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Evaluation of Alkaloids and their Pharmacological Activity from *Leptadenia pyrotechnica* (Forsk) Decne

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Leptadenia pyrotechnica is fibre yielding plant and member of Asclepiadaceae family. Commonly all parts of *Leptadenia pyrotechnica* are used in folk medicines by local people. The present study gives qualitative estimation of alkaloids and its pharmacological properties. The qualitative and analysis of alkaloids carried out by using GCMS and spectral studies. Antimicrobial properties evaluate by well diffusion method. Fifty compounds found in GCMS analysis of shoots of *Leptadenia pyrotechnica* á,á Carotene, 6',7'-didehydro-5,6-epoxy-5,5', 6,6',7,8hexahydro3,3', 5'trihydroxy8oxo (3.56%) found to be maximum area observed at the retention time of 28.15. Shoots and roots both showed remarkable antimicrobial activity against selected microbial agents. Maximum zone of inhibition was observed in roots (15 mm) against *Staphylococcus aureus* and in fungal strains shoot showed (17 mm) zone of inhibition against *Penicillium funiculosum.* The findings of this study illustrate that *Leptadenia pyrotechnica* have remedial capability.

Keywords: Antimicrobial; GCMS; Leptadenia pyrotechnica; well diffusion method.

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1. INTRODUCTION

Leptadenia pyrotechnica found in dry habitat .it occurs in the Rajasthan, Punjab, and Western Uttar Pradesh [1]. *Leptadenia pyrotechnica* is bushy shrub generly occurs without leaves [2]. world's oldest system of medicine is called Ayurveda . The ayurvedic drugs mainly based on plants, which provides health and help to prevent illness [3]. Plants secondary metabolites are used as medicine. Alkaloids are naturally occurring compounds containing carbon, hydrogen, nitrogen, and usually oxygen and are primarily found in plants, especially in certain flowering plants [4]. At least 3000 years ago alkaloid containing plants have been used by people as medicines, teas [5]. Alkaloids produced by plants, micro- and marine organisms, and fungi. Alkaloid contain an unlimited structural frameworks and N atom in their molecules, so they are highly variable [6]. In Chinese folk medicine plants that contain protoberberine alkaloids used as antiseptics analgesics, sedatives and also used by Indian and Islamic folk medicine for bleeding disorders and eye diseases alkaloids possess many biological and therapeutic effects [7]. The opium, was first crude drug to be investigated chemically and used for its analgesic and narcotic properties [8]. Alkaloids structure is complex their nitrogen atom is part of a heterocyclic system and they possess a significant pharmacological activity [9].

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

The plant material of *Leptadenia pyrotechnica* (shoot and root) was collected from the field of Heerapura (Jaipur). The plant was identified and voucher specimen was deposited to the Herbarium of Department of Botany, University of Rajasthan,Jaipur. The voucher number is (RUBL*No.211572).

2.2 Extraction of Alkaloids

Plants parts of *Leptadenia pyrotechnica* (shoots and roots) were taken in 100 mL Erlenmeyer flasks containing distilled water (50 mL/g) and 5 mL of 0.05 N sulphuric acid was added to it. Mixture was macerated for 3-4 h and boiled gently for 25 minutes. Heavy magnesium oxide (2.5 g/g) was added to the mixture and again boiled gently for 20 minutes. It was cooled at room temperature and an equal amount of distilled water was added to make up for loss of distilled water during boiling. Alcohol was added to remove the mucus. Mixture was filtered throu gh Whatman filter paper-1 (having diameter -12.5 cm) [10]. Filtrate was evaporated to dryness *in vacuum,* reconstituted in distilled water for further analysis.

2.3 Qualitative Analysis

2.3.1 Gas chromatography and mass spectroscopy (GC-MS)

The extract and the standard samples were analyzed by GC-MS of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with using Agilent 7890A/5975C GC HP-5. Capillary column (phenyl methyl siloxane, 25 m×0.25 mm i.d) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 100°C (3 min) to 280°C at 1 to 40°C/min; detector temperature, 250 to 280°C; carrier gas, He (0.9 mL/min). Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions [11]. The components of the standard and plant samples were identified by comparison of their mass spectra and retention time with those given in literature and by comparison with the mass spectra of the Wiley library or with the published mass spectra.

2.3.2 Method for antimicrobial activity

Plant parts of *Leptadenia pyrotechnica* (Shoots and roots) were shade dried and their alkaloid extract were used for evaluate antimicrobial properties.

2.4 Preparation of Extract

The unrefined concentrate was acquired by macerating 30 g of dried plant powder in 95% in separate solvents and kept on a turning shaker for 24 h. The concentrate was separated, centrifuged at 5000 rpm for 15 min. also, was dried under decreased weight. The concentrate was put away at 4ºC in water/air proof containers.

2.5 Culture and Maintenance of Clinical Isolates

Unadulterated cultures of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli*, *Streptomyces griseus, Aspergillus niger, Fusarium oxysporum, Trichoderma reesei* and *Penicillium funiculosum* got from S.M.S. Restorative College, Jaipur, India were utilized

as pointer life forms. Each culture was additionally kept up on a similar medium after each 48 h of transferring. A crisp suspension of test living being in 0.9% saline NaCl arrangement was set up from a newly developed agar incline before each antimicrobial test.

2.6 Determination of Antibacterial Assay

Determination of Antifungal Assay Antibacterial action of the unrefined methanol concentrate was considered against Gram positive and Gram negative bacterial strains by the agar well dispersion method [12]. Mueller Hinton agar no. 2 (Hi Media, India) was utilized as the bacteriological medium. The concentrates were weakened in 100% Dimethylsulphoxide (DMSO) at the convergences of 40 mg/mL. The Mueller Hinton agar was liquefied and cooled to 48 - 50ºC and an institutionalized inoculum (1.5x108 CFU/mL, 0.5 McFarland) was then added aseptically to the liquid agar and filled sterile petri dishes to give a strong plate. Wells were set up in the seeded agar plates. The test compound (40 µL) was presented in the well (4 mm). The plates were brooded medium-term at 37ºC. The antimicrobial range of the concentrate was resolved for the bacterial species regarding zone measures around each well. The widths of zone of restraint delivered by the operator were contrasted and those created by the business control anti-toxins, Ciprofloxacin. For each bacterial strain controls were kept up where unadulterated solvents were utilized rather than the concentrate. The control zones were subtracted from the test zones and the subsequent zone distance across was estimated with anti-toxin zone peruser to closest mm. The trial was performed multiple times to limit the blunder and the mean qualities are exhibited**.**

Against fungi action of the trial plant was researched by agar well dissemination method [13]. The saprophytic fungi was subcultured onto Sabouraud's dextrose agar, SDA (Merck, Germany) and brooded at 25°C for 2 - 5 days. Suspensions of contagious spores were set up in sterile Phosphate Buffer Saline and acclimated to a grouping of 106 cells/mL. Plunging a sterile swab into the contagious suspension and moved on the outside of the agar medium. The plates were dried at room temperature for 15 min. Wells of 4 mm in breadth and around 7 mm separated were punctured in the way of life media utilizing sterile glass tube. 40 µL test sample and 40 µL standard ketoconazole were filled in respective wells, which are labeled. Plates were brooded at 37°C. After hatching of 24 h bioactivities were controlled by estimating the width of restraint zone (in mm). All tests were made in triplicate for exact outcome. The activity index is calculated by:

$$\text{AI - Activity Index} = \frac{\text{Sample Zone}}{\text{Standard Zone}}$$

3. RESULTS AND DISCUSSION

Qualitative analysis is done by GCMS, in alkaloid extract of the shoot of *Leptadenia pyrotechnica* fifty compounds were found á,á Carotene, 6',7'-didehydro-5,6-epoxy-5,5', 6,6',7,8hexahydro3,3', 5'trihydroxy8oxo (3.56%) found to be maximum area observed at the retention time of 28.15 and phosphorodithioic acid, O,S, Strimethyl ester (0.94%) found to be minimum area at retention time of 20.86 **(**Table 1 and Fig. 1).

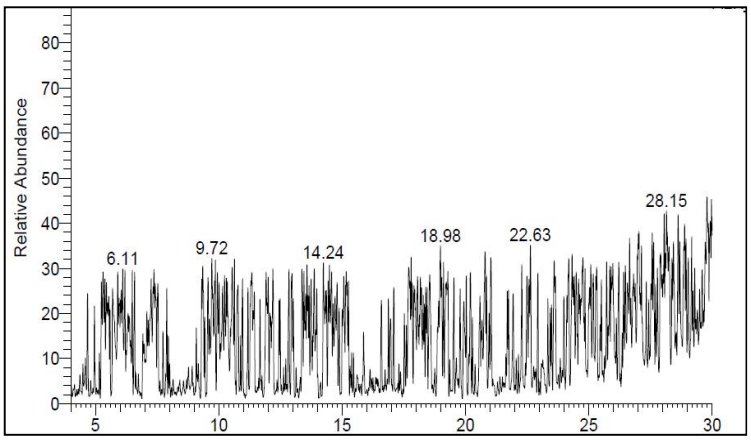

Fig. 1. GC MS analysis of alkaloids from shoots of *Leptadenia pyrotechnica*

Table 1. GC MS analysis of alkaloids from shoots of *Leptadenia pyrotechnica*

S. No.	RT	Compound name	Area	Area %
1	6.20	Cisligustilide	37347	1.25
2	6.49	2Ethylprop1ene (13) sultine	33301	1.11
3	6.59	Benzenamine, N[1(dimethoxymethyl)2-methylpropylidene]	56801	1.89
4	7.89	2,4Dimethylamphetamine	35645	1.19
5	9.31	D:CFriedoB': A'neogammacer9(11)en25oicacid, 2,3,23trihydroxy,methyl ester, (2à,3á,4à)	40635	1.36
6	9.35	1,2Diphenylcyclopropene3carboxylicacid	79747	2.66
7	9.86	4Chloro4'[(1hydrozinocarbonylethyl)oxy]Estilbene	57457	1.92
8	10.63	1,2,4Triazolo[4,3a]pyridin8amine,3methyl	37695	1.26
9	10.78	Benzene, [(2,4dimethylphenoxy)methyl]pentafluoro	85638	2.86
10	10.96	Pyrrolidine,1(5,5dimethyl3methylene1cyclohexen1yl)	48112	1.60
11	11.30	Beryllium,pentakis[æ(acetatoO:O')]æhydroxyæ4oxotetra	57722	1.93
12	11.34	Diethyl1(5ethoxy5oxopentyl)2,4,6trimethyl1,4-dihydro-3,5pyridinedicarboxylate	72645	2.42
13	11.92	Benzyl 3hydroxypropanoate	79369	2.65
14	12.19	Ethyl isocyanide	75916	2.53
15	12.84	2',3',4',5,7,8Hexamethoxyflavone	59878	2.00
16	13.37	3,4Heptadien2one,3cyclopentyl6methyl	66881	2.23
17	13.97	Benzenethiol, 2(phenylamino	43536	1.45
18	14.24	Heptanamide, NhexylN[3(methylamino)propyl	85308	2.85
19	14.43	1Phenyl5methylheptane	35017	1.17
20	14.49	N(2Thiono4oxo1,2,3,4tetrahydroquinazolin3yl)2(4methylphenoxy) acetamide	63199	2.11
21	15.26	1,2Epoxy5,5dimethyl1phenyl3hexyne	29234	0.98
22	17.80	9OMethyl4,5deoxymaytansinol	83901	2.80
23	18.45	3Bromobenzalacetone	41949	1.40
24	18.98	2(4Chlorophenyl)4[(2,3dibromopropyl)thio]quinazoline	83182	2.77
25	19.04	5Cyano6ethylsulfanyl2methyl4phenyl1,4dihydropyr idine3carboxylicacid phenylamide	83182	2.77
26	19.11	Propanoic acid, 3,3'thiobis,dibutyl ester	38274	1.28
27	19.29	[1]Benzothiopyrano[4,3b]benzo[e]indole	73610	2.46
28	19.53	1,3Dithiane4,6dione	46051	1.54
29	20.04	5Phenylthiazolidine	34686	1.16
30	20.21	4Chloro3(N(4methoxyphenyl)iminomethyl)(2H)benzothiapyran	68852	2.30
31	20.86	Phosphorodithioic acid, O,S,Strimethylester	28188	0.94
32	21.03	Benzene, 1fluoro4(2methoxyethenyl)	71362	2.38
33	22.28	álArabinopyranoside,methyl	44292	1.48
34	22.63	Spiro[cyclopentane1,3'[3H]indole], 2'methyl	62159	2.07
35	22.93	Tetrazolo[1,5b]pyridazine, 8methyl	47957	1.60
36	23.60	Ethanone, 1(3pyridinyl),oxime	89635	2.99
37	24.19	Octadecanoic acid, 6chloro7trimethylsilyloxy,methylester	85652	2.86
38	24.32	2H1Benzopyran,3,4dihydro6,7dimethoxy3(2,3,4,5-tetramethoxyphenyl	79462	2.65
39	24.85	Azuleno[5,6c]furan1(3H)one,4,4a,5,6,7,9hexahydro3,4-dihydroxy6,6,8trimethyl	29452	0.98
40	26.22	Dimethyl1(2,4dimethylphenyl)naphthalene-2,3-dicarboxylate	62609	2.09
41	27.57	9,9Diphenyl2methyl9sila9,10dihydro3azaantracen10one	51959	1.73
42	27.63	Benzene, (1nitropropyl	59848	2.00
43	28.15	á,áCarotene,6',7'didehydro-5,6-epoxy5,5',6,6',7,8-hexahydro3,3',5'trihydroxy8oxo	106745	3.56
44	28.43	Difluoro(bicyclo[2.2.1]hept2en7yl)aminocarbonylacetic acid, methyl ester	62830	2.10
45	28.62	Ethanol, 2(phenylsulfonyl	79338	2.65
46	28.88	2,6-Diazaspiro(4,4)nonane3,7dione	98522	3.29
47	28.95	(3E,5Z)3,5Undecadien1yne	42509	1.42
48	29.17	Dimethyl1(2,4dimethylphenyl)naphthalene-2,3-dicarboxylate	43767	1.46
49	29.79	Ethanol, 2(phenylsulfonyl	84770	2.83
50	29.97	3-Methyl7phenylhepta1,3,4triene	68260	2.28

Table 2. Antibacterial activity of alkaloids isolated from *Leptadenia pyrotechnica* against various bacterial strains

S. No.	Name of bacterial strain	Shoot	Root	Standard As ciprofloxacin
		(zone in mm)		
1	*Escherichia coli*	6 AI-0.27	nil	22
2	*Staphylococcus aureus*	12 AI-0.6	15 AI-0.75	20
3	*Streptomyces grisveus*	nil	nil	24
4	*Bacillus subtilis*	12 AI-0.46	nil	26

Table 3. Antifungal activity of alkaloids isolated from *Leptadenia pyrotechnica* against various fungal strains

S. No.	Name of fungal strain	Shoot	Root	Standard as ketoconazole
		(zone in mm)		
1	*Trichoderma reesei*	nil	10 AI-0. 5	20
2	*Aspergillus niger*	nil	nil	24
3	*Penicillium funiculosum*	17 AI-0.77	nil	22
4	*Fusarium oxysporum*	15 AI-0.57	10 AI-0.38	26

3.1 Antimicrobial Activity of Alkaloids

Alkaloid extract of both sample (shoot and root) were analyzed for antimicrobial assay by well diffusion method. Maximum zone of inhibition was observed in roots (15 mm) against *S. aureus*. Minimum zone of inhibition observed by shoot (6mm) against *Escherichia coli* and root extract showed no activity in *Escherichia coli.* while no activity was observed against *S. grisveus* (Table 2). Antifungal activity was found against *F. oxysporum* in both plant parts but a maximum zone of inhibition was observed in shoots (17 mm) against *P. funiculosum* hereas roots not show any activity against *P. funiculosum* .Test sample not show any zone of inhibition against *A. niger* (Table 3).

4. CONCLUSION

At present the enthusiasm of individuals in ayurveda is expanded. Leptadenia pyrotechnica indicated noteworthy potential for pharmacological properties and bioactive mixes (alkaloids).These capacity is useful to treat different maladies, the finding of this examination will further grow for progressively remedial capability of Leptadenia pyrotechnica and it will give a significant help for its future clinical use in current drug.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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