Potential of *Euphorbia caducifolia* Haines as a renewable source for biofuel

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Abstract

The shortage in the availability of fossil fuels leading to adverse effects on atmosphere has led to search for alternate crops as a source of biofuels. *Euphorbia caducifolia* Haines (family Euphorbiaceae), a latex yielding plant, grows abundantly in arid and semiarid regions. The phylloclades of this species are tough, succulent and fleshy, covered with divaricated sharp spiny stipules. The sticky milky latex flows out in abundance when any slight injury is caused to the plant. For the first time study was undertaken to extract biocrude from phylloclade dried biomass. This was to study its elemental analysis, gross calorific value and chemical analysis of biocrude for use as a possible biofuel crop. It was observed that the percentage of moisture content was lower in phylloclades collected from Nandimandalam (84.2 ± 3.7%) followed by Panyam (85.8 ± 0.7), Goothi (88.0 ± 0.7) and Gurramkonda (90.1 ± 0.5). Correspondingly the higher dry matter (15.8 ± 0.7%) was obtained from the phylloclades of Nandimandalam region when compared to the forest areas of other districts of Rayalaseema region. The ash content of dried biomass was reported to be 4.9 ± 1.0% for Nandimandalam followed by 5.3 ± 1.9% for Panyam, 5.7 ± 3.1% for Goothi and 6.2 ± 2.1% for Gurramkonda. Cyclohexane (CHE) biocrude obtained from dried biomass of Nandimandalam region (15.1 ± 0.4%) was higher followed by Goothi (14.4 ± 0.6%), Panyam (14.1 ± 1.3%) and Gurramkonda (14.1 ± 0.3%). The hexane biocrude (HE) also reported to be higher from the phylloclades collected from Nandimandalam (14.2 ± 0.4%) as compared to Goothi (13.6 ± 0.6%), Panyam (13.5 ± 0.6%) and Gurramkonda (13.0 ± 0.5%). Cyclohexane biocrude contained 81.1% carbon, 10.9% hydrogen and 0.2% nitrogen, while hexane biocrude contained 75.4% carbon, 10.7% hydrogen and 0.1% nitrogen. Gross calorific values for cyclohexane and hexane biocrude was 9, 465.9 cal/g and 8516.8 cal/g respectively and were almost comparable to crude oil (10, 505 cal/g). GC analysis indicating the presence of long aliphatic carbons. In proton NMR, the presence of peaks at the range of 0.7 to 1.2 ppm, 1.1 to 2.0 ppm and 4.6 to 5.2 ppm representing –CH3 group, –CH2 group and –CH group respectively, corresponded to the presence of poly isoprenoid structures. FTIR spectral analyses showed a broad peak at 3449.04 cm^{-1} indicated the presence of –OH group, peaks at 2918 cm^{-1} and 2849 cm^{-1} indicating the presence of amide and methylene groups. A peak at 1463 cm^{-1}, 1377 cm^{-1}, and 1239 cm^{-1} indicated the presence of C = C, –CH3 and ether linkage (C–O–C). 804 cm^{-1} and 828 cm^{-1} peaks correspond to double bonded CH and tri substituted CH bending's respectively indicating the presence of isoprene and polyprene units. From these observations, this species may be considered as a potential and possible source of biofuel and chemical feed stocks.

Keywords: Euphorbia caducifolia Haines, Biocrude, Gas chromatography, Nuclear magnetic resonance spectroscopy, Fourier transforms infrared spectroscopy, Elemental analysis and Gross calorific value

1. Introduction

The combustion of fossil fuels led to massive emissions of greenhouse gasses and irreversible change to the global climate [1]. Therefore to resolve the dependence on crude oil which led to increasing impairment of the environment by identifying of competitive and sustainable and competitive alternative fuels [2]. Furthermore, the phenomenal growth of number of automobiles and vehicles, and the sophistication of technology has also necessitated awareness of biofuels. This led to a renewed interest in discovering and developing alternate crops to meet the growing demand for fuels, chemicals, and industrial feed stocks. The plants grown conveniently on either underutilized or marginal soils thereby not competing with the agricultural crops are most preferred feedstock for production of biofuels.

Certain plant species with ability to reduce carbon dioxide to high molecular weight hydrocarbon material prompted the idea of utilising them as sources for producing biofuels and chemical raw materials [3, 4, 5, 6 and 7]. The Earth has vast areas of land unsuitable for food and fodder crops, coupled with experience with growing hydrocarbon-yielding plants suggests that the possibility to use marginal soils for harvesting plants which yield substitutes for conventional hydrocarbons. There were several compelling reasons for seriously exploring the prospects of hydrocarbon plantations, although presently no substantive claims have been listed on the viability of this option. Various workers have conducted extensive screening programs attempting to identify potential biocrude and botanochemicals feed stocks [8].

Biocrude is the hydrocarbon fraction extracted from plants by organic solvents and upgraded to either liquid fuels or other useful chemical feed stocks [6]. The extractable biocrude may contain a complex mixture of triglycerides, waxes, phytosterols, terpenes, and other modified isoprenoid compounds that can be a good source of hydrocarbons [3, 9 and 10]. Botanical laticiferous families such as Asclepiadaceae, Asteraceae, Anacardiaceae, Convolvulaceae, Caprifoliaceae, Euphorbiaceae, Lamiaceae and Moraceae are being researched as possible and potential biofuel crops [8, 11 and12]. Calvin and co-workers (1979) screened several species of the genus *Euphorbia* (family: Euphorbiaceae).Species of this genus may grow naturally under semi-arid and arid conditions producing abundant latex rich in hydrocarbons. So far there have been reports on the possible and potential use of species like *E. abyssinica* [13], *E. resinifera* [14], *E. tirucalli* [15 and 16], *E. lathyris* [15], *E. rigida* and *E. antisyphilitica* [9] for biofuel production.

Euphorbia caducifolia Haines is a latex yielding shrub, grows abundantly on gravel and rock habitat of arid and semi-arid areas. This species grows to a height of 5-6 m, branching repeatedly and producing clumps of phylloclades. The phylloclades are tough, succulent and fleshy, covered with divaricated sharp spiny stipules. The sticky milky latex flows out in abundance on slight injury caused to any part of the plant. The present study was focused for the first time to extract biocrude from phylloclade dried biomass and to study elemental analysis, gross calorific value and chemical analysis of energy extracts to utilise this species as a possible biofuel crop.

2. Materials and methods

2.1 Plant materials

Euphorbia caducifolia Haines plant (of approximately 5-6 m height) growing in the forest areas of Goothi of Anantapur, Gurramkonda of Chittoor, Nandimandalam of Kadapa and Panyam of Kurnool Districts of Rayalaseema region of Andhra Pradesh, India; was selected for the present study. The clumps of phylloclades were separated from the plants of various areas of these districts. Huge spreading of this plant was observed in Nandimandalam forest area of Kadapa district of Andhra Pradesh.

2.1.1 Processing of plant material

Phylloclades were kept in paper bags and shade dried at room temperature for more than 20 days until constant mass was obtained. Dried phylloclades were milled to a sieve mesh of 2 mm size. Further the dried powder was again desiccated in incubator at 35 ^{0}C for 24 h till constant mass was obtained and stored at ambient temperature in sealed polythene bags.

2.2 Proximate analysis

2.2.1 Moisture content

The moisture content of phylloclades was determined using formula of Association of Official Analytical Chemists [17]. The percentage of moisture content was calculated as follows;

$$\text{Moisture (\%)} = (W_2 - W_3)/ (W_2 - W_1) \times 100 \qquad \text{(A.1)}$$

Where W_1 = initial weight of empty paper dish; W_2 = weight of empty paper dish + phylloclade sample before drying and W_3 = Final weight of paper dish + phylloclade sample after drying to constant mass and cooling

2.2.2 Ash content

The dried powdered biomass was weighed before and after ashing to determine the concentration of ash present in the samples. The ash content can be expressed on dry matter basis using with the following equation [17].

$$\text{\% Ash (dry basis)} = M_{ash}/ M_{Dry} \text{ X } 100 \qquad \text{(A.2)}$$

Where M_{ash} = ash obtained after heating at high temperature; M_{Dry} = powder obtained after sieving the dried phylloclade

2.2.3 Extraction of biocrude

Milled dry powder (100g) of various forest areas were individually extracted in soxahlet apparatus with 250 ml of cyclohexane or hexane separately at 35 ^{0}C for 48 h. The subsequent cyclohexane and hexane extracts were concentrated by rota-evaporator at 35 ^{0}C under reduced pressure where the solvent extracts and residues were separated carefully and the extracts were dried at room temperature, weighted for yield per gram of dry sample. The percentage of extracts and residues of these solvents were calculated.

2.3 Biocrude analysis by Gas chromatography

Gas chromatography (GC) was used to determine hydrocarbon fractions of cyclohexane and hexane biocrude using an Agilent 5890 S2 gas chromatograph on an AR and DGC capillary column (30 m×0.53 mm i.d. ×3.00 µm). Nitrogen was the carrier gas and held at a constant flow of 3.5 kg/cm^2. The oven temperature was ramped from 50 ºC to 240 ºC in 19 min, and held at 240 ºC for the remaining 2 min of analysis. The inlet temperature was kept constant at 50 ºC and the detector (FID) at 230 ºC. An aliquot (0.2 µl) of sample was injected manually into the gas chromatograph using a 5 µl gas tight syringe. Standard curves were constructed of the GC peak area ratios relative to various concentrations of hydrocarbon standards of C10 and C18 carbon chain lengths. From the standard curves, the composition of hydrocarbon chain length of these extracts was determined.

2.4 Biocrude analysis by nuclear magnetic resonance spectroscopy

NMR-Spectral analysis was done with Bruker Biospin Fällanden, Switzerland *AVANCE III*, 500 MHz, 1mm TXI - Probe head,. (500.13 MHz); 5mm BBO - Probe head (125.78 MHz) at University of Basel, Switzerland. The results were analysed using Topspin 2.1 PL 3 Software. Cyclohexane and hexane biocrude were individually dissolved in $CDCl_3$ (^{13}C = 0.5 ml). The numbers of scans (ns) were 2048 for ^{13}C NMR spectra.

2.5 Biocrude analysis by Fourier transforms infrared spectroscopy (FTIR)

FTIR spectra were recorded for cyclohexane and hexane biocrude on a Perkin Elmer instrument; model SPECTRUM 2, using KBr as a dispersion medium. About 2 mg of the hexane and cyclohexane extracts samples were individually grounded thoroughly with KBr and pellets were prepared using a hydraulic press under a pressure of 600 kg/cm^2. Spectra were scanned between 4000 and 450 cm^{-1}.

2.6 Ultimate analysis and gross calorific value

The percentage of carbon (C), hydrogen (H) and nitrogen (N) in phylloclade dried biomass, biocrude and residues were determined by CHN analyser (Series II) at Research and Development Centre, Indian Oil Corporation, Faridabad. The gross calorific values were determined by bomb calorimeter (TECHED Instrument) at Department of Mechanical Engineering, Yogi Vemana University Engineering College, Proddutur.

3. Results and discussion

3.1 Proximate analysis

Extensive survey was conducted throughout the Rayalaseema region for the distribution of latex yielding plants and found that *E. caducifolia* Haines shrubs was spreading abundantly in Nandimandalam forest area of Kadapa district than the other forest areas of Rayalaseema. The proximate analysis (moisture content, dry matter and ash content) was carried out in dried biomass and results were summarized in Table 1.

Table 1. *Proximate analysis of phylloclade biomass of E. caducifolia collected from various regions of Rayalaseema*

Parameters	Nandimandalam	Gurramkonda	Panyam	Goothi
Proximate analysis [a]				
Moisture content (%)	84.2 ± 3.7	90.1 ± 0.5	85.8 ± 0.7	88.0 ± 0.7
Dry matter (%)	15.8 ± 0.7	9.9 ± 0.2	14.2 ± 2.1	12.0 ± 3.1
Ash (%)	4.9 ± 1.0	6.2 ± 2.1	5.3 ± 1.9	5.7± 3.1
aValues are means of three replications ± SD				

3.2 Moisture content

The percentage of moisture content was carried out for phylloclades collected from four regions namely Nandimandalam, Panyam, Goothi and Gurramkonda. It was found that the percentage of moisture content was lower in phylloclades collected from Nandimandalam (84.2 ± 3.7%) followed by Panyam (85.8 ± 0.7), Goothi (88.0 ± 0.7) and Gurramkonda (90.1 ± 0.5). Correspondingly the higher dry matter (15.8 ± 0.7%) was obtained from the phylloclades of Nandimandalam region when compared to the forest areas of other districts of Rayalaseema region (Table 1). In general, more the moisture contents in the plant biomass lesser the dry matter. The less dry matter has negative relation with heating value of the biomass. Variation in percentage of moisture content may be dependent on climate, plant density, phenology and other physical parameters.

3.3 Ash content

The ash content of dried biomass was reported to be 4.9 ± 1.0% for Nandimandalam followed by 5.3 ± 1.9% for Panyam, 5.7 ± 3.1% for Goothi and 6.2 ± 2.1% for Gurramkonda. Comparative data was presented in Table 1. Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures between 500 and 600 ºC. Water and other volatile materials are vaporized and organic substances are burned in the presence of the oxygen in air to CO_2, H_2O and N_2. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates. Although most minerals have fairly low volatility at these high temperatures, some are volatile and may be partially lost e.g., iron, lead and mercury. High ash content has negative effect on the calorific value [18]. Ash content for *E. pulcherrima*, *E. neerifolia* [8], *E. hirta* and *E. splendens* [19] was found to be 3.4%, 4.76%, 0.1%, 2.9%, respectively.

3.4 Extraction of biocrude

Biocrude was extracted from the dried biomass of plant material collected from different areas using non-polar solvents namely cyclohexane and hexane in a soxahlet apparatus. Comparative data of these extractables and residues were presented in Table 2. Cyclohexane (CHE) biocrude obtained from dried biomass of Nandimandalam region (15.1 ± 0.4%) was higher followed by Goothi (14.4 ± 0.6%), Panyam (14.1 ± 1.3%) and Gurramkonda (14.1 ± 0.3%). The hexane biocrude also reported be higher from the phylloclades collected from Nandimandalam (14.2 ± 0.4%) as compared to Goothi (13.6 ± 0.6%), Panyam (13.5 ± 0.6%) and Gurramkonda (13.0 ± 0.5%). From this data, it can be considered that, cyclohexane is more efficient to extract the maximum yield of biocrude from plant biomass than hexane solvent due to its properties. Cyclohexane and hexane solvents are shown slight variations in their physical and chemical properties. It might be because of high polar nature of cyclohexane (C_6H_{12}) and having one extra C - C bond and two less C - H bond than hexane (C_6H_{14}). Cyclohexane is cyclic in nature and hexane is linear but both are alkanes. Biocrude was extracted from *E. lathyris* by heptane solvent followed by methanol solvent. Heptane soluble extract contains hydrocarbons and methanol soluble extract contain carbohydrates [20]. This species can yield up to 20,000 kg dry matter (DM) ha^{-1}, of which between 5 - 8% are hydrocarbons and 20% fermentable sugars [21]. *E. tirucalli* can produce a biomass of 25 tonnes /hectare/year without any special agronomy and biocrude was extracted by heptane solvent followed by methanol solvent. The yield of biocrude was about 8% on dry weight biomass [22]. *E. antisyphilitica* contains a maximum biocrude yield of 8.8% on dry weight biomass [23]. From all these observations, biomass collected from Nandimandalam region has shown better characteristics than the other areas and hence we focused on this area for further analysis.

Table 2. *Percentage of biocrude and spent residues extracted from dried phylloclade biomass of E. caducifolia collected from various regions of Rayalaseema*

Region	Cyclohexane biocrude[a]	Cyclohexane-residue[b]	Hexane biocrude[a]	Hexane-residue[b]
Nandimandalam	15.1 ± 0.4	84.3 ± 0.5	14.2 ± 0.4	85.1 ± 0.3
Gurramkonda	14.1 ± 0.3	85.3 ± 0.3	13.0 ± 0.5	86.4 ± 0.5
Panyam	14.1 ± 1.3	85.3 ± 1.3	13.5 ± 0.6	85.9 ± 0.6
Goothi	14.4 ± 0.6	85.0 ± 0.8	13.6 ± 0.6	85.9 ± 0.4
a, b Values are means of three replications ± SD				

3.4.1 Gas chromatography

Gas chromatogram analysis was performed for both cyclohexane and hexane biocrude and represented as Fig 1, 2. GC peaks of cyclohexane biocrude was analyzed and found that proportions of 56% of C4 – C8; 8% of C12 – C14; 6% of C18; 10% of C24; 8% of C28; 4% of C32; 2% of C34 and 6% of other multiple peaks. In hexane biocrude analysis, 48% of C2 – C6, 4% of C18, 22% of C22–C24, 16% of C26 – C32 and remaining 10% of other carbon chains were present. Normally, plants have C16 and C18 as the chief fatty acids, but the latex bearing plants has long chain fatty acids, such as C20 – C30 in the form of di, tri and sesquiterpenes [24].

Fig. 1 *GC analysis of cyclohexane biocrude extracted from dried phylloclade biomass of E. caducifolia*

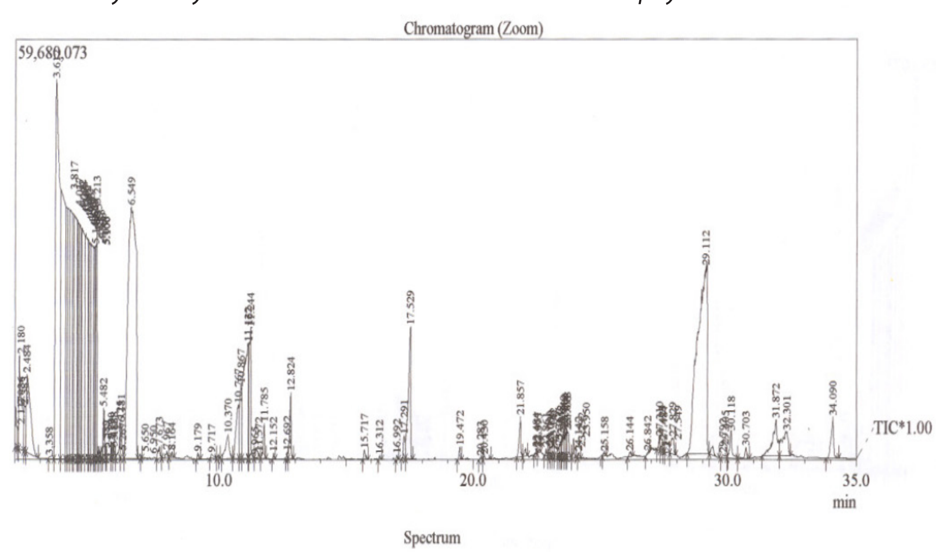
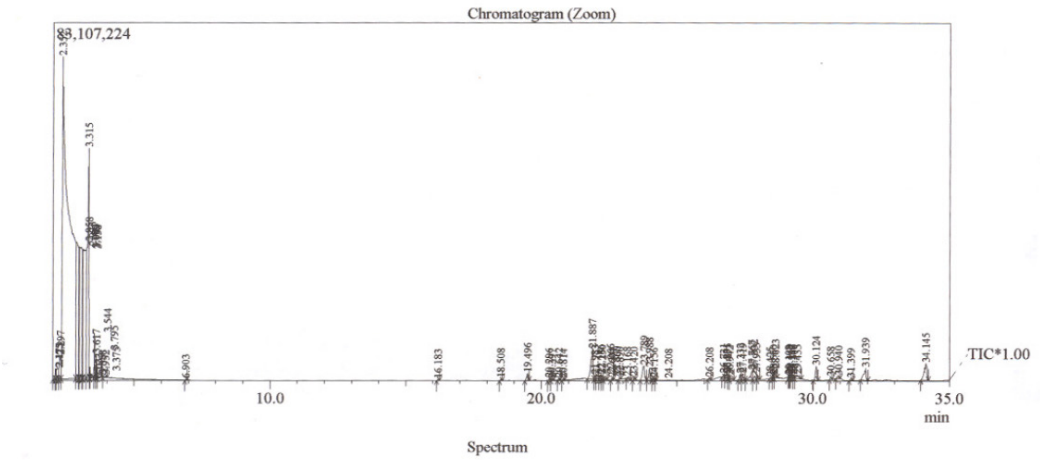


Fig. 2. *GC analysis of hexane biocrude extracted from dried phylloclade biomass of E. caducifolia*



3.4.2 NMR studies

This study was almost similar in both cyclohexane and hexane biocrude. In proton NMR, the presence of peaks at the range of 0.7 to 1.2 ppm, 1.1 to 2.0 ppm and 4.6 to 5.2 ppm representing –CH3 group, –CH2 group and –CH group respectively, corresponded to the presence of poly isoprenoid structures in the biocrude of hexane and cyclohexane as shown in Fig 3, 4. In ^{13}C NMR, the presence of peaks mainly between 12 and 70 ppm indicated the presence of alkyl groups, alkyl chains (11–23 ppm) and longer alkyl chains (22 to 38 ppm) and –CH2 groups in cyclic rings (35 – 65 ppm) as shown in Fig 5, 6. In case of *E. hirta* and *E. splendens* had showed peaks at 1.68 and 2.03 ppm matching peaks produced by cis-polyisoprene (natural rubber) [25].

Fig.3. *H NMR analysis of cyclohexane biocrude extracted from dried phylloclade biomass of E. caducifolia*

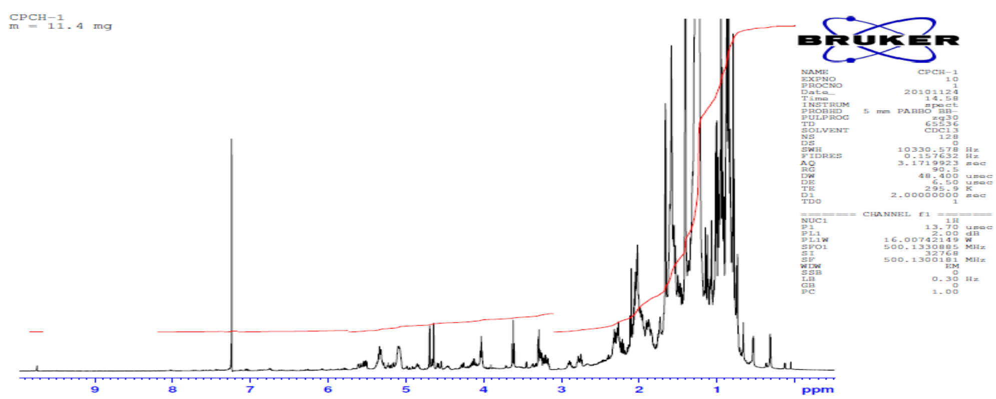


Fig.4. *1H NMR analysis of hexane biocrude extracted from dried phylloclade biomass of E. caducifolia*

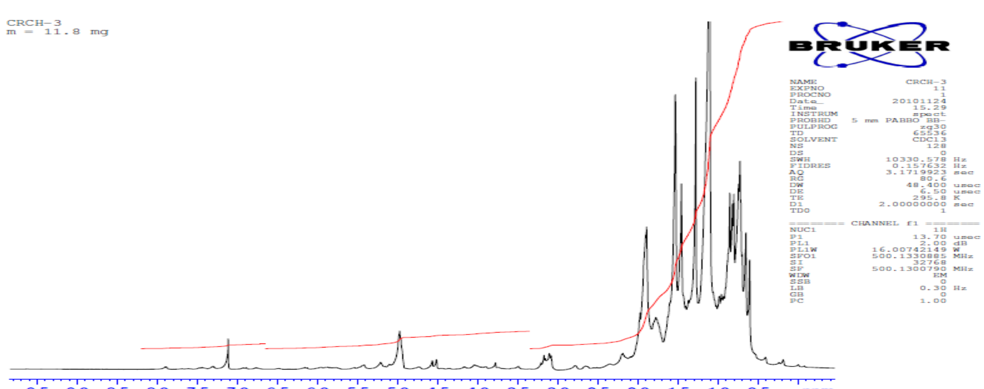


Fig.5. *C NMR analysis of cyclohexane biocrude extracted from dried phylloclade biomass of E. caducifolia*

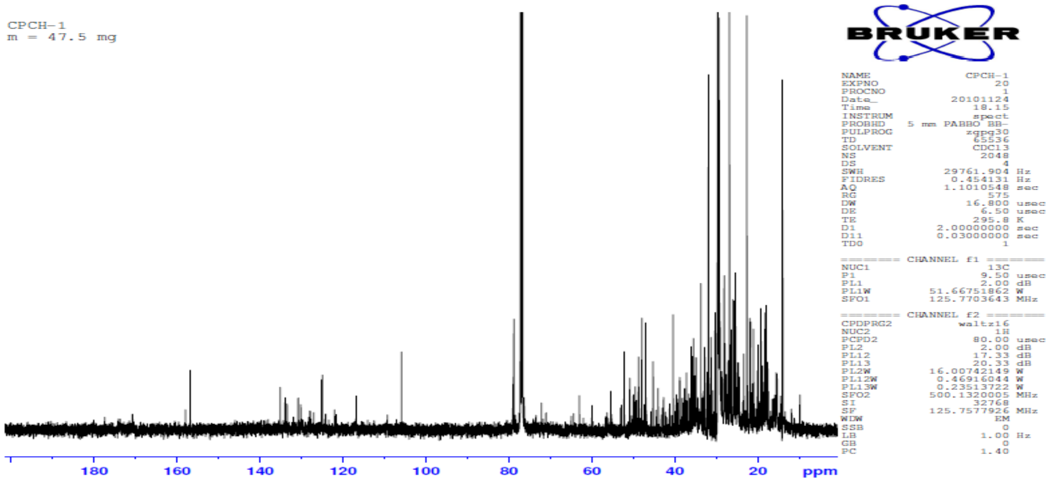
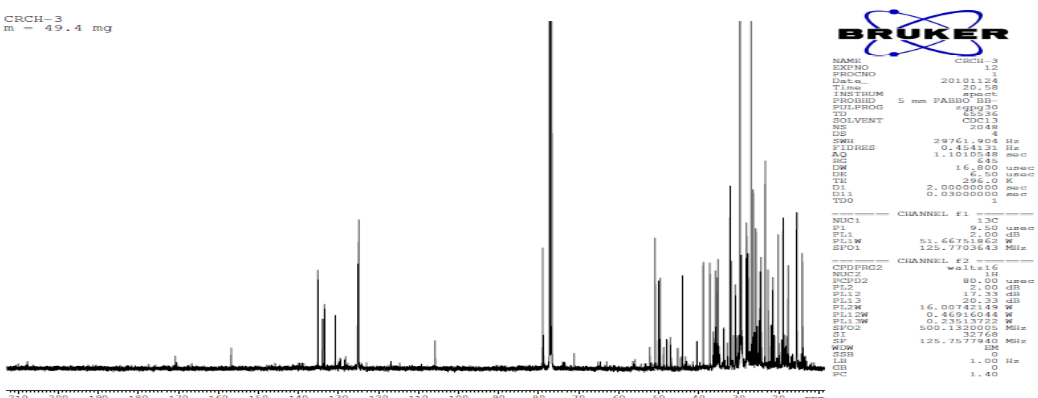


Fig.6. *C NMR analysis of hexane biocrude extracted from dried phylloclade biomass of E. caducifolia*



3.4.3 FT-IR

FTIR analyses of cyclohexane and hexane biocrude are shown similar spectra in Fig 7, 8. A broad peak at 3449.04 cm^{-1} indicated the presence of –OH group, peaks at 2918 cm^{-1} and 2849 cm^{-1} indicating the presence of amide and methylene groups. Peaks at 1736 cm^{-1} indicated the presence of keto group or aldehyde. A peak at 1463 cm^{-1}, 1377 cm^{-1}, and 1239 cm^{-1} indicated the presence of C = C, –CH3 and ether linkage (C–O–C). 804 cm^{-1} and 828 cm^{-1} peaks correspond to double bonded CH and tri substituted CH bending's respectively indicating the presence of isoprene and polyprene units. Similarly the biocrude was analyzed by FTIR in *E. antisyphilitica* [9]) and *E. rigida* [26].

Fig. 7. *FTIR analysis of cyclohexane biocrude extracted from dried phylloclade biomass of E. caducifolia*

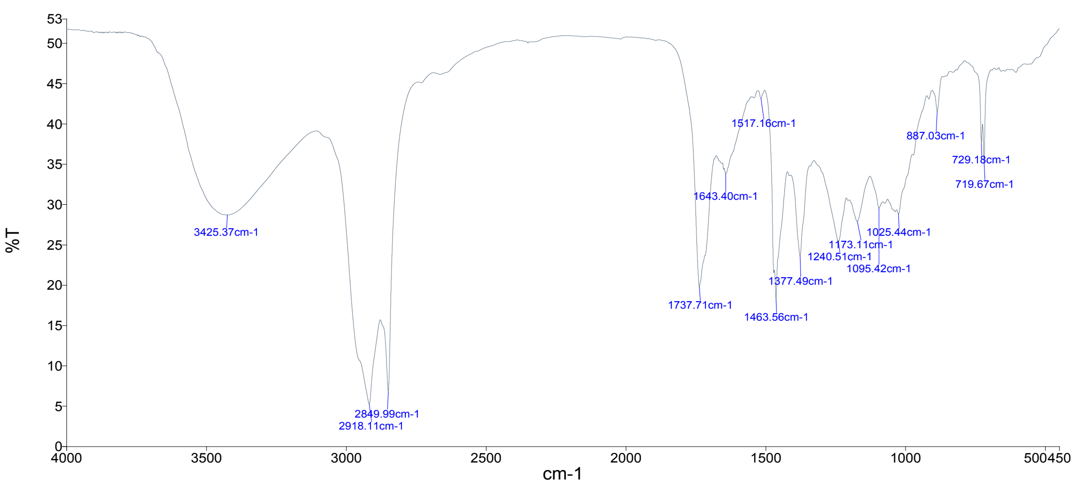
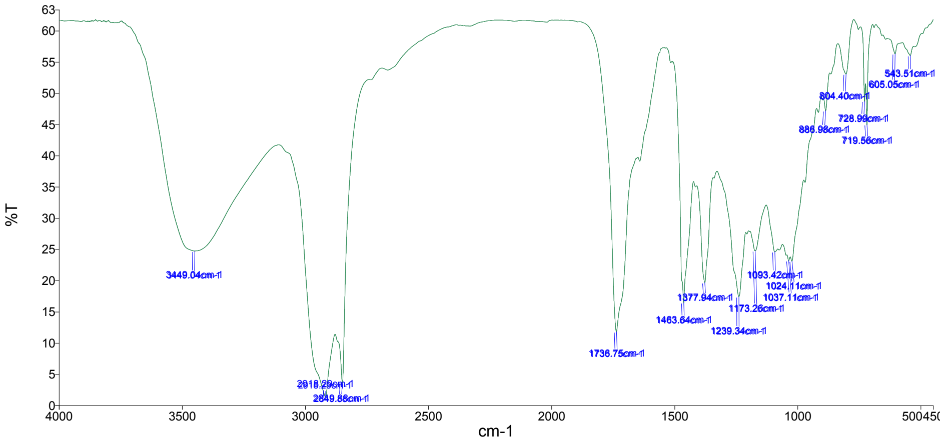


Fig.8. *FTIR analysis of hexane biocrude extracted from dried phylloclade biomass of E. caducifolia*



3.5 Elemental analysis and gross calorific value

The characteristics such as total percentage of C, H, N and calorific values of hexane and cyclohexane biocrude and residues are presented in Table 3 and compared with those of coal and petroleum products. Cyclohexane biocrude contained 81.1% carbon, 10.9% hydrogen and 0.2% nitrogen. Similar percentages of elemental contents were also observed by hexane extract. Here gross calorific value for cyclohexane biocrude (9, 465.9 cal/g), Hexane biocrude (8516.8 cal/g) and elemental composition of both solvents were almost similar to crude oil. *E. hirta* and *E. splendens* have 3769 cal/g and 3684 cal/g [19]. Calorific values mainly depend on the composition of hydrocarbon fraction and its content of biocrude and their molecular weights [27]. The hydrogen to carbon ration is not only an indicator of the elemental composition, but also the conversion capability of biomaterials that have hydrocarbon or hydrocarbon like compounds for low molecular weight fuels or chemical raw materials [28].

Table 3. *Analytical values of carbon, hydrogen and nitrogen fractions and gross calorific value (gcv) of biocrude and spent residues of E. caducifolia phylloclade biomass*

Sample	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Gross calorific value (Cal/g)
Dried biomass	39.6	5.65	1.41	2372.70
Cyclohexane biocrude	81.1	10.9	0.2	9465.9
Hexane biocrude	75.4	10.7	0.1	8516.8
Cyclohexane Residue	34.1	4.9	1.2	3671.4
Hexane Residue	35.6	5.3	1.6	1973.1
Anthracite Coal	79.7	2.9	6.1	7115.0
Lignite Coal	40.6	6.9	45.1	3888.0
Crude oil	84.0	12.7	1.2	10,505.0
Gasoline	84.9	14.7	0.0	10,764.0
Fuel oil	85.6	11.9	0.6	11,527.0

4. Conclusion

E. caducifolia has its ability to grow profusely in marginal arid and semiarid soil without agronomic management reducing production costs compared to other conventional agricultural crops. Biocrude extracted by cyclohexane has high calorific value, low ash content, rich in aliphatic hydrocarbons and almost comparable to crude oil.This plant can be used as a substitute for the production of biofuels and other chemical feedstocks. In future studies, spent residues,during extraction,can be consideed as a lignocellulosic source for production of bioethanol.

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