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STUDIES ON PHYSICOCHEMICAL PROPERTIES AND FATTY ACID PROFILE OF SEED OIL FROM TWO *HIBISCUS SPECIES*

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ABSTRACT

A study on the seed oil of two *Hibiscus species- Hibiscus cannabinus* and *Hibiscus sabdariffa* were analyzed to establish their physicochemical properties and fatty acid using standard methods. The oil was extracted using hexane (40-45°) by Soxhlet apparatus and fatty acid profile was determined using Gas chromatography (GC) analyser. The present study shows that, *Kenaf* and *roselle* had golden yellowish oil color and oil content was found to be 24% (*Kenaf*) and 22% (*Roselle*). The acid value (3.02: 2.80 mg KOH/g), specific gravity (0.88:0.86), refractive index (1.46: 1.45), moisture content (1.88: 1.46%), viscosity (53.00:52.70mPas), FFA (0.77: 0.65%) and saponification values (172:170mgKOH/g) were higher in *Hibiscus cannabinus* when compared to that of *Hibiscus sabdariffa* whereas iodine value (133:177gI/100g), peroxide value (13.3: 26.6meq/kg) and Unsaponifiable matter (0.052: 0.85%) were lower in *Hibiscus cannabinus* when compared to that of *Hibiscus sabdariffa*. Both plant seed oils were rich in oleic and linoleic acids. The seed oil with highest amount of polyunsaturated fatty acids can find an application in surface coating industries biolubricant base oil applications, whereas the high amount of monosaturated fatty acid can find an application as a biodiesel feedstock. The evaluation of fatty acid composition using gas chromatography revealed that, *Hibiscus cannabinus* contained higher amounts of oleic acid (28.91%) and linoleic acid (38.49%) and significantly lower amounts of stearic (3.96%) and palmitic acid (20.75%) than *Hibiscus sabdariffa* seeds (25.16%, 44.72%, 18.52% and 4.31%, respectively).Hence, two plants of *Hibiscus species* seed oil has a great potential for industrial applications such as in paint and surface coatings, biolubricant and production of biodiesel. Therefore, it is crucial to have more research on these two plants seed oil in the future to explore its potential as a future biodiesel oilseed crops.

KEYWORDS: *Hibiscus cannabinus*, *Hibiscus sabdariffa*, physicochemical properties, fatty acids, oil and seed characterization.

1. INTRODUCTION

Hibiscus species are the plants which belong to Malvaceae family and are native to Southern Asia and West Africa respectively. These two plants can grow well under such adverse climate because of their low moisture demands, fertility requirements and tolerance to high temperatures and are a drought-resistant warm season annual or biennial, herbaceous plants growing to about 16-20 ft tall with a woody base in a wide range of soils.[15] The stems are 1-2cm in diameter and often but not always branched. The leaves are 10-15cm long, variable in shape with leaves near to the base of the stems. The flowers are 15-18cm in diameters, white yellow or purple, and when white or yellow the centre is still dark purple. The fruits are capsule of 2cm in diameter containing around 20-25 seeds.[16]

These are quick growing crops and seeds become capable of having their oil extracted after 4-5 months of plantation depending on the soil quality and rainfall. The annual yield of these two plants is in the range from 0.5 to 12 tons and can also grow at low and high altitude areas that have an average annual temperature above 20°C and can tolerate slight frost. The cultivation of these two plants is successful in the tropics with the annual rainfall of 2500,000 mm.[10] The marginal soil quantities with a low nutrient content[18] are sufficient for growing of *Hibiscus cannabinus* and *Hibiscus sabdariffa* plants.

Hibiscus cannabinus and *Hibiscus sabdariffa* are the plants with multifunctional properties with many attributes and considerable potential. The various parts of these two plants have may useful applications[19] like Stems are used in making rope, cordage, canvas, sacking, carpet backing, fishing nets, jute fibers and domestic purposes. Leaves are used as vegetables in preparing salad and for cooking purpose. Seeds contain oil which is used as good lubricant oil and making soap, flowers or calyces are used in preparing tea. And can be used as medicine in treating diuretic, mild laxative, relieve dysuria, bruises, fever, puerperium, anemia, fatigue, blood and throat disorders, treating acidity and treatment for cardiac and nerve diseases and cancer. The oil cake, a byproduct remaining after the extraction of oil can be used in the form of food for cattle and as an organic fertilizer. These two plants oil is nowadays considered as on-edible oil due to the presence of anti-nutritional factors. The cultivation of *Hibiscus cannabinus* and *Hibiscus sabdariffa* may be advantageous to farmers

due to the fact of soil erosion prevention, their ability to act as living fence and reclamation of waste land.[18]

2. MATERIALS AND METHODS

A. Collection of Plant Materials

The seeds and the leaves sample of two *Hibiscus species* were collected from Kadganchi, Tengli, Aland and supermarket of Gulbarga district, Karnataka, India. The oilseeds and leaves had some foreign materials and dirt, which were then removed by thorough washing followed by sun-drying for 5 days. The oilseeds were winnowed to remove the chaffs. Finally, the cleaned oilseeds and leaves were made into powder by grinding with an electric grinder.

B. Extraction of Oil

A 500ml soxhlet apparatus and hexane as solvent were used for this study. Initially the apparatus was charged with known (50g) weight of two *Hibiscus species* oilseeds powder which was placed in a thimble of soxhlet apparatus. A round bottom flask containing known volume (150ml) of hexane was fixed to the end of the apparatus and a condenser was tightly fixed at the bottom end of the extractor. The whole set up was heated up in a water bath at temperature of 45ºC. The excess solvent in the oil was recycled by heating in a heating mantle at the temperature 45ºC after the extraction. Quantity of oil extracted was determined gravimetrically. The oil yield was evaluated as the ratio of the weight of the extracted seed oil to the weight of the two plants of *Hibiscus species* oil seed powder sample as described below:-

$$\textbf{\% Oil yield (w/w)} = \frac{\text{Wt. in g of extracted oil}}{\text{Wt. in g of oilseed powder sample}}$$

The obtained seed oil was stored appropriately for further physicochemical analysis.

C. Physicochemical Analysis

The evaluation of the following physicochemical properties of the extracted seed oil was determined by the following methods:

1. Oil content/ percentage

The oil was obtained by using Soxhlet extraction method. And the oil yield was evaluated as the ratio of the weight of the extracted seed oil to the weight of the oilseed powder sample as described below.

OIL YIELD (%) - $\frac{\text{Weight in gram of extracted oil}}{\text{Weight in gram of oilseed powder sample}}$

2. Specific gravity

Specific gravity of *Hibiscus cannabinus* and *Hibiscus sabdariffa* seed oil was determined by specific gravity bottles method using IS 1460-2000. The experiment was conducted at 25°C and specific gravity of the sample was calculated by using the formula-

Specific gravity = (W3-W1)/ (W2-W1)

Where, W1-weight of empty specific gravity bottle, W2-weight of water + specific gravity bottle, W3-weight of test sample + specific gravity bottle.

3. Refractive index

The refractive index of *Hibiscus cannabinus* and *Hibiscus sabdariffa* was determined by a standard instrument employing the principle of the critical angle using diffused daylight as suggested by the standard methods of AOAC (1997). The experiment was conducted at 20°C.

4. Moisture content

The seeds were cleaned manually to remove all foreign matter such as dirt, dust, stones and chaff as well as immature and broken seeds. The initial moisture content of seeds was determined by oven drying at 105°C for 24hrs. The initial moisture content of seeds was 0.02±0.01 d.b. The samples of desired moisture contents were prepared by adding the amount of distilled water as calculated from the following relation (Sacilik et al. 2003).

Q= Wi (Mf-Mi)/100-Mf

Where Wi- initial mass of sample in kg, Mi- initial moisture content of sample in % d.b and Mf- final moisture content of sample in % d.b.

The samples were then poured into separate polythene bags and tightly sealed. The samples were then kept at 5°C in a refrigerator for a week to enable moisture to distribute uniformly throughout the sample. Before starting a test, the required quantity of the seeds were taken out of the refrigerator and allowed to equilibrate to the room temperature for about 2 hrs (Singh and Ghoswami, 1996; Cokun et al. 2006).

5. Viscosity

The oilseeds viscosity was calculated according to the Standard AOAC methods (1997).

6. Acid value

Standardization of KOH

20 ml of 0.1 N oxalic acid solution was taken in a 250 ml conical flask. Add 1-2 drops of phenolphthalein indicator was added to this solution. And finally, the oil sample was titrated against KOH and the appearance pink colour indicates the end point. Later, the by knowing the volume of the KOH solution in burette, the normality of KOH was determined.

Procedure

5gm of oil was weighed and transfer to a 250 ml conical flask. Later, 50 ml of neutralized alcohol solution was added to the oil solution and then incubated in hot waterbath for about 10 minutes. After incubation, 1-2 drops of phenolphthalein indicator was added and finally, titrated against KOH solution KOH and the appearance pink colour indicates the end point.

Standardisation of Potassium hydroxide was calculated by using the formula

Normality of KOH consumed (N2) = V1 N1 / V2

Where V1-Volume of oxalic acid, N1- Normality of oxalic acid, V2- Volume of KOH consumed and N2- Normality of KOH

The Acid value of the oil sample was calculated using the formula-

Acid value = (Volume of KOH x Normality of KOH x Eq. wt x 1000) / Weight of Oil sample.

7. Free fatty acids

10gm of oil was weighed and dissolved in 40ml isopropanol in a 250ml conical flask. Then 2-3 drops of phenophthalene indicator was added to the oil sample and then the sample was titrated against 0.1 N NaOH till the formation of pink colour appears.

Acid value= Titre value x normality of NaOH x 28.2/wt. of the sample (g).

8. Saponification value

2g of oil was taken in a 250 ml of conical flask and then 25ml of alcoholic KOH was added to the sample to dissolve the oil completely. Then, incubate the test tube containing sample for about 30min, cooled to room temperature. Later, few drops of phenophthalene indicator was added and mix vigorously. And finally, the oil sample was titrated against 0.5N HCL till pink colour disappears. Treat the blank similarly in absence of oil.

Saponification value= 28.05 x (Titre value of blank- Titre value of sample/wt. of sample (g).

9. Iodine value

0.1g of oil was taken in a 250 ml of conical flask and then 10ml chloroform was added followed by 25ml of iodine solution. And incubate the tubes in dark for about 30minutes. After incubation, add 10ml of potassium iodide (KI) followed by adding 100ml distilled water along with few drops of starch indicator. And finally, the oil sample was titrated against 0.1N sodium thiosulphate to get the clear solution. Treat the blank similarly in absence of oil.

Iodine value= (B-S) x N x 126.9/gm of oil sample

Where B- ml of sodium thiosulphate used in titration of blank, S- ml of sodium thiosulphate used in titration of sample, N- Normality of sodium thiosulphate solution.

10. Peroxide value

3g of oil was weighed in a 250ml of conical flask and then 18ml of acetic acid : chloroform mixture was added to the oil sample followed by adding 0.5ml of saturated KI solution and allowed the test tubes to stand for 1minute. Later, around 260 ml of water along with 3-4 drops of starch indicator was added and mixed well. Finally, the sample was titrated against standard 0.01 N sodium thiosulphate and observed till the disappearance of dark brown colour. Treat the blank in absence of oil. Peroxide value of the sample was calculated by using the formula-

Peroxide value=AxNx1000/wt. of oil meq / kg oil

Where A- ml of sodium thiosulphate (Test- Blank) and N- Normality of sodium thiosulphate solution

11. Unsaponifiable matter

Unsaponifiable matter content of oil samples was determined by the following IUPAC method (1979). 100mg of unsaponifiable fraction was dissolved in 2ml of chloroform. To 1ml aliquot, 4ml of a trifluoroacetic- chloroform(1:3, v/v) solution was added. And then the absorbance of the sample was measured at 620nm using spectrophotometer.

D. Fattyacid Composition by Gc Analysis

Fatty acid composition of the seed oil of two plants were determined by using an Agilent 7890A GC equipped with a flame ionization detector (FID) and an auto sampler. Peak separation was performed on a DB-23 capillary coloumn from Agilent Technologies. The carrier gas was helium set o a constant inlet pressure of 12,000psi. A Fatty acid methy ester (FAME) standard mix was used to establish pek retention times for 20 unique fatty acids. All

the standards were purchased from sigma Aldrich.1µl of sample was injected at 30:1 split ratio into the column with the following thermal profile: 185°C for 3min; 185-190 at 5°C/min; 190-240 at 10°C/min. The inlet and the detector were set to 280 and 300°C respectively. Total run time for each sample was 9min. Fatty acid composition of the seed oil of two plants were determined by identifying and calculating relative peak areas.

3. RESULTS AND DISCUSSION

The data collected from the study of the physical and chemical properties of the test sample of two *Hibiscus species* was determined by dry weight. Oil content of *Hibiscus cannabinus* and *Hibiscus sabdariffa* was found to be good which is 24% and 22% respectively. High oil content of *Hibiscus species* indicates that these two tropical species are suitable as edible vegetable oil feed stock in oleo chemical industries, biodiesel, fatty acid, soap, fatty nitrogenous derivative, surfactants and detergents etc. The specific gravity of two *Hibiscus species* is 0.88 and 0.86. Experimental result showed that the two *Hibiscus species* oil seed has FFA content of 0.77% and 0.65%. The moisture content of these two plants was found to be 1.88% and 1.46% which have significant effects on the transterification of glycerides with alcohol using catalyst. Saponification values of the studied oil samples were 172 mg KOH/g and 170.8 mg KOH/g respectively. The iodine value measured of the unsaturation of fats and oils was higher which indicates that the sample with higher unsaturation of fats and oils and the values of *Hibiscus cannabinus* and *Hibiscus sabdariffa* were found to be 133I/100g and 177 I/100g respectively and these values place the two plants in the semi drying oil group. The peroxide values of two *Hibiscus species* were found to be 13.3meq/Kg and 26.6meq/Kg and the unsaponifiable matter was found to be 0.052% and 0.85% respectively. The refractive index, acid value and viscosity of *Hibiscus cannabinus* values were high when compared to that of *Hibiscus sabdariffa* **(Table 1).**

Table 1. Physicochemical properties of two *Hibiscus species*

S.No.	Parameters	*Hibiscus canabinus*	*Hibiscus sabdariffa*
1	Colour	Light Yellowish green	Dark yellowish green
2	Seed oil content	21-24%	22%
3	Specific gravity	0.88	0.86
4	Refractive index	1.46	1.45
5	Moisture content	1.88%	1.46%
6	Viscosity	53.00 mPas	52.70 mPas
7	Acid value	3.02 mgKOH/g	2.80 mg KOH/g
8	Free fatty acid	0.77%	0.65%
9	Saponification value	172 mgKOH/g	170.8 mgKOH/g
10	Iodine value	133 gI/100g	177 gI/100g
11	Peroxide value	13.3 meq/kg	26.6meq/kg
12	Unsaponifiable matter	0.052%w/w	0.85%w/w

Fatty acid composition of these two plants was determined by GC analysis **(Figure 1).**

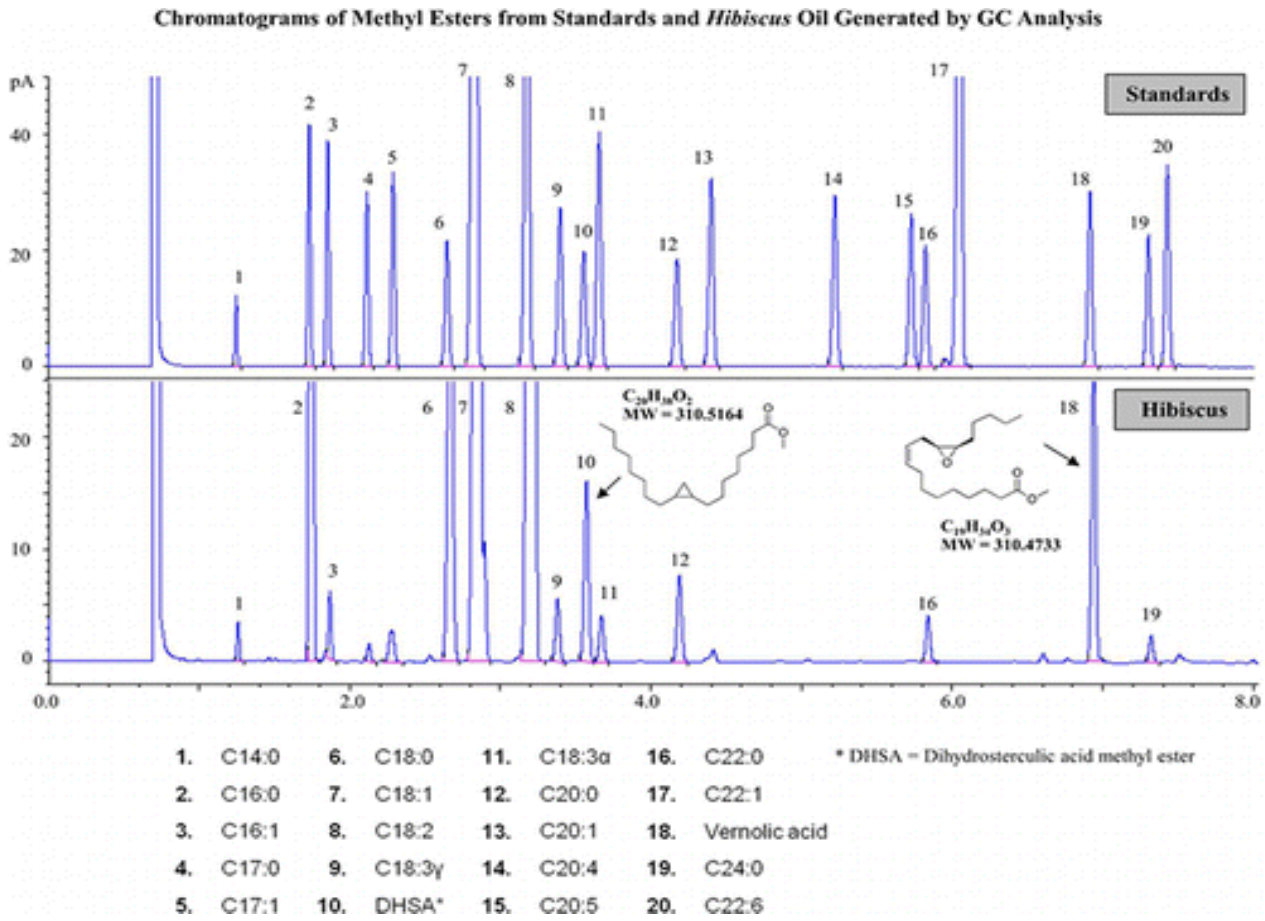

Fig1. Fatty acid profile generated by GC analysis.

There were significant differences in investigated seed traits between *Hibiscus cannabinus* and *Hibiscus sabdariffa*. *Hibiscus cannabinus* contained higher amounts of oleic acid (28.91%) and linoleic acid (38.49%) and significantly lower amounts of stearic (3.96%) and

palmitic acid (20.75%) than *Hibiscus sabdariffa* seeds (25.16%, 44.72%, 18.52% and 4.31%, respectively). From the investigated seed traits, overall *Hibiscus cannabinus* is a better potential source of oil than *Hibiscus sabdariffa* for production of biodiesel and industrial utilization **(Table 2)**.

Table2. Fatty acid composition of two *Hibiscus species*

S.No.	Fatty acids		*Hibiscus cannabinus* (%)	*Hibiscus sabdariffa* (%)
1	Oleic acid	C18:1	28.91	25.15
2	Linoleic acid	C18:2	38.49	44.72
3	Plamitic acid	C16:0	20.75	18.52
4	Stearic acid	C18:0	3.96	4.31
5	DHSA		1.08	1.57
6	Vernolic acid		4.16	3.52

CONCLUSION

The present study shows that, *Hibiscus cannabinus* and *Hibiscus sabdariffa* seed oils are rich in oleic and linoleic acids. The seed oils of these two plants with the highest amount of polyunsaturated fatty acids like linoleic can find the application in surface coating industries and biolubricant based oil applications, whereas the high content of monounsaturated fatty acid can find an application as biodiesel feedstock.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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