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# Isolation and identification of few fatty acid esters from the aerial roots of *Rhaphidophora aurea* twined over different host trees

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Plants are the store houses of various secondary metabolites and bio-essential natural products. Several therapeutic agents are isolated from *Rhaphidophora aurea*. The aerial roots of the mentioned plant twined over different trees were explored. Sequential extraction (Polar to non-polar solvents) was carried out for the aerial roots of *Rhaphidophora aurea* twined over different host trees. Each extract was subjected to solvent-solvent fractionation. Through Thin-layer chromatography and column chromatography, the fatty acid compounds were identified and isolated. The isolated compounds were characterized through recording UV, IR, GC-MS/MS (Thermo), and 1D and 2D NMR techniques. Six fatty acids *viz*. 4-oxo-tricosanoic acid icosyl ester, butyl octadecanoate, dodecanoic acid dodec-3-enyl ester, octacosa-23, 26-dien-12-one, ethyl cis-6-octadecenoate and 15,18- dotriacontadienoic acid and methyl ester was isolated and characterized by analytical characterization techniques.

**Keywords**: *Areca catechu, Azadirachta indica*, *Cocos nucifera*, Fatty acid ester, *Lawsonia inermis, Rhaphidophora aurea.*

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## Introduction

One of the major phytoconstituents in the plant cells is fatty acids, which serve as storage products, membrane compounds, as a source of energy and metabolites[1,2]. Fatty acids commonly occur in dietary oils and natural fat. In living organisms, fatty acids play a major role as nutritious substances and metabolites[3] which may mediate the chemical protection against microorganisms[2]. Antibacterial and antifungal properties of fatty acids are already reported[4].

The phytochemical screening of the aerial roots of *Rhaphidophora aurea* twined over different host trees shows the presence of several secondary metabolites[5]. This has prompted us to take up the isolation of compounds from the aerial roots of *R. aurea*. This plant has a characteristic host-guest relationship. The weird property of the aerial roots to twine over the host and suck its nutrients has persuaded us to concentrate on this specific part of the plant. Hence the aerial roots were chosen for the present study and hence isolation was attempted.

With the advancements in Science and Technology, the methods of screening plant extracts have also gained momentum and taken new forms. Two methods namely, chromatographic separation and fractionation remain be most frequently used methods for isolation and separation of compounds from extracts. Column chromatography or preparative thin layer chromatography are separation techniques used to purify chemical compounds from mixtures of different compounds. Solvent fractionation or solvent–solvent fractionation is a process of separating various metabolites or compounds from plant extracts[6]. Four different methods were adopted for the isolation of compounds from the plant extracts *viz.* (i) Solvent-solvent fractionation isolation, (ii) Solvent fractionation isolation, (iii) Preparative thin layer chromatographic isolation, and (iv) Column chromatographic isolation.

## Materials and Methods

### Collection of plant material

Aerial roots of *Rhaphidophora aurea* (Linden ex Andre) intertwined over *Lawsonia inermis* (MM) and *Azadirachta indica* (MN) were collected from Coimbatore District, 11.0782° N, 76.8851° E and *R. aurea* intertwined over *Areca catechu* (MB) and *Cocos nucifera* (MC) was collected from Palakkad District, 10.7964° N, 76.6434° E in the month of June 2010. The botanical identification of aerial roots of

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*Rhaphidophora aurea* was carried by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Coimbatore (Authentication number - BSI/SC/5/ 23/09-10/Tech- 1534).

## Extraction

Plant materials MM (570 g), MC (360 g), MN (290 g) and MB (740 g) were defatted using petroleum ether and the defatted plant residue was then sequentially extracted by conventional refluxing with solvents acetone, chloroform, ethyl acetate, ethanol, and aqueous for 12 h. The procedure was repeated to complete extraction as noted from the colour of the solvents. The solvents were then filtered and distilled to get the extracts. The yield of the extracts ranges from 0.3 to 20 mg depending on different solvents.

### Isolation of compounds

In the present study, four different methods were taken for isolation of compounds from the extracts. The methods are given below.

#### *Compounds from solvent-solvent fractionation*

The petroleum ether extract of MM (PEMM) showed several blue fluorescence spots under long UV and iodine absorbing spots. Hence Solvent-solvent fractionation was employed. Around 1 g of PEMM was subjected to Solvent-solvent fractionation using water, chloroform, and ethanol. PEMM was partitioned between chloroform and water. The organic layer and the aqueous layers were separated and solvent was distilled off to get a residue which was treated with distilled alcohol and refrigerated. The solid thrown out on refrigeration was filtered and the filtrate further fractionated to yield UK (Compound 1).

#### *Compounds from solvent fractionation*

Isolation of compounds from crude extracts through solvent fractionation minimizes the usage of solvents and the procedure involves the use of only one solvent. This method is based on the hydrophobicity/ hydrophilicity of the chemicals taken for the study[6]. The choice of solvent is thus important in isolation and separation of compounds. The present study deals with the petroleum ether extracts of MC and MN they were taken for solvent fractionation.

The TLC of the petroleum ether extract of MC (MCPE) showed a yellow fluorescence spot at $R_f$ 0.82 and gave iodine absorption. Additional iodine absorption spots were noted; hence MCPE was recrystallized with ethanol. The colourless solid thrown out was filtered and labelled as MCPE2 (Compound 2).

The TLC of the petroleum ether extract of MN showed a yellowish blue fluorescence spot at $R_f$ 0.96 and a yellow fluorescence spot at $R_f$ 0.519, which also gave iodine absorption. Additional iodine spots at $R_f$ 0.36 and 0.37 were noted. Hence PEMN was recrystallized with ethanol. The solid thrown out was filtered and the filtrate on further concentration yielded MNPE1 (Compound 3).

#### *Compounds from preparative thin layer chromatography isolation*

The ethanol extract of MM was extracted with acetone. The acetone soluble portion was evaporated and the solid obtained fractionated by preparative thin-layer chromatography isolation in petroleum ether: ethyl acetate (4:1) to yield MDP2 (Compound 4).

#### *Compounds from column chromatography isolation*

Owing to the tedious column chromatographic isolation procedures and difficulty in the procurement of the samples only one extract was taken for isolation studies by column chromatographic studies and also rest of the other plant extracts (MM, MC, and MN) are obtained in low yields, hence column chromatography isolation was not attempted.

Column chromatography isolation is a standard method for the isolation of compounds. In this method, the ethanol extract slurry of MB was taken for column chromatography isolation. The separation of ethanol crude extract was dispensed by the different mobile systems (Petroleum ether, ethyl acetate, ethanol, and methanol). The fractions were spotted onto a TLC and eluted with a suitable mobile phase. The TLC spots of the fractions were visualized in a UV chamber at 356nm. The iodine absorption of the fraction spots was monitored in an iodine chamber. Based on the TLC observations the fractions were combined.

The fraction P4 obtained from 1% ethyl acetate on concentration yielded a yellow liquid compound P4 (Compound 5). The fraction P9 obtained from 10% ethyl acetate showed a bluish red fluorescence spot ($R_f$ 0.50) and good iodine absorption. Further recrystallization gave only a yellow wax solid P9b (Compound 6).

**Characterization of isolated compounds**

The isolated compounds (1-6) were characterized through recording UV (Systronics PC based double beam spectrophotometer-2202), IR (Shimadzu 87005, ATR), GC-MS/MS (Thermo TSQ 8000) and 1D NMR and 2D NMR (BrukerAvance III 500MHz).

## Results

The physical and chemical characteristics of isolated fatty esters from the plant materials are given below Table 1.

**Spectral characterization of UK (Compound 1)**

The UV spectrum of UK showed an absorption maximum at 260.73 nm owing to n-π* transition. The mass spectrum of UK exhibited a molecular ion peak $[M]^+$ *m/z* 634 suggesting the molecular weight 634. The subsequent fragment at *m/z* 566 might be due to the loss of $C_5H_8$ [M- $C_5H_8$] and the fragment at *m/z* 270 might be due to the loss of $C_{26}H_{52}$ [M- $C_{26}H_{52}$].

The proton spectrum of UK (Table 2) revealed the presence of two terminal methyl groups at $\delta_H$0.90 (6H, t) and thirty-two methylene protons in an identical environment at $\delta_H$1.25 (64H, broad). A four proton triplet observed at $\delta_H$1.65 corresponds to two methylene resonances, one α to carbonyl carbon and the other β to ester oxygen. The resonance observed at $\delta_H$2.31 is assigned to one methylene group linked between carbonyl carbons. A proton triplet observed at $\delta_H$4.07 is assigned to the methoxy group. Thus UK was partly identified to be an aliphatic ester containing two terminal methyl groups and one methoxy molecule.

The $^{13}C$ NMR spectrum (Table 2) showed the presence of 42 resonances, which comprised of two methyl, thirty-seven methylene, one methoxy, and two quaternary carbon atoms confirmed from DEPT-45, DEPT-90 and DEPT-135 spectral analysis. The signals at $\delta_C$178.74 and 173.73 were attributed to quaternary carbons confirmed from DEPT-45 and assigned to C-1 and C-2 positions respectively. DEPT-135 confirmed the presence of 38 methylene carbons and two methyl carbons, as seen from the downward and upward peaks respectively.

The proton peak at $\delta_H$4.07 ppm showed COSY correlation to signal at $\delta_H$1.65. The peak at $\delta_H$2.36 showed COSY correlation to $\delta_H$1.65. The proton resonance at $\delta_H$1.65 showed COSY correlation to $\delta_H$1.25 and peak at $\delta_H$1.25 to the signal at $\delta_H$0.90. These H-H correlations indicate the presence of -CO-$CH_2$-$CH_2$-CO-O-$CH_2$-$CH_2$-$(CH_2)_{14}$-$CH_2$-$CH_2$-$CH_3$ chain (Fig. 1a).

The peak at δ4.07 showed HSQC correlation to the signal at δ64.42. The peaks at δ2.31, 2.36, and 1.65(4H) ppm showed HSQC correlation to peaks at δ34.43, 33.73, 25.04, and 24.71 ppm, respectively. The peak at δ4.07 showed an HMBC correlation to signal at δ173.73. The peak at δ2.36 showed HMBC correlation to peaks at δ1.65, 1.25, and 2.31. The peak δ2.36 showed an HMBC correlation to δ34.43. The

Table 2 — 1D NMR records of 4-oxo-tricosanoic acid icosyl ester

| C. No | $^{13}C$ (ppm) | $^{1}H$ (ppm) | C. No | $^{13}C$ (ppm) | $^{1}H$ (ppm) |
|---|---|---|---|---|---|
| 1 | 178.74 | - | 22 | 29.44 | 1.25(2H) |
| 2 | 173.73 | - | 23 | 29.44 | 1.25(2H) |
| 3 | 64.42 | 4.07(2H) | 24 | 29.44 | 1.25(2H) |
| 4 | 34.43 | 2.31(2H) | 25 | 29.44 | 1.25(2H) |
| 5 | 33.73 | 2.36(2H) | 26 | 29.44 | 1.25(2H) |
| 6 | 31.93 | 1.25(2H) | 27 | 29.36 | 1.25(2H) |
| 7 | 31.93 | 1.25(2H) | 28 | 29.36 | 1.25(2H) |
| 8 | 29.70 | 1.25(2H) | 29 | 29.36 | 1.25(2H) |
| 9 | 29.66 | 1.25(2H) | 30 | 29.36 | 1.25(2H) |
| 10 | 29.59 | 1.25(2H) | 31 | 29.24 | 1.25(2H) |
| 11 | 29.59 | 1.25(2H) | 32 | 29.24 | 1.25(2H) |
| 12 | 29.59 | 1.25(2H) | 33 | 29.17 | 1.25(2H) |
| 13 | 29.59 | 1.25(2H) | 34 | 29.17 | 1.25(2H) |
| 14 | 29.48 | 1.25(2H) | 35 | 29.07 | 1.25(2H) |
| 15 | 29.48 | 1.25(2H) | 36 | 28.65 | 1.25(2H) |
| 16 | 29.48 | 1.25(2H) | 37 | 25.04 | 1.65(2H) |
| 17 | 29.48 | 1.25(2H) | 38 | 24.71 | 1.65(2H) |
| 18 | 29.48 | 1.25(2H) | 39 | 22.69 | 1.25(2H) |
| 19 | 29.48 | 1.25(2H) | 40 | 22.69 | 1.25(2H) |
| 20 | 29.48 | 1.25(2H) | 41 | 14.12 | 0.90(3H) |
| 21 | 29.44 | 1.25(2H) | 42 | 14.12 | 0.90(3H) |

Table 1 — Physical and chemical characteristics of isolated compounds

| Physical and chemical characteristics | Compound 1 | Compound 2 | Compound 3 | Compound 4 | Compound 5 | Compound 6 |
|---|---|---|---|---|---|---|
| Yield | 11 mg | 14 mg | 16 mg | 21 mg | 40 mg | 15 mg |
| Solvent system | PE:EA | PE:EA | PE:EA | PE:EA | PE: EA | PE:EA |
| TLC- Rf | 0.29 | 0.82 | 0.51 | 0.56 | 0.76 | 0.50 |
| UV –356 nm (Fluorescence) | - | Yellow | Yellow | Intense blue | Intense blue | Bluish red |
| Iodine ($I_2$) observation | Strong $I_2$ absorption | Strong $I_2$ absorption | Strong $I_2$ absorption | - | Strong $I_2$ absorption | Strong $I_2$ absorption |

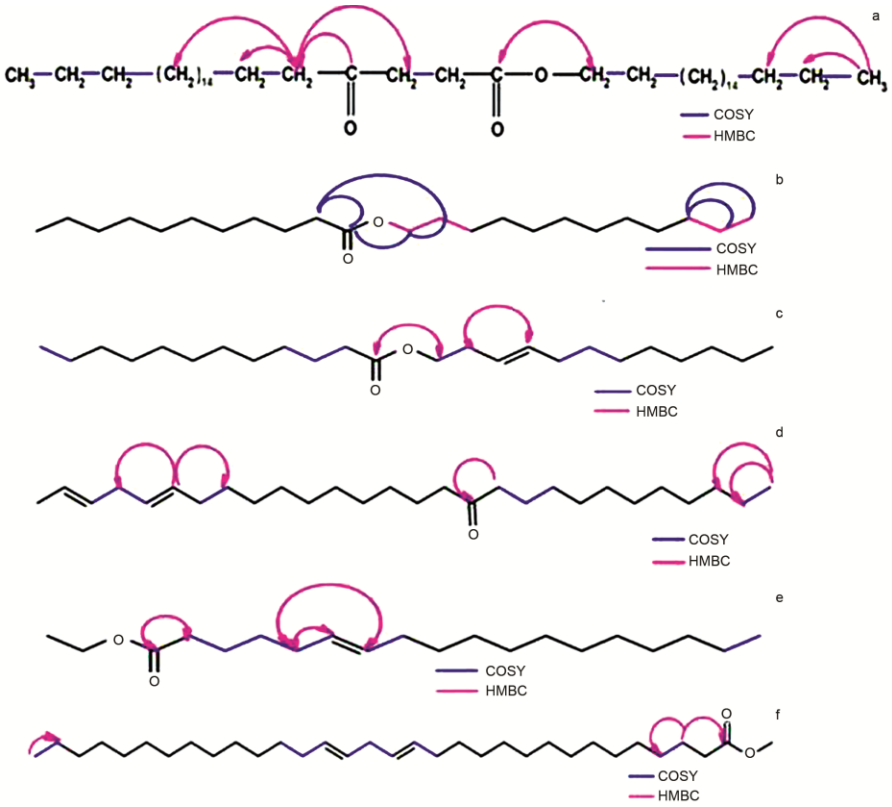


Fig. 1 — Structure of a) 4-oxo-tricosanoic acid icosyl ester, b) butyl octadecanoate, c) dodecanoic acid dodec-3-enyl ester, d) octacosa-23, 26-dien-12-one, e) ethyl cis-6-octadecenoate, and f) 15, 18- dotriacontadienoic acid methyl ester.

proton resonance at δ0.90 showed an HMBC correlation to δ22.69 and 31.93. The data obtained from the mass and NMR spectra (Table 2) confirm the structure of UK to be of 4-oxo-tricosanoic acid icosyl ester with the molecular formula $C_{42}H_{82}O_3$.

**Spectral characterization of MCPE2 (Compound 2)**

The IR spectrum of compound MCPE2 exhibited an absorption band at 1720 $cm^{-1}$ characteristic of C=O stretching. Absorption bands at 2920 and 2873 $cm^{-1}$ are characteristic of aliphatic C-H stretching. The band at 1261 $cm^{-1}$ is attributed to C-O-C stretching.

The $^1H$ NMR spectrum of MCPE2 (Table 3) exhibited the presence of two-terminal methyl protons at δ0.88. The methylene resonance observed at δ2.22 is assigned to C-3 protons, α to the carbonyl group. The resonance at δ4.05 corresponds to methoxy proton and a methylene resonance at δ1.61 corresponds to C-4 α to the methoxy proton. A broad singlet was observed at δ1.2, which is assigned to fourteen methylene protons in the structure. These assignments were based on the integration values and 2D NMR spectral analysis.

The $^{13}C$ NMR spectrum (Table 3) showed the presence of 22 resonances, which is comprised of two

Table 3 — 1D NMR records of MCPE2 (Butyl octadecanoate)

| C. No | $^{13}C$ (ppm) | $^1H$ (ppm) | C. No | $^{13}C$ (ppm) | $^1H$ (ppm) |
|---|---|---|---|---|---|
| 1 | 174.06 | - | 12 | 29.44 | 1.25 |
| 2 | 64.42 | 4.05 | 13 | 29.36 | 1.25 |
| 3 | 34.43 | 2.22<br>2.34 | 14 | 29.26 | 1.25 |
| 4 | 25.04 | 1.61 | 15 | 29.17 | 1.25 |
| 5 | 24.71 | 1.61 | 16 | 29.07 | 1.25 |
| 6 | 29.70 | 1.25 | 17 | 31.93 | 1.25 |
| 7 | 29.66 | 1.25 | 18 | 31.93 | 1.25 |
| 8 | 29.66 | 1.25 | 19 | 22.69 | 1.25 |
| 9 | 29.60 | 1.25 | 20 | 22.69 | 1.25 |
| 10 | 29.54 | 1.25 | 21 | 14.12 | 0.88 |
| 11 | 29.48 | 1.25 | 22 | 14.12 | 0.88 |

methyl signals, eighteen methylene signals, one methoxy signal, and one quaternary carbon atom confirmed from DEPT-45, DEPT-90, and DEPT-135 spectral analysis. The signal at δ174.06 ppm is characteristic of quaternary carbon (C-1), which was confirmed from its absence in DEPT-45. DEPT-90 revealed the presence of nineteen methylene carbons at δ64.42, 25.04, 34.43, 24.71, 29.70, 29.66, 29.66, 29.60, 29.54, 29.48, 29.44, 29.36, 29.26, 29.17, 29.07, 31.93, 31.93, 22.69 and 22.69 which is assigned to

C-2, C-3, C-4, C-5 to C-16, C-17, C-18, C-19, C-20, respectively. The resonance at δ14.12 is assigned to C-21 and C-22 carbons (Table 3). DEPT-135 confirms the presence of methylene and methyl as seen from downward and upward signals.

The proton peak at δ4.05 showed COSY correlation to signal at δ1.61. The peak at δ2.22 showed COSY correlation to signal at δ1.61. The proton resonance at δ1.61 showed COSY correlation to signal at δ1.25 and the peak at δ1.25 showed COSY correlation to signal at δ0.88. These correlations depicted the presence of -CO-$CH_2$-$CH_2$- and -$CH_2$-$CH_2$-$CH_3$ chain (Fig. 1b). The peak at δ4.05 (H-2) showed HSQC correlation to resonance at δ64.42 (C-2). The H-3 and H-4 protons showed HSQC correlation to signals at C-3 and C-4. The peak at δ0.88 showed HSQC correlation to the resonance at δ14.12. The proton peak at δ4.05 showed an HMBC correlation to the resonances at δ174.06 and 25.06. H-3 signal showed an HMBC correlation to signals at C-1 and C-4. The data obtained from the NMR spectra (Table 3) confirms the structure of MCPE2 to be butyl octadecanoate. Butyl octadecanoate is a class of fatty acid ester and a superclass of lipids. The synonyms are butyl actadecylate, butyl stearate, and N-butyl stearate. The molecular formula is $C_{22}H_{44}O_2$.

**Spectral characterization of MNPE1 (Compound 3)**

The IR spectrum of compound MNPE1 exhibited absorption bands at 2920.2 and 2850.79 $cm^{-1}$ characteristics of –C-H– aliphatic stretching. The bands at 1735.9 $cm^{-1}$ are characteristic of the ester carbonyl region and the band at 1465.9 $cm^{-1}$ is characteristic of -$CH_2$-stretching.

The proton spectrum of MNPE1 (Table 4) exhibited a triplet at δ0.88 corresponding to methyl proton. The proton peak at δ1.25 corresponds to methylene protons and integrated into 9 methylene groups. A fine triplet observed at δ1.61 corresponds to the methylene group, β to the carbonyl carbon. The resonances observed at δ2.09 and 2.04 are assigned to methylene α to the olefinic protons. A triplet observed at δ2.34 corresponds to methylene proton. A triplet at δ4.05 corresponds to methoxy proton α to the carbonyl carbon. The resonances observed at δ5.13 and 5.34 correspond to olefinic protons.

The carbon spectrum of MNPE1 (Table 4) showed the presence of 24 resonances, which comprises of two methyl signals, eighteen methylene signals, one methoxy signal, two olefinic signals, and one quaternary carbon, confirmed from DEPT-45, DEPT-90, and DEPT-135 spectral analysis. The signal at δ174.06 was attributed to the quaternary ester carbon. The resonances observed at δ146.12 and 127.40 correspond to olefinic carbons. A signal observed at δ64.42 is characteristic of methoxy carbon α to the carbonyl group. DEPT-135 revealed the presence of 19 methylene carbons, two olefinic carbons, and two methyl carbons. The resonances observed at δ64.42 and 24.72 correspond to methoxy and methylene carbon α and β to the carbonyl carbon respectively and the resonance at δ34.43 corresponds to methylene carbon alpha (α') to the carbonyl carbon. The resonances observed at δ33.80 and 28.66 correspond to methylene α and β to the olefinic carbons respectively. The resonances at δ31.93 – 29.08 ppm correspond to methylene assigned to C-7 to C-16 positions. The resonance observed at δ22.70 and 14.02 are assigned to methylene and methyl carbons C-21 and C-24 respectively.

Table 4 — 1D NMR records of MNPE1

| C. No | $^{13}C$ (ppm) | $^{1}H$ (ppm) | C. No | $^{13}C$ (ppm) | $^{1}H$ (ppm) |
|---|---|---|---|---|---|
| 1 | 174.06 | - | 13 | 29.37 | 1.25 |
| 2 | 146.12 | 5.13 | 14 | 29.26 | 1.25 |
| 3 | 127.40 | 5.34 | 15 | 29.17 | 1.25 |
| 4 | 64.42 | 4.05 | 16 | 29.08 | 1.25 |
| 5 | 34.43 | 2.29 | 17 | 28.66 | 1.61 |
| 6 | 33.80 | 2.34 | 18 | 25.94 | 2.04 |
| 7 | 31.93 | 1.25 | 19 | 25.04 | 1.25 |
| 8 | 31.93 | 1.25 | 20 | 24.72 | 1.61 |
| 9 | 29.71 | 1.25 | 21 | 22.70 | 1.25 |
| 10 | 29.61 | 1.25 | 22 | 22.70 | 1.25 |
| 11 | 29.54 | 1.25 | 23 | 14.02 | 0.88 |
| 12 | 29.48 | 1.25 | 24 | 14.02 | 0.88 |

The proton peak at δ4.05 showed COSY correlation to signal at δ1.61. The resonances at δ1.61 ppm showed COSY correlation to signals at δ4.05, 2.34, 2.29, and 1.25. The peak at δ2.04 showed correlation to signal at δ1.25 and δ1.25 showed correlation to signal at δ0.88. These H-H correlations indicated the presence of -CH3–(CH2)7-CH2-CH2-OCO-CH2-CH2- chain. The carbon signals at δ146.12, 127.40, and 64.42 showed HSQC correlation to δ5.13, 5.34, and 4.05 respectively. These correlations were indicative of carbon-proton connectivity. The proton peak at δ4.05 showed an HMBC correlation to the carbon signal at δ174.06. The proton resonance at δ2.29 showed an HMBC correlation to signals at δ25.94 and 29.71. The resonance at δ1.61 showed an HMBC correlation to the resonances at δ127.40. These HMBC correlations

indicated the presence of long range couplings in the $–CH_2-CH_2-CH=CH-CH_2-CH_2-$ chain in the structure (Fig. 1c). The data obtained from the IR and NMR spectra (Table 4) confirm the structure of MNPE1 to be dodecanoic acid dodec-3-enyl ester with molecular formula is $C_{24}H_{46}O_2$.

**Spectral characterization of MDP2 (Compound 4)**

The proton NMR spectrum of MDP2 (Table 5) revealed the presence of two methyl protons, twenty-one methylene protons, and four olefinic protons. The resonances at δ0.88 and 0.91ppm correspond to methyl protons. The proton signal observed at δ2.80 ppm corresponds to the methylene proton assigned between olefinic protons. The resonance observed at δ2.05 ppm corresponds to methylene proton alpha to the olefinic proton and a proton observed at δ2.36 and 1.64 ppm corresponds to methylene protons α and β to the carbonyl carbon. The proton peak observed at δ1.27 ppm corresponds to nineteen methylene protons.

The carbon NMR spectrum of MDP2 (Table 5) revealed the presence of twenty-eight resonances, which comprises of two methyl carbons, twenty-one methylene carbons, four olefinic carbons, and one quaternary carbon atom. The resonance observed at δ179.9 corresponds to quaternary carbon. The resonances observed at δ130.21, 129.72, 128.06, and 127.90 corresponds to olefinic carbons. The peak observed at δ25.63 is assigned to methylene carbon between olefinic carbons. The resonance observed at δ27.20 is assigned to the methylene proton alpha to the olefinic carbon and NMR peaks at δ34.21 and 24.79 are assigned to methylene carbons α and β to the carbonyl carbon respectively. The carbon signals observed at δ31.92, 31.91, 31.52, 29.76, 29.65, 29.60, 29.52, 29.45, 29.45, 29.36, 29.32, 29.26, 29.16, 29.10, and 22.68 are assigned to the methylene carbons C-8 to C-21 and C-26 respectively. The resonances observed at δ22.57 and 14.10 were assigned to C-27 and C-28 respectively. These assignments were made based on the downward peaks of methylene groups in DEPT-135.

Table 5 — 1D NMR records of MDP2

| C. No | $^{13}C$ (ppm) | $^{1}H$ (ppm) | C. No | $^{13}C$ (ppm) | $^{1}H$ (ppm) |
|---|---|---|---|---|---|
| 1 | 179.9 | - | 15 | 29.45 | 1.27 |
| 2 | 130.21 | 5.38 | 16 | 29.45 | 1.27 |
| 3 | 129.72 | 5.38 | 17 | 29.36 | 1.27 |
| 4 | 128.06 | 5.38 | 18 | 29.32 | 1.27 |
| 5 | 127.90 | 5.38 | 19 | 29.26 | 1.27 |
| 6 | 34.21 | 2.36 | 20 | 29.16 | 1.27 |
| 7 | 34.21 | 2.36 | 21 | 29.10 | 1.27 |
| 8 | 31.92 | 1.27 | 22 | 27.20 | 2.05 |
| 9 | 31.91 | 1.27 | 23 | 27.16 | 2.05 |
| 10 | 31.52 | 1.27 | 24 | 25.63 | 2.79 |
| 11 | 29.76 | 1.27 | 25 | 24.79 | 1.64 |
| 12 | 29.65 | 1.27 | 26 | 22.68 | 1.27 |
| 13 | 29.60 | 1.27 | 27 | 22.57 | 0.91 |
| 14 | 29.52 | 1.27 | 28 | 14.10 | 0.88 |

The proton peak at δ5.38 showed COSY correlation to peaks at δ2.80 and 2.05. The signal at δ2.36 showed correlation to signal at δ1.64 and the signal at δ1.64 showed correlation to signal at δ1.27. The signal at δ1.27 showed COSY correlation to signals at δ0.91 and 0.88. These COSY correlations indicate the presence of $–CH=CH-CH_2-CH=CH-(CH_2)n-OC-(CH_2)n-CH_3$ chain (Fig. 1d).

The resonances observed at δ5.38 showed HSQC correlation to signals at δ130.21, 129.72, 128.06, and 127.90. The resonances observed at δ2.79, 2.36, 2.05, and 1.64 showed HSQC correlation to signals at δ25.63, 34.21, 27.20, and 24.79 respectively. The proton signal at δ2.79 showed HMBC correlation to signals at δ129.7 and 130.2. The resonance at δ2.36 showed an HMBC correlation to signal at δ179.9 and the signal at δ128.06 showed correlation to signal at δ1.64. The resonance observed at δ0.88 showed an HMBC correlation to signals at δ31.9 and 22.6. These HMBC correlations, indicative of long-range coupling confirm the $–CH_2-CH=CH-CH_2-$ chain. The data obtained from the NMR spectra (Table 5) confirm the structure of MDP2 to be octacosa-23, 26-dien-12-one.

**Spectral characterization of P4 (Compound 5)**

The IR spectrum of compound P4 exhibited absorption bands at 2924.3 and 2854.6 $cm^{-1}$ characteristic of aliphatic C-H stretching. The band at 1739.3 $cm^{-1}$ is characteristics of a carbonyl group. The band at 1454.3 $cm^{-1}$ is characteristic of methylene stretching. The band at 1369.4 $cm^{-1}$ is attributed to –C-H- stretching and the band at 1242.1 $cm^{-1}$ is characteristic of –C-O-C– stretching. The bands at 1087.9, 1033.85, and 833.25 $cm^{-1}$ are characteristic of -C-C-, –C-H-, and–C-O– stretching respectively. The fingerprint region of P4 exactly matched with the stretching frequencies of ethyl cis-9-octadecenoate[7].

The $^{1}H$ NMR of P4 (Table 6) revealed the presence of two methyl protons, two olefinic protons, one methoxy protons, and fourteen methylene protons. The resonance observed at δ5.37 corresponds to olefinic proton and the resonance at δ4.15 is assigned to the methoxy proton α to the carbonyl

Table 6 — 1D NMR records of P4

| C. No | $^{13}C$ (ppm) | $^{1}H$ (ppm) | C. No | $^{13}C$ (ppm) | $^{1}H$ (ppm) |
|---|---|---|---|---|---|
| 1 | 173.89 | - | 11 | 29.35 | 1.30 |
| 2 | 130.21 | 5.37 | 12 | 29.26 | 1.30 |
| 3 | 128.04 | 5.37 | 13 | 29.11 | 1.30 |
| 4 | 60.15 | 4.15 | 14 | 27.20 | 2.06 |
| 5 | 34.39 | 2.30 | 15 | 27.16 | 2.06 |
| 6 | 32.20 | 1.30 | 16 | 26.40 | 1.69 |
| 7 | 31.90 | 1.30 | 17 | 25.63 | 2.79 |
| 8 | 29.77 | 1.30 | 18 | 24.99 | 1.62 |
| 9 | 29.59 | 1.30 | 19 | 22.68 | 1.30 |
| 10 | 29.46 | 130 | 20 | 22.57 | 0.90 |

carbon. The resonances observed at δ2.79 and 2.06is assigned to the methylene protons, alpha to the olefinic protons. The resonance observed at δ1.62 corresponds to methylene proton β to the carbonyl carbon. The resonances observed at δ1.30, 1.04, and 0.90 corresponds to -$CH_2$ and -$CH_3$ proton in the structure.

The $^{13}C$ NMR of P4 (Table 6) revealed the presence of 20 resonances which comprised two olefinic carbons, two methyl carbons, 14 methylene carbons, one methoxy carbon, and one quaternary carbon as confirmed from the DEPT-45, 90 and 135 spectral analyses. The resonance observed at δ173.89 corresponds to the carbonyl carbon characteristic of the ester group. Resonances observed at δ130.21 and 128.04 correspond to that of olefinic carbons. The resonance at δ60.15 corresponds to methoxy carbon. The resonances observed at δ25.63 and 26.40is assigned to the methylene carbons alpha to the olefinic carbons. The resonance observed at δ24.99 is assigned to the methylene carbon β to the carbonyl carbon. The resonances observed at δ32.20 - 29.11 correspond to methylene and methyl carbons and are assigned to C-6 to C-13 (Table 6) in the structure. The resonances observed at δ22.68 and 22.57 are assigned to C-19 and C-20 respectively.

The proton peak at δ5.37 showed COSY correlation to signal at δ2.79. The proton peak at δ4.15 showed correlation to peak at δ1.62 and a peak at δ2.30 showed a correlation to the signal at δ1.62. The resonance at δ2.06 showed COSY correlation to resonance at δ1.30 and a signal at δ1.30 showed correlation to signal at δ0.90. These COSY correlations indicate the presence of –OCO-$(CH_2)$n-CH=CH-$(CH_2)$n-$CH_3$ chain (Fig. 1e).

The resonances observed at δ5.37 showed HSQC correlation to signals at δ130.21 and 128.04. The resonances observed at δ2.79, 2.30, 2.06, and 1.62 showed HSQC correlation to signals at δ25.63, 34.39, 27.20, and 24.99 respectively. These HSQC correlations indicate the presence of carbon-proton connectivity.

The proton signal at δ2.79 showed an HMBC correlation to signals at δ128.04 and 130.21. The resonance at δ2.30 showed HMBC correlation to signal at δ173.89 and the signal at δ4.15 showed correlation to the signal at δ173.89. These HMBC correlations indicate the presence of long-range coupling in the structure. The results obtained from the NMR spectra (Table 6) confirm the structure of P4 to be ethyl cis-6-octadecenoate.

**Spectral characterization of P9b (Compound 6)**

The IR spectrum of compound P9b exhibited an absorption band at 2924.0 and 2854.6 $cm^{-1}$ characteristics of aliphatic C-H stretching. The band at 1708.9 $cm^{-1}$ is attributed to C=O stretching and the band at 1465.1 $cm^{-1}$ is characteristic of cyclic -$(CH_2)_n$-. The absorption band at 1381.0 $cm^{-1}$ may be attributed to C-H bending, 1176.5 and 837.1 $cm^{-1}$ due to C-H vibrations.

The proton spectrum of P9b (Table 7) exhibited the presence of two terminal methyl groups at δ0.89 (6H) and twenty two methylene protons units in an identical environment at δ1.27. The olefinic resonances observed at δ5.36 (4H) corresponded to methine proton. A proton triplet observed at δ1.64 ppm corresponds to two methylene resonances β to the carbonyl carbon. The resonance observed at δ2.36 ppm is assigned to methylene proton α to the carbonyl carbon. The resonance observed at δ2.06 ppm is assigned to methylene proton α to the olefinic protons. The resonance observed at δ2.78 ppm was assigned to the proton in the ‘c’ between the olefinic protons. A proton doublet of doublet was observed at δ3.96 ppm was assigned to the methoxy group, attached to the ester group.

The $^{13}C$ NMR spectrum (Table 7) showed the presence of 33 resonances, which comprises of two methyl signals, twenty-five methylene signals, four methine signals, one methoxy signal, and one quaternary carbon atom, confirmed from DEPT-45, DEPT-90 and DEPT-135 spectra analysis. The signal at δ179.77 is characteristic of quaternary carbon (C-1), which was confirmed from DEPT-45. The signals at δ130.21, 127.90, 130.02, and 128.06 were assigned to olefinic carbons confirmed from DEPT-90. DEPT-135 confirms the presence of methine, methylene, and methyl carbons (Table 7).

Table 7 — 1D NMR records of 15, 18- dotriacontadienoic acid, methyl ester

| C. No | $^{13}$C (ppm) | $^{1}$H (ppm) | C. No | $^{13}$C (ppm) | $^{1}$H (ppm) |
|---|---|---|---|---|---|
| 1 | 179.77 | - | 18 | 29.32 | 1.27 |
| 2 | 56.20 | 3.96 | 19 | 29.24 | 1.27 |
| 3 | 130.21 | 5.36 | 20 | 29.14 | 1.27 |
| 4 | 130.02 | 5.36 | 21 | 29.07 | 1.27 |
| 5 | 128.06 | 5.36 | 22 | 29.03 | 1.27 |
| 6 | 127.90 | 5.36 | 23 | 28.95 | 1.27 |
| 7 | 34.03 | 2.36 | 24 | 27.20 | 2.06 |
| 8 | 31.92 | 1.27 | 25 | 27.18 | 2.06 |
| 9 | 31.85 | 1.27 | 26 | 25.63 | 2.77 |
| 10 | 31.52 | 1.27 | 27 | 24.69 | 1.64 |
| 11 | 29.76 | 1.27 | 28 | 24.68 | 1.64 |
| 12 | 29.66 | 1.27 | 29 | 22.68 | 1.27 |
| 13 | 29.64 | 1.27 | 30 | 22.65 | 1.27 |
| 14 | 29.58 | 1.27 | 31 | 22.57 | 1.27 |
| 15 | 29.52 | 1.27 | 32 | 14.10 | 0.89 |
| 16 | 29.43 | 1.27 | 33 | 14.06 | 0.89 |
| 17 | 29.35 | 1.27 | - | - | - |

The proton peak at δ5.36 showed COSY correlation to peaks at δ2.78 and 2.06. The peak at δ2.36 showed COSY correlation to peak at δ1.64. The proton resonance at δ1.64 showed COSY correlation to resonance at δ1.27 and peak at δ1.27 showed COSY correlation to peak at δ2.06 and 0.89. These H-H correlations indicated the presence of -$CH_2$-$CH_2$-$CH_2$- and -$CH_2$-$CH_2$-CH=CH-$CH_2$-CH=CH-$CH_2$-$CH_2$- chain in the structure (Fig. 1f).

The peak at δ3.96 showed HSQC correlation to peak at δ56.2. The peaks at δ2.77, 2.36, and 1.64 (4H) showed HSQC correlation to peaks at δ25.63, 34.03, and 24.69 respectively. The proton resonances at δ5.36 (4H) showed HSQC correlation to signals at δ130.21, 127.90, 130.02, and 128.06 respectively. The peak at δ1.64 ppm showed an HMBC correlation to signals at δ179.77 and 1.29 (methylene carbons). The proton resonance at δ0.89 showed an HMBC correlation to signals at δ22.68 and δ31.92. HSQC indicated the presence of one bond coupling and HMBC indicated the presence of long-range coupling in the structure. The data obtained from the NMR spectra (Table 7) confirm the structure of P9b to be 15, 18- dotriacontadienoic acid, methyl ester and the molecular formula is $C_{33}H_{62}O_2$.

## Discussion

Natural products from plants offer unlimited opportunities for brand new drug leads[8]. Bioactive substances in plants are secondary plant metabolites eliciting toxicological or pharmacological effects in animals and man. The aliphatic compound heptacosanyl-5-hydroxypentadec-2-enoate obtained from the leaves of *Cassia fistula* L.[9] closely resembled the structure of UK. There are no reports on the isolation of 4-oxo-tricosanoic acid icosyl ester from the aerial roots. This is the first report of isolation and characterization of this aliphatic ester from MM. Tricosanoic acid icosyl ester is one of the major ester used in the preparation of lubricant and also the US patent has been applied using this ester for the preparation of lubricant[10]. The compound butyl stearate (MCPE2) is used as a direct food additive as synthetic flavor[11], solvent for flavours like butter and banana[12], as plasticizer for laminated fiber products, cellulose acetate butyrate, polystyrene, cellulose nitrate, and ethylcellulose, molding of polyvinyl chloride and as a lubricant in extrusion, as emollient in creams, lotions, and lipsticks[13], as a solvent, spreading agents and softening agent in plastics, textiles, cosmetics, and rubber industries[14,15], to make leather varnishes in polishes[16], special lubricants and coating, in carbon paper and inks, as damp-proofer for concrete, as waterproofing material, in propellants[17,18] and defoamer[19]. A kind of shape-stabilized phase modified material made of butyl octadecanoate impregnated by capillary forces in an absorbent graphite matrix is used as a suitable material for the wall[20].

Oral doses of 32 g/kg of Butyl stearate was tolerated by rats without any lethal effects[21]. It has been quantified in the fruits of *Mandragora autumnalis* and *Daturametel* by GC-MS analysis[22,23], in the aerial parts of *Eomeconchionantha hance*[24] and

isolated from *Ocimumbasilicum*. It possesses an anti-sickling activity[25]. The compound decanoic acids (MNPE1) are used in the manufactures of perfumes and artificial fruit flavours. Drugs existing as a decanoate ester/ salt include vanoxerine, fluphenazine, nandrolone, haloperidol, and bromperdol[26]. Dodecanoic acids (Lauric acid) are used in the treatment of acne[27,28]. Lauric acids are used as vegetable shortening agents, manufacture of shampoo and soap, treating infections namely, swine flu, cold, influenza, and preventing the transmission of HIV from mother to child[29].

Octacosa-23, 26-dien-12-one closely resembles the part structure of MDP2, it is one of the derivative products of linoleic acid and also it should be consumed for good health, otherwise, it causes poor wound healing, hair loss and mild skin scaling[30,31]. The compound P4 (Ethyl oleate) is used as a flavouring agent, as stabilizers, nutrients, emulsifiers and surfactants[32]. Ethyl oleate has a highly favourable safety profile[33]. It has been isolated from *Phyllanthus amarus* and possesses antimicrobial activity[34]. It is used in the packaging of mulberries[35], pharmaceutical drug preparations[36], food additives, lubricants, and plasticizers. The part structure of the P9b resembled the structure of linoleic acid which is typically used in the manufacture of beauty products, because of its beneficial properties on the skin and also possesses acne reductive, anti-inflammatory, moisture-retentive[37,38], and antioxidant properties[39,40,41].

## Conclusion

In the present study, a total of six fatty acid esters have been isolated from the solvent extracts of aerial roots of *Rhaphidophora aurea* twined over different host trees. The fatty acids esters were characterized as 4-oxo-tricosanoic acid icosyl ester, butyl octadecanoate, dodecanoic acid dodec-3-enyl ester, octacosa-23, 26-dien-12-one, ethyl cis-6-octadecenoate and 15, 18- dotriacontadienoic acid, methyl ester. Most of these fatty acid esters are reported to possess antibacterial, antioxidant, antifungal, and anti-inflammatory activity. Also, these fatty acid esters are used as a flavouring agent, food additives, plasticizers, lubricants, softening agents, nutrients, etc.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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