

Chloroform Trituration Process-Assisted Extraction of Chemical Compounds from Male Flowers of *Carica Papaya*

Research Article

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ABSTRACT

The male flowers of *Carica Papaya* were brought from Bangladesh Agriculture Development Corporation in Gazipur. Long chain unsaturated hydrocarbon, long chain unsaturated ester, unsaturated hydroxyl compounds, unsaturated hydroxyl ketones, and unsaturated hydroxyesters were isolated for the first time through chloroform trituration of an ethyl alcohol extract from the male flowers of *Carica Papaya*.

Keywords: Column Chromatography, *Carica Papaya*, Male flowers, Chloroform trituration process

Introduction

Genus *Carica* is one of the four genera under the family Caricaceae. There are 45 species in the genus *Carica* of which *C. Papaya* is reported by Kirtikar and Basu 1935). *Carica Papaya* is a large perennial tree and is found throughout Bangladesh. It is said to have come from Mexico and Brazil. Locally it is known as *Papay*. Various parts of the tree namely roots, leaves, flowers, fruits, seeds and other parts are used as remedy of many diseases, like skin diseases, fever, urinary complaints, and many other complications. Its fruits also have medicinal value

such as to cure inflammations and insanity, astringent to the bowels, diuretic, enlargement of the spleen, relieves obesity. The milky juice collected from the unripe fruit has long been used for killing parasitic worms, pimples cleansing and potentially used in the expulsion of lumbrici. Anthelmintic and emmenagogue effect are usually found in the seeds of *Papaya*. Moreover, the seeds are usually prescribed for bites and stings from poisonous insects. They are given as a thirst quencher, and they form a component part of a drink used in fevers. The leaves have potential impact on reducing inflammation, blood sugar

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controlling, skin care, hair care, and cancer prevention. The leaves extract of *Carica Papaya* increases the platelet count in dengue fever rapidly and prevents the complication of thrombocytopenia (Ahmad et al. 2011).

Ascorbic acid was isolated from the leaves of *C. Papaya* (Victor and Juan 1957). Phytoanthin, violaxanthin, zeaxanthin, fructose, glucose, carotene, niacin, hexanoic acid and octanoic acid were separated from the fruits and vegetables (Siddappa and Bhatia 1954; Peters 1959; Flath and Forrey 1977; Schwab and Schreier 1988). Phosphatidyl choline, phosphatidyl ethanol amine, phosphatidyl inositol, lysophosphatidyl choline, cardiolipin were isolated from the seeds (Prasab et al. 1947). Cotinine, myosmine, citric, malic, tartaric, galacturonic acids were identified in the plants (Smalergger et al. 1968; Brekke et al. 1971). Histidine, asparagines, arginine were isolated from the female flowers of *C. Papaya*. (Howard and Fenical, 1976; Ayyadet al., 1990). Carotene, thiamine, riboflavin, ascorbic acid were found in the fruit juice (Victor et al. 1944). Papain enzyme that present in the root can catalyze the breakdown of proteins by hydrolysis (Kara R. 2006).

Although various parts of the plant have been extensively examined, a few study is found on literature by the male flowers of the plant *C. Papaya*. Only few reports described the presence of aspartic acid, β -sitosterol, long-chain hydroxyl ketone, and long-chain ester, long-chain hydrocarbons, bromine substituted terpenoid compound in the male flowers of the plant are available in the literature (Kasinathan et al. 1965; Mesbah et al. 2001, Hossain et al. 2021). In this paper, our findings on the chemical examination of the male flowers of the plants are discussed.

Materials and Methods

General: Methanol (MeOH), ethanol (EtOH), ethyl acetate (AcOEt), n-hexane ($n\text{-C}_6\text{H}_{14}$) and chloroform (CHCl_3) were used as solvents. These were purified by distillation at the boiling temperature of the respective solvent. The IR spectra were recorded on a Shimadzu FTIR DR 8001 spectrophotometer, in most cases as liquid films and in other cases as KBr pellet. $^1\text{H-NMR}$ spectra were run on an Ac 250 MHz Digital NMR spectrometer as solution in CDCl_3 with TMS as the internal reference at the chemistry department of the Universität Bayreuth, Bayreuth, Germany. The Mass spectra (MS) were taken by a kratos MS-25 using DH88 data system at the Department of

Chemistry of the Universität Hannover, Germany. Precoated thin-layer chromatography (TLC) plates with silica gel 60 Kieselguhr F254 (Layer thickness 0.2 cm, E-MERCK) were used. For flash Column chromatographic separations, column was prepared by slurry method using silica gel 40 (70-230 mesh, E-MERCK) as the stationary phase.

Extract preparation from the Male Flowers of *C. Papaya*

The male flowers of *C. Papaya* were collected from Bangladesh Agriculture Development Corporation in Gazipur. The flowers were washed with water. The flowers (1.6 kg) were macerated with EtOH (>98%, 5 dm³) for 24 hrs at room temperature. The macerated flowers were then mashed in a blender and again allowed to stand in EtOH for 24 hrs. The extract was then collected and the residue was soaked with a fresh quantity of EtOH for 24 hrs. The extract was collected again. The extraction was repeated four more times. Upon solvent evaporation, a yellowish-red gummy crude material (80 g) was obtained from the extract mixtures.

Study on Crude Material (Chloroform trituration)

The EtOH extract (80 g) was subjected to treatment with CHCl_3 ($3 \times 100 \text{ cm}^3$). The combined CHCl_3 triturate was dried through anhydrous Na_2SO_4 and a greenish brown gummy substance (13 g) was obtained after evaporating the solvent to dryness under reduced pressure. The extract yielded five pure compounds on column chromatographic separation.

Compound 1 (25 mg)

IR (KBr film): ν_{max} 2920, 2849, 1462, 1377, 1242, 719 ($\text{C}=\text{C}$ bending) cm^{-1}

$^1\text{H-NMR}$ (CDCl_3): δ 0.85 (t, $J = 12.5$ Hz), 1.20 (t, $J = 6.25$ Hz), 1.6 (m), 2.2 (br), 5.1 (s).

Mass Spectrum: m/z 364 (M^+), 281, 253, 239, 211, 183, 153, 149, 143, 125, 109, 95, 81, 69, 57, 43 (100%).

Compound 2 (2.0 mg)

IR (KBr film): ν_{max} 3010, 2922, 2851, 1738 ($>\text{C}=\text{O}$), 1462, 1379, 1169, 760 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3): δ 0.85 (t, $J = 12.5$ Hz), 1.24 (s), 1.5 (m), 1.67 (br), 2.1 (t, $J = 8.9$ Hz), 2.3 (t, $J = 8.8$ Hz), 4.6 (d, $J = 7.2$ Hz), 5.35 (br).

Mass Spectrum: m/z 436 (M^+), 351, 323, 307, 293, 278 (100%), 253, 225, 197, 141, 127, 123, 97, 71, 57, 43.

Compound 3 (9.0 mg)

IR (KBr film): ν_{\max} 3450(-OH), 3100, 2926, 2850, 1335 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3): δ 0.85 (d, $J = 12.5$ Hz), 1.23 (s), 1.62 (d, $J = 6.5$ Hz), 2.2 (br), 5.0 (m), 5.9 (t, $J = 7.5$ Hz).

Mass Spectrum: m/z 378 (M^+), 323, 298, 279, 239, 237, 211, 139, 125, 111, 97, 83, 71, 57, 43 (100%)

Compound 4 (4.0 mg)

IR (KBr film): ν_{\max} 3400 (-OH), 3010, 2926, 2850, 1716 ($>\text{C}=\text{O}$), 1458, 1103 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3): δ 0.85 (bd, $J = 12.5$ Hz), 1.23 (s), 1.6 (d, $J = 6.5$ Hz), 2.2 (s), 2.3 (br), 4.0 (s), 5.11 (m), 5.7 (m).

Mass Spectrum: m/z 294 (M^+), 279, 276, 251, 239, 221, 206, 181, 158, 153, 139, 135, 125, 111, 109, 95, 71, 57, 43 (100%).

Compound 5 (25 mg)

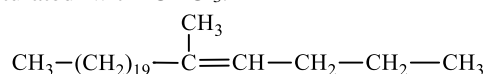
IR (KBr film): ν_{\max} 3316 (-OH), 2926, 2851, 1732 ($>\text{C}=\text{O}$), 1468, 1379, 1180 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3): δ 0.80 (t, $J = 12.5$ Hz), 1.27 (s), 2.0 (br), 2.35 (t, $J = 8.8$ Hz), 3.6 (t, $J = 6.9$ Hz), 3.9 (quin.), 4.15 (m), 5.35 (distinct distorted triplet).

Mass Spectrum: m/z 356 (M^+), 325, 299, 271, 255, 241, 239, 227, 213, 201, 195, 183, 169, 157, 155, 143, 129, 115, 101, 85, 83, 69, 57, 55 (100%), 31.

Results and Discussion

The male flowers of *C. Papaya* were soaked in EtOH and followed by completely extracted with EtOH which showed yellowish-red gummy material. The extract was triturated with CHCl_3 .

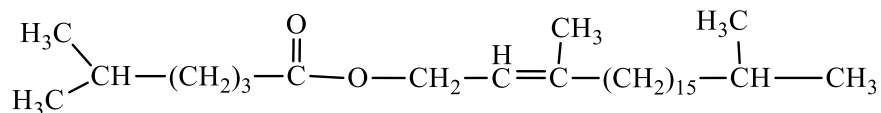
**Compound 1**

Compound 2 was also a colorless liquid and showed one spot on the TLC (R_f 0.26 in pet. ether.) IR spectrum of the compound showed an absorption band at 1738 cm^{-1} for $>\text{C}=\text{O}$ of an ester. The other absorption bands at 2922, 2851, 1462, 1379 cm^{-1} are due to the C-H stretching and bending vibrations respectively. The band at 3010 cm^{-1} is easily ascribed to $=\text{C}-\text{H}$ stretching. The $^1\text{H-NMR}$ spectrum of the compound is also in agreement with that expected from an ester. The 2H triplet at δ 2.3 and a 2H doublet at δ 4.6 are appropriate for the protons of the type $\text{CH}_2-\text{CH}_2-\text{C}=\text{O}$ and $\text{CH}-\text{CH}_2-\text{O}-\text{C}=\text{O}$ respectively. It also showed a 1H multiplet, a 3H unsplit singlet, one triplet and broad singlet at δ 1.5, 1.67, 2.1 (2H) and

The CHCl_3 extract showed five spots at R_f 0.85, 0.74, 0.58, 0.49 and 0.37 respectively along with tailing from the base line in $n\text{-C}_6\text{H}_{14}:\text{AcOEt}$ (4:1) The extract was gave five pure compounds (**1 to 5**) on column chromatographic separation.

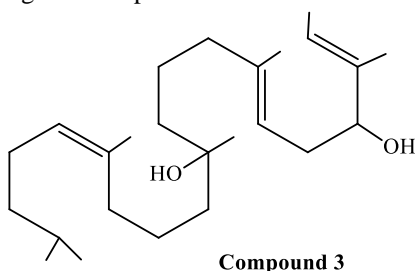
Compound 1 was a light green colored liquid (R_f 0.64 in pet. ether) indicating the compound to be fairly non polar. This was borne out by the IR absorptions of the compound which showed strong absorptions at 2920, 2849, 1462, 1377, 1242 and 719 cm^{-1} indicating it to be a long chain hydrocarbon. The compound gave a waxy solid when allowed to stand in the refrigerator but eluted soon after it was taken out from the freezer. These observations also support the view that the compound is likely to be a long chain hydrocarbon. The $^1\text{H-NMR}$ spectrum showed a triplet at δ 0.85 for the terminal methyl groups; a huge broad singlet at δ 1.2 which may be assigned to them ethylene protons of a long alky chain. The spectrum also showed the presence of an olefinic proton with absorption at δ 5.1 as unsplit singlet and a doublet at δ 1.6. The mass spectrum of the compound **1** showed the highest mass at m/z 364 which corresponds to the molecular formula $\text{C}_{26}\text{H}_{52}$ (m/z 364). The mass spectrum of the compound also showed the presence of long alkyl chain (m/z 43, 57, 71, 85, 99, 113, 127, etc. with consecutive loss of 14 mass units). The TLC properties of Compound **1** and its IR, $^1\text{H-NMR}$ and mass fragments proposed to be unsaturated hydrocarbon with the following tentative structure:

5.37 which is appropriate for the $>\text{CH}-$, $\text{C}=\text{C}-\text{CH}_3$, $\text{CH}_2-\text{C}=\text{C}$, and $\text{CH}=\text{C}$ respectively. The absorption at δ 0.85 and huge absorption at δ 1.24 are consistent with the methyl type protons and methylene type protons. The mass fragments at m/z 43, 57, 71, 85, etc. The presence of long alkyl chain in the compound was confirmed through its consecutive loss of 14 mass units and diminishing intensity. If the mass at m/z 436 is considered as the molecular ion peak then the molecular formula satisfying the groups present in the molecule is determined to be $\text{C}_{29}\text{H}_{56}\text{O}_2$. Various combinations of acid and unsaturated alcohol fractions of ester led to the following structure **2** for the compound.



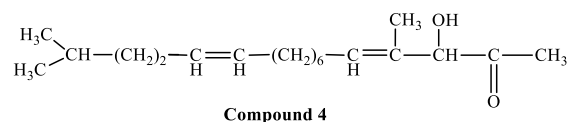
Compound 2

Compound 3 was a yellowish liquid. It showed one spot on the TLC (R_f 0.43 in pet. ether:AcOEt (9:1)). The IR spectrum of the compound showed an absorption band at 3450 cm^{-1} for hydroxyl group besides bands at 2926 , 2850 and 1335 cm^{-1} for C-H stretching and bending vibrations respectively. An absorption band at 3100 cm^{-1} for the olefinic type proton ($=\text{C}-\text{H}$ stretching) is also seen in the IR spectrum. The $^1\text{H-NMR}$ spectrum of the compound showed two doublets at δ 0.85 and 1.62 for the terminal methyl groups and methyl protons of the type $\text{CH}_3-\text{C}=\text{CH}$ respectively. The olefinic proton of the type $\text{CH}_2-\text{CH}=\text{C}-\text{C}$ is seen at δ 5.9. The huge absorption at δ 1.23 is consistent with methylene protons of alkyl chain. It also showed an unsplit singlet and weak peak at δ 2.2 and δ 5.0 for the methylene protons of the type $\text{CH}_2-\text{C}=\text{C}-$ and methyne protons of the type $\text{H}-\text{C}=\text{C}-$ respectively. If the highest ion peak at m/z 378 is considered as the molecular ion peak then computing the number of hydroxy substituents, the number of carbon-carbon double bond. The molecular formula of the compound is determined to be $\text{C}_{25}\text{H}_{46}\text{O}_2$. The presence of a number of methyl groups suggested that the compound may be terpenoid in nature. Working on terpene skeleton (C_{25}) and assigning positions for the OH groups and $\text{C}=\text{C}$, the following structure seems to satisfy the spectral findings, **3**. The structure can nicely explain the mass fragments at m/z 323, 279, 237, 211, 125, 111, 97, 71 and 57 including the base peak at m/z 43.



Compound 4 was a colorless liquid, soluble in CHCl_3 , AcOEt and MeOH but sparingly soluble in pet.ether. It showed one spot on the TLC (R_f 0.13 pet.ether:AcOEt (7:3)). IR spectrum of the compound showed an absorption band at 1716 cm^{-1}

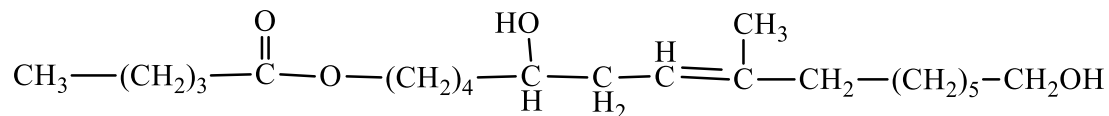
for $>\text{C}=\text{O}$ besides absorptions at 2926 & 2850 , 1456 & 1338 cm^{-1} for C-H stretching and bending vibrations respectively. A broad band at 3400 cm^{-1} in the IR spectrum confirmed the presence of O-H group. Moreover, its $^1\text{H-NMR}$ spectrum showed a broad doublet at δ 0.85, a huge unsplit singlet at δ 1.23 and a doublet at δ 1.6 for the terminal methyl groups, methylene protons of a long alkyl chain and methyl protons adjacent to the $-\text{C}=\text{C}-$ respectively. The $^1\text{H-NMR}$ spectrum also showed a singlet at δ 2.2, a singlet at δ 4.0 for the methyl protons adjacent to the carbonyl group, methyne protons adjacent to the hydroxyl group; the weak peaks at δ 5.11 and 5.7 indicated the presence of olefinic protons. The mass spectrum of the compound showed the peak maximum at m/z 294. Besides, the presence of alkyl chain in the compound was suggested due to the presence of mass fragments at m/z 43, 57, 71 etc. and consecutive loss of 14 mass units. The nature of its IR, $^1\text{H-NMR}$ and mass fragments of compound **4** suggested it to be an unsaturated hydroxy ketone. The position of the double bonds of the ketonic and hydroxyl groups have been determined to explain various mass ions. The following structure **4** can explain the mass fragments at m/z 279, 206, 181, 153, 139, 135, 125, 111, 109, 97, 71, 57, 43 very nicely matches with the molecular ion at m/z 294.



Compound 5 was a waxy white solid material, soluble in CHCl_3 , AcOEt and MeOH but sparingly soluble in pet.ether. It showed one spot on the TLC (R_f 0.26 pet. ether: AcOEt (1:1)). IR spectrum of the compound showed absorption bands at 3316 and 1732 cm^{-1} for hydroxy and ketonic groups respectively. The $^1\text{H-NMR}$ spectrum of the compound showed a triplet at δ 0.8, a huge unsplit singlet at δ 1.27 which could be assigned for the terminal methyl groups and methylene groups of an

alkyl chain. The absorption at δ 2.0 is appropriate for the proton of the type $-\text{CH}_2-\text{C}=\text{O}$; the spectrum also showed three triplets at δ 2.35, 3.6 and 4.15 for the methylene protons of the type $-\text{CH}_2-\text{CH}_2-\text{C}=\text{O}$, $-\text{CH}_2-\text{OH}$ and $-\text{CH}_2-\text{CH}_2-\text{O}-\text{C}=\text{O}$ respectively. The olefinic protons are seen as a distinct distorted triplet at δ 5.35 and a $-\text{CH}-\text{OH}$ is seen at δ 3.9 as a

quintet. The mass spectrum of the compound showed the highest peak at m/z 356. The following structure **5** can explain the mass fragments at m/z 299, 239, 227, 201, 169, 155, 101, 83, 69, 57, 41 including the base peak at m/z 55 very nicely matches with the molecular ion at m/z 356.



Compound 5

Conclusions

EtOH extract of male flowers was triturated with CHCl_3 and gave five compounds, **1-5** such as long chain unsaturated hydrocarbon, long chain unsaturated ester, unsaturated hydroxyl compounds, unsaturated hydroxyl ketone, and unsaturated hydroxy ester respectively using column chromatography technique. The structures of the compounds were confirmed by spectroscopic analysis, e.g., IR, NMR and Mass spectrometry.

Acknowledgement

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