EXPLORING THE ANTIOXIDANT, ANTIDIABETIC, ANTIMICROBIAL, CYTOTOXIC AND ANTI-INFLAMMATORY PROPERTIES OF *LEPTADENIA PYROTECHNICA* - CHEMICAL PROFILING BY FTIR AND HPLC

N. U. Ain[1], J. Hafeez[1], Ajwa[1], M. Gull[1], A. Khizran[1] and F. Hussain[1*]

[1]Department of Biochemistry, Faculty of Sciences, University of Agriculture, Faisalabad, Pakistan
[*]Correspondence author email: fatmauaf@yahoo.com

ABSTRACT: Plants employed in medicine have opened up opportunities for pharmacological advancement. One of these was *Leptadenia pyrotechnica,* utilized in conventional medicine due to its pharmacological potential. *L. pyrotechnica* was processed to synthesize *n*-hexane, methanol and aqueous extracts (yields were 4.52%, 2.004% and 7.45%). Anti-bacterial activity exhibited better results with methanol (17mm with *E. coli* and 15.2mm with *S. auerus*) as compared to *n*-hexane (14mm and 11mm) and aqueous (0mm and 10mm). Total phenolic compound in *n*-hexane, methanolic and aqueous extracts were 348.92±0.9, 569.03±1.6 and 446.24±0.9 mg GAE/mL respectively. Total flavonoids content in methanol, *n*-hexane and aqueous extracts were 156.67±8.5, 35.09±3.4 and 163.53±7.8 µg CE/mL respectively. DPPH percentage inhibitions were 7.739%, 12.72%, 19.71% and 97.8% of *n*-hexane, methanol, aqueous and control respectively. Hemolytic percentage inhibitions by extracts were 55.31%, 32.34%, 61.85% and 97.84% of *n*-hexane, methanol, aqueous and control respectively. Anti-inflammatory percentage inhibition of *n*-hexane, methanol, aqueous and control was 1.32%, 1.31%, 4.44% and 67.87%. Percentage inhibitions in antidiabetic assay were 12.82%, 22.77%, 24.27% and 21.21% of *n*-hexane, methanol, aqueous and control correspondingly. Fourier transformed infrared spectroscopy showed peaks at 3278.2 to 669.1cm^{-1} and presence of phenols**,** sulfates, chloride, alcohol, flavonoid, carboxylic acid, sulfone, sulfonyl, primary and secondary amines, sulfonamides and aromatic compounds. Chlorogenic acid, HB acid, p-coumaric acid, kaempherol, caffeic acid, ferulic acid and coumarin detected by high performance liquid chromatography having retention time ranges from 2.8 to 16.5 minutes. One way ANOVA was applied. Further study is required to fully assess the outstanding characteristics and therapeutic potential of this herb.

Keywords: Antioxidant, Hemolytic, Cytotoxic, Antidiabetic, Phenols.

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INTRODUCTION

Plants have a tendency to yield pharmaceutical compounds with significant pharmacological potential and immense value in the improvement of healthcare services. Nations that are both developing and industrialized have immense potential for using herbal medicines for curing a broad spectrum of human ailments (Poddar *et al.,* 2020). Ethnopharmacology is a transdisciplinary and collaborative method of developing pharmaceuticals which includes defining, monitoring and analyzing the biological processes associated with conventional procedures. In order to preserve historical applications of medicinal plants and conduct bio-prospecting, ethnopharmacological investigations are an essential step (Ahmad *et al.,* 2018).

Asclepiadaceae, a subfamily of Apocynaceae, is a beneficial genus for discovering unique chemicals that are bioactive. The two features that have received the most attention of this family are the intricate nature of flower structures, their relevance to pollinator connections and the variety of strategies for defense from specialized herbivores (Wang *et al.,* 2023).

The application of plants in conventional healthcare has created new avenues for the development of pharmacology. Among those one is *Leptadenia pyrotechnica* which is utilized in conventional medication on account of its pharmacological properties, which involve antioxidant, antidiabetic, hemolytic, antibacterial and anti-inflammatory properties. Serve as an effective and beneficial natural remedy. *L. pyrotechnica* is a xerophytic species that produces an abundance of biomass in sandy arid regions (Fitiany *et al.,* 2023).

According to a structural perspective, it is a shrub that rises vertically and reaches heights of around 0.50 to 2.60 meters. The bushes of *L. pyrotechnica* possess numerous branches and are capable of growing up to 3 meters tall. The shrub blossoms and yields fruit in the months of January and August. The plant's roots may extend as deep as 12 meters (Verma *et al.,* 2018).

About 4% of *L. pyrotechnica* is made up of fats, 4% of fibers, 28% of carbs, 23% of proteins, vitamin E and minerals including phosphorus, calcium and iron

(Masood *et al.,* 2023). The elemental analysis of *L. pyrotechnica* was as follows: N > Na > K > Ca > Mg > Al > Si (ElGawad *et al.,* 2022).

It is medicinal herb that yields potent components that have historically made it well-known for a variety of uses. In Ayurveda, *L. pyrotechnica* is used as an astringent, bowel movements, soothing and stimulant. It is advised to use *L. pyrotechnica's* fresh or recently collected stem as a flavoring ingredient (Alkaltham *et al.,* 2023). The residents of Saudi Arabia along with other Arab nations utilize *L. pyrotechnica* as an agricultural product. The stem was historically utilized for the relief of an array of illnesses such as cancer, skin irritation, psoriasis, cough, smallpox, urinary retention, diarrhea, renal problems and diabetes (Mahassni *et al.,* 2022).

Despite its traditional use, *L. pyrotecnica* extract has been largely overlooked in scientific research, warranting a comprehensive investigation into its bioactive compounds and therapeutic potential. Therefore current investigation was undertaken to assess phytochemistry along with antioxidant, antidiabetic, antimicrobial, cytotoxic and anti-inflammatory potentials of *L. pyrotecnica.*

MATERIALS AND METHODS

Collection of Sample: Collection of *L. pyrotechnica* sample was done from Kot Mithan. Authentication of *L. pyrotechnica* was done from the Department of Botany, University of Agriculture, Faisalabad, Pakistan. Voucher specimen (110-2-23, dated 30-09-2023) was saved at Herbarium.

Preparation of extracts: The plant was crushed into a fine powder after drying process and kept in a labeled polythene bag. Extraction was performed at room temperature using microwave assisted method. Aqueous, methanol and *n*-hexane were three solvents used in experiment. The mixtures of sample (50g) having solvents (250mL) of definite ratio (1:5) were heated in microwave oven thrice for 30 seconds. Sample-filled flasks were covered using aluminum foil, kept at room temperature for a whole day. Following this, filtration was done. The filtered samples were put on a water bath at 52-55^{o} C until semi solid extracts were formed and transferred into labeled sample container and refrigerated for use in further experiment. Semi–solid extracts were reconstituted by using dimethylsulfoxide (DMSO) with 1:4 ratios for further examination (Noreen *et al*., 2020).

Antimicrobial Evaluation: Antibacterial characteristics of *L. pyrotechnica* extracts were evaluated through the agar well diffusion technique. In an eppendrof tube, sample was formed through the combination of 25 g of aqueous, *n*-hexane and methanolic extracts in 1mL of dimethylsulfoxide (DMSO).

In 2 different flasks, 8.05 g agar and 350 mL distilled water were mixed to produce agar solution. Petri plates and agar solution were autoclaved. After cooling, agar solution and petri plates to 40°C, 100µL of the bacterial strains *Staphylococcus aureus* (gram +ve) and *E. coli* (gram –ve) were incorporated to both flasks. After that, bacterium-containing agar solution was shifted in 2 petri dishes in laminar air flow and allowed to solidify. By using micropipette tip of 1 mL, four 8 mm wells were made and three wells on each plate were loaded with 80µL of sample, while fourth well contained ciprofloxacin as a positive control. After incubation (37°C) for 24 hours, growth inhibitory zone's diameter was calculated (Riaz *et al.,* 2019).

Antioxidant contents: $AlCl_3$ colorimetric technique was employed to calculate total flavonoids contents (TFC) as µg CE (catechin equivalent)/mL. *L. pyrotechnica* extracts solution (25 µL), 5 µL of 1M potassium acetate and 140µL of distilled water were mixed and for 10 minutes incubated. Then 5 µL of 20% $AlCl_3$ was added and incubated for 5 minutes at room temperature. At 415 nm, the absorbance was determined by ELISA reader. Total phenolic content (TPC) as mg GAE (gallic acid equivalents) per mL was assessed by Folin-Ciocalteu (FC) reagent. In *L. pyrotechnica* extracts (25µL), 100µL 20 % sodium carbonate solution and 125µL of FC reagent were added and incubated for 2 hours. Absorbance was measured at 765 nm (Hussain *et al.,* 2021).

Free Radical Scavenging Assay: For evaluation of antioxidant capacity, DPPH (2,2-diphenyl-l-picrylhydrazyl) scavenging was done. In 2.5 µL of sample, 250 µL (0.004%) DPPH solution was added and incubated for 30 minutes (Hussain *et al.,* 2021). At 570 nm, absorbance (A) was taken. Ascorbic acid was used as a standard. % DPPH scavenging = [A (control) – A (sample) /A (control)] × 100.

Cytotoxic Potential - Hemolytic Assay: Three mL of recently drawn, human blood were taken from the Pathology Laboratory, Mujahid Hospital, Faisalabad, Pakistan. Centrifugation of blood was carried out to eliminate plasma from blood samples at 1000 rpm for five-minutes. The RBC pellet was rinsed three times in 1 ml of chilly (4^{o}C), uncontaminated and isotonic phosphate buffer saline (PBS; pH 7.4), after that plasma was discarded. Ultracentrifugation was done at 1000 rpm for 5 minutes. Chilled PBS (1.3 mL) was added to make volume. In eppendrof tubes, 20µL of each test sample (smpl) and 180 µL of diluted blood was added and incubated for 30 minutes. Centrifugation was done at 1000 rpm for 3 minutes. Then, supernatant (100 µL) was extracted and diluted with 900 µL of chilled PBS. Prepared solutions (200µL) were loaded in 96 wells plate (Shahzadi *et al.,* 2019). PBS and 0.1% triton X-100 were

negative control (NC) and positive control (PC) respectively. At 576 nm, absorbance (Absr) was determined to measure percentage inhibition of hemolysis as: [Absr smpl – Absr NC/ Absr PC] × 100

Anti-inflammatory Evaluation: Bovine serum albumin (BSA) denaturation technique was used. Test samples (20 µL) and 2mL of 1% BSA were added to test tubes. Test tubes were incubated for 5 minutes at 37°C and after that test tubes were kept in water bath for 5 minutes at 72°C. After cooling, prepared solution (200µL) was loaded in 96 wells plate. At 255 nm, absorbance was observed. In positive control diclofenac (20 µL) was used. For preparation of diclofenac solution, 0.1g of diclofenac was added in 10mL of distilled water. In negative controls (C), 2mL of 1% BSA was added in 20 µL of PBS, and samples (S) methanol, *n*-hexane, aqueous extracts (Williams *et al.*, 2008).

$$\text{Anti-inflammatory activity was calculated as: } \frac{\text{Absorbance of C} - \text{Absorbance of S}}{\text{Absorbance of C}} \times 100$$

Antidiabetic Evaluation

Alpha-Amylase Inhibition Assay: Small volume (10 µL) of sample and 10 µL of enzyme solution (source of enzyme was *Bacillus subtilis* and was prepared by adding 0.5 mg/mL of enzyme in 0.02 M sodium phosphate buffer) were added and incubated for 10 minutes on ELISA plate. After incubation, 20 µL (1%) starch solution was added and incubated for 30 minutes. Then 10 µL (1M) HCl was added. Enzymatic procedure was stopped after acid treatment. Iodine solution (40 µL) was added into each well. An ELISA reader was employed for determining absorbance (A) at a wavelength of 570 nm. Acarbose was used as a positive control. For comparison, a negative control was blank sample devoid of substrate (Hussain *et al.*, 2021). Percentage inhibition (%) of alpha amylase was calculated as: $\frac{1-\text{A (negative control)}}{\text{A (test sample)}} \times 100$

Structural Analysis

Fourier Transform Infrared Spectroscopy (FTIR): FTIR is a quick, inexpensive, safe means of acquiring information that does not produce laboratory waste, enables identification and measurement of organic groups with functional properties that reveal the biochemical makeup of flora (Pereira *et al.*, 2024). *L. pyrotechnica* fine powder extract was used for FTIR analysis.

High performance liquid chromatography (HPLC): Some modifications were implemented to this analysis for the current study. The Zorbax SB-C18 (250 × 4.6 mm, 5 µm) column was utilized in an HPLC (Agilent Technologies) with a DAD-1260 VL detector at 30°C. The mobile phases were acetic acid (HPLC grade) and methanol. The DAD was adjusted to 280 nm and 11 µL sample was introduced into the HPLC apparatus (Bagad and Khan, 2015).

Statistical Analysis: Results for all the activities were expressed as mean ± S.D or percentage (n=3). Analysis was performed by using Minitab statistical software (version 17) with $p<0.05$ as level of significance.

RESULTS AND DISCUSSION

Results were computed by using calculations of processed extracts of *L. pyrotechnica*. Weight of plant sample used was 50g and weights of aqueous, methanol and *n*-hexane extract were 3.725 g, 2.26 g and 1.002 g respectively. Yields are given in table 1.

Table 1. Yield percentage.

Plant name	Plant part	Aqueous	Methanol	*n*-hexane
Leptadenia pyrotechnica	Whole plant	7.45%	4.52%	2.004%

Kumari *et al*. (2024), reported *L. pyrotechnica* extracts' percentage yields (% w/w) using an array of solvents, which included ethanol, hexane, aqueous, petroleum ether, ethyl acetate and chloroform. According to the results, the polar solvent ethanol had an exceptionally high extractive yield (7.37 % and 5.7%) for both stem and root parts while in current study aqueous extract showed high yield ranges up to 7.45% (w/v) as compared to methanol (4.5%) and n-hexane (2.004%). Previously, Masood *et al*. (2023) stated that the yield of methanol extract was around 8.22% (w/v), but the yield in the present study was 4.5% (w/v).

Antibacterial Activity: In case of *E. coli L. pyrotechnica* showed antimicrobial activity, aqueous extract showed no zone of inhibition but methanol and *n*-hexane extract showed a zone of inhibition 17mm and14 mm respectively while control shows zone of inhibition of 38.5 mm as shown in fig 1. Similarly, in case of *Staphylococcus aureus L. pyrotechnica* showed antimicrobial activity, aqueous, methanol and *n*-hexane extract showed zone of inhibition 10mm, 15.2mm and11mm respectively while control gave the inhibition zone of 38mm (table 2). Current study showed outcomes ranges from 0 to 38.5mm in case of *E. coli* and 10 to

38mm in case of *S. aureus* while, Fitiany *et al.* (2023) observed 6 mm *S. aureus* and 26 mm *E. coli*. Multiple sources demonstrated *L. pyrotechnica* potent antibacterial capabilities. According to the findings, different extracts and fractions were effective against the tested bacterial strains at different concentrations.

Table 2. Antibacterial activity of *L. pyrotechnica* extracts.

Strains	Sample			
	Control	Zone of inhibition (mm)		
	(Ciprofloxacin)	Aqueous extract	Methanol extract	*n*-hexane extract
E. coli	38.5	0	17	14
S. aureus	38	10	15.2	11

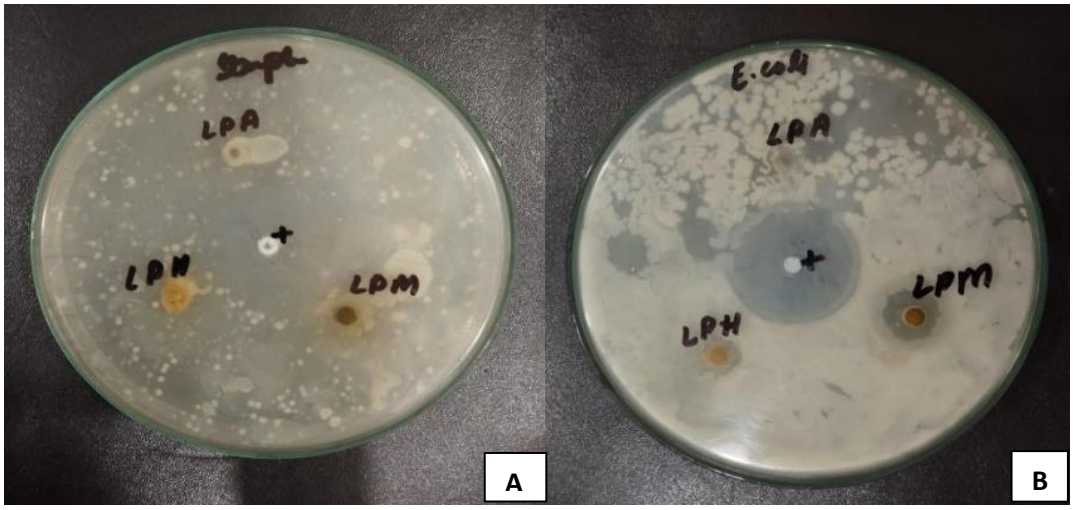

Fig. 1 Antimicrobial activity - Zones of inhibition (mm) by *Leptadenia pyrotechnica* (A) *S. aureus* (B) *E.coli*
LPA: aqueous extract, LPH: *n*-hexane extract, LPM: methanol extract

Antioxidant activity: Results (table 3) showed presence of more phenolic content in methanolic extract. After that aqueous showed presence of TPC which were followed by *n*-hexane extract. Order of total phenolic content was methanol > aqueous > *n*-hexane. Highly significant difference was observed in TPC of *n*-hexane, methanol and aqueous extracts of *L. pyrotechnica.* Zubair *et al.* (2022) and Alkaltham *et al.* (2023), observed TPC level ranging from 8 to 26 mgGAE/g and 95 to 215 mg GAE/mL while current study showed TPC level ranging from 348 to 569 mg (GAE)/mL. Equation enabled calculation of values of every absorbance, from which TFC was derived by taking the mean and standard deviation was also calculated. Results showed good activity with *n*-hexane extract then of aqueous and methanolic extract. Order of TFC was aqueous > methanol > n-hexane. Highly significant difference was observed in TFC of *n*-hexane, methanol and aqueous extracts of *L. pyrotechnica*. Alkaltham *et al.* (2023) observed TFC level ranging from 1.38 to 3.32 mg CE/g while current study showed TFC level ranging from 37 to 163 µg CE/mL. The TFC readings from the aerial sections and origins of *L. pyrotechnica* have been estimated to be 139.45±8.68 and 76.87±2.27 mg QE/g, correspondingly. A remarkable variation ($p<0.05$) has been observed in the TFC of both plant section (Munazir *et al.,* 2015). Current study showed DPPH percentage (%) inhibition of whole plant of *L. pyrotechnica* which ranges from 7.5 to 62.9% and control showed 93.6%. The aqueous extract produced outstanding outcomes which were followed by the *n*-hexane extract. However, the DPPH inhibition test findings for the methanolic extract were extremely negative. Highly significant difference was observed in DPPH % inhibitition by *n*-hexane, methanol and aqueous extracts of *L. pyrotechnica*. Alkhaltham *et al.* (2023), observed DPPH % inhibition ranging from 22 to 78% while current study showed observed DPPH % inhibition ranging from 7 to 62%. Investigation revealed that *L. pyrotechnica* has a higher capacity to scavenge free radicals (DPPH) than several other plant species that Munazir *et al.* (2015) has observed.

Table 3 Different activities of *L. pyrotechnica* extracts.

Contents/Activities	Samples			
	Control	Extracts		
		n-hexane	Aqueous	Methanol
		Antioxidant Profile		
TPC (mg GAE /mL)	-	348.92±0.9	446.24 ±0.9	569.35±1.6*
TFC (µg CE/mL)	-	35.09±3.4	163.53±7.8*	156.67±8.5
DPPH (%)	93.6±1.4	7.53±4.1	11.9±5.7*	7.83±1.7
		Antidiabetic Profile (% inhibition)		
Alpha-amylase	77.5±5.3	11.6±2.1	24.3±1.1*	23.7±1.5
		Cytotoxicity (% inhibition)		
Hemolytic activity	99±1.7	56.7±54.9	62.8±1.4*	32.3±1.3
		Anti-inflammatory Activity (% inhibition)		
Albumin denaturation	67.87±0.67	1.32±0.39	4.44±0.31*	1.31±0.65

Data are represented as mean ± SD or percentage (n=3). * Significant at $p < 0.05$.

TPC: total phenolic contents expressed as mg GAE (gallic acid equivalents)/ mL, TFC: total flavonoid contents expressed as µg CE (catechin equivalent)/mL, DPPH: 2,2-diphenyl-l-picrylhydrazyl scavenging (antioxidant activity) expressed as percentage. Positive controls for; DPPH assay: ascorbic acid, alpha-amylase inhibitory assay: acarbose, hemolysis assay: Triton X-100, anti-inflammatory activity: diclofenac sodium.

Antidiabetic Activity: Alpha amylase inhibition potentials of *n*-hexane, methanol and aqueous were 23.7%, 11.6% and 24.3% respectively (table 3). While acarbose exhibited 77.5% inhibition. Highly significant difference was observed in alpha amylase restriction by *n*-hexane, methanol and aqueous extracts. **A**queous extract showed better results in comparison with other extracts.

Overall percentage inhibition ranges among 11 to 24%. Previously Chaudhry *et al*. (2011) determined an inhibition that ranged from 27-56% for alpha amylase by *L. pyrotechnica*.

Hemolytic Activity: The aqueous extract exhibited significant effects which were followed by *n*-hexane and methanol (table 3). Highly significant difference was observed in hemolytic potential of *n*-hexane, methanol, and aqueous extracts of *L. pyrotechnica.* Current study showed outcomes ranging from 32 to 62% of % hemolysis. Asad *et al.* (2014) observed hemolytic activity whose concentration ranges from 4 to 67%. At 576 nm, *n*-hexane demonstrated highest absorbance as compared to methanol and aqueous.

Anti-Inflammatory Activity: Highly significant difference was observed in anti-inflammatory activity of *n*-hexane, methanol and aqueous extracts of *L. pyrotechnica* (table 3). For *n*-hexane (1.32±0.39), methanol (1.31±0.65) and aqueous (4.44±0.31) extracts, percentage ranges between 1 to 4%. Hadi *et al.* (2021) determined 17% anti-inflammatory activity.

FTIR Evaluation: The chemical compounds found in *L. pyrotechnica* powder are graphically shown in this interferogram produced by FTIR (Fig 2, table 4). The absorption values for multiple chemical compounds as determined by FTIR analysis were displayed in the table. Carboxylic acid was present, according to medium peak measured at 3278.2cm^{-1} (also demonstrated presence of primary and secondary amines and amides that are stretch) , strong peaks at 1235.6cm^{-1}, medium-strong peaks at 1017.6 cm^{-1}, 1032.5 cm^{-1} and 1051.1 cm^{-1}. Stretched alkanes were also detected at peak of 2920.4 cm^{-1}. Spectral analysis of O-H predicted that bond was stretched and usually very broad whereas C-O predicted that stretch occurred at medium intensity. Strong peaks measured at 2160.0 cm^{-1} and 2201.0 cm^{-1} showed presence of alkynes. Sulfones, sulfonyl chlorides, sulfates and sulfonamides were also present ensured by peak observed at 1235.6 cm^{-1}. In alcohols stretching vibrations measured in the range 1260-1000 cm^{-1} (1235.6cm^{-1}, 1017.6 cm^{-1}, 1032.5 cm^{-1} and 1051.1 cm^{-1}). Ethers exhibit a minimum of one C-O band between the 1300–1000 cm−1. The C-O band enables basic aliphatic ethers to be recognized among alkanes. Medium-strong peak measured at 1051.1 cm^{-1} demonstrated presence of alcohol, ethers, esters, carboxylic acid, anhydrides, amines and fluoride. Strong peak measured at 896.4 cm^{-1} demonstrated presence of aromatic compounds (C-H) which were out of plane band. Strong peak at 669.1 cm^{-1} demonstrated presence of alkenes (out of plane bend).

Current study revealed presence of compounds having characteristic absorption ranges from 3278.2 to 669.1 cm^{-1} while Eltyeb *et al.* (2024), observed peaks at 3564 to 607 cm^{-1}.

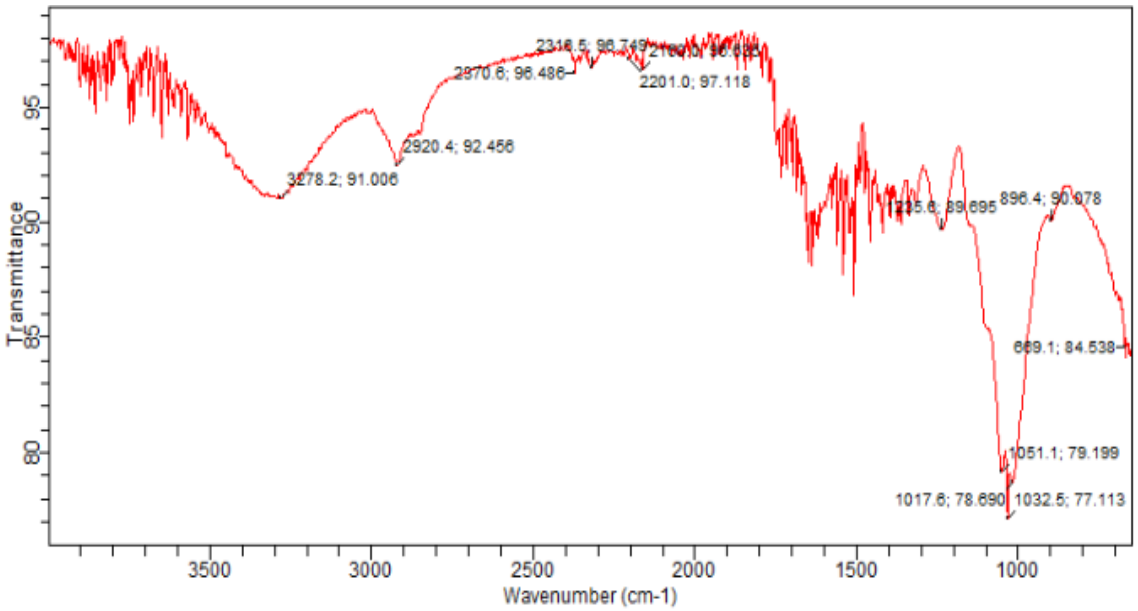

Fig.2. FTIR spectra of *L. pyrotechnica*

Table 4: FTIR Spectra.

Sr. no.	Characteristic Absorption	Compounds Class	Identified Functional group	Absorption intensity
1	3278.2	Primary and secondary amines and amides(stretch), carboxylic acids	N-H, O-H	Medium
2	2920.4	Alkanes (Stretch)	C-H	Strong
3	2201.0	Alkyne	C≡C	Medium-weak
4	2160.0	Alkyne	C≡C	Medium-weak
5	1235.6	Alcohol, ethers, esters, carboxylic acid, anhydrides. Sulfones, sulfonyl chlorides, sulfates, sulfonamides	C-O, S=O	Strong,
6	1051.1	Alcohol, ethers, esters, carboxylic acid, anhydrides. Amines, Fluoride	C-O, C-N, C-X	Strong, Medium-Strong, Strong
7	1032.5	Amines, Fluoride Amines, Alcohol, ethers, esters, carboxylic acid, anhydrides. Fluoride.	C-N, C-O, C-X	Strong Strong, Medium-Strong, Strong
8	1017.6	Alcohol, ethers, esters, carboxylic acid, anhydrides.	C-O, C-N, C-X	Strong, Medium-Strong, Strong
9	896.4	Aromatics (out of plane bend)	C-H	Strong
10	669.1	Alkenes (out of plane bend)	C-H	Strong

HPLC Evaluation: The compounds found in *L. pyrotechnica* powder are graphically shown in this graph produced by HPLC in fig 2 and table 5. Absorbance was measured in mili-absorbance unit, represented as mAU.

In this study ten compounds were identified by HPLC in whole plant of *L. pyrotechnica*. The results were represented in histogram (fig 3) and table 5. Chlorogenic acid was observed at retention time 2.845 min having area and height of 1,619,135.0 and 148,405.7. The retention time of P-Coumaric acid and HB acid (haemoglobinic acid) were 3.292 min and 6.735 min having area and height of 569,537.7 mV.s, 584,167.2 mV.s and 50,336.4, 36,285.6. Similarly, caffeic acid and kaempherol were observed at retention time of 7.467 min and 11.007 min with peak area and height 437,130.4 mV.s, 268,598.1 mV.s and 46,509.0, 20,242.7 respectively. Retention time for ferulic acid and Coumarin were 12.464 min and 16.538 min with peak area and height of 677,585.9 mV.s, 7,881,533.2 mV.s and 50,502.0 and 470,451.1 respectively. Preet *et al.* (2018), evaluated ten phenolic compounds from whole extract of *L. pyrotechnica* in which the most significant quantity of caffeic acid (3.30%) was recorded. Alkaltham *et al.* (2023), identified ten compounds including salicylic acid,

caffeic acid, tannic acid, chlorogenic acid, vanillin, resorcinol, acetylsalicylic acid, 3,5-dinitro salicylic acid, quercetin and 1,2-dihydroxybenzene in *L. pyrotechnica* samples by incorporating certain modifications. Masood *et al*. (2023), performed HPLC for the evaluation of bioactive components present in methanolic *L. pyrotechnica* extract. Results showed presence of compounds along concentration inculding vanillic acid (1.1), rutin (2.0), catechin (6.0) and sinapinic acid (0.1). Retention time of four detected compound ranges from 12.11 to 25.21 while current study showed retention time of seven detected compounds ranges from 2.84 to 16.53.

Table 5. HPLC Histogram.

Sr. No	Compound Name	Retention time (min)	Peak Area [mV.s]	Height
1	Chlorogenic Acid	2.845	1,619,135.0	148,405.7
2	p-Coumaric Acid	3.292	569,537.7	50,336.4
3	HB Acid	6.735	584,167.2	36,285.6
4	Caffeic Acid	7.467	437,130.4	46,509.0
5	Kaempherol	11.007	268,598.1	20,242.7
6	Ferulic Acid	12.464	677,585.9	50,502.0
7	Coumarin	16.538	7,881,533.2	470,451.1

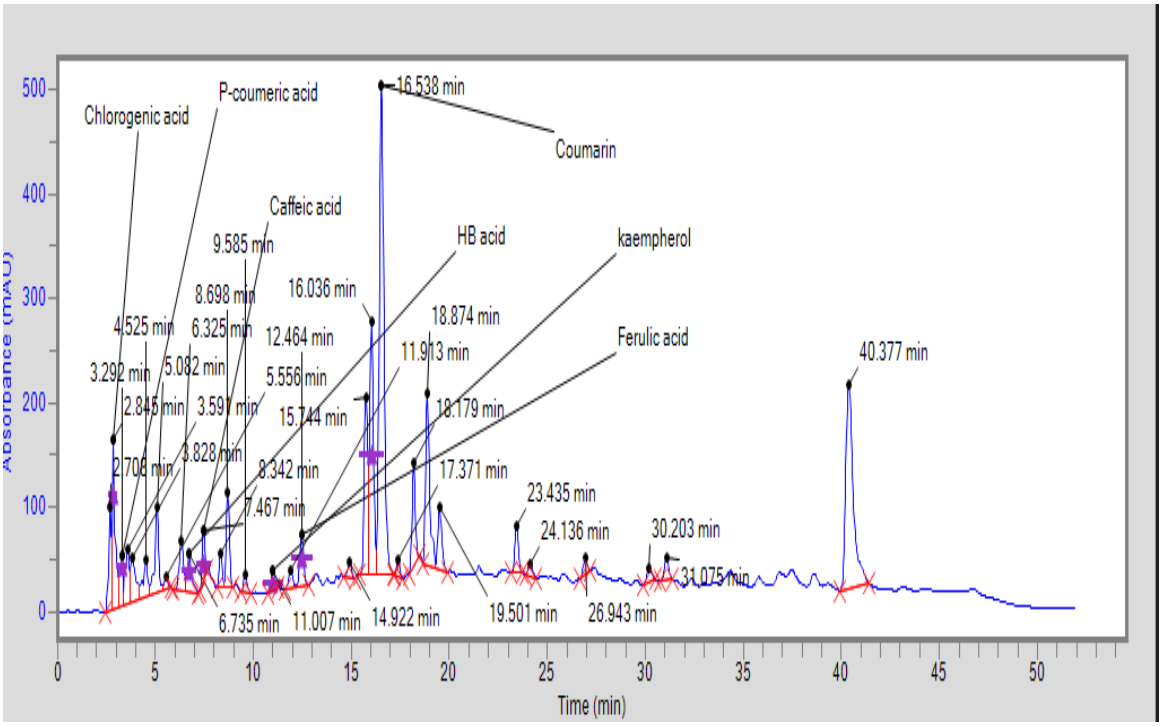

Fig 3 HPLC histogram

Conclusion: Examining the biochemical processes and bioactive elements present in aqueous, methanol and *n*-hexane extracts of *L. pyrotechnica* plant was the ultimate objective of this research. According to results of this investigation *L. pyrotechnica* extracts demonstrated exceptional pharmaceutical properties. Further study is required to fully assess the outstanding characteristics and therapeutic potential of this herb.

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