



Research Article

Chemical investigation on the male flowers of *Carica Papaya* using acid-base separation process

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ABSTRACT

Five long-chain hydrocarbons and a new brominated nitrogenous terpenoid were isolated from the acidic and basic fraction respectively of an ethanol extract of the male flowers of *Carica Papaya* for the first time. It was collected from Bangladesh Agriculture Development Corporation, Gazipur.

Introduction

Carica Papaya is a small soft wooded, fast-growing tree with large glabrous palmatifid and palminerved leaves belonging to the family Caricaceae grows throughout Bangladesh. Locally it is known as papaya. *C. Papaya* has many medicinal properties (Vij and Prashar, 2015; Asghar et al. 2016). The ripe fruit is stomachic, appetizer, digestive, carminative. The milky juice of the unripe fruit is applied locally to the osuteri to procure abortion. The seeds are also said to be vermifuge, but they are mostly used as an emmenagogue. It is a popular belief that they may cause abortion. It has been reported that the seed extracts of *C. Papaya* have hypolipidaemic, anticancer, anti-inflammatory, and antifertility activities (Singh and Ali, 2011; Li et al., 2012; Julaha et al., 2015). The root is ground up and mixed with water, and the resulting solution is used as an enema. The dry leaves, when placed in water, form yellowish-red liquor, which is drunk to cure stomach troubles.

Dengue fever treatment was carried out with *C. Papaya* leaves extracts (Ahmad et al., 2011). Tocopherol (Engel and de Vries, 1946) and Pseudocarpaine (Govindachari et al., 1954) were separated in the leaves of *C. Papaya*. Vitamin A,

Vitamin B, Vitamin C, volatile components, volatile acids, Aryl β -d-glucosides were identified in the fruits and vegetables (Miller, 1926; Flath and Forrey, 1977; Idstein et al., 1985; Schwab and Schreier, 1988). Glutamine, cyclotransfesase, papain were collected in the dried papaya latex (Messer and Ottesen, 1964). Benzyl isocyanate was found in the fruit pulp (Tang, 1971). Carpasemine, benzyl thiourea, benzyl isothiocyanate, hentriacontane, β -sitosterol, β -D-galactosidase, α -D-mannosidase, benzylglucosinolate, essential oils were isolated in the seeds (Panse and Paranjpe, 1943; Ettlinger and Hodgkins, 1956; Kimiko and Akira, 1983, Curl and Fenwick, 1983; He et al., 2017). Nonvolatile acids were collected in the plant (Brekke et al., 1971). Asparagines, histidine, arginine was extracted in the female flowers of *C. Papaya*. (Howard and Fenical, 1976; Ayyad et al., 1990).

Literature survey exhibits that few chemical investigations on the male flowers of the plant *C. Papaya* has been carried out. To the best of our knowledge, the only report on the male flowers of *C. Papaya* described the isolation of aspartic acid, β -sitosterol, long-chain hydroxyl ketone, and long-chain ester (Kasinathan et al., 1965; Mesbah et al., 2001). The present work was undertaken to carry out a systematic study on the male flowers of *C. Papaya*.

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Materials and Methods

General: All reagents were used after distillation. Ethanol, chloroform, hexane, ethyl acetate, methanol were used as reaction solvents. Melting points were recorded by the thin disc method on a Fisher Johns electrothermal melting point apparatus. The IR spectra were taken on a Shimadzu FTIR- 8101 spectrophotometer as KBr disc and at times as thin film. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were recorded as a solution in CDCl_3 with TMS as the internal reference on an AC250 MHz digital NMR spectrophotometer at the chemistry department of the Universität Bayreuth, Bayreuth, Germany. Mass spectra (MS) were measured by a kratos MS-25 using DH.88 data system at the chemistry department of the Universität Hannover, Germany. Analytical thin-layer chromatography (TLC) was Merck aluminium oxide 60 F254 neutral or silica gel 60 F254 coated on 25 TCC aluminium sheets (20×20 cm). Flash column chromatographic separations were performed on silica gel 60 N (neutral, 40-100 μM), or activated alumina oxide (75 μM).

Collection and Extraction of the Male Flowers of *C. Papaya*

The male flowers of *C. Papaya* were supplied from the gardens under Bangladesh Agriculture Development Corporation at Kasimpur in Gazipur. The flowers were cleaned, and removed all impurities. The flowers (1.6 kg) were soaked with 5.0 L ethyl alcohol (>98%) at room temperature for 24 hours. The extract was collected, and the process was repeated four more times. The extract was combined and evaporated to dryness by removal of the solvent, and a deep yellowish-red gummy crude Mass CP (45 g) was obtained.

Acid-Base Separation of Mass CP

Crude Mass CP (45 g) was treated with 5% aqueous sulphuric acid (50 mL), and the acidic solution was then extracted by shaking with chloroform four times (4x50 mL) in a separatory funnel. The chloroform extract was then dried over anhydrous Na_2SO_4 and subsequently evaporated to dryness on a rotary

evaporator to yield a greenish-brown non-basic fraction (2.5 g). The residual aqueous acidic extract was made alkaline with 5% sodium hydroxide solution to pH 12 or above, and this was then extracted by shaking with chloroform in the usual way. The chloroform extract was dried over anhydrous sodium sulfate, and the solvent was removed at the pump below 40°C to give a reddish-yellow basic fraction (15 mg).

Results and Discussion

The macerated male flowers of *Carica Papaya* were exhaustively extracted with ethanol which gave a deep yellowish-red gummy product. The extract was separated into an acidic fraction and a basic fraction to isolate pure compounds.

The acidic fraction on column chromatographic separation gave one compound, which was shown by TLC and designated as compound **I**. The basic fraction was found to be a mixture of at least two compounds, as shown by TLC. Column chromatography of the basic fraction was carried out and gave one pure compound, which was designated as compound 6.

Acidic fraction gave one spot on TLC plate with R_f 0.72 in n-hexane. It was presumed to be a pure compound **I**. It was a colorless gummy solid, melted at $51-52^\circ\text{C}$. It was highly non-polar, as shown by its behavior on TLC plates. The IR spectrum (Fig. 1) of the compound showed absorption in the 2849 cm^{-1} region, owing to $\text{Sp}^3\text{C-H}$ stretching vibrations and C-H bending vibrations observed at 1464 and 1472 cm^{-1} region suggesting it to contain a long-chain alkyl group.

This was further confirmed by $^1\text{H NMR}$ (Fig. 2) of the compound, which showed a triple at δ 0.98, a huge unsplit multiplet at δ 1.55 for the methyl ($-\text{CH}_3$), methylene ($-\text{CH}_2-$) and methyne ($>\text{CH}-$) protons. $^{13}\text{C NMR}$ spectrum (Fig. 3) of the compound showed peaks between 14.106 to 31.937, suggesting the presence of only an alkyl carbon atom in the compound. TLC behavior of compound **I** and the nature of its IR, $^1\text{H NMR}$, and ^{13}C spectra suggested it to be a saturated hydrocarbon.

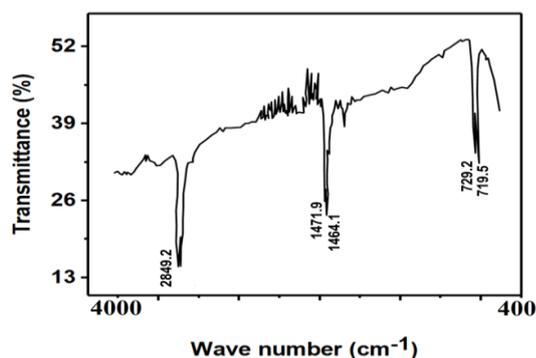


Fig. 1. IR spectrum of compound I

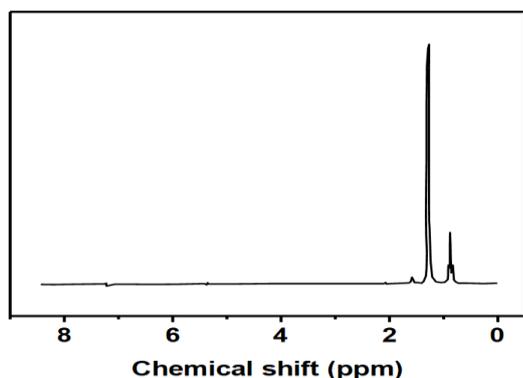


Fig. 2. ¹H NMR spectrum of compound I

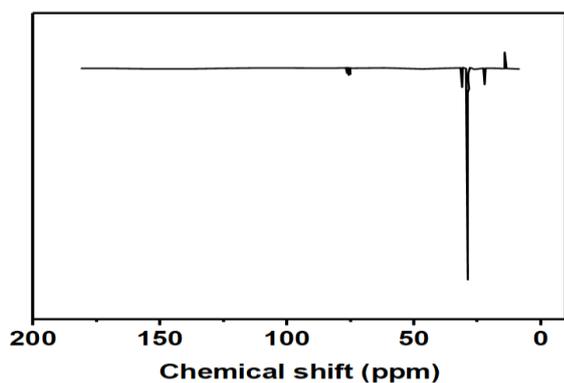


Fig. 3. ¹³C NMR spectrum of compound I

Although the TLC of the compound suggested it to be a pure compound I, the GC-mass spectrum (Fig. 4) showed it to be a mixture of at least five compounds with scan nos. 41, 45, 47, 48, and 49.

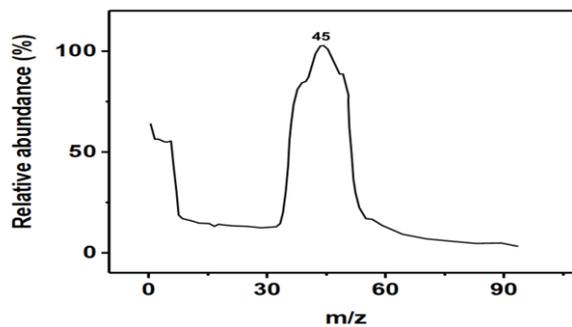


Fig. 4. GC-Mass spectrum of compound I

The mass spectrum corresponds to scan no. 41 (Fig. 4a) showed the highest mass peak at m/z 324. The mass fragments at m/z 295, 267, 239, 197, 183, 155, 127, 113, 99, 85, 71, 57 (base peak), 43 with consecutive loss of 14 mass units and increasing intensity confirmed the presence of long alkyl chain in the compound. However, since the mass ion at m/z 57 is the base peak, it tends to support the presence of the 3-methyl propyl group at one end of the hydrocarbon chain. The hydrocarbon is therefore proposed to be (1).

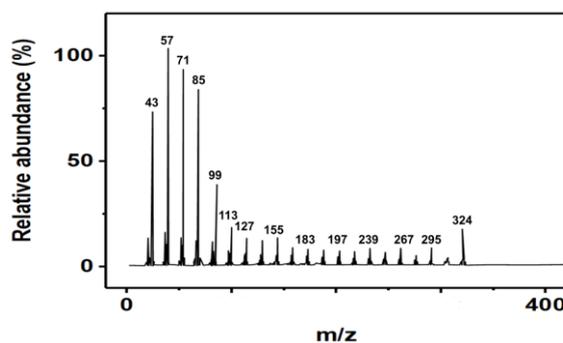
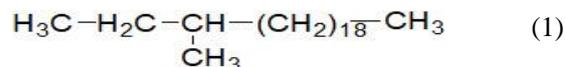


Fig. 4a. Mass spectrum of compound 1 corresponding to scan no. 41

The mass fragmentation pattern corresponds to scan no. 45 (Fig. 4b) showed the molecular ion peak at m/z 352. It further showed the first mass fragment at m/z 309, indicating a loss of mass 43 (isopropyl unit) followed by successive loss of fourteen mass units. The molecular ion peak at m/z 352, base peak at m/z 57, and fragmentation pattern are consistent with that expected for a branched-chain hydrocarbon like (2).

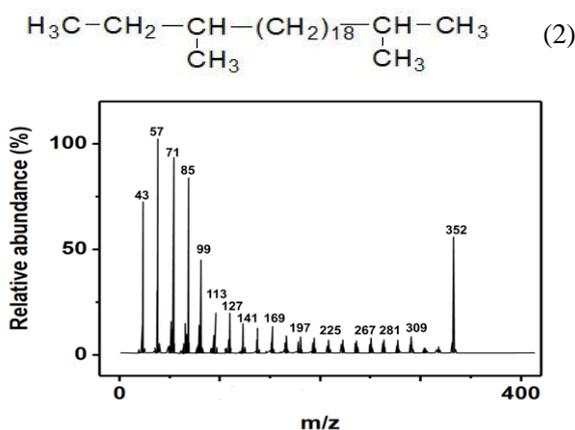


Fig. 4b. Mass spectrum of compound 2 corresponding to scan no. 45

The mass spectrum of the compound corresponding to scan no. 47 (Fig. 4c) showed the highest mass peak at m/z 380. The mass fragments also showed mass ions at m/z 295, 267, 239, 197, 183, 155, 127, 113, 99, 85, 71, 57 (base peak), 43. It is clearly seen that the molecular ion peak is at m/z 380, and the first mass ion fragment at 295, indicating a loss of 85, is followed by successive loss of fourteen mass units. The molecular ion peak at m/z 380, base peak at m/z 57, and fragmentation pattern can be readily explained if the hydrocarbon has the following structure (3).

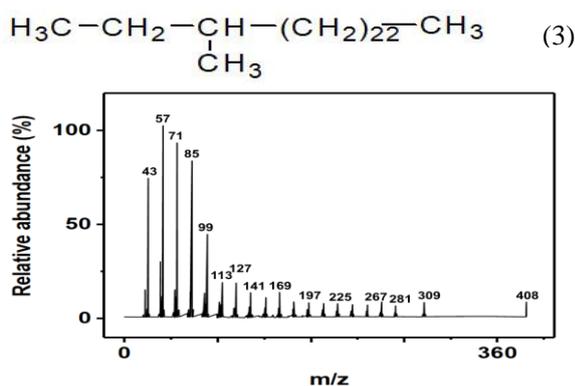


Fig. 4c. Mass spectrum of compound 3 corresponding to scan no. 47

Similar analysis of the mass spectrum corresponding to scan no. 48 (Fig. 4d) with molecular ion at m/z 408 suggest the hydrocarbon to have the structure (4).

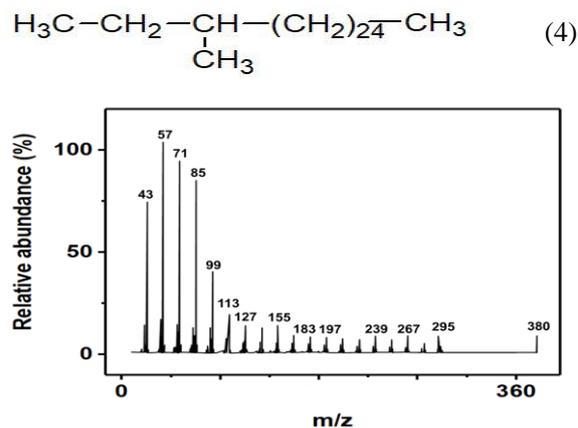


Fig. 4d. Mass spectrum of compound 4 corresponding to scan no. 48

The analysis of the mass spectrum of the compound corresponding to scan no. 49 (Fig. 4e) showed the molecular ion peak at m/z 436, a base peak at m/z 57, and the first mass fragment from molecular ion at m/z 393, indicating a loss of isopropyl group. These observations can be rationalized in the hydrocarbon has the structure (5).

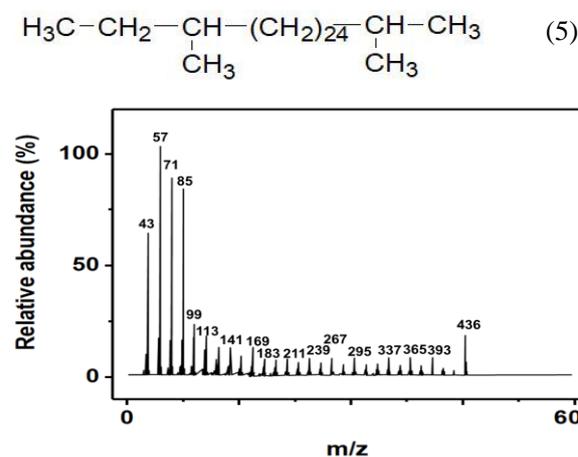


Fig. 4e. Mass spectrum of compound 5 corresponding to scan no. 49

The basic fraction showed two spots on TLC plates in different solvent systems and had R_f 0.74, 0.53 in EtOAc: MeOH (19:1) with tailing from the baseline. A compound 6 (2 mg) in the pure state was isolated by column chromatography and gave one spot at R_f 0.54 in EtOAc:MeOH (19:1). Compound 6 was a brown colored gummy substance, and it was soluble in chloroform, ethyl acetate, and methanol.

The compound's IR spectrum (Fig. 5) showed two bands at 3308 and 3200 cm^{-1} for unsymmetrical and symmetrical N-H stretching vibration, respectively, of a primary amino group. The band at 1620 cm^{-1} could be ascribed to N-H bending. The absorption at 2926 cm^{-1} suggested the compound to contain C-H stretching vibration of olefinic or aromatic type. The band at 1666 cm^{-1} confirmed the presence of C=C. The proton NMR spectrum (Fig. 6) of the compound showed a doublet at δ 0.85, an unsplit singlet at δ 1.23, and multiplet at δ 1.5 for the methyl, methylene and methyne type protons, respectively. In contrast, protons of the amino group (-NH₂) appeared as a broad doublet at δ 2.0. The absorption in the region at δ 5.3-5.7 indicated the presence of several olefinic protons, which could not be quantified because of broad absorption.

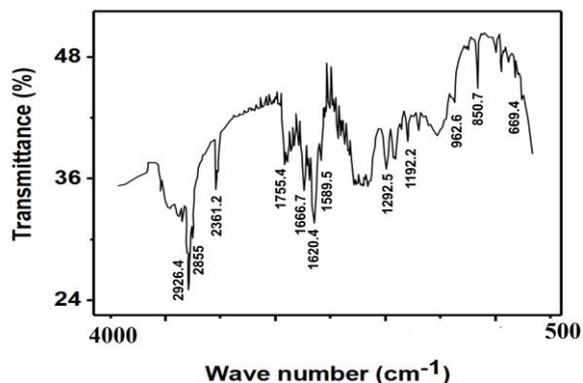


Fig. 5. IR spectrum of compound 6

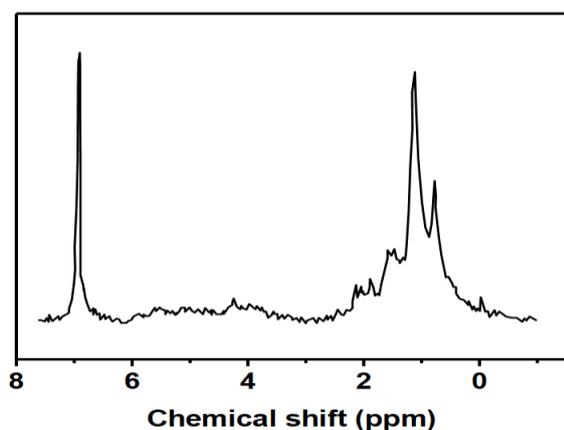


Fig. 6. ¹H NMR spectrum of compound 6

The compound's mass spectrum (Fig. 7) showed two mass peaks at m/z 396/398 (1:1), suggesting the

presence of a bromine atom in the molecule. The base peak was observed at m/z 326/328 and other peaks at 353/355, 286/288, 242/244 in the ratio 1:1 showed the attachment of the bromine atom with the corresponding fragment.

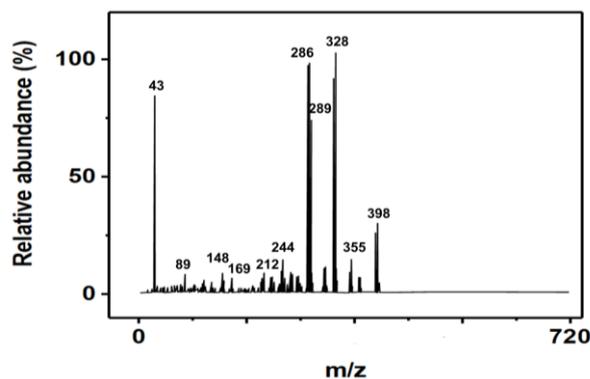
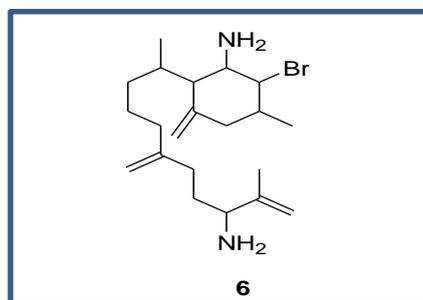


Fig.7. Mass spectrum of compound 6

If the highest mass ion at m/z 396/398 of the compound is considered as the molecular ion peak, various combinations of C, H, N, Br to fit in the molecular weight leads to the molecular formula C₂₁H₃₇N₂Br during this workout attention was, however, given to the biogenetic route to terpenoid compounds. The exercise led to the following structure (6).



Conclusions

Ethanol extract of male flowers was separated into an acidic and basic fraction with 5% aqueous sulphuric acid and 5% sodium hydroxide solution, respectively. The acidic fraction gave long-chain hydrocarbons. The GC Mass spectrum indicated a mixture of five compounds 1, 2, 3, 4, and 5. The basic fraction yielded bromine substituted terpenoid compound, 6. The structure of the compounds was elucidated by spectroscopic analysis, e.g., IR, NMR, and Mass.

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Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this article.

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