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Identification of Phenolic compounds from degraded *Prosopis juliflora* by *Oscillatoria laetevirens*

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ABSTRACT: Large amount of lignocellulosic waste are generated through forestry and agricultural practices, which pose environmental pollution and can be converted into various different 'value added' products. Bioconversion of lignocellulosic waste by marine cyanobacteria could make a significant contribution to the production of organic chemicals. Cyanobacteria which are phototrophs and show high flexibility to varied environment because of their trophic independence to carbon and even nitrogen in a number of cases and play potential role in biodegradation. Thus biodegradation of *P. juliflora* using marine cyanobacterium *O. laetevirens*, result in release of phenolic compounds which were detected using phenol estimation followed by spectral analysis, TLC, HPLC,GC-MS and FTIR. The results clearly showed the presence of catechin like compound without any noticeable impurities in the extract of degraded *P. juliflora* when compared with control *P. juliflora* alone.

Keywords: *Prosopis juliflora*; *Oscillatoria laetevirens*; Biodegradation; Phenols;

1. INTRODUCTION

Biomass can be considered as the mass of organic material from any biological source and by extension any large mass of biological matter. A wide variety of biomass resources are available on our planet for conversion into bioproducts. The clear understanding of these material composition and sources could help in design of new biomaterials and also accounts for the production of desired bioproducts. Biosynthesis of the numerous organic compounds including complex secondary metabolites like sterols, alkaloids, pigments, phenols, oils, volatile compounds, hormones, rubber, wax etc is an important aspect of plant life. This present work emphasizes the presence of phenolic compounds in degraded *P. juliflora* [1]. The free radical scavenging abilities and anti-oxidant activities of the plant phenolic compounds has increased its demand in the healthcare industries. In this line, flavonoids are widely distributed and most common among plant phenolic compounds as aglycones and glycoside derivatives. Also, flavones and flavnols and glycosides are the most common compounds of higher plants with vital importance to mankind [2]. Many plant species have become weed by being transferred by human action to locations where they compete with other plants for space. *Prosopis juliflora,* native of West Indies, Central America and Northern South America. In India it is an invader species that compete with native species and becoming an aggressive weed in several states due to its invasiveness and subsequent ecological, economic and social impacts. Invasion of irrigation channels and arable land has affected the agricultural community [3].

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Competing interests
The authors have declared that no competing interests exist.

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2. MATERIALS AND METHODS

2.1 *Prosopis juliflora* Degradation

Prosopis juliflora and Oscillatoria laetevirens were taken in 0.1: 0.3 (wet:dry) ratio in ASNIII medium [4]. The experimental set up was incubated at 25±2° C under white fluorescent light of 1500 lux with 10/12hr light/dark cycle for 30 days. After incubation the culture was filtered to separate the pellet and supernatant. Thus collected pellet was dried, powdered and subject to biochemical analysis.

2.2 Preparation of extract

The degraded *P. juliflora* by *O. laetevirens* was ground fine powder, passed through a 115-mesh sieve and dried at 60 ºC before extraction. Soxhlet extraction was done using ethanol in which 10 g of sample powder was extracted with 180 mL of the solvent for 15 hours at a rate of 10 to 12 cycles per hour. After extraction, the solvent was

evaporated under reduced pressure in a Buchi rotavapor and the crude extract dried under vacuum in a desiccator over P_2O_5.The mass of the remaining extract was measured and used for compound identification [5].

2.3 Analytical methods

Spectral analysis was measured using a Jasco UV- 550 spectrophotometer (Japan) in wavelength ranges from 200-800nm. Phenolic compounds were separated using Thin Layer Chromatography (TLC) (Touchstone, 1992). High Performance Liquid Chromatography (HPLC) was recorded in HPLC instrument (UFLC), Fourier Transform Infrared Spectroscopy (FTIR) was recorded in Perkin Elmer Spectrum one FTIR and Gas Chromatography Mass Spectroscopy (GC-MS) was recorded in Jeol Gcmate II GC-MS [5].

3. RESULTS AND DISCUSSION

3.1 Spectrum analysis

The UV-visible absorption spectra and retension time of phenolic compound enable its identification using chromatographic peaks. The phenolic molecules can absorb light by their functional elements and the benzene ring system found in simple phenols absorbs in 260-280nm region [6]. The spectrum of ethanolic extract of different days of degraded *P. juliflora* extract confirmed absorption bands at 270nm and the peak indicates that the compound present in the extract was of aromatic in nature (Figure 1).

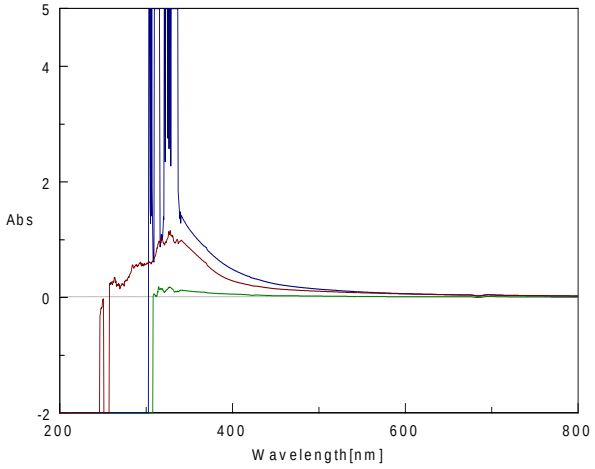

Fig.1: UV Spectral Analysis of degraded *P. juliflora* extract during 30th day

According to literature all the flavanols present are quite similar in UV absorption pattern, but appear at distinct retention times [5]**.** Similar result was reported in which degradation of coir pith by *Oscillatoria annae* shows an appreciable amount of phenolic compounds released into the surrounding medium which was determined by spectral analysis [7]. From this it is evident that *O. laetevirens* degrades degrades *P. juliflora* and releases phenolic compounds and other metabolites into the media.

3.2 Thin Layer Chromatography (TLC)

The ethanolic extract of control and test samples showed the presence of major phenolic compounds in plates eluted with Hexane : Ethyl acetate (6:4) (Fig. 2). The retention factor (R_f**)** value of separated spots of both control and test were measured and matched with standard phenols which showed the presence of 4- ethoxy benzoic acid (0.24), 3,4- diethoxy benzoic acid (0.62) and catechin (0.65) in both samples.

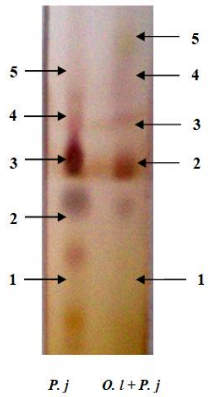

Fig. 2: Separation of compound from *O. laetevirens* treated *P. juliflora* Sample by Thin Layer Chromatography (TLC) (30th day sample)

Also a few other compounds at R_f value of 0.60, 0.66, 0.74 were observed (Table. 1). Similar results were reported that the presence of juliprosin and isojuliprosine in the polar fraction of *P. juliflora* leaf extract [8]. Later, catechin was isolated and identified as the main compound in the methanol extract of *Prosopis alba* trunk [9]**.** Also the presence of two alkaloids, which were identified as 3-oxo-juliprosopine and secojuliprosopinol from the methanolic extract of *Prosopis juliflora* bark [10].

Table. 1: Standard chemicals matching with compounds separated from *O. laetevirens* treated *P. juliflora* Sample

S. No	Standard Chemicals	Rf Value	Matching Standard	
			P. j	***O. l + P. j***
1	4-ethoxy benzoic acid	0.24	0.23	0.24
2	Vanillic acid	0.58	-	-
3	-	0.60	unknown	-
4	3,4 diethoxy benzoic acid	0.62	0.62	0.62
5	Catechin (reference standard for mesquitol)	0.65	0.64	0.64
6	-	0.66	unknown	unknown
7	P-coumaric acid	0.68	-	-
8	Syringaldehyde	0.72	-	-
9	-	0.74	-	unknown
10	4-hydroxy benzaldehyde	0.76	-	-
11	veratraldehyde	0.87	-	-

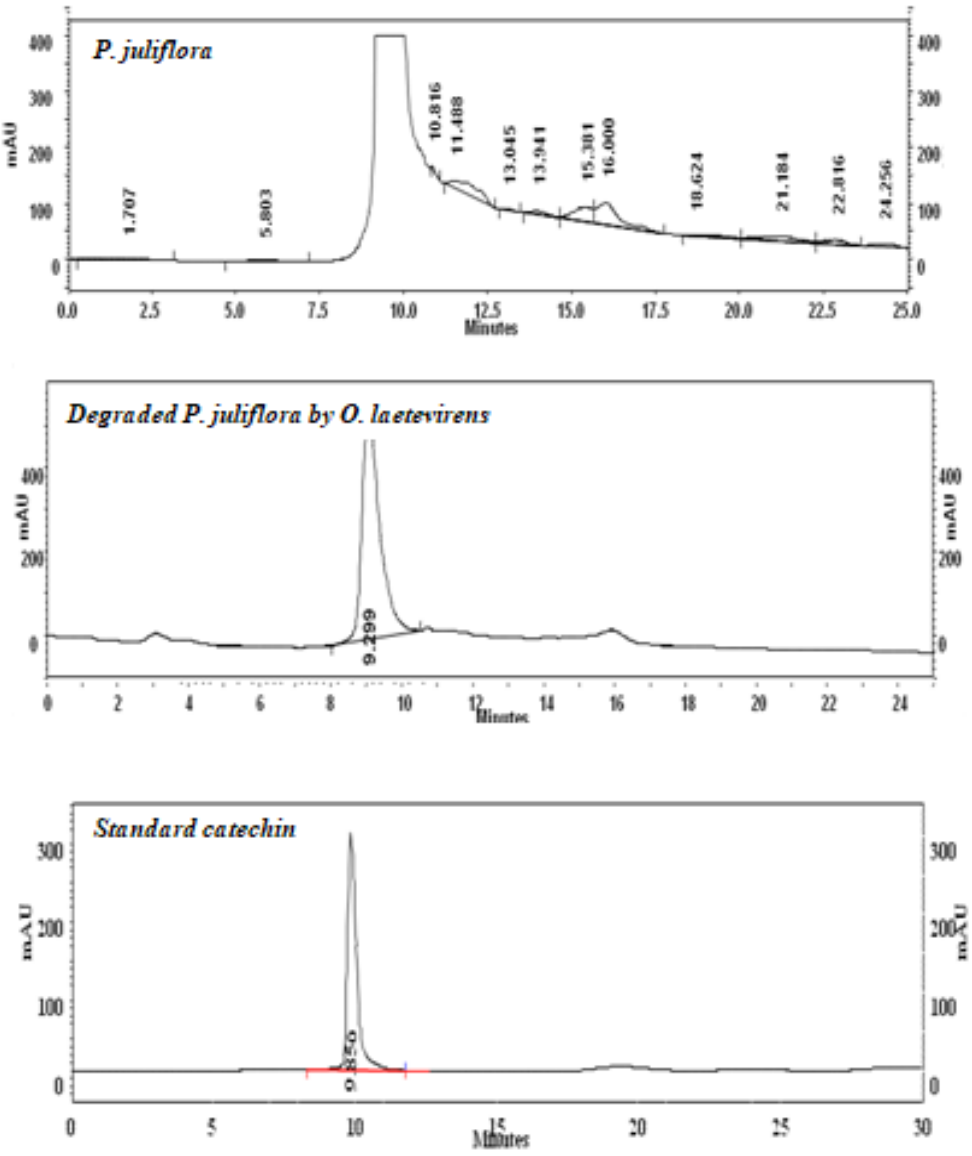

Fig. 3 Identification of compound by High Performance Liquid Chromatography (HPLC) (30th day sample)

3.3 High Performance Liquid Chromatography (HPLC)

The methanolioc extract of control and test sample was subject to HPLC analysis The phenolic compounds present in both sample were identified by comparing chromatographic peaks with the retention time (R_t) of individual standard catechin. The obtained results showed the presence of catechin (R_t = 9.8) like compound in *Prosopis juliflora* (R_t = 9.9) and degraded *Prosopis juliflora* (R_t = 9.2) (Fig. 3). Similar resul ts were obtained in HPLC analysis of crude acetonic extract of *P. juliflora* heartwood which indicated the presence of mesquitol as sole compound which corroborate with the present results [11]. Supporting evidence showed that catechin was identified as the main free radical scavenger from the extract of *Prosopis alba* [12]. Similar reports shows the presence of major compounds in acetone and water extracts of *Prosopis laevigata* were identified as (-)-epicatechin, (+)-catechin and taxifolin which are quantitatively determined by liquid chromatography (RP-HPLC-UV) [13].

3. 4 Gas Chromatography Mass Spectrometry (GC-MS)

The mass spectrum showed the m/z ratio (mass to charge) at 292 for *P. juliflora* and 291 for degraded *P. juliflora* which match with the molecular mass of mesquitol which is 291. These results confirmed the presence of mesquitol like compounds in both the control and test samples (Figure 4). Further confirmation and identification of the compound was done by FT-IR and NMR analysis. Supporting evidence showed that GC-MS analysis of toluene or ethanol bark extractives of *P. juliflora* indicates the presence of numerous compounds among which 4-O-methylgallocatechin as the main component.

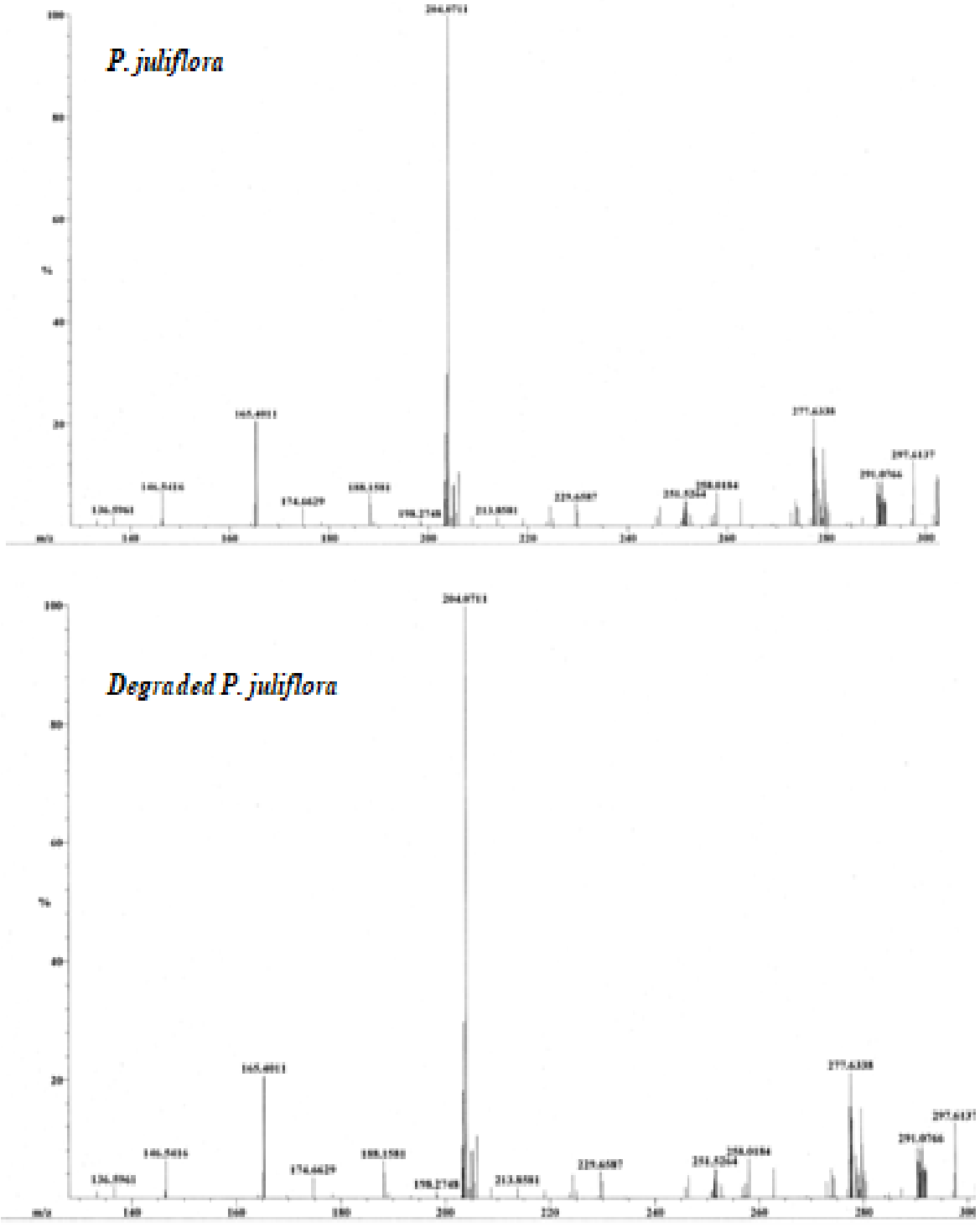

Fig.4: Gas chromatography mass spectrometry (GC-MS) of ethanolic extract of degraded *P. juliflora* (30th day sample)

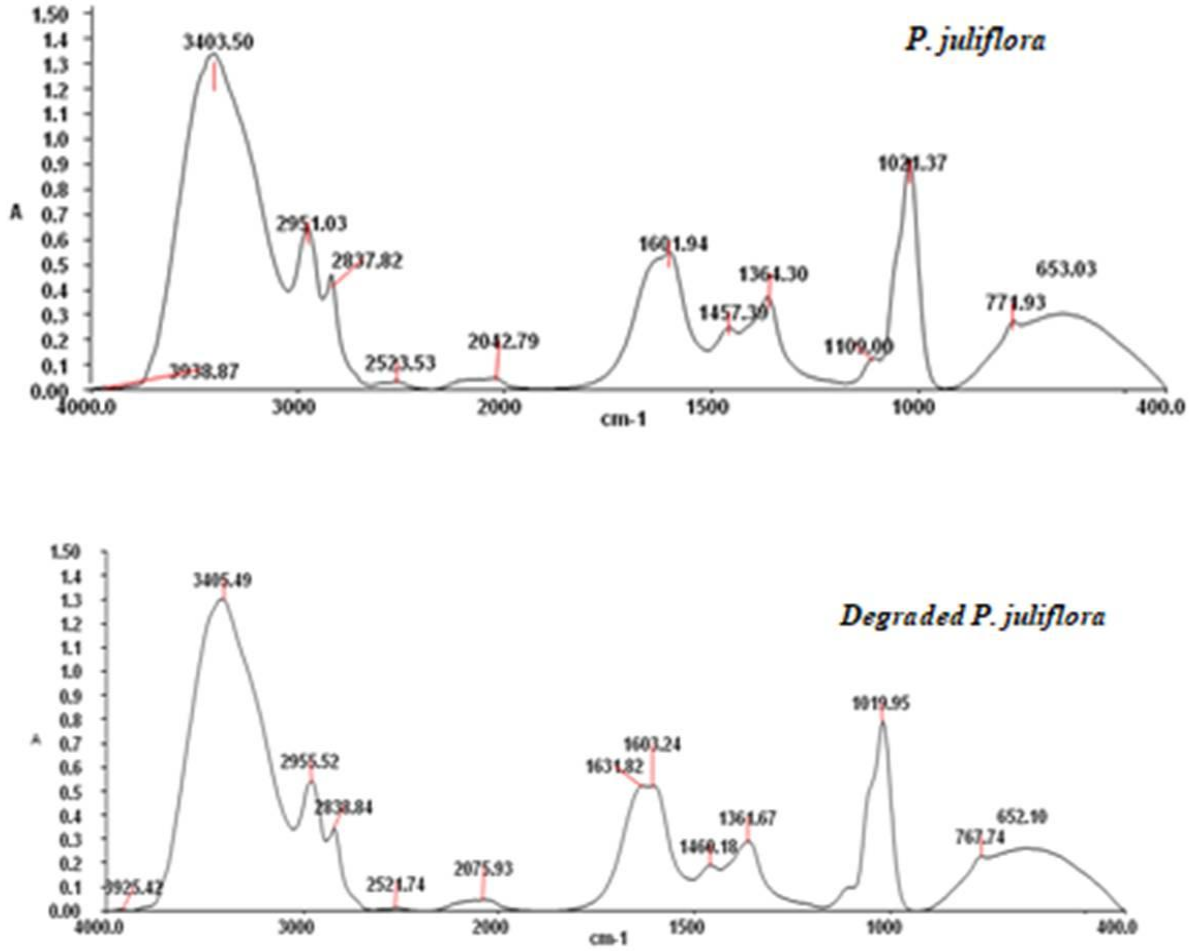

Fig. 5 Fourier Transform Infrared (FTIR) spectrometry spectrum of ethanolic extract of degraded *Prosopis juliflora* (30th day sample)

Also identifications of different compounds from *P. juliflora* bark extracts using National Institute of Standards and Technology (NIST) library and literature information indicate the presence of epicatechins, catechins, methylgallocatechins, gallocatechins, fatty acids and sugars [5]. Also it is reported that catechins, as the main constituent from exudates of *Prosopis flexuosa* [14].

3.5 Fourier Transform Infrared Spectrometry (FTIR)

FTIR spectrum for *P. juliflora* showed characteristic hydroxyl group absorption at 3405 cm-1. Fatty acids groups were identified by the presence of C-H vibrations between 2955 and 2838 and carbonyl bands at 1631cm-1. Aromatic C=C skeletal vibrations at 1603, 1460 and 1361 cm-1 are typical of aromatic structure in flavanols. A strong absorption at 1019cm-1 is characteristic of C-O vibrations in sugar units. Similarly, hydroxyl group absorption at 3396 cm-1, C-H vibration between 2948 and 2835, aromatic C=C skeletal vibration at 1599, 1456, 1364 cm-1 and C-O sugar unit at 1024 cm-1 were observed for degraded *P. juliflora* (Figure 5).

Similar results were observed in FTIR spectrum of acetone extractives of *P. juliflora* heartwood spectrum, indicated hydroxyl group absorption at 3350 cm-1 and aromatic C=C skeletal vibrations at 1613, 1514 and 1475 cm-1 which resembles flavanol structure [5]. Also FTIR spectrum of acetone extractives of *P. juliflora* leaves showed absorption bands at 3400 cm-1 characteristic of OH group and bands at 1119 and 1071 cm-1, which could be ascribed to sugars units. A strong absorption band at 2917 and 2849 cm-1 characteristic of C-H vibrations and 1728 cm- characteristic of C=O vibrations can be attributed to the presence of fatty acids in their free or esterified form. Also, bands at 1510 and 1450 cm-1 are ascribable to aromatic structures [5].

3.6 Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectrum showed a higher complexity indicating the presence of different families of product such as fat, sugar along with typical flavonol signal in control *P. juliflora* while spectrum of degraded *P. juliflora* extract showed the presence of main component of flavanol signal. From the spectrum data it is revealed that mesquitol is the main

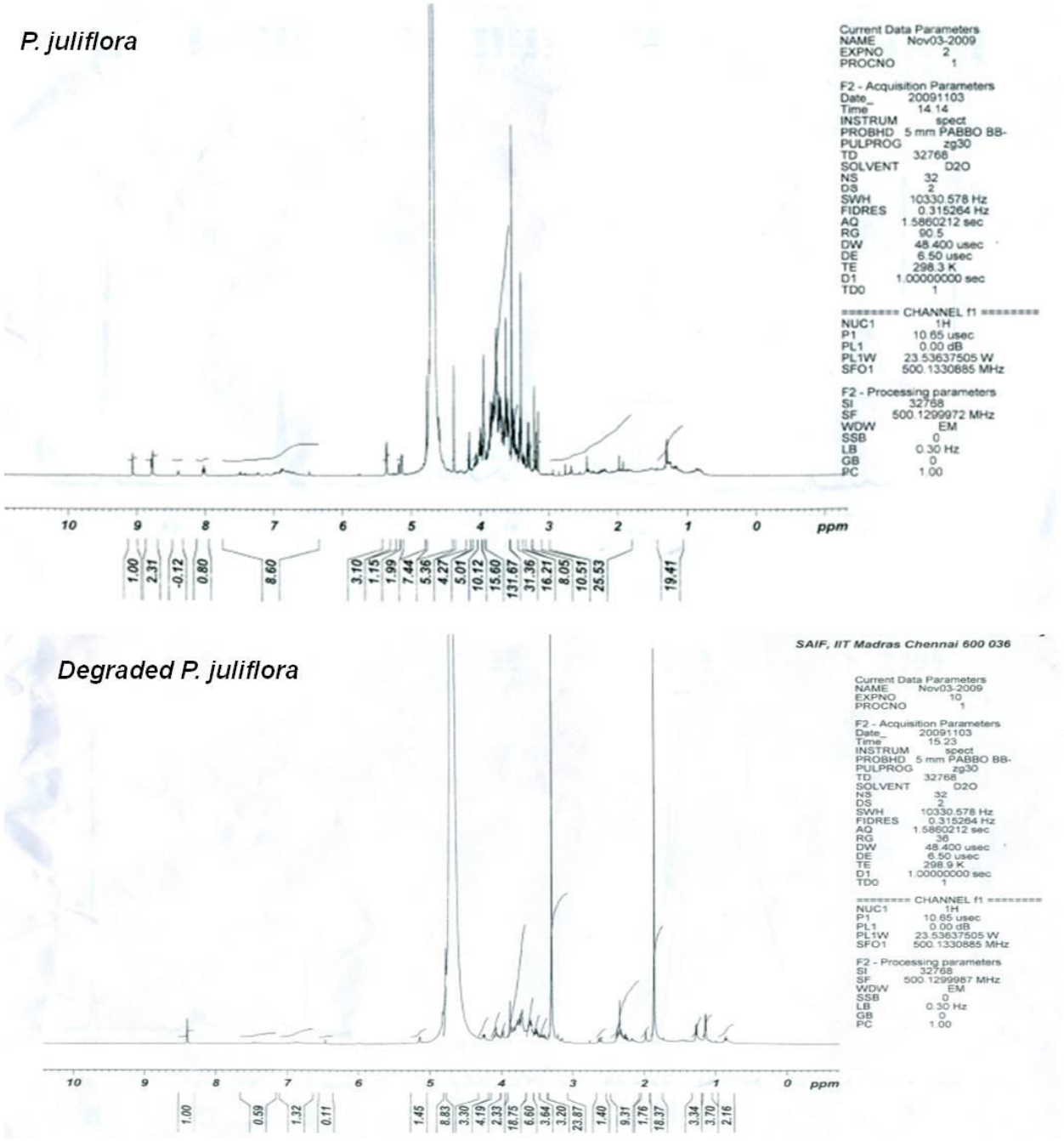

Fig. 6 Nuclear Magnetic Resonance (NMR) spectroscopy spectrum of ethanolic extract of degraded *P. juliflora* (30th day sample)

component of ethanolic extract without any noticeable impurities in degraded *P. juliflora* (Figure 6). Supporting evidence showed that high amounts (8%) and purity of the rare flavonoid (-)-mesquitol was identified as the major metabolite in heartwood extractives, while (+)-epicatechin, (+)-catechin, gallocatechins, methylgallocatechins, fatty acids and free sugar are present in the bark of *P. juliflora* [10]. Pods contain important quantity of galactomannans, mannose, saturated and unsaturated fatty acids and free sugar. Leaves of *P. juliflora* contain alkaloids such as tryptamine, piperidine, phenethylamine and juliprosopine [14].

4. CONCLUSION

Phytocomponent analysis by TLC revealed that a higher number of phenolic compounds were separated in plates eluted with Hexane: Ethyl acetate (6:4). The Rf value of the test samples when matched with the Rf value of the standard which showed the presence of phenolic compounds like 3, 4 diethoxy benzoic acid 4-ethoxybenzoic

acid and catechol. This result was further confirmed by HPTLC analysis. Structural and physicochemical elucidation based on HPLC, GC-MS and FTIR analysis showed the presence of catechin like compound in the extract of degraded *P. juliflora* when compared with control. The major amount of mesquitol present in the ethanolic extract of degraded *P. juliflora by O. laetevirens* put forth that these degraded P.juliflora as an potential renewable source of antioxidants..

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