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ANTI-INFLAMMATORY ACTIVITY AND METABOLITE PROFILING OF ROOT ENDOPHYTIC *LASIODIPLODIA PSEUDOTHEOBROMAE* ASSOCIATED WITH *ICHNOCARPUS FRUTESCENS*

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ABSTRACT

The roots of *Ichnocarpus frutescens* of the family Apocyanaceae are known to have several secondary metabolites with antioxidant, anti-inflammatory and anti-cancer activities. During the present study, *Lasiodiplodia pseudotheobromae* was isolated from the roots of the plant and was identified based on colony morphology, conidial characteristics and ITS1- ITS4 sequences of ribosomal DNA. The ethyl acetate fraction of the endophyte exhibited promising levels of phenolics. The metabolites were analysed by High Resolution Liquid Chromatograph Mass Spectrometer (HR-LCMS). Timnodonic acid, derivatives of indomethacin, matricin, hydroxy cinnamic acid, citraconic acids, furans were predominantly observed. Both the crude and partially purified fractions of endophyte extracts have been found to inhibit lipoxygenase with IC_{50} values 48 µg and 55µg respectively. The study reported the presence of indomethacin from the root endophyte for the first time as confirmed by ^{13}C and ^{1}H NMR analysis. Thus, the fungus can be further exploited as a potent candidate to be used against inflammation and serve as an alternative to plant-derived bioactive compounds.

Keywords: *Ichnocarpus frutescens; Lasiodiplodia pseudotheobromae*; HR-LCMS; NMR; anti-inflammatory

1.INTRODUCTION

Plant derived natural products are the rich sources of lead molecules and represent a huge chemical diversity. They contain biologically active groups that play a decisive role in synthesis of novel therapeutic molecules **[1]**. But, large -scale exploitation of medicinal plants for drug development led to gradual extinction. Thus, endophytic fungi which are promising as prodigious, untapped and partially explored sources of bioactive molecules are exploited. Endophytes inhabiting specialised ecological niches like medicinal plants are the producers of diverse secondary metabolites, which are produced during biotic or abiotic stress to the plant, similar to its host **[2, 3]**. Secondary metabolites like alkaloids, flavonoids, quinines, steroids and several others are produced by plant-endophyte association. Thus, bioprospecting of fungal endophytes associated with medicinal plants propels the development and use of novel compounds to be exploited in various fields of biology. High Resolution Liquid chromatography coupled to mass spectrometry (HR-LCMS) is a robust tool used for the analysis of potential, natural novel compounds of plant or the endophytic extracts **[4]**. Further, Nuclear magnetic resonance (NMR) is a popular tool often used in natural product research and is effective in elucidating the structures associated with small molecules present in a tangled mixture. There is a great need for the development of anti-inflammatory compounds from natural sources as several of the existing non-steroidal anti-inflammatory drugs (NSAIDs) have adverse effect on health. Inflammation is a defence mechanism triggered by enzymes like Lipoxygenases (LOXs) and Cyclooxygenase-2 (COX-2) catalyse the biosynthesis of leukotrienes (LTs). Inhibiting LOX and COX-2 reduce the formation of leukotrienes. Phomol from *Phomopsis* sp., an endophyte of *Erythrina crista-galli* was the first anti-inflammatory metabolite reported **[5]**.

Ichnocarpus frutescens (L.) is a woody creeper belonging to the family Apocynaceae. The leaves of the plant are used to treat jaundice and diabetes. This plant is attributed majorly with anticancerous and anti-inflammatory properties **[6, 7]**. The roots are rich in phenolic acids, flavonoids, glycosides and triterpenoids. The present study aimed at isolation of root endophytes of *I. frutescens,* screening them for the production of secondary metabolites having anti-inflammatory potential.

2.MATERIALS AND METHODS

2.1. IDENTIFICATION, COLLECTION OF PLANT SAMPLE AND ISOLATION OF ROOT ENDOPHYTES FROM *ICHNOCARPUS FRUTESCENS*

The plant sample was collected from Chamundi Hill, Mysuru, Karnataka, India. The identity of the plant was confirmed by a taxonomist. The samples were stored in plastic bag, processed within 24-48h of collection. Isolation of endophytes from *I. frutescens* was done according to **[8]**. The isolated endophytic fungi were preserved as glycerol stocks and stored at -80^{0}C.

2.2 GENOMIC DNA EXTRACTION AND PCR

The identification of the endophyte was done primarily based on their colony characters and spore morphology using standard identification manuals **[9]**. *L. pseudotheobromae* was identified as the predominant endophyte colonizing *I. frutescens*. DNA was extracted from pure culture of *L. pseudotheobromae* grown on PDA medium by CTAB (cetyl trimethyl ammonium bromide) method **[10]**. The Extracted DNA was amplified using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') universal primers for fungi **[26]**. The sample was sent for sequencing, analysed and compared using BLAST tool available in NCBI and sequences have been deposited at the GenBank.

2.3 EXTRACTION OF SECONDARY METABOLITES FROM THE *L. PSEUDOTHEOBROMAE*

A piece of mycelial mat (~0.5-1cm^2) from actively growing fungal colony was aseptically inoculated to sterile potato dextrose broth and incubated in static conditions at 25±2^{0}C with alternate light(8h) and dark cycles (16h) for 28days. This was homogenised and filtered. The filtrate was extracted by two-phase solvent extraction with solvents in the order of their increasing polarities (hexane>ethyl acetate> chloroform > methanol), which was dried in a flash evaporator and concentrated in a vacuum concentrator. The extracts were evaluated for various bioactivities and scale up of secondary metabolites.

2.4 METABOLITE PROFILING AND ANALYSIS OF CRUDE EXTRACTS BY HIGH RESOLUTION LIQUID CHROMATOGRAPH MASS SPECTROMETRY (HR-LCMS) Ethyl acetate extract was analysed by HR-LCMS (Agilent Technologies, USA, 1290) at Indian Institute of Technology-Sophisticated Analytical Instrumentation Facility (IIT-SAIF), Bombay. Ultra-high-performance liquid chromatographic-photodiode array (UHPLC-PDA) system was used as the detector. The patterns of the compounds were correlated with National Institute of Standards and Technology Mass Spectral database (NIST-MS).

3.CHARACTERIZATION

3.1 EXTRACTION AND PURIFICATION OF COMPOUNDS BY COLUMN CHROMATOGRAPHY

The endophyte extract was partially purified by silica gel column chromatography (60-200 mesh size). The column was pre-equilibrated with ethyl acetate overnight. About 5g of the crude material was dissolved in 20mL of ethyl acetate and was coated to silica. The material was packed and the fractions were gradient eluted using a combinatorial series of hexane and ethyl acetate (100% hexane, 9:1, 8:2, 7:3, 6:4, 1:1 and 3:7 and 100% ethyl acetate with a flow rate of 1 ml/min; the fractions were collected and active fractions were analysed by thin layer chromatography

3.2 LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS) AND NUCLEAR MAGNETIC RESONANCE (NMR) ANALYSIS OF PARTIALLY PURIFIED FRACTION OF *L. PSEUDOTHEOBROMAE*

Partially purified extract of *L. pseudotheobromae* was analysed for LC-MS by electrospray ionization (ESI), and NMR for determining the structure of the compound. ^{1}H and ^{13}C spectra of NMR were obtained on Agilent Technologies (USA) (400mHz instrument) using DMSO-d6 (deuterated dimethyl sulfoxide) and chloroform (deutereted) as solvents. Trimethyl silane (TMS) was used as internal standard. Mass spectrometric analysis was done in Bruker MicroTOF QII mass spectrometer in positive mode with a relaxation delay of 1s.

4.BIOACTIVITY OF CRUDE AND PARTIALLY PURIFIED FRACTIONS OF *L. PSEUDOTHEOBROMAE*

4.1 DETERMINATION OF TOTAL PHENOLICS FROM THE CRUDE AND PARTIALLY PURIFIED ENDOPHYTE EXTRACTS

Different solvent fractions of crude endophyte extracts (Hexane, ethyl acetate and methanol) and partially purified fractions were analysed for total phenolics by Folin-Ciocalteu method **[11]**. The values were expressed as Gallic acid equivalents per milligram of the sample.

4.2 DETERMINATION OF ANTI-INFLAMMATORY ACTIVITIES OF ENDOPHYTE EXTRACTS

The anti-inflammatory activity of the extracts was determined by inhibition of protein denaturation and 15- lipoxygenase inhibition assay.

4.2.1 COLLECTION OF BLOOD SAMPLES AND PREPARATION OF PACKED RED BLOOD CELLS (PRBCS)

Fresh blood samples were collected from a slaughter house in Mysore (Karnataka, India) and was citrate stabilized. To this, 20mL of Phosphate buffered saline (pH 7.0) was added, centrifuged at 10000rpm for 10min at $4^{0}C$. The collected RBCs were again washed twice with PBS. After the last wash, the Packed RBCs (PRBCs) were diluted to a 10% (v/v)

solution with PBS, stored at 4^0C for further use.

4.2.2 EFFECT OF ETHYL ACETATE EXTRACTS OF *L. PSEUDOTHEOBROMAE* ON STRESS INDUCED HAEMOLYSIS

The reaction mixture involved 0.5mL of RBCs, 5mL of hypotonic solution containing 50 mM sodium chloride dissolved in 10 mM phosphate buffered saline at pH 7.4. Both the crude and partially purified extract of *L. pseudotheobromae* at concentrations ranging from 25-100µg/mL was added to the reaction mixture, incubated for 10min at room temperature and centrifuged at 3000 rpm for 10min at 4^0C. The supernatant was collected and absorbance was measured at 540nm.

The percentage of haemolysis inhibition was calculated as follows:

$$\% \text{ inhibition of haemolysis} = \frac{A_1 - A_2}{A_2} \times 100$$

A_1= Absorbance of the control; A_2= Absorbance of the test

4.2.3 INHIBITION OF ALBUMIN DENATURATION

Both the crude and partially purified extracts of various concentrations (2-10mg/mL) were prepared, mixed with bovine serum albumin (BSA) and incubated at 37^0C for 20min. The mixture was heated at 51^0C for 20min, cooled and the turbidity of the sample was measured at 660nm in a spectrophotometer and the inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = \frac{A_C - A_T}{A_C} \times 100$$

Where A_C= Absorbance of the control; A_T= Absorbance of the test

4.2.4 INHIBITION OF PROTEINASE

The activity was tested according to the method described by **[12]**. The absorbance of the supernatant was recorded at 280nm. The tube containing the buffer was taken as blank. The experiment was done in triplicates and the percentage of protein denaturation was calculated.

4.2.5 15-LOX INHIBITION ASSAY

Anti-lipoxygenase activity of ethyl acetate fractions of endophyte extracts was performed according to the method described by **[13]**. The experiment was performed in duplicates. A 50% inhibitory concentration of LOX was determined and the percent inhibition was calculated as follows:

% Inhibition of lipoxygenase = [(difference in the absorbance without extract – difference in the absorbance with extract)/ difference in the absorbance without extract] X 100

5. RESULTS

5.1 ISOLATION OF ROOT ENDOPHYTES FROM *ICHNOCARPUS FRUTESCENS*

The habit characteristic of the medicinal plant *I. frutescens* is shown in **Figure 1**. The isolated endophytes were analysed for colonization frequency, dominance and density of colonization in different parts of the plant. The results are shown in **Table 1**.

During the present study, the endophytic fungi *Lasiodiplodia pseudotheobromae* was a dominant colonizer in the root parts of *I. frutescens* with rD% 88.37. The percentage colonization and relative dominance was high when compared to other endophytes isolated from stem and leaf parts of the plant. Other fungal endophytes like *Colletotrichum gleosporioides*, *Fusarium* sp. and *Lecanicillium aphanocladii* were isolated from stem and leaf parts of the plant. The colony and spore morphology of the *L. pseudotheobromae* is shown in **Figure 2a, Figure 2b** respectively**.**

The isolated root endophyte was authenticated by molecular tools. The sequence of PCR amplified regions of ribosomal DNA of the fungus was compared with the reference sequence (KY569616.1) and the accession number was obtained for *L. pseudotheobromae* (MH015227).

5.2 CHARACTERIZATION OF SECONDARY METABOLITES BY HIGH RESOLUTION- LIQUID CHROMATOGRAPHY MASS SPECTROMETRY (HR- LCMS)

Chromatographic analysis of dried, ethyl acetate fractions of *L. pseudotheobromae* (Lp-010) unveiled the compounds which belonged to the phenolics and the fatty acid derivatives. High levels of phenolics or its derived compounds reveal the efficacy of extracts as promising sources of antioxidants and anti-inflammatory compounds. The HR-LCMS chromatogram obtained from the crude ethyl acetate extracts of *L. pseudotheobromae* is shown in the **Figure 3**. Analyses of compounds were done based on their chemical nature and reported biological activities.

Out of 50 compounds, the peaks of 13 major compounds were numbered according to their respective retention times. Occurrence of Desmethyl deschlorobenzoyl Indomethacin is reported for the first time from the endophyte extracts of *L. pseudotheobromae*, indicating the efficacy of this endophyte extract as promising anti-inflammatory agent. Major classes of compound synthesised from the root endophyte extracts of *L. pseudotheobromae* is shown in **Table 2**.

Figure 1: Habit characteristics of *Ichnocarpus frutescens*

Table 1: Percentage colonization and dominance of endophytic fungi from different tissues of *I. frutescens*

Taxa	Total isolates of each fungus	%CF	Dominance of fungi	rD%		
				Root	Stem	Leaves
L. pseudotheobromae	38	21.11	43.18	88.37	-	-
C. gleosporioides	30	16.66	34.09	-	6.97	-
Fusarium sp.	10	5.55	11.36	-	2.32	-
F. solani	05	2.77	5.68	-	1.16	-
L. aphanocladii	05	2.77	5.68	-	-	1.16
TOTAL	88	48.86	99.99	88.37	10.45	1.16

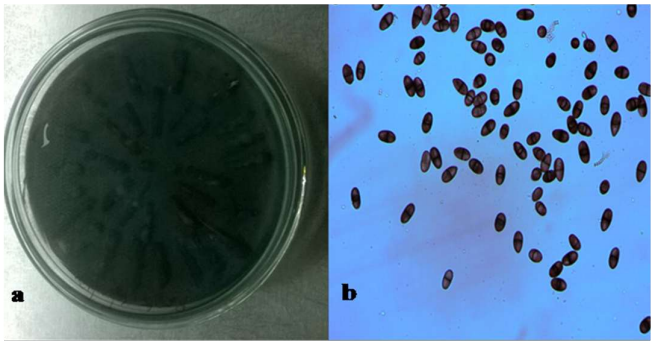
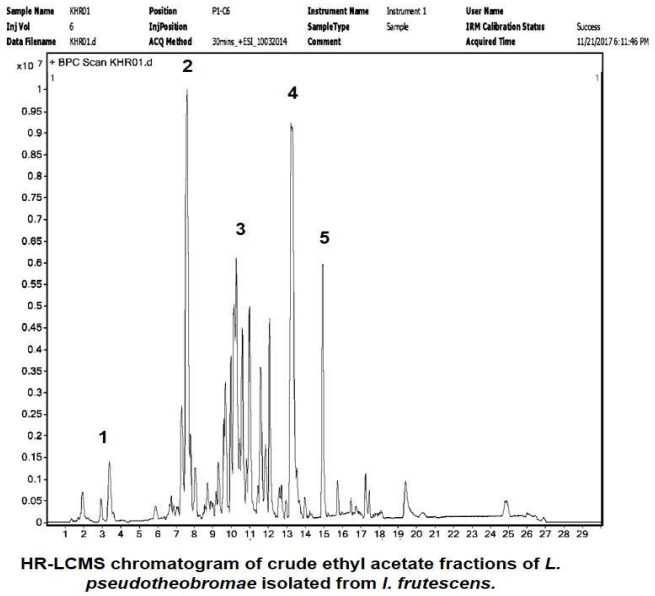


Figure 2: (a) PDA plate showing the emergence of *L. pseudotheobromae* from the surface sterilized root bits of *I. frutescens* (b) conidial characteristics of *L. pseudotheobromae*



HR-LCMS chromatogram of crude ethyl acetate fractions of *L. pseudotheobromae* isolated from *I. frutescens*.

Major metabolites identified

Peak no. 1
- Desmethyl deschlorobenzoyl indomethacin

Peak no. 2
- Timnodonic acid
- Citraconic acid dimethyl ester
- 2,4,6,8-decatetraenal

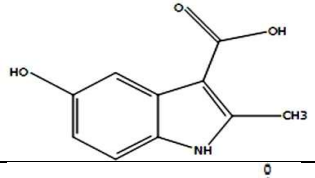
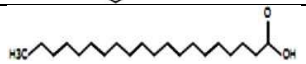
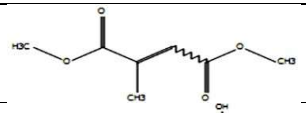
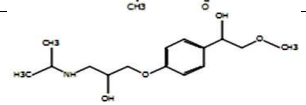
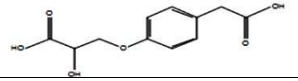
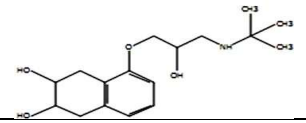
Peak no. 3
- alpha-Hydroxymetoprolol
- 5-tert-Butyl-4-hydroxymethylfuran-2 carboxylic acid
- Dihydrosphingosine
- 2-Hydroxy-3-(4methoxyethylphenoxy)-propanoic acid
- Methyl 7-Desoxypurpurogallin-7-Carboxylate trimethyl Ether
- Cetylpyridinium

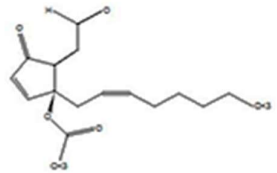
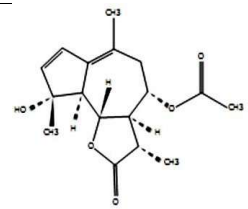
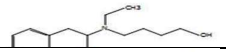
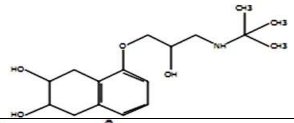
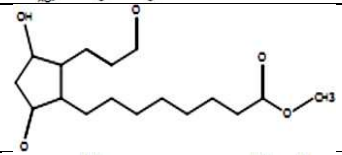
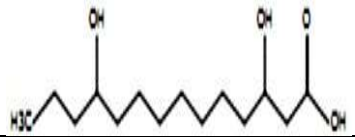
Peak no. 4
- Nadolol
- methyl 8-[2-(2-formyl-vinyl)3-hydroxy-5-oxo-cyclopentyl]octanoate

Peak no.5
- 3,11-dihydroxy myristoic acid

Figure 3: HR-LCMS chromatogram of crude ethyl acetate fractions of *L. pseudotheobromae* showing major peaks, corresponding to major metabolites

Table 2: HR-LCMS based metabolite characterization of ethyl acetate fractions of *L. pseudotheobromae* and the reported biological activities

Rt	Mass	m/z ratio	Name of the compound	Structure	Molecular formula	Biological activities	References
3.401	191.05	174.05	Desmethyl deschlorobenzoyl Indomethacin		C_{10} H_9 N O_3	Anti-inflammatory responses, an inhibitor of prostaglandin synthetase	[14]
7.304	302.22	307.20	Timnodonic acid		C_{20} H_{30} O_2	Inhibitor of prostaglandin biosynthesis	[15]
7.595	158.05	141.05	Citraconic acid dimethyl ester		C_7 H_{10} O_4	The derivatives of the compound used as anticancer agents	[16]
10.164	283.17	284.18	alpha-Hydroxymetoprolol		C_{15} H_{25} N O_4	Anti- hypertensive activity and a cardio selective beta blocker	[17]
10.447	240.09	223.09	2-Hydroxy-3-(4-methoxyethylphenoxy)-propanoic acid		C_{12} H_{16} O_5	Cell proliferation and growth inhibitor	[18]
10.458	309.19	310.20	Nadolol		C_{17} H_{27} N O_4	Regulation of glucose metabolism	[19]

11.578	**290.15**	**291.1587**	**Clavirin I**		**$C_{17}H_{22}O_4$**	**Antimicrobial activity**	**[20]**
12.056	**306.14**	**289.14**	**Matricin**		**$C_{17}H_{22}O_5$**	**Anti-inflammatory activity**	**[21]**
13.265	**265.20**	**266.21**	**Mebeverine metabolite**		**$C_{16}H_{27}NO_2$**	**An antimicrobial agent.**	**[22]**
13.316	**309.19**	**310.20**	**Nadolol**		**$C_{17}H_{27}NO_4$**	**Used to treat high blood pressure**	**[23]**
14.930	**310.17**	**293.17**	**Methyl 8-[2-(2-formyl- vinyl)3-hydroxy-5-oxo- cyclopentyl]octanoate**		**$C_{17}H_{26}O_5$**	**A metabolite observed during leukemic conditions**	**[24]**
15.742	**260.20**	**265.17**	**3,11-dihydroxy myristoic acid**		**$C_{14}H_{28}O_4$**	**Used as flavor active lactones**	**[25]**

5.3STRUCTURAL CHARACTERIZATION OF BIOACTIVE COMPOUND FROM *L. PSEUDOTHEOBROMAE*.

Partially purified extract of *L. pseudotheobromae* obtained as white, amorphous powder was analysed for mass by LC-MS. The extract showed an intense base peak at 341.0793 m/z and produced a protonated molecule [M+H]+ at m/z of 356.0921 as shown in **Figure 4** corresponding to the mass of 356.029.

5.4 NMR ANALYSIS

The ^{1}H NMR and ^{13}C NMR spectra indicated the presence of functional groups like -CH_3, and –COOH in the extract. The results are as shown in the **Figure 5**. ^{13}C spectrum of the extract showed the presence of fifteen carbon signals and exhibited ten signals for aromatic ring (100.6, 112.20, 113.40, 114.5, 128.70, 129.30, 131.10, 135.10, 137.60 and 140.0ppm). Signals at 13.20 and 29.60ppm indicate the presence of methyl groups and signal at 174.20ppm indicate carboxyl groups, while, ^{1}H NMR spectra revealed the presence of four singlets from 12.15 to 2.14ppm. Based on this, the structure of the compound was proposed as shown in **Figure 6**.

5.5 DETERMINATION OF TOTAL PHENOLICS FROM CRUDE AND PARTIALLY PURIFIED EXTRACTS OF *L. PSEUDOTHEOBROMAE* (LP-010)

The phenolic content for the crude extract was found to be higher than partially purified extracts. The total phenolic content in the crude extract was found to be 71.12±0.50mg GAE/mg of the extract, while in the partially purified extract, it was found to be 45±0.50 GAE/mg of the extract. The correlation coefficient was found to be R^2=0.9261.

5.6 ANTI-INFLAMMATORY ACTIVITY OF ETHYL ACETATE EXTRACTS OF *L. PSEUDOTHEOBROMAE*

Both the crude and partially purified extracts of *L. pseudotheobromae* showed dose-dependent inhibition of the enzyme. Higher inhibition was exhibited by crude extracts with the percentage inhibition 32.18±0.47% corresponding to the concentration of 25 µg/mL and IC_{50} value of 48 µg, while, the percentage inhibition and IC_{50} value of partially purified fraction was found to be 25 and 55 µg respectively. The IC_{50} value of standard *i.e.* Quercetin was 40 µg with higher inhibition percentage of 56.60. Crude extracts of *L. pseudotheobromae* showed the highest trypsinase inhibitory activity and prevented albumin denaturation corresponding to 56.78±0.76% and 51.75±0.1%. (**Table 3**).

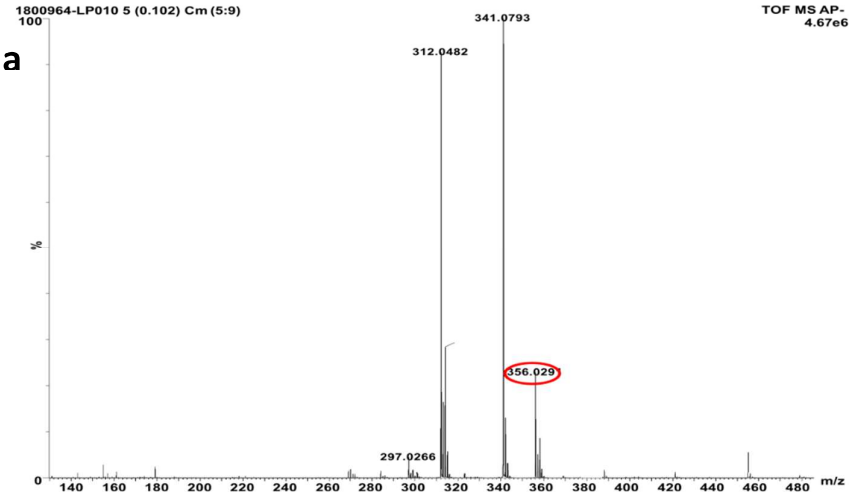
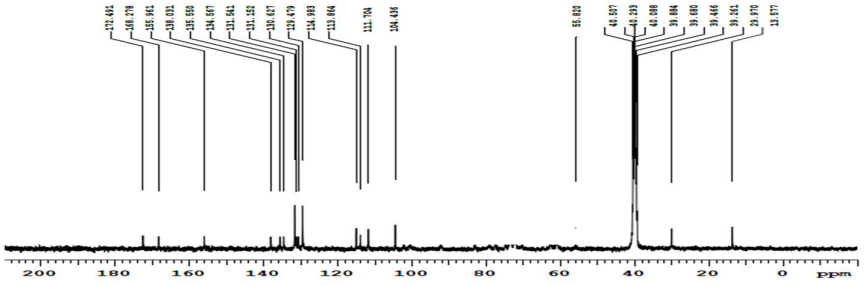
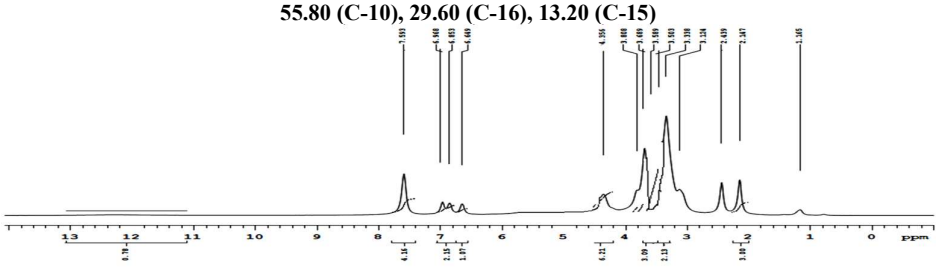


Figure 4: MS spectra of partially purified, ethyl acetate extracts of *L. pseudotheobromae*

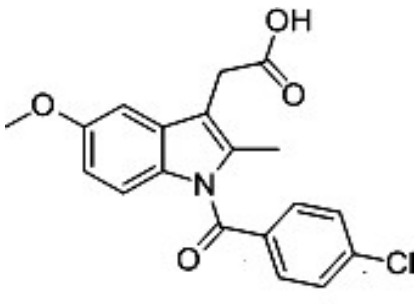


^{13}C NMR DMSO-d6, 100MHz: 174.20 (C=O, -17), 167.70 (C=O, -5), 154.00 (C-9), 140.0 (C-1), 137.60 (C-12), 135.10 (C-14), 131.30 (C-3), 131.10 (C-4), 129.30 (C-2), 128.70 (C-6), 114.5 (C-13), 113.40 (C-7), 112.20 (C-8), 100.6 (C-11), 55.80 (C-10), 29.60 (C-16), 13.20 (C-15)



NMR, DMSO-d6, 400MHz: 12.15 (1H, S, -COOH), 7.59-6.64 (7H, M, Ar-H), 3.80-3.68 (3H, S,-OCH_3), 3.58-3.50 (2H, S, -CH_2), 2.14 (3H, S, -CH_3)

Figure 5: Structural characterization of bioactive compound from the extracts of *L. pseudotheobromae* by ^{13}C and ^{1}H NMR



Chemical formula: $C_{19}HN_{16}ClO_4$

Molecular weight: 357.79

Systematic name: 2-[1-(4-chlorobenzoyl)-5- methoxy-2-methylindol-3-yl] acetic acid

Common name: Indomethacin

Figure 6: Proposed structure of the bioactive compound from *L. pseudotheobromae*

Table 3: Total phenolics and 15-Lox inhibition assay of crude and partially purified fractions of *L. pseudotheobromae.*

Ethyl acetate extracts of root endophyte	Conc. of the extracts/Standards (µg/mL)	Total phenolics		15-lox inhibition assay		Inhibition of albumin denaturation (%)		Proteinase inhibition assay* (%)		Stress induced haemolysis * (%)	
		Crude fraction	Partially purified fraction	Crude fraction	Partially purified fraction	Crude fraction	Partially purified fraction	Crude fraction	Partially purified fraction	Crude fraction	Partially purified fraction
Lp-010	25	71.12±0.50	45±0.50	32.18±0.47 (48)*	25±0.50 (55)*	49.58±0.28	35.60±0.45	54.00±0.57	48.60±0.55	75.18±1.42	61.15±0.68
	50	71.56±0.90	45.12±0.50	33.41±0.50 (50)*	25.50±0.69 (57)*	51.59±0.76	36.65±0.88	55.20±1.04	49.50±0.65	76.03±1.53	62.26±0.69
	100	71.90±0.60	45.15±0.50	33.56±0.78 (51)*	26±0.65 (58)*	51.75±0.14	38±0.83	56.78±0.76	50.05±0.73	78.98±0.05	64.58±0.83
Quercetin* /Aspirin*	25	-	-	56.60±0.35 (40)*		58.98±0.29		62.15±0.49		85.12±0.54	
	50	-	-	56.62±0.32 (42)*		59.32±0.35		63.02±0.05		86.23±0.38	
	100	-	-	56.69±0.34 (43)*		59.78±0.12		63.55±0.25		86.45±0.34	

***Total phenolic content was expressed as Gallic acid equivalents/mg of the extract and the results are expressed as mean±SD**
Figures inside the paranthesis represent the IC_{50} values *Quercetin- positive control for LOX inhibition assay and Aspirin- positive control for proteinase inhibitory assay

6. DISCUSSIONS

L. pseudotheobromae was isolated as the dominant endophyte from the roots of *I. frutescens*. This work is the first report on the presence of indomethacin producing endophytic *L. pseudotheobromae* from the root parts of *Ichnocarpus frutescens*. Endophytic *L. pseudotheobromae* was also known to produce p- hydroxyl benzoic acid, mullein and hydroxymellein which are involved in the pathogenesis caused by the fungus **[26]**. The genus *Lasiodiplodia* is known to produce compounds like lasiojasmonates A, B and C, which are effective in controlling pathogens. **[27]** reported that the metabolites from *L. pseudotheobromae* can be exploited as potent biocontrol agents against anthracnose disease in mango tree, caused by *Colletotrichum gleosporioides*.

L. pseudotheobromae existed as an endophyte of *Aegle marmelos*, entrusted with potent fibrinolytic activity **[28]**. The culture filtrate of *L. pseudotheobromae* exhibited inhibition of enzymes involved in anti-inflammatory mechanism as reported in the present study, which is similar to that of its host, *I. Frutescens* **[29]**. Thus, chemical profiling of the plant and its endophytes provide a valuable insight to consortia of metabolites produced by them, which could be the future candidates for the synthesis of lead molecules or backbones of novel therapeutic molecules. Both the crude and column fraction of the endophytic extract exhibited promising levels of anti-inflammatory activities, comparable with the standard. [30] reported the inhibition of LOX by endophytes like *Aspergillus fumigatus* and *Fusarium* spp. isolated from *Garcinia* species. The endophyte is also known to synthesise volatile organic compounds (VOCs) like sesquiterpenes which have anti-inflammatory, anti-bacterial, anti-irritant and anti-allergic activities **[31]**. Species of *Lasiodiplodia*, isolated from mangrove trees are known to produce Dichloro isocoumarins which can be used as potent anti-inflammatory agents **[32]**.

7. CONCLUSION

Fungal endophytes are the reservoirs of bioactive compounds. The HR-LCMS based metabolite characterisation of dried, ethyl acetate fractions of *L. pseudotheobromae* facilitated identification of compounds which are earlier reported to have various bioactivities as that of the host. The crude and partially purified fraction exhibited promising levels of phenolics which conveys that the extracts are the potent candidates to be used as antioxidant and anti-inflammatory agents. The extract also showed a good inhibition of lipoxygenase as confirmed by *in vitro* studies. Presence of indomethacin is reported from the endophyte of *I. frutescens* for the first time during this study. Thus, it

can be affirmed that the root endophyte *L. pseudotheobromae* isolated from *I. frutescens* could be a promising source of several therapeutic molecules.

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