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Fatty acids composition analysis of seed-oil of the medicinal weed *Cleome rutidosperma* DC. (Cleomaceae) by Gas-Chromatography

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Abstract
Fringed spider flower or purple cleome (*Cleome rutidosperma*), an important wild weed, has long been known for medicinal and nutritional activities. A close similarity of the seed of it with the mustard seed leads us to investigate the lipids extracted from the seeds of purple cleome. Initial qualitative analysis of seed extract showed a characteristic oil marks in filter paper. Next the moisture content of the fresh seeds of Cleome was determined and it was found to be 84.10±1.28 %. Then the fresh seeds were dried, refluxed in 3N HCL and then subjected to solvents extraction using n-Hexane. Fatty acid composition of the extracted lipid through Gas Chromatographic (GC) analysis highlighted that the n-hexane fraction contains 41 peaks. Among them, the ester of 20 fatty acids were identified. Linolelaidic acid methyl ester, Cis9-oleic acid methyl ester, Methyl Palmitate, Methyl Octadecanoate are the major ones. Relative abundance of low amount of saturated fatty acid (SFA: 25.18%) and predominance of unsaturated fatty acids (MUFA: 23.20% and PUFA: 44.09%) may also make the Cleome seed oil useful for different anthropogenic uses including biodiesel production.

Keywords: Biodiesel; *Cleome rutidosperma;* gas chromatography; lipid, seed oil; medicinal weed

Introduction
Cleome rutidosperma DC. (CR) (Figure 1) is an annual herbaceous medicinal weed belongs to the family Cleomaceae and it is commonly known as Fringed Spider Flower. It is native to Tropical Africa [1]. The seeds of CR (Figure 2 & 3) have a stark resemblance in appearance with the seeds of an oil producing plant *Brassica nigra*DC.

Fig 1: Seed Containing Plant of *Cleome rutidosperma*, Fig 2 & 3 Seeds of CR

The seeds of CR were found to be eaten by ants and when tested, the seed extracts left a characteristic oil mark when passed through filter paper. Intrigued by the findings, it was decided to analyse the fatty acid composition of the seed-oil to find a new source of edible oil.

Materials and Methods
Chemicals
Fatty acid methyl esters (FAME) used as reference standards and BF_3-Methanol (20%) and other chemicals and reagents were analytical grade and purchased from Sigma-Aldrich, USA. All other chemicals and reagents used for this experiment were of analytical grade (AR) and were purchased from SRL Laboratory, Mumbai.

Collection and Identification
Fresh seed containing plants were collected from Salt Lake, West Bengal, India and were authenticated (Specimen No. PG-03) by Central National Herbarium, Botanical Survey of India.

Proximate Analysis
Moisture Content: Using Standard method, moisture content of the fresh seed was determined, by heating the seeds in microwave oven and noting the weights down at 30 seconds interval [2, 3].

$$\text{Moisture Content} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}}$$

Analysis of Fatty Acids Composition by Gas Chromatography (GC)
Solvent Extraction of Lipid
Dried Seed Samples were first refluxed in 3N HCL and then subjected to solvent extraction using n-Hexane.

Sample Preparation for GC Analysis
Fatty acid methyl esters were synthesized according to AOAC method. Briefly, Seed lipids (100 mg) of CR was refluxed with 2 ml of 8% methanol KOH for 30 min at 80°C and cooled.
Then 2 ml of BF_3-Methanol was added to the mixture and warmed for 20 min.
Then it was cooled and the methyl esters were obtained in n-heptane by phase separation.
The heptane solution was concentrated and filtered through 0.22 µm filter and it was ready for analysis.

GC Analysis

Fatty acid analysis was performed in a 7890B GC (Agilent Technologies, USA) equipped with a Flame Ionisation Detector (FID).

Separation was achieved using a fused silica capillary glass column HP-INNOWAX (30 m, 0.25 mm ID, 0.25 µm thickness) with an oven temperature programmed initially at 170°C, held for 1 min, raised to 200°C at the rate of 1°C/min and increased to 225°C during 5 min and was hold for 5 min. The injector and detector temperature were set at 250°C and 270°C, respectively. The sample volume injected was 1 µl with a split ratio 5:1. The carrier gas was nitrogen flowing at the rate of 1 ml/min. Hydrogen and compressed air used for FID were maintained at 275.6 KPa. The identification and quantification was performed by external standard method using Open Lab software for instrument control and data evaluation [4].

Results

The seeds of CR was found to contain around 84.10±1.28 % moisture. Upon GC Analysis, the result showed the relatively greater abundance of Methyl Palmitate, Methy Octadecanoate, cis-9-Oleic Acid methyl Ester, Linolelaidic acid methyl ester, along with trace amounts of Methyl Octanoate, Methyl Laurate and Methyl Undecanoate (Figure-4, Table-1) etc.

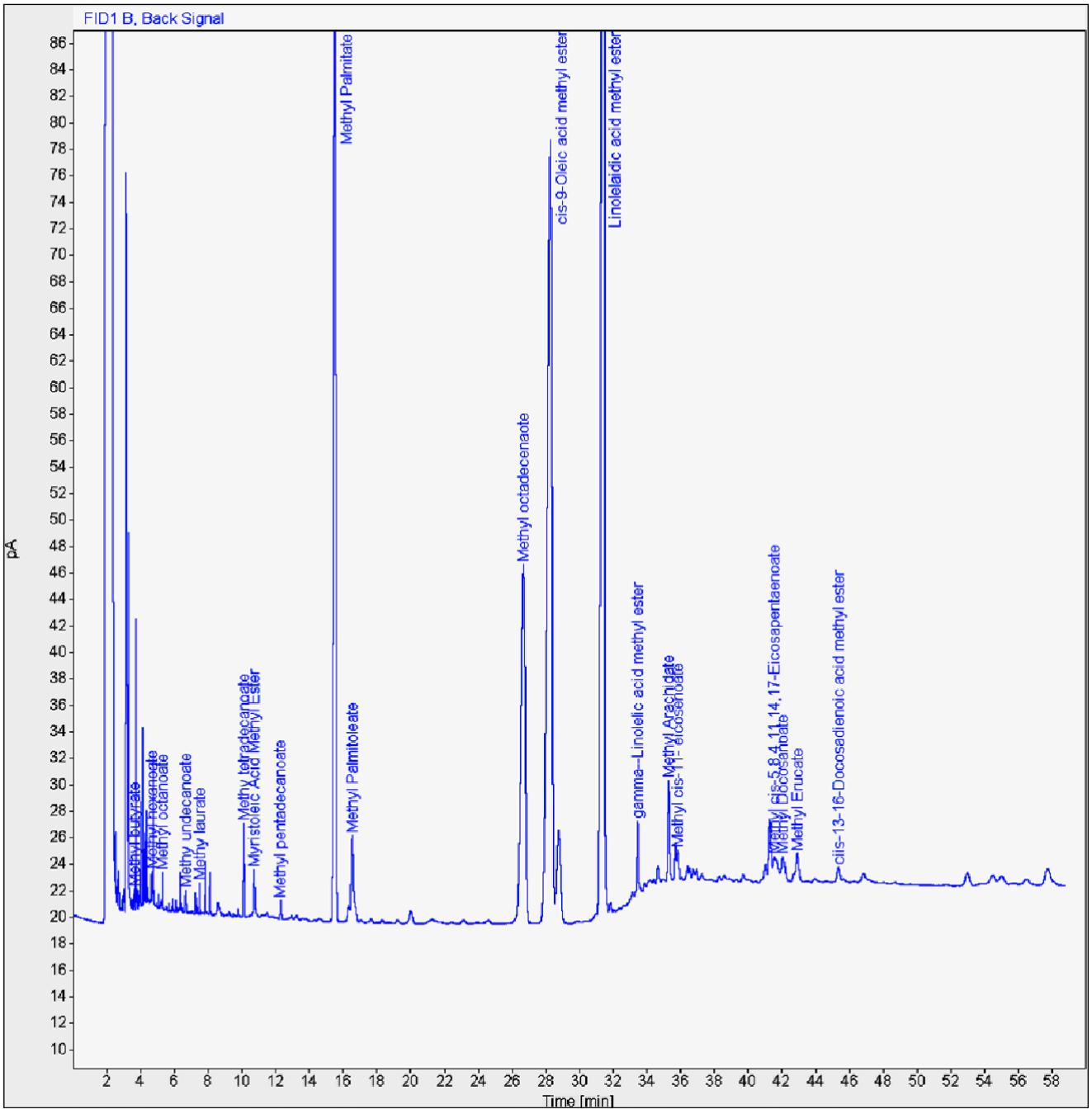

Fig 4: GC Plot for n-Hexane Fraction

Table 1: Fatty acid composition of *C. rutidosperma* seed oil

Sl. No.	Fatty acids	RT	Composition (%)
1	C4:0	3.61	0.06
2	C6:0	4.65	0.06
3	C8:0	5.29	0.05
4	C11:0	6.66	0.12
5	C12:0	7.51	0.08
6	C14:0	10.11	0.45
7	C14:1(n-9)	11.72	0.40
8	C15:0	12.30	0.13
9	C16:0	15.51	13.72
10	C16:1(n-9)	16.52	1.33
11	C18:0	26.67	9.04
12	C18:1(n-9)	28.27	20.59
13	C18:3(n-6)	31.44	42.50
14	C18:3(n-3)	33.45	0.62
15	C20:0	35.27	0.92
16	C20:1(n-9)	35.83	0.32
17	C20:5(n-3)	41.54	0.72
18	C22:0	42.03	0.55
19	C22:1(n-9)	42.90	0.56
20	C22:2(n-6)	45.34	0.25
Summary			
SFA			25.18
MUFA			23.20
PUFA			44.09
Others unidentified			7.53

Conclusion

The lipid content of the seed is low to moderate[5] but its non-toxic nature as evident from ant's affinity towards seed, creates an aura of enthusiasm. Though the two essential fatty acids are not detected in GC data, conditionally essential fatty acid gamma-linolenic acid (an omega-6 fatty acid) is present.

Substantially low content of saturated fatty acids theoretically negated the risk factor for consumption. For detailed insights of fatty acid composition, a GC-MS analysis of the lipid sample is needed to be performed. Quantitative phytochemical, nutritional value determination and antioxidant status and *in vitro* pharmacological and cytotoxicity studies were performed in different solvent extracts by previous studies which confirmed the potentiality of the plant leaves as medicinal uses for future purposes [6-10].

Hence *Cleome rutidosperma* may be considered as a potent source of food and nutrition subject to successful cytotoxicity tests of seed extracts on human cell lines. The oil can be used in pharmaceutical purposes as well. Also, unsaturated fats tending to remain in liquid state at room temperature, lipids obtained from seeds of *Cleome rutidosperma* can be a potent feedstock for biodiesel production.

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Conflict of Interest

The author declares no conflict of interest.

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