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Phytochemical Estimation of the Biocrude of *Pedilanthus tithymaloides*- A Petrocrop with Pharmacognostic Properties

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Abstract

Researchers have taken an interest in finding crop species as alternative sources of nonconventional energy as fossil fuels or petroleum-derived fuels are quickly being reduced with the economic and political development and increasing use of automobiles. Hence, there is a scarcity of petroleum-derived fuels and industrial feedstock it has become essential to search for renewable sources of organic compounds. Solar energy is transformed into a by-product by green plants which are at par with synthetic petrochemicals. *Pedilanthus tithymaloides* L. Poit (Euphorbiaceae) has considerable potential as hydrocarbon yielding plant with its pharmacognostic properties also. Analysis of latex has revealed the presence of a large number of secondary metabolites. Extraction of bio crude was done in hexane using Soxhlet apparatus followed by column chromatography using solvents serially on the basis of their increasing polarity. The solvents used were hexane, benzene, ethyl acetate, acetone, methanol and chloroform in a sequence. Twenty-one fractions were collected from the extract and analysis of fractions was done using GLC. Fraction obtained in hexane: benzene (1:1) showed four major peaks at retention time 4.90, 5.10, 6.76 and 8.86 minutes at chart speed 1.0 cm/per minute. Fraction obtained in benzene and fraction obtained in benzene: ethyl acetate in the ratio of 1:1 and 1:3 showed various peaks. Major peaks obtained were at 2.98, 4.36, 5.71, 6.79, 8.76 and 9.11 minutes at chart speed.1.0 cm/min. in the fraction obtained in pure ethyl acetate. Qualitative phytochemical analysis of the plants revealed flavonoid, triterpenoids, sterols, alkaloids, saponins and anthraquinones in all the three varieties of *Pedilanthus tithymaloides* i.e. var. green, cuculatus and variegate

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Introduction

Increasing energy demands due to rapid industrialization, expansion of agriculture and enormous increase in population have not only resulted in the extensive depletion of non-renewable petroleum resources but also increased global warming and climate change. This environmental deterioration and loss of fossil fuels has promoted the research in search of alternative renewable energy. Hence, it is essential to look for new sources of organic compounds which can act as substitute of petroleum-based substances.[1,2] These sources are plants which are called Petrocrops as they produce petroleum substances as supplementary to petrol. These plants produce hydrocarbon compounds or ethanol. *Calotropis procera, Pedilanthus tithymaloides, Copifera langsdorfii, Euphorbia lathyris* and *Jaropha curcas* are examples of petro-crops. It has been reported that gasoline and identical compounds are obtained from plant hydrocarbons with the help of catalyst like Zeolite (ZSM-5 type).[3,4] In *Grindelia squarrosa* (Pursh) whole plant methylene chloride extract has been reported to be highly converted into liquid fuels.[4]. Such closely developed methods change the scope of fuels and biochemicals.

Most of the phytochemical crop studies by Calvin,5 are based on latex- bearing species, particularly *Euphorbia* species that can also be grown in arid and semi-arid regions of the world. A popularly known ornamental plant *Pedilanthus tithymaloides* (L.) Poit. (Euphorbiaceae), new scientific name *Euphorbia tithymaloides* (L.) Poit. is a succulent shrub commonly found in America and Asia. It is 0.4m to 3m tall and 40-60 cm wide. Some common names for *Pedilanthus tithymaloides* include Red Bird Flower, Devil's Backbone, Bock thorn, Christmas Candle, Fiddle Flower, Slipper Plant, Violin Flower, and so on. It thrives in various soil types, especially those with abundant micronutrients like Boron, Copper, Iron, Manganese, Molybdenum and Zinc. while it struggles to grow in highly saline soil. The plant can flourish when supplemented with fertilizers. Stem is olive-green with ovate leaves and beak-shaped flowers. laticifers are present in the whole plant. It is known as petro-crop and has medicinal importance also. Investigations have been carried out in the field of developing agro-technology for enhancing biomass and bio crude productivity of *Pedilanthus tithymaloides*.[6,7,8,9,10] There was a need to develop proper extraction technique and to identify the hydrocarbon compounds present in the plant. Research was carried out on extraction procedure of hydrocarbons in the University of Arizona. Analysis of latex has revealed the presence of a large number of secondary metabolites in *Calotropis procera*[11,12,13,14,15,16] *Euphorbia sikkinensis* Boiss[17] *Croton bonplandianum* Baill,[18] *Euphorbia hirta* [19,20,21,22] *Euphorbia lathyris*[23] Major classes of hydrocarbon like substances present in bio-crude include terpenoid, isoprenoid, polymers and long chain aliphatics. Hydrocarbons from *Pedilanthus macrocarpus* have been analyzed by H'-NMR indicating the alkane range $C_{27}H_{56}$ to $C_{35}H_{72}$.[24] Gas-liquid chromatography of *E. antisyphilitica* revealed the presence of $C_{29}H_{60}$, $C_{31}H_{62}$ and $C_{33}H_{68}$ compounds.[24] A new tetracyclic triterpene (3-epi-cyclolaudenal-22-en-3 alpha-ol) cyclolaudenol has been reported in *E. cauducifolia*.[25] Alcoholic extract of roots of *E. ferganensis* B. Fedtsh yielded on chromatography an aromatic ethyl ether ($C_{29}H_{1008}$, m.p. 158-160 degree Celsius) and a coumarin, scopoline.[26]

Phytochemical analysis was carried out on *P. tithymaloides* var. green, var. *cuculatus* and var. variegatus to find out the occurrence of various metabolites that might have potential pharmaceutical applications as well as use in biomass derived hydrocarbon like material of high-grade transportation fuel.

Materials and Methods

Plants of *P. tithymaloides* var. green, var. *cuculatus* and var. *variegatus* were harvested in the month of August and identified in the herbarium of Rajasthan University. Aboveground portions were dried and powdered. Thimbles of this powder were used for soxhlet extraction using low-boiling nonpolar hexane as solvent. The hexane extract of *P. tithymaloides* var. green obtained was then fractionated using the column chromatographic technique. Some of the fractions thus obtained were subjected to gas-liquid chromatography at the University of Rajasthan.

Column Chromatography

Silica gel H Glaxo grade was used for the packing of the column measuring 1 cm x 60 cm. The bio crude obtained by soxhlet extraction was filtered twice or thrice on Whatman filters paper No. 1. The filtrates obtained were mixed with silica gel and loaded on

a packed silica gel column. This was covered by a thin cotton layer. Different solvents were passed through the column serially according to their increasing polarity. The solvents used in the present study were hexane (having polarity 0.0), benzene (having polarity 2.7), ethyl acetate (having polarity 4.4), acetone (having polarity 5.1), methanol (having polarity 6.6) and chloroform (having polarity 9.4) in a sequence in the combination of pure solvent, 3:1, 1:1 and 1:3. Twenty-one fractions were collected from the extract based on colour.

Gas Liquid Chromatography

The fractions obtained from column chromatography were studied using the GLC technique. The chromatography used was Hewlett-Packard 5890A gas-liquid chromatography. 0.5µl fraction was injected on the 10-meter long HPL (Methyl Silicon gum column) of 0.53 mm diameter with a film thickness of 2.65 µm. The chart speed was 0.50-1.0 cm. per minute. Nitrogen was used as carrier at a flow rate of 40 ml/minute. The flow of hydrogen was 35 ml/minute and of air was 360 ml/minute. The column was initially set at 50˚C and it gradually increased to a final temperature of 300˚C at a rate of 15˚C per minute.

Qualitative phyto-chemical estimation of biocrude and plant dried samples–

To estimate the occurence of different hydrocarbons in the biocrude and plant-dried samples qualitative phyto-chemical assay was carried out using established methods proposed by Gibbs[27] and Harbone.[28]

Results and Discussion

In the present investigation ground plant parts including leaves and stems of all three varieties of *Pedilanthus tithymaloides* collected in urban areas were processed to obtain the profile of the phytochemicals present. The assay resulted in the detection of Flavonoids, Triterpenoids, Sterols, Alkaloids, Anthraquinones and Saponins. However, Tannins, Iridoids, Juglone, Leucoanthocyanins and Catechol were not detected (Table 1).

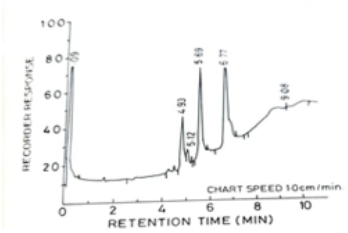
Table 1: Response of *Pedilanthus tithymaoloides* var. *green*, var. *cuculatus* and var. *variegatus* to different phytochemical tests

Phytochemicals	var. *green*	var. *cuculatus*	var. *variegatus*
Tannins	Not existing	Not existing	Not existing
Anthraquinones	Existing	Existing	Existing
Triterpenoids	Existing	Existing	Existing
Sterols	Existing	Existing	Existing
Alkaloids	Existing	Existing	Existing
Flavonoids	Existing	Existing	Existing
Iridoids	Not existing	Not existing	Not existing
Juglone	Not existing	Not existing	Not existing
Leucoanthocyanins	Not existing	Not existing	Not existing
Saponins	Existing	Existing	Existing
Catechol	Not existing	Not existing	Not existing

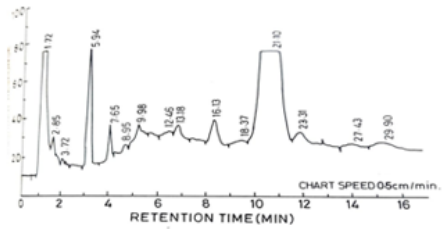
Column chromatography fractions of the bio crude when analyzed through GLC revealed peaks at different retention times for a large number of compounds that need further characterization. Fraction obtained in hexane: benzene (1:1) showed four major peaks at retention time 4.90, 5.10, 6.76 and 8.86 minutes at chart speed 1.0 cm/per minute. Fraction obtained in benzene and fraction obtained in benzene: ethyl acetate in the ratio of 1:1 and 1:3 showed various peaks. Major peaks obtained were at 2.98, 4.36, 5.71, 6.79, 8.76 and 9.11 minutes at chart speed.1.0 cm/min. in the fraction obtained in pure ethyl acetate. Gas-liquid chromatograms (1 -5).

Other workers also found similar results as hexane, ethyl acetate and ethanol extracts from leaves of *Pedilanthus tithymaloides* were tested phytochemically resulting in the detection of

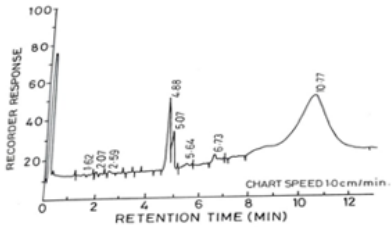
triterpenes, steroids, saponins, tannins and coumarins which were effective for antimicrobial activities and other biological properties.[29,30,31] Sandjo and coworkers[32] reported that nine coumarin derivatives derived from *Pedilanthus* were able to stop the germination of *Magnaporthe orizae* a phytopathogenic sporulating fungus. Antimicrobial activities of diterpenoids extracted from *Pedilanthus tithymaloides* were reported to be effective against *Mycobacteryum tuberculosis*.[33] The major components extracted from *Euphorbia* latex were tetracyclic triterpenoids mainly cycloartenol, 24-methylene cycloartenol and lanosterol.[34] It was reported that the latex of *E. lathyris* converts acetate and mevalonate to many tetracyclic triterpenes, mainly triterpenols and their fatty acid esters which are structurally different.[35,36] Detailed investigations carried out to find out the source of triterpene biosynthesis have revealed that an osmotically sensitive organelle of the plant cell and possibly calmodulin are involved in the triterpene biosynthesis.[34,37,38,39]



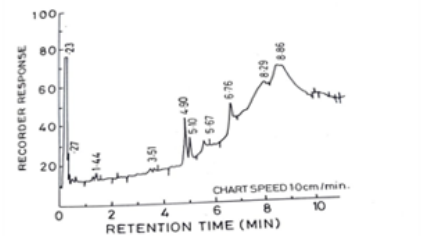
1. Gas –Liquid Chromatogram of fraction obtained in benzene : Ethyl Acetate (1:3)



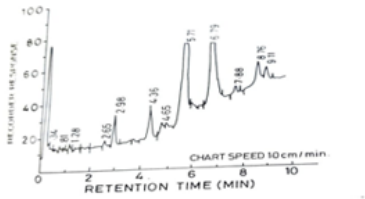
2. Gas –Liquid Chromatogram of fraction obtained in benzene : Ethyl Acetate (1:1)



3. Gas –Liquid Chromatogram of fraction obtained in benzene



4. Gas –Liquid Chromatogram of fraction obtained in Hexane : benzene (1:1)



5. Gas –Liquid Chromatogram of fraction obtained in Ethyl Acetate

Pedilanthus tithymaloides var. *green*

The experiments show that triterpenol and triterpene esters are synthesized at separate sites in a plant. Triterpene ester with triterpenols of the latex are synthesized in the wall lining cytoplasm of the laticifers in *Hoya diversifolia* Blume. and other *Hoya* species, (Asclepiadaceae),[40,41,42]

An assortment of solvents were utilized to extract pertinent phytochemicals, such as coumarins, tannins, steroids, triterpenes, and saponins from hexane and ethyl acetate extracts.[43] Kaempferol 3-O-o-D-glucopyranoside-600-(3-hydroxy-3-methylglutarate), quercitrin, isoquercitrin, and scopoletin were the antioxidants found in a bioassay. A total of 76 point 0 mg of gallic acid equivalents/g extract[44] was determined to be the phenolic and flavonoid contents. *Pedilanthus tithymaloides* was found to contain a set of four coumarin derivatives.[45] Steroids, cardenolides, anthraquinones, alkaloids, unsaturated steroids, phenolics, leucoanthocyanin

etc. were reported from *Pedilanthus tithymaloides*.[46] Plant chemicals such as flavonoids, terpenoids, phenols, tannins, saponins, glycosides, sterols, amino acids, and reducing sugars were found in the water filtrate of *Pedilanthus* leaves and stems. These secondary metabolites are useful in the creation of substitute bio-control agents that target mosquito vectors and agricultural insect pests.[47]

Twenty-one latex-bearing plants in the Indian state of Maharashtra were the subject of analysis. It was found that the secondary metabolites of these plants, which included *Pedilanthus tithymaloides*, were primarily alkaloids, flavonoids, terpenoids, cynogenic glycosides, phenolics, tannins, and saponins. These findings are consistent with the current study. The main finding was that all latex-bearing plants possessed pharmaceutically important secondary metabolites.[48] Hence, the experimental plant has pharmacognostic and energy potential.

Conclusion

The findings derived from phytochemical analysis of the three varieties of *P. tithymaloides* revealed that the plants possess flavonoids, anthraquinones, sterols, alkaloids, triterpenoids and saponins. In order to determine the presence of gutta, rubber, waxes, and other materials, a gas-liquid chromatogram of hydrocarbon compounds extracted in hexane, benzene, and ethyl acetate revealed a number of peaks that could be further identified by 1H NMR analysis, 13 C-NMR analysis, IR spectroscopy, Proton Magnetic Resonance (PMR) and Gel Permeation Chromatography (GPC). The plant species is a very powerful energy source because of the plant's readily extracted compounds, such as triterpenoids, which can be cracked to produce high-octane gasoline. It can be used as a feedstock for fuel, chemicals, and petroleum derivatives. All three varieties are promising for development as petrocrops as they provide high biomass and high hydrocarbon yield. Evidence from the literature also justifies the pharmacognostic potential of the plant for treating various ailments.

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Conflict of Interest

The author doesn't have any conflict of interest in the research.

Author's Contribution

The research and paper writing was performed by the author itself.

Data Availability Statement

The manuscript incorporate all datasets produced or examined throughout this research study.

Ethics Approval Statement

Experiment on no human or animal was performed during the study.

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