]. Since no work has been found to be reported of antimicrobial screening against *S. abony* and *K. pneumoniae*, hence it was thought worthwhile to screen the different extracts of the seeds of *P. pinnata* against the above two bacterial strains and isolate the active constituents responsible for the biological activity. The present study aims at screening for antimicrobial activity and biological activity guided isolation of phytochemical constituents.

MATERIALS AND METHODS:

Materials: The chemicals and standards used were obtained from Sigma–Aldrich, and are of Analytical grades. Filter papers were from Whatman, GE Healthcare companies, China. The bacterial strains were obtained from Integral Univerity, Lucknow, India.

Plant Sample: The dried seed pods were collected from local areas of Lucknow (UP), India and authentication of plant material was done at CSIR-National Botanical Research Institute, India (Voucher specimen no.– 118409).

Preparation of Samples: The plant seeds were separated from the pods and were carefully shredded into smaller pieces then ground into fine powder for extraction process.

Extraction and Fractionation Procedure for Powdered Seed Sample: 1Kg of dried and powdered *P. pinnata* seeds were extracted in 2.5L of methanol by the process of maceration [14] for a duration of 48 hrs. The extract was filtered and resultant filterate was concentrated to dryness on a rotary evaporator under reduced pressure and used as crude extract. The dry crude methanolic extracts was subjected to fractionation (liquid-liquid extraction) through separating funnel using different solvents in the order of increasing polarity (hexane, chloroform and butanol). These fractions and methanolic extract were used for phytochemical studies and antimicrobial activity.

Preliminary Phytochemicals Screening: A preliminary qualitative analysis of the plant extracts was performed to check for the presence of different class of compounds in the methanolic extract of seeds. The presence of tannins, steroids, flavonoids, terpenoids, proteins, phenol, saponin, alkaloids was detected by different test methods [15].

Antibacterial Activity:

Preparation of Inoculums of Microorganism for Testing: Two pathogens *Salmonella abony* and *Klebsiella pneumoniae* were used as test subjects to determine the antimicrobial activity of *P. pinnata* seed extract by disc diffusion assay as described by Ashraf *et. al.* [16]. These two pre-isolated bacterial cultures were obtained from Department of Biosciences, Integral University, Lucknow. The cultures were subcultured on NA slants and stored at 4°C until needed. For testing of sample, the inoculums were prepared from the stock culture and subcultured into nutrient broth (30ml) using a sterilized wire loop. The inoculate was further incubated overnight at 37^0C in a rotary shaker. These inoculates were further stored at 4^0C until usage.

Activity Analysis (Disc Diffusion Assay): The antimicrobial activity of fractions and methanolic extract was done by using agar well diffusion method under sterilized conditions. For this eight NA plates were prepared for fractions and

methanolic extract. 400µl inoculum of each selected bacterium was uniformly spread over agar plates with the help of sterilized glass spreader. After five minutes five wells, approximately 7mm in diameter were bored with the help of borer. The volume of fractions, methanolic extract (50 µl, 75 µl, 100 µl) and antibiotic Gentamycin (50 µl), was poured into the wells. The plates were incubated at 37 ^{0}C for 24 hrs in incubator. Next day, the results were observed and the antimicrobial potential was measured in terms of diameter of zone of inhibition.

HPLC Analysis of Methanolic Extract of Seeds: HPLC analysis of methanolic extract was performed on Shimadzu prominence system equipped with an autosampler (SIL-20 AC), LC Solution 1.0 software and DAD detector. The methanolic extract of the seeds was analyzed in triplicate at the concentration of 10 mg/ml and method as described in **Table 1** under the following conditions: stationary phase: C_{18} column (dimensions 250mm x 4.6mm and particle size of 5 µm). The injection volume and the flow rate were set at 10 µl and 1ml/min respectively at the constant temperature of 25°C. Before, analysis the solutions were degassed and filtered through 0.22 µm filter membrane. The wavelength was set at 254 nm for analysis. All the reagents used for chromatographic analysis were of HPLC grade.

TABLE 1: GRADIENT ELUTION METHOD FOR HPLC ANALYSIS OF PHENOLICS IN *P. PINNATA* SEEDS

Time (min)	Solution A: water+ 1% (v/v) acetic acid (% v/v)	Solution B: Acetonitrile (ACN) (% v/v)
0.01	79	21
13.00	64	36
38.00	50	50
50	79	21
55.00	79	21

Gas Chromatography- Tandem Mass Spectrometry (GC-MS/MS) Analysis: The volatile and semi-volatile components of hexane fraction were analyzed using an Agilent 8890GC system and an Agilent 5977A Mass Selective Detector (MSD), column HP-88 diameter 0.250 mm, 100 mm length and 0.20 µm film. Helium was used as the carrier gas and a flow rate of 1 mL/min.

The temperature of the injector was operated at 240°C and set at 60 °C for the oven, then the temperature was increased gradually for 50 min. the identification of compounds of hexane fraction of seeds was based on the attached NIST library with GC-MS instruments.

ADMET Prediction: *In-silico* ADMET study refers to a computational or computer based approach used in drug discovery and development to predict and assess the compound's absorption, distribution, metabolism, excretion, and toxicity in and through the human body are all considered ADMET characteristics. Chem Draw Ultra 16.0 was used to illustrate the structure of selected compounds for the pharmacokinetic studies. According to the established standard protocol, the legands were transformed into SMILES format, and ASwissADME website was used for ADME to estimate the drug like and pharmacokinetic features of the chosen compounds [17].

Acute Rat Toxicity Prediction: *In-silico* prediction of LD50 values for rats with four types of administration (oral, intravenous, intraperitoneal, subcutaneous, inhalation) by GUSAR software. The training sets were created on the basis of data from SYMYX MDL Toxicity Database. They include the information about 10000 chemical strucrures with data on acute rat toxicity represented on the LD50 values (log 10 (mmol/kg) [18].

RESULTS AND DISCUSSION:

The Percentage Yield and Phytochemical Analysis of Crude Methanolic Extract: The percentage yield of methanolic extract of the seeds was 15.4 %, this extract was further fractionated into four fraction using different solvents (hexane, chloroform, butanol). Phytochemical screening of *P. pinnata* seeds methanolic extract revealed the presence of several bioactive compounds, including alkaloids, flavonoids, saponins, and terpenoids, which may contribute to its therapeutic effects.

Antimicrobial Activity of the Various Extracts of the Seeds of *P. pinnata*: The methanol and

hexane extract of *P. pinnata* showed antimicrobial activity against *S. abony* bacterial pathogens as shown in table 2. The hexane extract exhibited the highest activity against *S. abony* at the highest concentration (100 µl), which suggests that *P. pinnata* seed extract have potential as a natural antimicrobial agent for treating infections caused by Gram-positive bacteria. While the activity against *K. pneumoniae* was less pronounced, the extract showed less significant potential, even at higher concentrations i.e. 100µl in the chloroform and hexane extract. The findings are consistent with previous studies that have reported the antimicrobial properties of *P. pinnata*. Further research is needed to isolate and identify the specific compounds responsible for these effects.

TABLE 2: ANTIMICROBIAL ACTIVITY OF *P. PINNATA* FRACTIONS AND METHANOLIC EXTRACT, AGAINST THE MICROORGANISMS *S. ABONY* AND *K. PNEUMONIAE* , THE RESULTS WERE EXPRESSED AS ZONE OF INHIBITION IN MM BY MEAN ± SD (STANDARD DEVIATION)

S. no.	Bacterial strain	Extract/ fractions	Concentration (µg/mL) of NPs (mm)			Gentamycin (Standard) (mm)	Control (DMSO)
			50 µg/mL	75 µg/mL	100 µg/mL	50 µg/mL	50 µg/ml
1	*S. abony*	Hexane	0.0±0.0	8.0±0.3	9.0±0.2	14.5±0.5	0.0±0.0
2		Chloroform	0.0±0.0	0.0±0.0	7.0±0.5	15.0±0.2	0.0±0.0
3		Butanol	0.0±0.0	0.0±0.0	0.0±0.0	13.0±0.4	0.0±0.0
4		Methanol	0.0±0.0	6.0±0.1	7.5±0.3	14.0±0.5	0.0±0.0
5	*K.*	Hexane	0.0±0.0	6.5±0.1	7.0±0.2	13.0±0.2	0.0±0.0
6	*pneumoniae*	Chloroform	0.0±0.0	0.0±0.0	6.5±0.5	15.5±0.4	0.0±0.0
7		Butanol	0.0±0.0	0.0±0.0	0.0±0.0	14.0±0.4	0.0±0.0
8		Methanol	0.0±0.0	0.0±0.0	0.0±.00	15.0±0.5	0.0±0.0

HPLC Analysis of the Methanolic Extract of the Seeds of *P. pinnata*: The methanolic extract showed the activity against *S. abony* therefore characterization of the extract was done for identification of major phenolic compounds present in the extract. During this analysis twelve most abundant phenolic compounds found in the plants were chosen out of which five phenolic compounds Gallic acid, Chlorogenic acid, Caffeic acid, Rutin and Quercetin were identified to be present in the methanolic extract with the help of reference standard as shown in **Fig. 1** and **2**. The Rt of identified phenolic compound were obsereved to be nearly same as Rt of their reference standard. The quantification of the compounds was done to find out the percentage content of the particular compound in the extract as shown in **Table 3.**

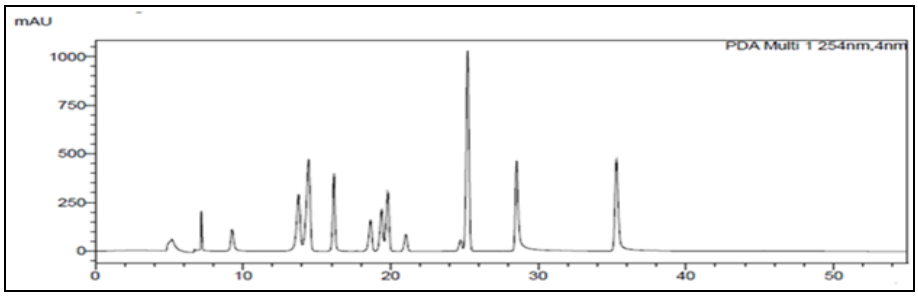


FIG. 1: HPLC CHROMATOGRAM SHOWING REFERENCE STANDARDS OF PHENOLIC COMPOUNDS AT DIFFERENT RT IN MIN

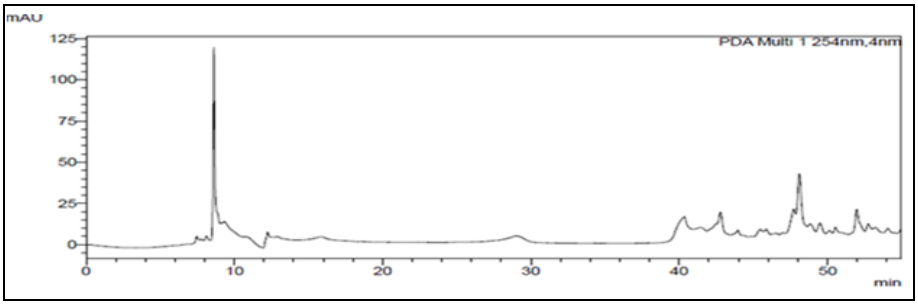
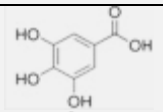
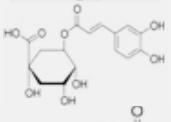
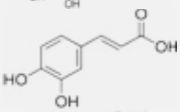
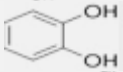
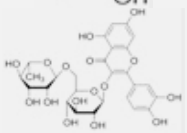
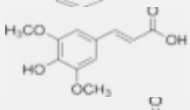
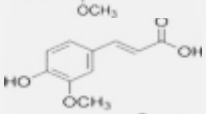
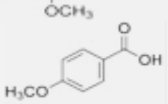
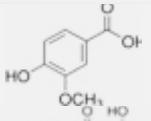
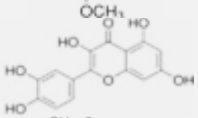
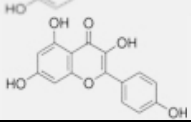


FIG. 2: HPLC CHROMATOGRAM OF METHANOLIC EXTRACT OF *P. PINNATA* SEEDS FOR PHENOLIC COMPOUNDS IDENTIFICATION AT DIFFERENT RT IN MIN

TABLE 3: DIFFERENT PHENOLIC COMPOUNDS WITH THEIR RETENTION TIME AND PERCENTAGE CONTENT IN THE METHANOLIC EXTRACT

S. no.	Phenolics	Rt. (min.) (standard)	Rt. (min.) (extract)	Formula	Structure	Area %
1	Gallic acid	7.18	6.96	$C_7H_6O_5$		13.66
2	Chlorogenic acid	9.26	9.32	$C_{16}H_{18}O_9$		1.33
3	Caffeic acid	13.77	12.88	$C_9H_8O_4$		19.23
4	Catechol	14.45	ND	$C_6H_60_2$		ND
5	Rutin	16.16	15.86	$C_{27}H_{30}O_{16}$		30.19
6	Coumaric acid	18.64	ND	$C_9H_8O_3$		ND
S7	Sinapic acid	19.40	ND	$C_{11}H_{12}O_5$		ND
8	Ferulic acid	19.81	ND	$C_{10}H_{10}O_4$		ND
9	Anisic acid	21.04	ND	$C_8H_8O_3$		ND
10	Vanillic acid	24.74	ND	$C_8H_8O_4$		ND
11	Quercetin	28.53	29.06	$C_{15}H_{10}O_7$		18.24
12	Kaempherol	35.29	ND	$C_{15}H_{10}O_7$		ND

*ND- not detected.

GC-MS/MS Analysis of Hexane Extract of *P. pinnata*: The GC-MS/MS analysis of hexane extract showed the presence of 42 volatile or partially volatile compounds **Fig. 3.** The major compounds with high percentage area are given in the **Table 4** among these compounds the highest percentage area was compound named Pongamol. The antimicrobial potential of the hexane extract may be due to the presence of these major compounds.

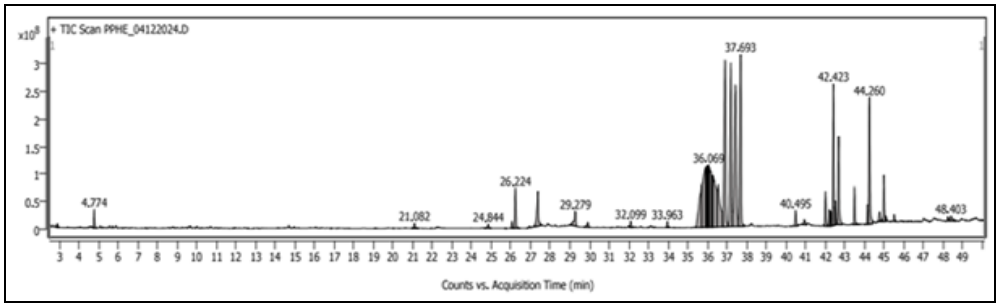
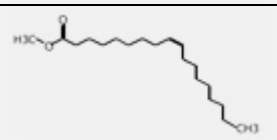
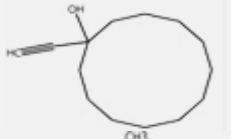
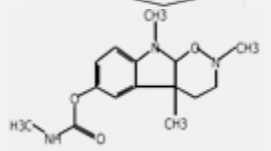
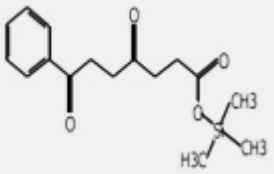
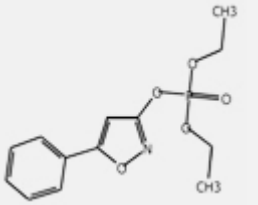
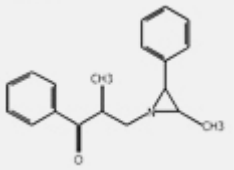
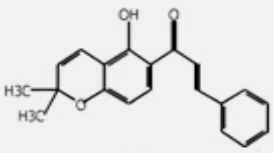
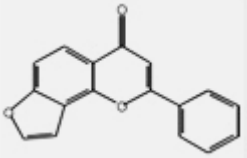
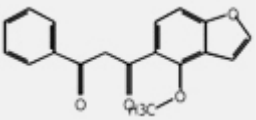
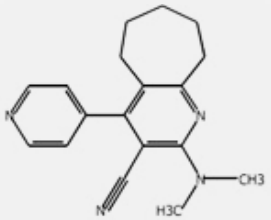
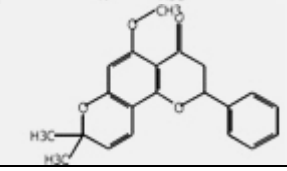
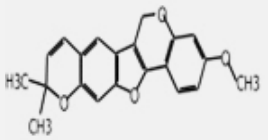
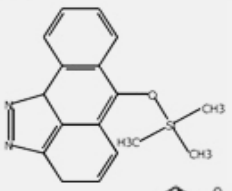
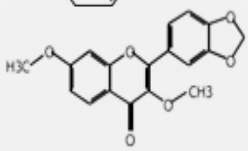
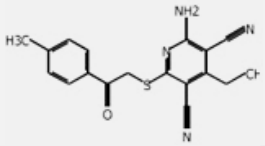
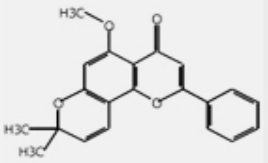


FIG. 3: GC-MS/MS CHROMATOGRAM OF HEXANE EXTRACT OF *P. PINNATA*

TABLE 4: COMPOUNDS FOUND IN HEXANE EXTRACT OF *P. PINNATA* SEEDS THROUGH GC-MS/MS

Peak no.	Compound	Formula	Mol. wt. (g/mol)	Rt. (min.)	Structure
6	9- Octadecenoic acid (z)-, methyl ester	$C_{19}H_{36}O_2$	296.48	26.22	
7	1- Ethynylcyclododecanol	$C_{14}H_{24}O$	208.34	27.36	
12	Eseridine	$C_{15}H_{21}N_3O_3$	179.22	35.70	
18	4,7- Dioxo- 7- phenylheptanoic acid, TMS	$C_{16}H_{22}O_4Si$	306.2	36.11	
19	Isoxathion- oxon	$C_{13}H_{16}NO_5P$	297.24	36.25	
21	alpha- Methyl- beta- N- (2- phenyl- 3- methylaziridyl) propiophenone	$C_{19}H_{21}NO$	279.38	36.56	
22	Lonchocarpin	$C_{20}H_{18}O_3$	306.36	36.90	
23	Lanceolatin B	$C_{17}H_{10}O_3$	262.26	37.19	
24	Pongamol	$C_{18}H_{14}O_4$	294.3	37.42	
25	2- Amino- 4 (pyridin- 4- yl)- 6, 7, 8, 9- tetrahydro- 5H- cyclohepta [b] pyridine- 3- carbonitrile, N, N- bis- methyl	$C_{18}H_{20}N_4$	292.4	37.69	
28	Pongachin	$C_{21}H_{20}O_4$	336.4	42.014	

31	10,10- Dimethyl- 6H, 10 H-chromeno[6', 7': 4, 5] furo[3, 2-C] chromen- 3- ye methyl ether	$C_{21}H_{18}O_4$	286.4	42.42	
33	6- [(Trimethylsilyl)oxy] dibenzo [cd,g] indazole	$C_{17}H_{16}N_2OSi$	292.0	42.68	
36	Dimethoxykanugin	$C_{18}H_{14}O_6$	326.3	44.26	
38	2- Amino- 4 ethyl- 6- {[2-(4-methylphenyl)-2- oxoethyl] sulfanyl} pyridine- 3,5-dicarbonitrile	$C_{18}H_{16}N_4OS$	336.4	44.99	
40	Isopongaflavone	$C_{21}H_{18}O_4$	334.4	45.51	

***In-silico* ADMET and Acute Rat Toxicity Prediction:** Comparative ADMET, radar, and toxicity analyses of the five major phytochemicals Lanceolatin B, Lonchocarpin, Eseridine, Pongamol, and Rutin highlight notable differences in their drug-likeness and pharmacokinetic properties. Radar plots **Fig. 4** revealed that Lanceolatin B and Pongamol showed well-balanced profiles with good bioavailability, solubility, blood–brain barrier (BBB) safety, and low toxicity, making them promising candidates for further development. Lonchocarpin and Eseridine displayed moderate properties, with acceptable solubility and absorption but higher probabilities of toxicity, suggesting potential limitations in their clinical applicability. Rutin, despite being a bioactive flavonoid, showed poor oral absorption, low bioavailability, and multiple violations of Lipinski's rule of five, consistent with its high molecular weight and excessive hydrogen bond donors/acceptors. The integrated ADMET **Table 5** further supports these findings: Lanceolatin B and Pongamol exhibited high human intestinal absorption (HIA), favorable oral bioavailability, and strong permeability, with Pongamol showing the highest half-life and distribution volume. Both also demonstrated balanced interactions with CYP enzymes, suggesting metabolic stability. In contrast, Rutin had poor permeability, limited absorption, and higher mutagenicity and hERG blocking probabilities, indicating restricted therapeutic potential as shown in **Fig. 5**. The most drug-like and pharmacologically advantageous options are Pongamol and Lanceolatin B, whereas Rutin is the least appropriate because of its poor pharmacokinetics and safety issues.

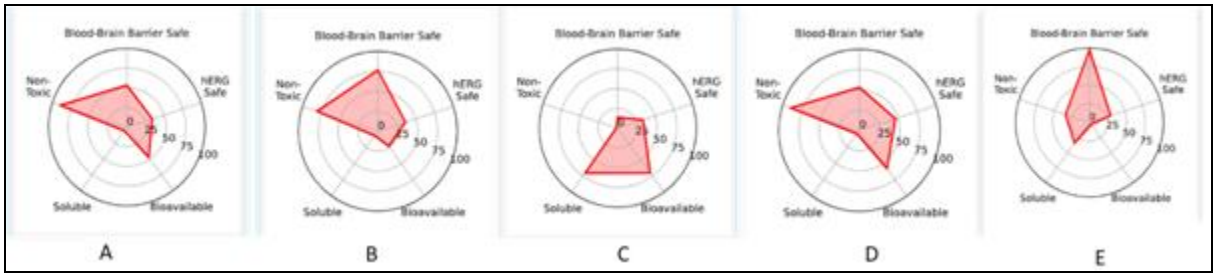


FIG. 4: RADAR PLOTS REPRESENTING ADMET SAFETY PROFILES OF (A) LANCEOLATIN B, (B) LONCHOCARPIN, (C) ESERIDINE, (D) PONGAMOL, AND (E) RUTIN

TABLE 5: ADMET AND PHYSICOCHEMICAL PROPERTIES OF SELECTED PHYTOCHEMICALS: (A) LANCEOLATIN B, (B) LONCHOCARPIN, (C) ESERIDINE, (D) PONGAMOL, AND (E) RUTIN

Category	Property	(A) Lanceolatin B	(B) Lonchocarpin	(C) Eseridine	(D) Pongamol	(E) Rutin
Physicochemical	Molecular Weight (Da)	292.29	354.32	310.33	294.31	610.52
	LogP	2.67	4.10	2.84	3.90	-1.69
	H-Bond Acceptors	4	6	5	4	16
	H-Bond Donors	0	2	1	0	10
	Lipinski Rule of 5 (violations)	0	1	0	0	3
	QED	0.51	0.46	0.48	0.53	0.14
	Stereo Centers	0	1	0	0	10
	TPSA (Å²)	56.9	94.2	72.4	56.5	269.4
Absorption	HIA	0.95	0.78	0.82	1.00	0.09
	Oral Bioavailability	0.62	0.55	0.60	0.84	0.18
	Solubility (log mol/L)	-4.75	-5.80	-5.10	-6.04	-3.86
	Lipophilicity	2.89	3.95	3.10	3.44	0.77
	Permeability (Caco-2/log cm/s)	-4.80	-5.10	-5.00	-4.40	-6.82
	PAMPA	0.80	0.65	0.72	0.98	0.09
	P-gp Inhibition	0.35	0.60	0.48	0.80	0.14
Distribution	BBB Penetration	0.42	0.25	0.33	0.71	0.06
	Plasma Protein Binding (%)	92.0	95.0	90.5	100.0	84.9
	Vd (L/kg)	3.2	4.6	4.0	5.04	6.39
Metabolism	CYP1A2 Inhibition	0.35	0.60	0.42	0.99	0.01
	CYP2C19 Inhibition	0.28	0.54	0.40	0.96	0.03
	CYP2C9 Inhibition	0.33	0.50	0.37	0.90	0.02
	CYP2D6 Inhibition	0.20	0.35	0.28	0.11	0.03
	CYP3A4 Inhibition	0.42	0.65	0.50	0.71	0.01
	CYP Substrate (Yes/No)	Yes	Yes	Yes	Yes	Partial
Excretion	Half-Life (h)	42.0	60.5	54.3	83.0	49.5
	Clearance (Hepatocyte)	32.5	41.8	38.2	73.8	25.6
	Clearance (Microsome)	44.2	52.3	47.0	51.8	40.1
Toxicity	hERG Blocking	0.28	0.40	0.34	0.33	0.65
	Mutagenicity	0.25	0.36	0.30	0.27	0.60
	DILI (Liver Injury)	0.70	0.85	0.75	0.94	0.75
	Carcinogenicity	0.08	0.10	0.09	0.05	0.02
	Acute Toxicity LD50 (log mol/kg)	2.85	2.88	2.86	2.91	2.90

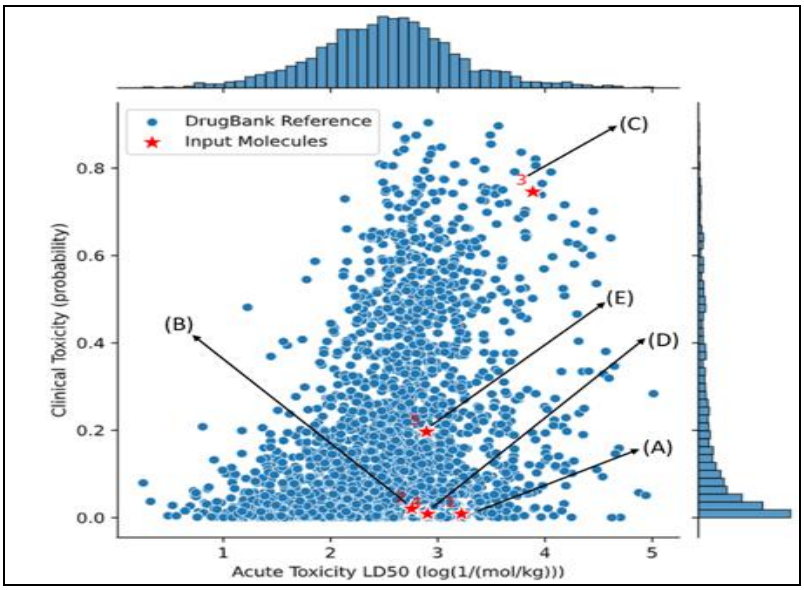


FIG. 5: ACUTE TOXICITY (LD_{50}) VERSUS CLINICAL TOXICITY PROBABILITY OF INPUT MOLECULES COMPARED WITH DRUGBANK REFERENCE COMPOUNDS. RED STARS REPRESENT THE INPUT MOLECULES: (A) LANCEOLATIN B, (B) LONCHOCARPIN, (C) ESERIDINE, (D) PONGAMOL, AND (E) RUTIN

CONCLUSION: The present research study focuses on antimicrobial activity-guided chemical profiling of phytomolecules present in *Pongamia pinnata* seeds, confirming the plant's potential as a source of bioactive compounds with antimicrobial properties. The study revealed that extract and fraction methanolic and hexane respectively, exhibited significant inhibitory effects against *S. abony* bacterial strain.

HPLC and GC-MS/MS analysis identified key bioactive constituents, including flavonoids, phenolics and alkaloids, which likely contribute to the observed antimicrobial activities. *In-silico* ADMET and acute toxicity prediction provide a scientific basis for the traditional use of *P. pinnata* and highlight its potential in the development of natural antimicrobial agents. Further studies, including *in-vivo* evaluations and isolation of individual active compounds, are required to explore the full therapeutic potential of *P. pinnata* seeds for pharmaceutical applications.

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Authors' Contributions: Vaishali Mishra and Shiwa Chaubey conceptualized the study. Dr. Swati Sharma and Shefali Singh conducted the anti microbial experiments and compiled data related to biological screening.

Mohd. Ashraf performed the formal analysis Meenu Verma wrote the initial draft of the manuscript, Dr. Mahesh Pal and Dr. Meenakshi Singh guided, reviewed and edited the final manuscript. All authors read and approved the final version of the manuscript.

REFERENCES:

1. Wasihun Y, Alekaw Habteweld H & Dires Ayenew K: Antibacterial activity and phytochemical components of leaf extract of *Calpurnia aurea*. Scientific Reports 2023; 13(1): 9767.
2. Al-Ansari M, Al-Dahmash ND, Angulo-Bejarano PI, Ha HA & Nguyen-Thi TH: Phytochemical, bactericidal, antioxidant and anti-inflammatory properties of various extracts from *Pongamia pinnata* and functional groups characterization by FTIR and HPLC analyses. Environmental Research 2024; 245: 118044.
3. Becerra SC, Roy DC, Sanchez CJ, Christy RJ & Burmeister DM: An optimized staining technique for the detection of Gram positive and Gram negative bacteria within tissue. BMC Research Notes 2016; 9: 1-10.
4. Lamichhane B, Mawad AM, Saleh M, Kelley WG, Harrington PJ, Lovestad CW & Helmy YA: *Salmonellosis*: An Overview of Epidemiology, Pathogenesis, and Innovative Approaches to Mitigate the Antimicrobial Resistant Infections. Antibiotics 2024; 13(1): 76.
5. Bastin MT, Neville NR, Parsons RE, Flannery AH, Tennant SJ & Johnson CA: An unusual case of Salmonella Enteritidis causing pneumonia, septic shock and multiple organ failure in an immunocompetent patient. ID Cases 2016; 6: 85-89.
6. Mäki-Lkola O & Granford K: Salmonella-triggered reactive arthritis. Scandinavian Journal of Rheumatology 1992; 21(6): 265-270.
7. Han X, Yao J, He J, Liu H, Jiang Y, Zhao D & Li X: Clinical and laboratory insights into the threat of hypervirulent *Klebsiella pneumoniae*. International Journal of Antimicrobial Agents 2024; 107275.
8. Priyanka A, Akshatha K, Deekshit VK, Prarthana J & Akhila DS: *Klebsiella pneumoniae* infections and antimicrobial drug resistance. Model organisms for microbial pathogenesis. Biofilm Formation and Antimicrobial Drug Discovery 2020; 195-225.
9. Navon-Venezia S, Kondratyeva K & Carattoli A: *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiology Reviews 2017; 41(3): 252-275.
10. Gyawali R & Ibrahim SA: Natural products as antimicrobial agents. Food Control 2014; 46: 412-429.
11. Al Muqarrabun LMR, Ahmat N, Ruzaina SAS, Ismail NH & Sahidin I: Medicinal uses, phytochemistry and pharmacology of *Pongamia pinnata* (L.) Pierre: A review. Journal of Ethnopharmacology 2013; 150(2): 395-420.
12. Kesari V, Das A & Rangan L: Physico-chemical characterization and antimicrobial activity from seed oil of *Pongamia pinnata*, a potential biofuel crop. Biomass and Bioenergy 2010; 34(1): 108-115.
13. Yasothkumar D, Jayaraman S, Ramalingam K & Ramani P: *In-vitro* Anti-Inflammatory and Antioxidant Activity of Seed Ethanolic Extract of *Pongamia pinnata*. Biomedical and Pharmacology Journal 2023; 16(4): 2187-2193.
14. Abubakar AR & Haque M: Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. Journal of Pharmacy & Bioallied Sciences 2020; 12(1): 1–10.
15. Pant DR, Pant ND, Saru DB, Yadav UN & Khanal DP: Phytochemical screening and study of antioxidant, antimicrobial, antidiabetic, anti-inflammatory and analgesic activities of extracts from stem wood of *Pterocarpus marsupium* Roxburgh. Journal of Intercultural Ethnopharmacology 2017; 6(2): 170–176.

16. Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, Sholkamy EN & Bakri MM: Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi Journal of Biological Sciences 2018; 25(2): 361-366.
17. Nisha CM, Kumar A, Nair P, Gupta N, Silakari C, Tripathi T & Kumar A: Molecular docking and *in-silico* admet study reveals acylguanidine 7a as a Potential Inhibitor of β-Secretase. Advances in Bioinformatics 2016; 1: 9258578
18. Roney M, Uddin MN, Sapari S, Razak FIA, Huq AKM, Zamri NB & Aluwi MFFM: *In-silico* approaches to identify novel anti-diabetic type 2 agents against dipeptidyl peptidase IV from isoxazole derivatives of usnic acid. 3 Biotech 2025; 15(5): 1-17.

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