



## Research article

# Analysis of bioactive compounds present in different crude extracts of *Benincasa hispida* and *Cucurbita moschata* seeds by gas chromatography-mass spectrometry

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## ARTICLE INFO

## Keywords:

GC-MS analysis  
Seed  
*Benincasa hispida*  
*Cucurbita moschata*  
Bioactive compounds

## ABSTRACT

Plant seeds are the resources of many different bioactive components. The chemical composition of the different crude extracts from *Benincasa hispida* (White pumpkin) and *Cucurbita moschata* (Pumpkin) seeds with three different polarity-based solvents (n-hexane, n-hexane-chloroform (2:1), and methanol) was analyzed to identify the biologically active compounds. Each of the extracts was analyzed by gas chromatography-mass spectrometry. Different extracts of targeted seeds showed different biologically active compounds that have different pharmacological potentialities. 9, 12-Octadecadienoic acid (ZZ) was the most potent bioactive compound present in three different extracts of both *B. hispida* and *C. moschata*. Another bioactive compound comparatively low percentage present in both plants was n-hexadecanoic acid. Other major pharmacologically active compounds present in both plants were 9- Octadecenoic acid (Z)-, methyl ester, and 9, 12-Octadecadienoic acid methyl ester (E, E). Besides these compounds, a few more biologically active compounds were present in the two plants separately. The findings of this study support the use of these seeds in modern functional foods, nutraceuticals, and medicinal purposes, and the whole seeds would give better health benefits rather than use any extract. Although further pharmacological examinations should be carried out to conclude the medicinal application of the seeds of these two plants as well as to understand the mechanism of the potential health benefits.

## 1. Introduction

Family Cucurbitaceae, commonly referred to as the “Gourd, pumpkin, or melon family” is a well-known plant family which contains a variety of economically important edible and medicinally valuable species [1]. *Benincasa hispida* (White pumpkin), locally known as Chal Kumra, is used as a raw material for the production of delicious jelly and preserved foods. This plant is well-known for its nutritional and medicinal properties. Besides these, fruits of this plant are traditionally used to treat several other diseases like diuretic, tonic, aphrodisiac, cardiotonic, urinary calculi, blood disease, insanity, epilepsy, and also in cases of jaundice, dyspepsia,

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<https://doi.org/10.1016/j.heliyon.2022.e12702>

Received 25 July 2022; Received in revised form 17 December 2022; Accepted 21 December 2022

Available online 29 December 2022

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fever, and menstrual disorders [2] *Cucurbita moschata* (pumpkin) which is locally known as misty kumra, is used as a vegetable. This is one of the greatest natural sources of vitamin A among vegetables. They are beneficial to human health. They purify the blood, relieve constipation, aid digestion, and provide energy. Cucurbitacins, a secondary metabolite, found in the seeds and fruit sections of various cucurbits, have been reported to have purgative, emetic, and anthelmintic activity [3]. *Cucurbita moschata* (Pumpkin seeds) are considered a source of essential amino acids in a balanced diet, minerals like zinc, magnesium, phosphorus, copper, potassium, vitamins and co-enzymes like niacin, folic acid, riboflavin, and thiamin. Trigonelline and nicotinic acid, isolated from pumpkin paste reduces the level of blood glucose, cholesterol, and triglycerides by a significant amount and improves diabetes [4]. The seed also possesses other pharmacological activities such as antioxidant, antifungal, antibacterial, and anti-inflammatory. Besides the seed oil contains mono and polyunsaturated fatty acids like palmitic acid, stearic acid, oleic acid, linoleic acid, etc. [5]. Whereas, white pumpkin seeds (*Benincasa hispida*) contain different phytochemicals such as alkaloids, polyphenols, tannins, and fixed oil [6]. The fruit is traditionally used as laxatives and antibacterial [7]. Considering the beneficial effects, it is necessary to identify the bioactive active compounds present in these two types of seeds of the above-mentioned plants that may help the researcher to isolate the bioactive compounds and examine their pharmacological potentiality for developing modern personalized foods, nutraceuticals, and novel classes of drugs.

Gas chromatography-mass spectrometry (GC-MS) is an important technique that is now being used to identify bioactive chemicals both qualitatively and quantitatively in the crude plant extract. No significant work has been done and there are no published papers regarding the analysis of *B. hispida* and *C. moschata* seed extracts in polarity-based solvents (n-hexane, n-hexane: chloroform (2:1), and methanol). The relative polarity of the solvent has an effect and ability to extract different physico-chemically active biomolecules. Only one study has been done on petroleum ether extract of *B. hispida* seed and other papers were on leaves, fruits, and pulp extract of both of these two plants. We tried to find out the chemicals using non-polar to polar solvents. Three different solvent systems viz. n-hexane, n-hexane-chloroform (2:1), and methanol have been selected with their increasing polarity because we know that some of the phytochemicals are non-polar, some are semi-polar and some are highly polar. So, to obtain most of the phytochemicals we used three different types of solvents that were containing different polarity. This study focuses on the analysis of bioactive compounds using GC-MS in n-hexane, n-hexane: chloroform (2:1), and methanol extract of both *B. hispida* and *C. moschata* seeds for the first time. The objective of the present study was to determine the bioactive compounds present in *B. hispida* and *C. moschata* seeds by using GC-MS analysis in different crude extracts of n-hexane, n-hexane-chloroform (2:1), and methanol so that it can understand the active compounds present in the seeds of *B. hispida* and *C. moschata* that are responsible for the health benefits. The findings of this study will help to formulate modern foods, nutraceuticals, and medicine using these nutritionally and phytochemically enriched seeds.

## 2. Materials and methods

### 2.1. Plant sample

Fresh fruit seeds of *B. hispida* and *C. moschata* were collected from Rajshahi district, Bangladesh on February 16–18, 2021. Plant samples were identified by a botanist Dr. Arfatun Nahar Chowdhury, Principal Scientific Officer, Bangladesh Council of Scientific and Industrial Research (BCSIR), Rajshahi Laboratories.

### 2.2. Extraction of crude extract

The Seeds of the fruit were air-dried at room temperature for about two days. The dried seeds were ground using a grinder. Dried and powdered samples were subjected to sequential extraction using solvents with increasing polarity namely, n-hexane, n-hexane: chloroform (2:1), and methanol.

### 2.3. Sample preparation for GC-MS analysis

Three falcon tubes of 50 ml capacity were marked serially. The powdered plant material was mixed with three different solvents at (1: 4.3 w/v). In each of the three falcon tube, 3.5 gm *B. hispida* seed powder were taken. In the 1st tube 15 ml n-hexane, in the 2nd tube 15 ml n-hexane- chloroform mixture, and in the 3rd tube 15 ml methanol was added. The tubes were vortexed until the mixture becomes colorless. Now the mixture was warmed at 50 °C for 2 h using a water bath. Then the organic part from the top of the mixture was taken out using a micropipette and analyzed by GC-MS. In the same way, the powder of *C. moschata* seed was extracted in the above-mentioned solvents and analyzed by GC-MS.

### 2.4. GC-MS analysis

The GC-MS analysis was performed on SHIMADZU GC-MS QP-2020 equipped with an auto-sampler (AOC- 20s) and auto-injector (AOC-20i) using SH Rxi 5MS Sill column (30 m × 0.25 mm; 0.25 μm). The helium was used as carrier gas at 1.72 ml/min flow pressure; oven temperature was programmed from 80 °C (hold time 2.00 min, raised at 5 °C/min) to 150 °C (hold time 5.00 min) and final temperature of 280 °C (hold time 5.00 min). The injector temperature was 220 °C, the ion source temperature was 280 °C and the injection volume was 5.0 μL at a 50:1 split ratio (injection mode was splitless). The ionization mass spectroscopic analysis was done with 70 eV. Mass spectra were recorded across the range from 45 *m/z* to 350 *m/z* for 50.0 min. The solvent cut time was 3 min and the total run time was 50.0 min. The bioactive compounds were identified based on retention time, MS fragment ions generated and the

percentage of these bioactive compounds was evaluated from the total peak area. The phytochemicals have been identified by comparing their mass spectra with those of NIST08s, NIST08, and NIST14 libraries.

### 2.5. Identification of chemical constituents

Bioactive compounds extracted from different extracts of *B. hispida* and *C. moschata* seeds were identified based on GC retention time on SH-Rxi 5Sill MS column and matching of the spectra with computer software data of standards NIST08s, NIST08, and NIST14.

### 2.6. Statistical analysis

Three different extracts (n-hexane, n-hexane: chloroform (2:1), and methanol) of both *B. hispida* and *C. moschata* seeds were assayed. All the experiments were performed in triplicate and statistical analysis of the data was performed by analysis of variance (ANOVA) followed by Tukey HSD test, using SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA). The  $P < 0.05$  was considered as statistically significant. All the data are presented as mean values  $\pm$  standard deviation (SD) for triplicate determinations.

## 3. Results

Bioactive compounds present in n-hexane, n-hexane: chloroform (2:1), and methanol extracts obtained from *B. hispida* and *C. moschata* seeds are shown in the chromatogram (Figs. 1–6), where the retention time and relative concentration of these bioactive compounds are given in Tables 1–2. Based on abundance, in the n-hexane extract of *B. hispida* which is shown in Table 1 and Fig. 1, the top two major compounds were 9, 12-Octadecadienoic acid (ZZ) (79.88%), and n-Hexadecanoic acid (16.951%). Whereas in n-hexane: chloroform mixture (Fig. 2) and methanol extracts (Fig. 3), 9, 12-Octadecadienoic acid (ZZ) (96.60%), n-Hexadecanoic acid (2.54%), and 9,12-Octadecadienoic acid (ZZ) (65.01%), 9,12-Octadecadienoic acid methyl ester (8.71%) were the main compounds respectively (Table 1). 9, 12-Octadecadienoic acid (ZZ) was the highest abundant compound in all three types of extract where significantly greater amount were determined in the semi-polar solvent (n-hexane: chloroform mixture).

For extract of *C. moschata* seeds which is shown in Table 2 and Figs. 4–6, the n-hexane extract containing top three major compounds were 9, 12-Octadecadienoic acid (ZZ) (59.71%), n-Hexadecanoic acid (37.38%), and supraene (2.39%) (Table 2 and Fig. 4). In n-hexane: chloroform mixture extracts (Fig. 5), 9, 12-Octadecadienoic acid (ZZ) (92.62%), 9-Octadecenoic acid (Z), methyl ester (2.80%), and 9,12-Octadecadienoic acid methyl ester (2.40%) were the major three compounds (Table 2). Methanol extract of *C. moschata* (Fig. 6) contains some bioactive compounds among them 9, 12-Octadecadienoic acid (ZZ) (53.23%), and n-Hexadecanoic acid (23.57%), and n-Decanoic acid (6.44%) were present in considerable amount (Table 2). Other compounds present in this extract comparatively in fewer amounts are shown in Table 2. Again, a significantly highest amount of 9, 12-Octadecadienoic acid (ZZ) compound was detected in the *C. moschata* extract in n-hexane: chloroform mixture. n-Hexadecanoic acid was the second highest abundant compound which were detected in greater amount in non-polar solvent (nHexane) both in the seeds of *B. hispida* and *C. moschata*.

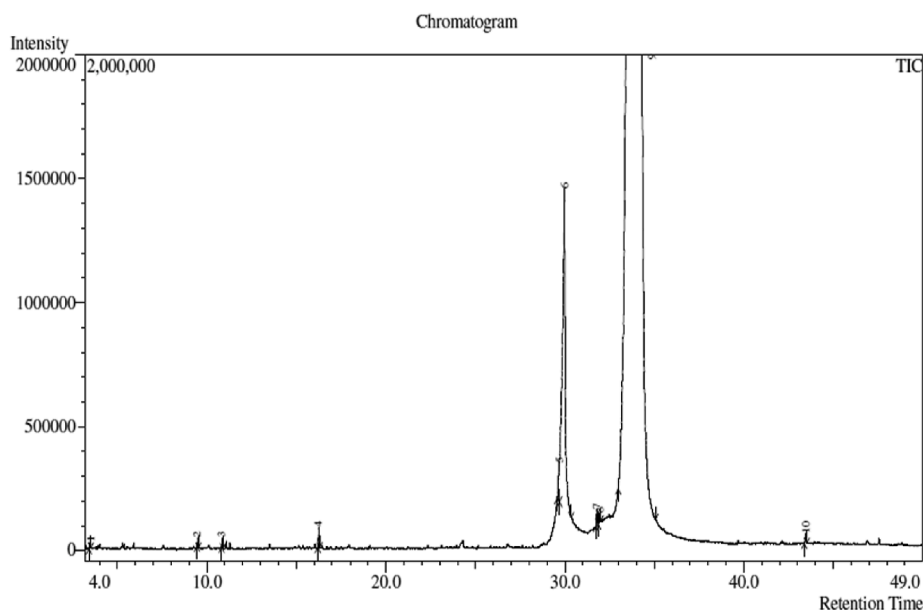


Fig. 1. GC-MS Chromatogram of bioactive compounds present in n-hexane crude extract of *B. hispida* seed.

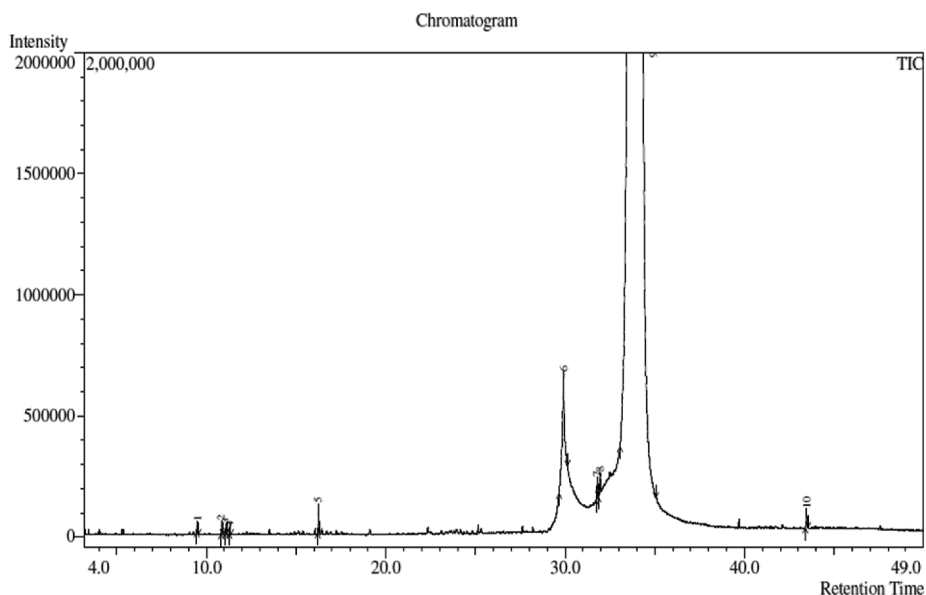


Fig. 2. GC-MS Chromatogram of bioactive compounds present in (n-hexane:chloroform-2:1) crude extract of *B. hispida* seed.

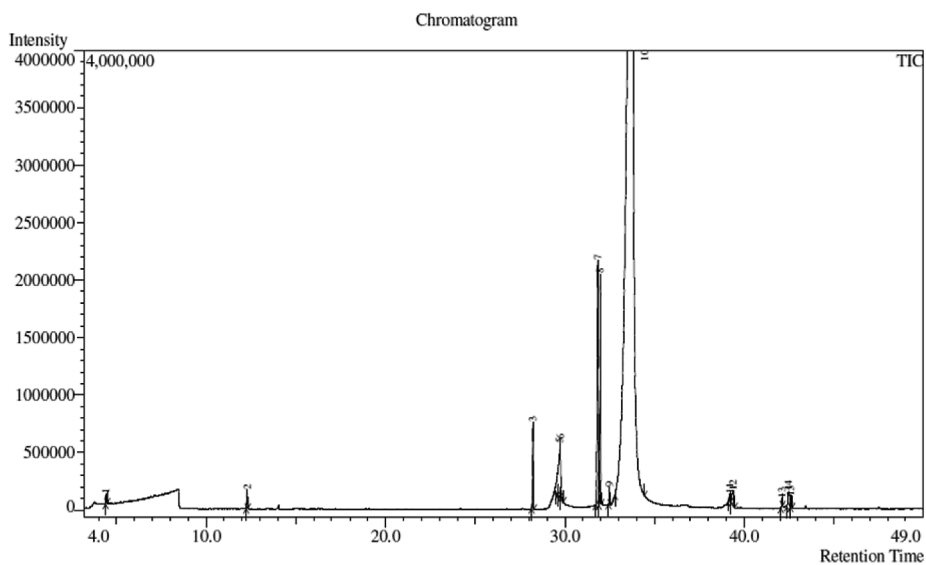


Fig. 3. GC-MS Chromatogram of bioactive compounds present in methanol crude extract of *B. hispida* seed.

#### 4. Discussion

The biologically active compounds were determined in seeds of *Benincasa hispida* (White pumpkin) and *Cucurbita moschata* (pumpkin) which are well known plant based food that are nutritionally enriched, have therapeutic, and medicinal properties. Polarity-based solvents (n-hexane, n-hexane: chloroform (2:1), and methanol) were used in the extraction to get the maximum quantity of compounds. Solvents that are containing different polarity have the impact in the extraction of biologically active molecules from plants. Here, three different solvents were used that were non-polar to polar in nature i.e. n-hexane, n-hexane-chloroform (2:1), and methanol respectively. GC-MS analysis of different crude extracts of these seeds showed various potential bioactive compounds (Figs. 1–6 and Tables 1 and 2). A total of 22 and 13 compounds were determined that were extracted using different solvents. Not all the compounds were found in the same solvent, only very few of the compounds were obtained in the same solvents with a significant variation. For *B. hispida* which is shown in Figs. 1–3 and Table 1, the top two major compounds were found in all three different crude extract with comparable percentage were 9, 12-Octadecadienoic acid (ZZ) and n-Hexadecanoic acid. 9, 12-Octadecadienoic acid (ZZ) was significantly highest percentage. The second most significantly abundant compound n-Hexadecanoic acid. For

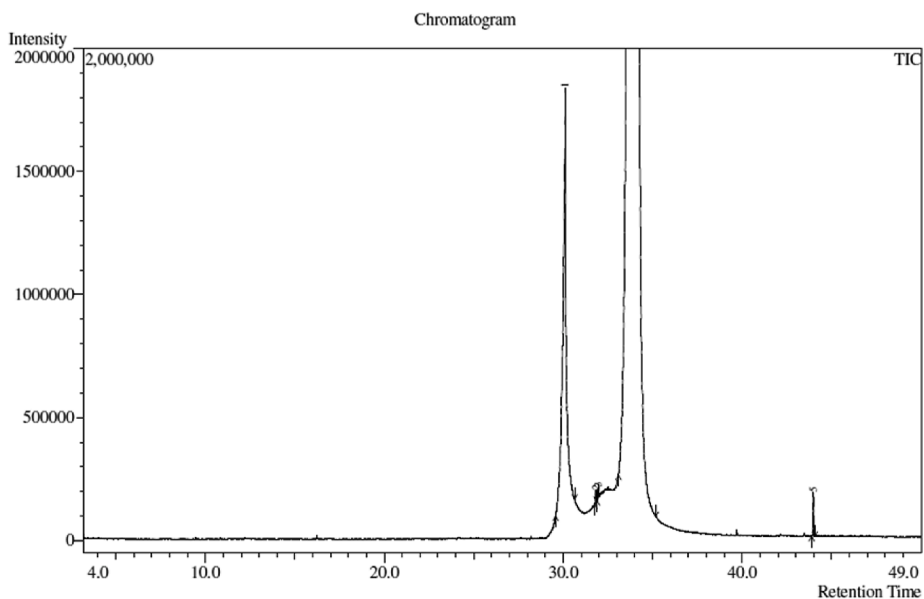


Fig. 4. GC-MS Chromatogram of bioactive compounds present in n-hexane crude extract of *C. moschata* seed.

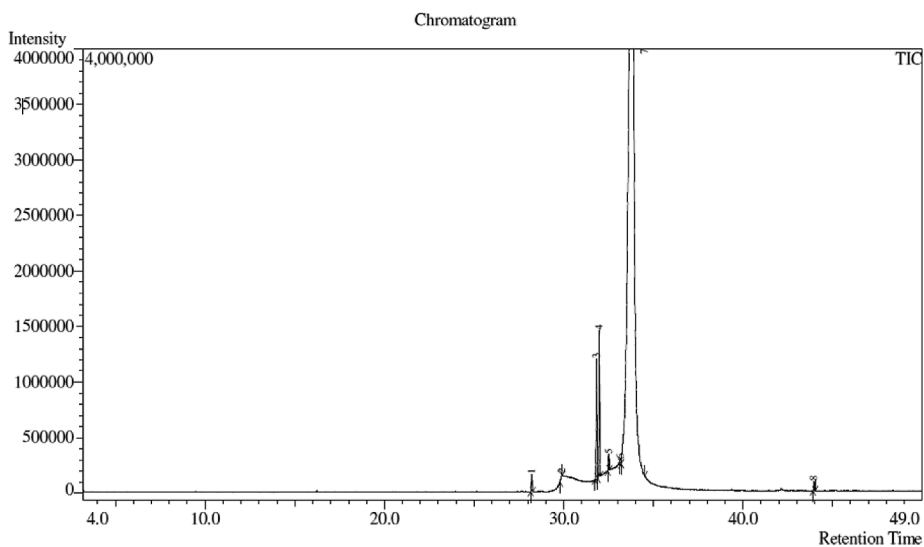


Fig. 5. GC-MS Chromatogram of bioactive compounds present in (n-hexane: chloroform-2:1) crude extract of *C. moschata* seed.

extract of *C. moschata* seeds also (Figs. 4–6 and Table 2) contained 9, 12-Octadecadienoic acid (ZZ) (59.71%) and n-Hexadecanoic acid as a major compound. Other compounds present in the extracts comparatively in fewer amounts.

The obtained biologically active chemicals have therapeutic value in human health. 9, 12-Octadecadienoic acid (ZZ) was the major bioactive compound found in all three extracts of both *B. hispida* and *C. moschata* (Tables 1 and 2). This compound plays an important role in prostaglandin biosynthesis of cell membranes with several biological functions such as anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective [8,9]. A significantly greatest percentage of 9, 12-Octadecadienoic acid (ZZ) was determined both in the seeds of *B. hispida* and *C. moschata*. This compound was present in all three different fractions too suggested the potential therapeutic importance of this two plants. Overall, the obtained result indicate that the combined therapeutic or medicinal effect of these seeds may contribute from the compound, 9, 12-Octadecadienoic acid (ZZ).

Another bioactive compound present in three fractions of both plant seeds was n-hexadecanoic acid. Studies on this compound showed antioxidant, hypocholesterolic, anti-androgenic, hemolytic, and 5-Alpha-reductase inhibitor activities [10]. A low percentage of 9-Octadecenoic acid (Z)-, methyl ester and 9, 12-Octadecadienoic acid methyl ester (E, E) were identified in the crude extract of *B. hispida* and *C. moschata*. These compounds have great potential antioxidant, anti-cancer, and anti-inflammatory properties [11]. Beenzene-1, 3-bis (1,1-dimethylethyl) was present in n-hexane and n-hexane-chloroform extracts of *B. hispida* which has lipid

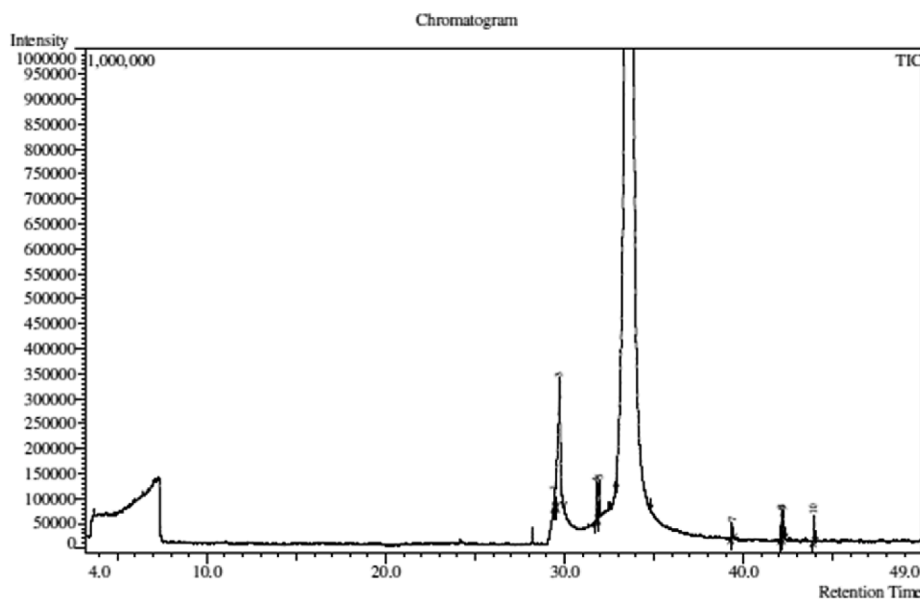


Fig. 6. GC-MS Chromatogram of bioactive compounds present in methanol crude extract of *C. moschata* seed.

oxidation properties in biology. The methanol extract contains Pentadecanoic acid, 14-methyl-, methyl ester, and Tetradecanoic acid which have antioxidant and antimicrobial properties. [12–13]). Three different extracts of *C. moschata* contained another biologically active compound supraene that has anesthetic activity [14]. n-hexane and n-hexane: chloroform extract of *B. hispida* contains three major bioactive compounds 2-Butyl-1-Octanol, 9-Octadecenamide, (Z)- and Phenol, 3, 5-bis(1, 1-dimethylethyl)-. 2-Butyl-1-Octanol which showed antimicrobial potentiality [15], 9-Octadecenamide, (Z)- showed anti-inflammatory and antibacterial activities [16] whereas Phenol, 3, 5-bis(1, 1-dimethylethyl)- showed Anti-cancer properties [17]. Four cholesterol derivative compounds such as 26-Hydroxycholesterol, beta-Sitosterol, Stigmasterol, and Stigmasta-5, 22-diene, 3-methoxy-(3,β.,22E)- are present in methanol extract of *B. hispida* having major role in cholesterol synthesis in the heart [18]. Z,Z-10,12-Hexadecadien-1-ol acetate is present in both methanol extracts of *B. hispida* and *C. moschata* which has a major role in the Biosynthesis of prostaglandins [19]. Additionally, methanol extracts of *B. hispida* contain Imidazole-4 acetic acid and Phenol, 2-methoxy-4-(2-propenyl)-acetate. Imidazole-4 acetic acid exhibits analgesic, sedative, hypnotics, platelet aggression, and hypotensive activities [20], and Phenol, 2-methoxy-4-(2-propenyl)-acetate exhibits antimicrobial and antioxidant activities [21]. A flavoring agent hexanoic acid is present in n-hexane extract of *B. hispida* contain that have anti-diabetic and anti-cancer properties [22]. Methanol extract of *C. moschata* contains n-Decanoic acid, Nonanoic acid, 13-octadecenal(Z), and Hexadecanoic acid, 2-hydroxy-1- (hydroxyme). Among them, nonanoic acid has been widely used to treat acute and chronic bronchitis, acute sinusitis, and lower respiratory tract infections. Antibacterial activity [23,24]. 13-octadecenal(Z) and Hexadecanoic acid, 2-hydroxy-1- (hydroxyme) have antibacterial activities [25] and antioxidant properties respectively [22].

The GC-MS analysis revealed the presence of bioactive compounds in *B. hispida* and *C. moschata* seed extracts. Some medicinally important chemicals like 9,12-Octadecadienoic acid (Z,Z), hexanoic acid, Nonanoic acid, 13-octadecenal (Z), hexadecanoic acid, 2-hydroxy-1- (hydroxyme) have been detected that justifies the traditional application and predicts the possibility of discovery of new drugs. Considering the biological activities of compounds present in different crude extracts supports the medicinal application of these plants. We are yet to isolate the major compounds with biological and chemical importance. For testing biological activity animal models should be set up. For conducting clinical trials inter-institutional cooperation and legal frameworks are needed. We are hopeful to do this work in the second phase of the project. However, the bio-active compounds we identified in the targeted seeds will be helpful to prepare personalized food and supplement as well as new drug discovery.

## 5. Conclusion

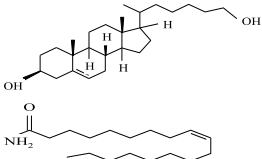
*B. hispida* and *C. moschata*, seeds contained bioactive compounds. This is the first work that revealed the profile of the biologically active compounds present in these targeted seeds using a polarity based extraction in three different solvents. The findings of this study supported the functional properties of these seeds that are using from ancient as food and herbal medicinal purposes. Considering the biological activities of compounds present in different crude extracts of *B. hispida* and *C. moschata*, seeds, it can be appropriate to use for nutraceuticals, supplemental foods as well as for the medicinal application. The study showed the major bioactive compounds present in all extracts of the seeds of these two targeted plants. Therefore, it can be concluded that the use of seeds of these vegetable plants in the food industry will enhance the health benefit even though phytochemical and pharmacological evaluation is required to understand the mechanism of health benefits of these plant seeds.

**Table 1**The relative concentration of chemical composition present in n-hexane, n-hexane chloroform and methanol crude extracts of *B. hispida* seed.

No.	Compounds Name	Structure	Retention Time (min)	Relative Concentration (%)		
				n-hexane	n-hexane: chloroform	Methanol
1.	Hexanoic acid		3.469	0.272 ± 0.002	ND	ND
2.	Imidazole-4 acetic acid		4.414	ND	ND	0.755 ± 0.004
3.	Beenzene,1,3-bis (1,1dimethylethyl)		9.476	0.299 ± 0.010 <sup>a</sup>	0.136 ± 0.002 <sup>b</sup>	ND
4.	1-octanol, 2-butyl-		10.845	0.170 ± 0.002 <sup>a</sup>	0.071 ± 0.003 <sup>b</sup>	ND
5.	1-Undecene,7-methyl-		11.084	ND	0.062 ± 0.002	ND
6.	1-Decanol, 2-hexyl-		11.304	ND	0.039 ± 0.001	ND
7.	Phenol,2-methoxy-4-, (2-propenyl)-acetate		12.271	ND	ND	0.449 ± 0.004
8.	Phenol,3,5-bis (1,1dimethylethyl)		16.240	0.463 ± 0.046 <sup>a</sup>	0.239 ± 0.002 <sup>a</sup>	ND
9.	Pentadecanoic acid, 14-methyl-, methyl ester		28.226	ND	ND	6.181 ± 0.335
10.	Pentadecanoic acid		29.685	1.508 ± 0.042	ND	ND
11.	Tetradecanoic acid		29.728	ND	ND	4.977 ± 0.273
12.	n-Hexadecanoic acid		29.985	16.95 ± 0.320 <sup>a</sup>	2.539 ± 0.008 <sup>b</sup>	1.832 ± 0.132 <sup>c</sup>
13.	9,12-Octadecadienoic acid, methyl ester, (EE)		31.797	0.163 ± 0.002 <sup>b</sup>	0.079 ± 0.002 <sup>c</sup>	8.699 ± 0.441 <sup>a</sup>
14.	9-Octadecenoic acid (Z), methyl ester		31.947	0.155 ± 0.002 <sup>b</sup>	0.088 ± 0.005 <sup>c</sup>	7.669 ± 0.087 <sup>a</sup>
15.	Heneicosanoic acid methyl ester		32.488	ND	ND	1.045 ± 0.005
16.	9,12-Octadecadienoic acid (Z,Z)		34.198	79.79 ± 0.265 <sup>b</sup>	96.59 ± 0.002 <sup>a</sup>	64.90 ± 0.089 <sup>c</sup>
17.	Stigmasterol		39.210	ND	ND	1.277 ± 0.008
18.	Stigmasta-5,22-diene,3-methoxy-(3beta,22)		39.383	ND	ND	0.759 ± 0.007
19.	Z,Z-10,12-Hexadecadien-1-ol acetate		42.102	ND	ND	0.428 ± 0.012
20.	beta-Sitosterol		42.486	ND	ND	0.619 ± 0.019
21.	26-Hydroxycholesterol		42.600	ND	ND	0.403 ± 0.007

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**Table 1** (continued)

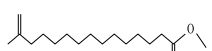
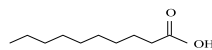
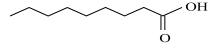
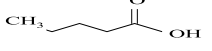
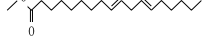

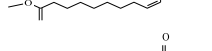
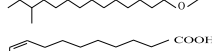





No.	Compounds Name	Structure	Retention Time (min)	Relative Concentration (%)		
				n-hexane	n-hexane: chloroform	Methanol
22.	9-Octadecenamide, (Z)-		43.455	0.217 ± 0.004 <sup>a</sup>	0.148 ± 0.005 <sup>a</sup>	ND

ND: Not detected.

Values (mean ± SD) are the average of three samples of each solvent extract which were analyzed individually in triplicate. The similar superscript letters contained in the same row did not differ significantly ( $p < 0.05$ ).

**Table 2**

The relative concentration of chemical composition present in n-hexane, n-hexane chloroform and methanol crude extracts of *C. moschata* seed.

No.	Compounds Name	Structure	Retention Time (min)	Relative Concentration (%)		
				n-hexane	n-hexane: chloroform	Methanol
1.	Pentadecanoic acid 14-methyl-, methyl ester		28.215	ND	0.874 ± 0.033	ND
2.	n-Decanoic acid		29.445	ND	ND	6.398 ± 0.120
3.	Nonanoic acid		29.615	ND	ND	3.152 ± 0.104
4.	n-Hexadecanoic acid		30.116	37.29 ± 0.485 <sup>a</sup>	0.178 ± 0.006 <sup>c</sup>	23.44 ± 0.232 <sup>b</sup>
5.	9,12-Octadecadienoic acid methyl ester		31.816	0.286 ± 0.054 <sup>a</sup>	2.386 ± 0.051 <sup>a</sup>	2.091 ± 0.040 <sup>a</sup>
6.	9-Octadecenoic acid (Z), methyl ester		31.962	0.337 ± 0.006 <sup>b</sup>	2.795 ± 0.038 <sup>a</sup>	2.290 ± 0.099 <sup>a</sup>
7.	Tetradecanoic acid 12- methyl-, methyl ester		32.509	ND	0.530 ± 0.035	ND
8.	Ethyl 9.cis.11. trans, -octadecadienoate		33.150	ND	0.037 ± 0.004	ND
9.	9,12-Octadecadienoic acid (Z, Z)		34.100	59.68 ± 0.407 <sup>b</sup>	92.58 ± 0.070 <sup>a</sup>	53.40 ± 0.694 <sup>c</sup>
10.	Hexadecanoic acid, 2-hydroxy-1-(hydroxy-methyl)ethyl ester		39.352	ND	ND	0.924 ± 0.052
11.	ZZ-10,12-hexadecadien-1-ol acetate		42.126	ND	ND	1.727 ± 0.029
12.	13-octadecenal(Z)		42.201	ND	ND	2.099 ± 0.049
13.	Supraene		43.977	2.403 ± 0.041 <sup>b</sup>	0.612 ± 0.015 <sup>c</sup>	4.467 ± 0.087 <sup>a</sup>

ND: Not detected.

Values (mean ± SD) are the average of three samples of each solvent extract which were analyzed individually in triplicate. The similar superscript letters contained in the same row did not differ significantly ( $p < 0.05$ ).



## Author contribution statement

Ali Ahsan Muzahid: Conceived and designed the experiments, performed the experiments, and wrote the paper. Samia Sharmin: Performed the experiments and wrote the paper. Md. Sakhawat Hossain: Performed the experiments. Kutub Uddin Ahamed: Performed the experiments. Nasim Ahmed: Performed the experiments. Most. Sarmina Yeasmin: Analyzed and interpreted the data. Nazim Uddin Ahmed: Conceived and designed the experiments. Dr. Barun Kanti Saha: Contributed reagents, materials, analysis tools or data. G.M. Masud Rana: Analyzed and interpreted the data. Bijoy Maitra: Analyzed and interpreted the data. Dr. Md Nurul Huda Bhuiyan: Conceived and designed the experiments, analyzed and interpreted the data and wrote the paper.

## Declaration of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships which can influence the results of this study and also do not have any conflict with any other research work.

## Acknowledgements

The whole work was conducted as a part of the fellowship program of BCSIR. We would like to acknowledge our colleagues in the BCSIR Rajshahi Laboratories for their support all the way to carry out the experimental work of this study.

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