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REGULAR ARTICLE

POTENTIALITY OF *CALOTROPIS PROCERA* ON THE YIELD OF BIOCRUDES AND BIOGAS PRODUCTION

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SUMMARY

The identification of plants rich in hydrocarobons and botanochemicals has gained worldwide interest now-a-days, since they can serve as potential renewable energy resources to alternate fossil fuels. The present study was aimed to screen the potentiality of the latex bearing plant *Calotropis procera* (L) W.T. Aiton as an alternative source of energy. The bio-crude yield of oil was varied from 1.54 to 3.97% and the polyphenol content was high in stem (S) 4.86% compared to whole plant (WP) (4.00%) and leaf (3.33%). The botanochemicals in the oil fractions were identified by thin layer chromatographic studies (TLC) as sterols, free fatty acids, waxes and hydrocarbons. The IR spectra of hydrocarbon fractions obtained from *C. procera* exhibited peaks at 2980cm^{-1} and 1955 cm^{-1} indicated the presence of C-H structure of methyl or methylene group (CH_3 & CH_4). The 1H-NMR spectral analysis confirmed the possible presence of potential bioactive compounds such as cis-polyisoprene (natural rubber), trans-polyisoprene (gutta), wax, ester and hydrocarbons. Experiments were also conducted at laboratory scale to evaluate the biomethanation property *C. procera*. The slurry of cow dung with the *C. procera* supplement (3:1) were used for the experiment and the results showed increased production of biogas as the age of digester increased over control. The nutrient composition was analysed in the left over slurry after digestion. The results showed that the fertilizer value of the digested slurry effluent in the test digester was significantly higher than the control slurry in terms of organic compounds as well as mineral content.

Key words: *Calotropis procera*, bio-crude, hydrocarbons, biogas production, IR, ^{1}H-NMR

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1. Introduction

The worldwide increase in the demand and depletion of fossil hydrocarbons created research interests to development alternate sources of fuels and petrochemical from plant sources. Biomass resources are potentially the world's largest renewable energy source and the conversion of the biomass to fuel and petrochemicals is the only alternative to replace the fossil fuel, oil and coal [1]. The green plants on the earth have the capacity to covert the solar energy into a wide range of by-products that are competitive with synthetic petrochemicals, especially secondary metabolites such as oil, hydrocarbons that are the most attractive sources for alternative energy [2]. The Assessment of sustainable biomass resource potential plays a major role in planning future energy activities of any country where biomass is the main renewable energy source [3]. Previous reports revealed that the biomass supply being one third of the developing countries energy varying from about 90% in countries like Uganda, Rwanda to 45% in India, 30% in China and 10% in Mexico [4]. Substitution of fossil fuels by plant biomass requires the right selection of plant species with high site suitability, an ecologically benign farming system and high yields [5]. The Potential biomass with high content of botanochemicals can also serve as feed stock for biogas to achieve economic success in energy harvest [6]. According to Tada et al., [7] and Demirci and Demirer [8] the Quantity and quality of biogas production mainly depend upon the feed stock characteristics.

There are several reports in which thousands of plant species that produce enormous amount of hydrocarbons have been identified and recommended for cultivation as bio-energy crops [9,10,11]. Since some plant species are strongly dependent on soil nitrogen availability, therefore their cultivation is expensive from a fossil energy point of view [5]. Due to various invasive effects of weed plant in agriculture, research studies have been focused to screen weed plants for their beneficial roles in agriculture and other related fields such as biomass and bio energy production. Several studies have been carried out on many hydrocarbon bearing plants in India, however as the plant resources of India are enormous and unique owing to varied topography and wide climatic conditions, there are a large number of weeds having wide distribution in arid climatic zones, which have not been systematically evaluated as potential sources of hydrocarbon and other valuable botanochemicals [12].

Calotropis procera, a latex yielding shrub commonly known as Milkweed belongs to the family Asclepiadaceae is common wasteland weed grows wild up to 2-5 m profusely without any management throughout the country on a variety of soils and in different climates, sometimes where nothing else grows. Several studies have been conducted in this plant species to evaluate its medicinal bioactive compounds and recently its latex content and wide availability draw the attention of researchers to evaluate its use in biomethanation [13]. However, studies were limited on the identification of potential hydrocarbons from this wide spread weed and its role in biogas production. The present study aimed to evaluate the presence of promising hydrocarbons from *C. procera* and also to correlate its potentiality as being a prominent supplement in biogas production.

2. Materials and Methods

Biomass collection and processing

The mature above-ground biomass (a total fresh weight of 2000 – 2500g) was collected from different arid wastelands around the foothill of Kolli Hills (Namakal District, Tamilnadu, India) which is an outlier of the Eastern Ghats (11º19′43″N and 78º17′42″E). The average annual precipitation in the area was 180 mm/year (2006-2007) and the average temperatures ranges from 29ºC to 38ºC. The collected plant materials were brought to the laboratory and allowed to air-dry in a sheltered area at 25 – 30C, then the subsamples (leaf, stem & whole plant) were ground in a Wiley mill to pass through a 1 mm screen.

Experimental procedure for bio-crude analysis

The sub-sampled air–dried materials were used for the analysis of harvest moisture content, dry matter production and ash content using standard procedures [14]. The sample were analysed for total N using Kjeldahl method and crude protein was calculated from the N value (6.25 x %N) and the percentage of C and H were determined by an elemental analyser (Perkin Elmer 2400) [12]. The milled sub-samples were subjected to acetone followed by hexane:aqueous ethanol (Water:Ethanol - 1:7) extraction to obtain 'oil' and 'polyphenol', and the residue was re-extracted with hexane to obtain 'hydrocarbon' fraction. The respective bio-crudes (oil, polyphenol and hydrocarbons) fractions were oven dried and the yields were calculated by weighing [2]. The extracted oil fraction was subjected to TLC using a carrier solvent mixture of hexane, diethyl ether and acetic acid (80:20:1) against a standard mixture of sitosterol, oleyl alcohol, oleic acid and oleyl laurate. Chromatographic plates were developed by placing them in an iodine chamber for 10-15 min and the respective R_f values were calculated based on observable spots. The hexane extraction of hydrocarbon fraction was subjected to IR spectroscopic analysis (Perkin Elmer 580B) and also to ^{1}H-NMR spectroscopic analysis (Varian T-60) using cadmium chloride as carrier solvent [12].

Experimental procedure for biogas production & estimation

The fresh leafy biomass of *C.procera* was collected and chopped in to pieces and mixed with cow dung in a 3:1 ratio on a fresh weight bases. The slurry with approximately 25% of total solids (TS) was placed in 2.5 litre glass digester bottles. 10% (v/v) of active inoculum was added and allowed for anaerobic bio-digestion at 28ºC for a period of 60 days. The inoculum was taken from a running biogas plant (KSR, Tiruchengodu) where FYM (cow dung with rice straw supplement) is used as raw material for biogas production. A separate experimental digester was also run without *C. Procera* biomass supplement as control. The biogas production was measured at 2 days interval by a water displacement method [6]. The composition of the slurry was analyzed for nutrients such as N, P, K and other organic components before and after the bio-digestion using standard procedures [6] to evaluate the fertilizer value of the slurry residue.

Statistical analysis

Statistical data analysis was carried out with the software package NCSS, version 2000. Sampling was replicated for three time for bio-crude and biomass analysis. Mean, standard deviation and standard error were determined. Variance analysis method (ANOVA) was used to find out significant differences in the treatment means for biogas production and slurry effluent analysis.

3. Results and Discussion

Biomass characterization

The harvest moisture content of *C. procera* was 67.60 % in leaf, 51.53 % in stem and 59.47 % in whole plant. The dry matter production was higher (140.37 g/kg fresh weight) in stem compared to other plant parts (Table 1). The results indicated the high content of crude protein (9.20 %) and N content (1.41 %) in stem. The highest carbon content was observed in stem (39.4 %) and hydrogen content was 5.83 % which can be a good indicator of the conversion capability of botanochemicals for low molecular weight fuels or petrochemicals [15]. The results on the elemental composition (Table 2) showed higher values for K (19040 ppm), available P (7120 ppm) and Na content (4256.67 ppm), expressing the possibility of *C. procera* biomass can be a good feed stock for fertilizer [16].

Table 1: Biomass and bio-crude composition of *C. Procera*

Biomass Components	Leaf (L)*	Stem (S)*	Whole Plant (WP)*
Harvest Moisture (%)	67.60 ± 0.8510	51.53 ± 0.4060	59.47 ± 0.5820
Dry Matter Production (g/kg FW)	68.77 ± 0.7760	140.37 ± 3.3830	106.57 ± 4.1600
Ash Content (%)	3.14 ± 0.0380	5.28 ± 0.0350	4.52 ± 0.0810
Crude Protein (%)	6.80 ± 0.1180	9.20 ± 0.0890	8.50 ± 0.0830
Oil (%)	1.54 ± 0.0473	3.97 ± 0.0551	3.14 ± 0.0379
Polyphenol (%)	3.33 ± 0.0436	4.86 ± 0.0393	4.00 ± 0.0732
Hydrocarbon (%)	1.65 ± 0.0459	3.16 ± 0.0417	2.65 ± 0.0471

Values are mean of three replicates ± SE

Table 2: Elemental composition of *C. Procera*

Elements	Leaf (L)*	Stem (S)*	Whole Plant (WP)*
Carbon (%)	32.5 ± 0.459	39.4 ± 0.530	36.30 ± 0.710
Hydrogen (%)	4.23 ± 0.033	5.83 ± 0.103	5.32 ± 0.063
Total Nitrogen (N) (%)	1.05 ± 0.041	1.41 ± 0.032	1.32 ± 0.035
Available P (ppm)	6330 ± 26.49	7120 ± 34.68	6796.67 ± 97.13
Potassium (K) (ppm)	12985 ± 74.08	19040 ± 88.98	16036.67 ± 114.2
Sodium (Na) (ppm)	3850 ± 37.90	4256.67 ± 53.70	4136.67 ± 32.86

Values are mean of three replicates ± SE

Bio-crude composition

The bio-crude yield was recorded as oil content (3.97 %), polyphenol content (4.86 %) and hydrocarbon content (3.16 %) (Table 1). The polyphenol content varied from 3.33 % to 4.86 % in *C. procera*, which were comparable to the data obtained for plants such as *Passiflora incornata* [17], *Euphorbia nerrifolia* [12], *Dodonaea viscose* [2]. Polyphenol is a generic term referring to a large complex of phytochemicals including polyphenolics, tannins, flavonoids, ligin and complex lipids. A moderate polyphenol content varying from 29 g/kg to 139 g/kg was reported in *Helianthus pumilus* and *H. Strumosus* respectively and it was suggested that large volume of polyphenols in low-cost plants can serve as important chemical feedstocks [18,19]. The oil content was observed significantly high in stem (3.97 %) compared to leaves and whole plant, which are comparable to *Aster ericoides* [20], in *C. procera* [12] and in some wild species of Western Ghats [2]. Even though the oil content of *C. procera* was not significantly high than other earlier reported species, it can be used as lubricant, ingredient for soap and raw material for candle industry [21]. The different components in the oil fraction extracted from *C.procera* were identified by TLC. The oil of *C. procera* exhibited 6 spots whose intensities were moderate at R_f 0.02, 0.05, 0.21, 0.41, 0.69 and strong at R_f 0.57. The standard mixture showed spots at R_f 0.06 (sitsterol), 0.10 (oleyl alcohol), 0.19 (oleic acid), 0.61 (oleyl laurate) and 0.68 (squaline). The R_f values of oil fraction when compared with the standard mixture clearly indicated the components were possibly sterols (at R_f 0.02 – 0.05), free fatty acids (at R_f 0.18 – 0.23), triglycerides (at R_f 0.42), wax esters (at R_f 0.60) and hydrocarbons (at R_f 0.66). The spot at 0.69, exhibited by the oil indicated the presence of a complex mixture of terpenes, waxes and low molecular weight polyisoprenes [12]. Similarly the spot at 0.21 exhibited the presence of oleic acid which indicates that the oil has a semi-drying property and hence, it may be potentially useful for the surface coating industry [22].

The IR spectra (Fig. 1) of hydrocarbon fraction obtained from *C. procera* exhibited peaks at 2980 cm^{-1} that showed the presence of C-H structure of methyl or methylene group. The split peaks observed at 1955 cm^{-1} and 1330 cm^{-1} could be due to the asymmetrical deformation vibration of –CH3 groups and due to the C-H bending absorption, respectively [12]. The ^{1}H-NMR spectrum of hexane extractable biocrude (Fig. 2) exhibited a strong singlet at 1.62 ppm comparable to the results of Kalita and Saikia (2003) (1.56 ppm) that confirmed the cis-configuration of the hydrocarbon fraction and the compounds showed peaks at 1.47 and 2.96 ppm could be assigned to alkyl groups. The similarity between the spectra of polyisoprene rubber and the hydrocarbon fractions suggested that the fractions were mostly polyisoprene rubber compounds [23].

Fig. 1. IR spectra of hexane extraction of hydrocarbon fraction of *C. procera*

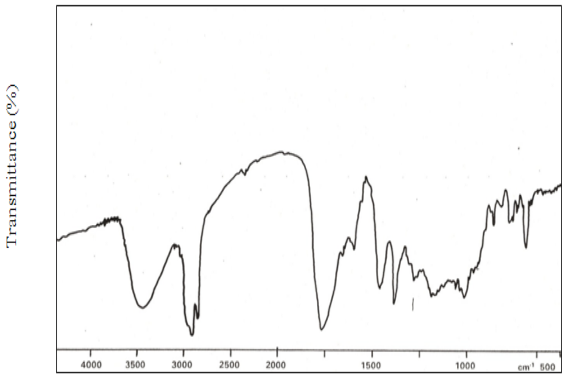
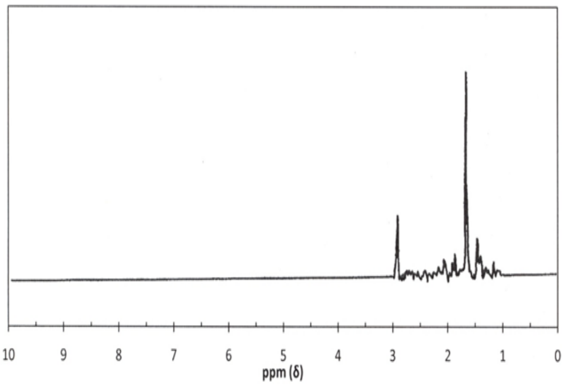


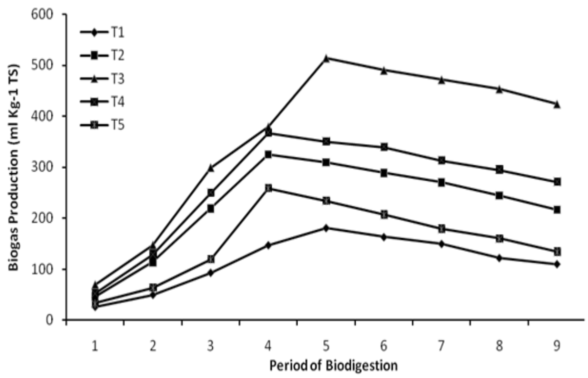
Fig. 2. 1H-NMR spectra of hexane extraction of hydrocarbon fraction of *C. procera*



Biogas production

The methane production through anaerobic digestion appears to be a competitive concept in both energy efficiency and environmental impact comparison studies [24]. The experimental results showed favourable effect of supplemented-digestion over control (Fig. 3). Significantly higher production of biogas was recorded in the test digesters than control digesters and the results also showed a steep increase in the gas production as the age of the digester increases. The possible reason for the higher gas production might be the organic and mineral composition of *C. procera* leaves that provided sufficient nutrients for microbes enabling increased anaerobic digestion. The availability of easily degradable compounds and various enzymes in the latex may also increase the digestion rate of feeding material in test digesters [13]. Similar positive effects of co-digestion of cattle dung and agricultural wastes have been reported in earlier studies. Biogas production from agricultural biomass is of growing importance as it offers considerable environmental benefits and it may be an additional source of income for farmers [11].

Fig. 3. Weekly biogas production from different treatments of *C. procera* and cowdung



The quality of biogas depends on the methane content and the proportion of gases (CH_4, CO_2, and O_2) depends on lipids and sugar content of the feeding slurry. Callaghan et al. [25] suggested that degradation of sugars give a mixture of equal volume of CO_2 and CH_4, whereas lipids give a greater percentage of methane. The results on chemical analysis of the slurry showed that fertilizer value of the digested slurry in the test digester was significantly higher than the control slurry in terms of organic as well as mineral content. According to Tafdrup [26] the resultant left over slurry having high N, P and K content can be used for agricultural application such as bio-fertilizers. The results of the present study on the nutrient analysis of the left over slurry showed significantly higher content of N, P and K content in the digested slurry with *C. procera* supplement than the control, possibly revealing the fertilizer value of the slurry (Table 3). According to Pakarinen et al. [27] the biogas production through anaerobic digestion is a widely applicable technology, as it can use agricultural wastes as substrates and the nutrients can also be recirculated. Weiland [28] has reported that the cattle manure has a lower potential to produce biogas and methane than the manure supplemented with biomass. The lower carbon content in the control digester might be due to the biodegradable carbon in cattle feed already digested in the rumen and in gut [29]. In our agreement with our results, Gunaseelan [30] has reported that different plant parts, harvest frequency, plant age, nutrient addition have a substantial effect on biogas and methane yield from sorghum.

Table 3: Nutrient contents of residual slurry of *C. procera* and its admixture

Treatments	Total Solids (TS) (%)		Volatile Solids (VS) (%)		Organic Carbon (%)		Nitrogen (N) (%)		Potassium (K) (%)		Available P (%)	
	BD	AD	BD	AD	BD	AD	BD	AD	BD	AD	BD	AD
T1 (1:0)	20.18	18.72	21.76	19.58	4.60	4.08	0.38	0.46	1.04	1.09	0.87	0.90
T2 (1:1)	26.22	24.81	24.11	22.92	6.41	6.03	0.44	0.69	1.17	1.21	1.09	1.12
T3 (2:1)	30.87	28.84	33.29	31.36	9.21	8.92	0.66	0.91	1.34	1.41	1.14	1.17
T4 (1:2)	27.88	26.11	29.13	28.16	7.36	6.92	0.57	0.66	1.24	1.30	0.97	1.14
T5 (0:1)	15.07	10.34	16.34	12.13	4.28	3.87	0.32	0.36	0.87	0.93	0.68	0.72
CD (0.05%)	0.125*	0.109*	0.082*	0.076*	0.122*	0.146*	0.162*	0.185*	0.091*	0.088*	0.087*	0.102*

*significance at 0.05 level BD – Before digestion AD – After digestion

4. Conclusions

The results of the present study demonstrated that milky weed *C.procera* can be used as a renewable source of hydrocarbon and intermediate energy resources. The present study also clearly demonstrated that a wide variety of chemical intermediates that are known to be major industrial feedstock such as sterols, long chain fatty acids, waxes, other hydrocarbons could be obtained from this whole plant. Moreover, it also exhibited high potential to serve as a supplementing material for biogas production. An extensive research is needed in future to evaluate the eco-physiology and hydrocarbon yield capacity of this weed on different agro-ecological conditions and efforts may be needed to increase the bio-crude potential of this species through genetic manipulation. Proper agro-technologies should be generated to cultivate this kind of weeds in large scale to harvest hydrocarbon materials as a substitute for the current fossil fuel, particularly in less developed areas of the world where a great deal of land are not suitable for food production.

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