# Technical

# Gas Chromatographic Identification of Fatty Acids, Fatty Alcohols, and Hydrocarbons of *Hibiscus rosa-sinensis* Leaves

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## ABSTRACT

*Hibiscus rosa-sinensis* leaves (family Malvaceae) were analyzed for their fatty acid, fatty alcohol, and hydrocarbon contents. Wax hydrocarbons ranging from $C_{16}$ to $C_{32}$ with $C_{23}$, $C_{25}$, $C_{27}$, and $C_{31}$ as major components and wax alcohols between $C_{21}$ and $C_{30}$ with $C_{26}$, iso-$C_{28}$, and iso-$C_{30}$ as major components were found to be present in the petroleum ether fraction of the leaves. Fatty acids ranging from $C_8$ to $C_{28}$ with $C_8$, $C_{12}$, $C_{14}$, $C_{16}$, and $C_{18:2}$ as major components were found in the combined form. Two cyclic acids, sterculic and malvalic, have also been identified.

## INTRODUCTION

*Hibisuc rosa-sinensis* is a common ornamental plant widely grown throughout India. Detailed chemical investigation of the plant was made because of its medicinal use (1) in the Indian system of medicine. The combination technique, thin layer (TLC) or column chromatography along with gas chromatography, has been used for the identification of fatty acids, alcohols, and hydrocarbons by a number of workers (2,3). We have also successfully employed the same technique for the identification of the waxy constituents of the leaves. However, the isolation and identification of the waxy constituents have merits of their own since a review of literature reveals none of these compounds reported earlier from the *Hibiscus rosa-sinensis* leaves.

## EXPERIMENTAL PROCEDURES

Dried powder OUOG) of the leaves was extracted with petroleum ether (40-60 C) in a soxhlet. The extract was concentrated to 500 ml, and sufficient quantity of 100 ml methanol was added till no further precipitation was observed. Total solution was kept overnight. The supernatent liquid was removed, and the residue (3 g) obtained was subjected to column chromatography. A small part of this syrupy mass was also used for TLC over Kieselgel G (0.25 mm thick plates) using petroleum ether:benzene (65:35) as a developing solvent. Spots were visualized by spraying with 70% $H_2SO_4$ containing 0.5% $CuSO_4$ and heating in an oven at 150 C for 15 min. Four spots of $R_f$ 0.92, 0.54, 0.12, and 0.03 were noted.

### Column Chromatography

Total residual mass was transferred to alumina column 20 cm long x 3 cm ID after dissolving in $CHCl_3$. Fractions (30 ml) of the eluents were collected, a small portion of each fraction was further run on TLC plates as before, and fractions with identical patterns were combined.

### Wax Hydrocarbons

Fractions 1 and 2 (Table I, 516 mg) were combined and crystallized with acetone. IR spectrum of this combined fraction showed it to be alkane in nature with absorption 725 and 715 $cm^{-1}$ and no absorption in the hydroxyl region. A known quantity of the mixture was used for gas liquid chromatography (GLC) analysis on Shimadzn Model GC-4 APF gas chromatograph using a 2 m long x 4 mm ID column packed with Shimolite W (80-100 mesh) coated with 1.5% OV-1 operating at 250 C. Hydrogen was used as carrier gas with flow rate at 66 ml/min, chart speed at 5 mm/min.

### Fatty Alcohols

Fractions 3 and 4 (Table I, 686 mg) were combined in

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TABLE I

Particulars of the Column Chromatographic Fractions

| Fraction number | Eluent | Weight (g) | $R_f$ |
|---|---|---|---|
| 1-2 | Petroleum ether | 0.516 | 0.92, 0.89 |
| 3-4 | Petroleum ether | 0.686 | 0.54, 0.50 |
| 5-7 | Petroleum ether | 0.121 | 0.50, Steak |
| 8-11 | Petroleum ether | 0.112 | 0.52, 0.12 |
| 12-13 | Petroleum ether:benzene | 0.080 | 0.12, 0.10 |
| 14-15 | Petroleum ether:benzene 75:25 | 0.170 | 0.12, 0.10 |
| 16-18 | Benzene: petroleum ether 50:50 | 0.421 | 0.03, 0.12 |
| 19-20 | Benzene:petroleum ether 75:25 | 0.516 | 0.03, Steak |
| 21-24 | Benzene | 0.060 | 0.03, Steak |
| 25-28 | Benzene:chloroform 90:10 | 0.0084 | 0.03, Steak |
| 29-33 | Benzene:chloroform 75:25 | 0.042 | 0.03, Steak |
| 34-38 | Benzene:chloroform 50:50 | 0.030 | 0.03, Steak |
| 39-43 | Benzene:chloroform 75:50 | 0.024 | 0.03, Steak |
| 44-48 | Chloroform | 0.021 | 0.03, Steak |

TABLE II

Distribution of Hydrocarbons, Fatty Acids, and Fatty Alcohols of *Hibiscus rosa-sinensis* [a]

| Carbon chain length | Hydrocarbon (%) | Acids (%) | Alcohols (%) |
|---|---|---|---|
| 8 | - | 25.3 | - |
| 10 | - | traces | - |
| 11 | - | traces | - |
| 12 | - | 50.5 | - |
| 13 | - | - | - |
| 14 | - | 10.2 | - |
| 15 | - | - | - |
| 16 | traces | 1.2 | - |
| 17 | - | - | - |
| 18 | - | 1.7 | - |
| 18.1 | - | - | - |
| 18.2 | - | 2.3 | - |
| Cyclo-$C_{18}$[b] | - | 3.2 | - |
| Cyclo-$C_{19}$[c] | - | 4.5 | - |
| 20 | - | - | - |
| 21 | traces | traces | traces |
| 22 | - | - | - |
| 23 | 16.2 | traces | - |
| 24 | - | traces | - |
| 25 | 1.9 | - | - |
| 26 | traces | - | 78.0 |
| 27 | 60.8 | - | - |
| 28 | - | - | - |
| iso-28 | - | traces | 8.6 |
| 29 | traces | - | - |
| iso-30 | traces | - | 12.8 |
| 30 | traces | - | - |
| 31 | 20.2 | - | - |
| iso-32 | traces | - | - |

[a]Component of < 0.1% has been indicated as "traces."
[b]Malvalic acid (2-octyl-1-cyclopropene-1-heptanoic acid).
[c]Sterculic acid (2-octyl-1-cyclopropene-1-octanoic acid).

chloroform (20 ml) and purified by repeated precipitation by adding methanol dropwise. IR spectrum of this fraction showed it to be alcoholic in nature with peak absorption at 3315 $cm^{-1}$ (OH stretching), 1055 $cm^{-1}$ (C-O, stretching for primary alcohol), and 725 and 715 $cm^{-1}$ (alkane chain). The fraction was analyzed on a Varian aerograph series 200 instrument using a stainless steel column (1.5 m length, 3.3 mm diameter) filled with acid washed Chromosorb W (60-80 mesh), detector temperature 340 C; column temperature 255 C, $N_2$ at a flow rate of 55 ml/min, as carrier gas; detector, flame ionization; chart speed 2.5 cm/min.

### Fatty Acids

The fractions (16-20) (Table I, 937 mg) were taken together and purified by dissolving in 20 ml hexane and then adding 85% aqueous alcohol dropwise till complete precipitation. IR spectrum of the purified mass showed absorption bands at 1735 $cm^{-1}$ (C=O, stretching), 1170 $cm^{-1}$ (C-O, ester stretching), and 710, 720 $cm^{-1}$ (alkane chain). Total mass was saponified with 20 ml 2N methanolic KOH for 24 hr at 40 C. Reaction mixture was concentrated, then diluted with water and washed with ether to remove unsaponifiable material. The remaining water solution was treated with dilute HCl to liberate fatty acids and then extracted with ether. Free fatty acids were converted to methyl esters by treating with $CH_2N_2$. The unsaponified material gave Liebermann-Burchard test, thus indicating that acids were mostly present as sterol esters. A known quantity of this methyl ester was subjected to a GLC analysis column 3.04 m long, 2 mm ID packed with Chromosorb W (60-80 mesh) coated with 20% diethylene glycol succinate; temperature programmed from 180 to 210 C at the rate of 0.2 C/min; chart speed 30 cm/hr; detector, flame ionization.

## RESULTS AND DISCUSSION

The petroleum ether extract of *Hibiscus rosa-sinensis* leaves has been found to contain, straight chain hydrocarbon ranging from $C_{16}$ to $C_{32}$ with $C_{23}$, $C_{25}$, $C_{27}$, and $C_{31}$ as major components (Table II). The odd numbered hydrocarbons were found to be present in higher concentration. Fatty acids were mostly of even numbered carbon atoms between $C_8$ and $C_{28}$. Major fatty acids were $C_8$, $C_{12}$, $C_{14}$, $C_{16}$, $C_{18}$, and $C_{18:2}$. Wax alcohols were mainly of even numbered carbon atoms between $C_{21}$ and $C_{30}$. $C_{26}$, iso-$C_{28}$, and iso-$C_{30}$ were the major wax alcohols. Two cyclic acids, sterculic and malvalic, were also identified in the extract.

The identifications of individual compounds were carried out by comparison with standard substance or by bromination (the unsaturated disappeared on GLC). The position of double bond in the unsaturated acids was not verified. Percentage of the compounds was determined by measuring the area of the individual peak.

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