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Chemical and biological activity studies of fruit of *Cucumis melo* L.

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ABSTRACT

Cucumis melo L. from the family Cucurbitaceae is native to Southeast Asian countries, and very well-liked fruit due to its ginormous medicinal and nutritive value. The DCM extract of the powdered fruit sample of *C. melo* L. was subjected to different chromatographic techniques to isolate secondary metabolites. α-spinasterol, α-spinesteryl acetate, trilinolein and oleic acid were isolated and their structures were elucidated by comparing the data obtained from ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra with reported data for corresponding compounds. The total phenolic content, total flavonoid content, total antioxidant capacity and DPPH radical scavenging activity were found to be the highest for methanolic extracts (14.25 ± 0.83 mg GAE/g, 9.2 ± 0.58 mg QE/g, 33.77 ± 1.58 mg AAE/g of dry extractives, and IC_{50} value of 11.13 µg/mL respectively) compared to *n*-hexane and DCM extracts.

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1. Introduction

Cucumis melo L. (commonly known as muskmelon) is a member of the Cucurbitaceae family (ITIS online database, 2022). The family consists of 95 genera and 965 species. (Christenhusz et al., 2016) The fruits and vegetables of this family (watermelon, honeydew melon, cucumber, squash, zucchini, pumpkin etc.) are of significant importance in ethnopharmacology and traditional medicine. They are used to treat conditions such as anemia, splenomegaly, inflammation, and indigestion (Mukherjee et al., 2022). As a member of this family, *Cucumis melo* L. possesses considerable medicinal value. In traditional medicine, it has been used to treat kidney dysfunction, diabetes, anemia, abdominal problems, obesity, amentia, and various other human ailments (Asif et al., 2014).

Different bioactive compounds have been isolated from various parts of the plant. Cucumol A, α-pinasterol and B:D-friedoolean-5-ene-3-β-ol have been isolated from the methanolic extract of the seed (Ibrahim et al., 2016). Cucurbitacin-type triterpenes have been isolated from stem (Chen et al., 2009). Several studies revealed ginormous biological importance of various parts of the plant, such as antidiabetic, antihyperlipidemic, anti-inflammatory, anti-ulcer, anti-Alzheimer, antibacterial and antioxidant activity (Srivastava et al., 2020; Singh and Devi, 2020; Moustafa et al., 2021).

A review of the literature on *Cucumis melo* (*C. melo*) revealed that limited research has been conducted on the edible part of the plant. Most chemical and biological studies to date have focused on the inedible parts, such as seeds, peels, and pulp. However, the fruit itself may possess rich nutritional and significant medicinal value, potentially contributing to the development of future therapeutic agents. In light of this, the present study focuses on the fruit of *C. melo* L., aiming to isolate and purify bioactive secondary metabolites from its extract and to elucidate the structures of the isolated compounds using various spectroscopic techniques, including FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. Biological studies of plants generally help identify various bioactive compounds, understand their associated health benefits or risks, and optimize dietary intake. To explore the bioactivity of the fruit of *Cucumis melo*, several bioassays were conducted, including total phenolic content, total flavonoid content, total antioxidant capacity, and DPPH radical scavenging activity. Additionally, antimicrobial and cytotoxicity assays were performed to evaluate the fruit's potential to inhibit bacterial growth and assess its toxicological effects, respectively.

2. Materials and methods

2.1. Collection, processing and extraction of plant materials

Fresh *Cucumis melo* L. fruits were collected from local markets in Dhaka, Bangladesh. The fruits were thoroughly washed to remove all undesirable particles. They were then cut into small slices and left to air-dry for several days, followed by drying in an oven at 40 °C. The dried fruit pieces were finely ground into a homogeneous powder using a grinder and stored in an airtight zip-lock bag for further analysis.

2.2. Compound isolation and structural elucidation

The powdered fruit sample weighing 523.0 g was taken into a 1000 mL conical flask. The successive extraction of the sample was conducted from non-polar (*n*-hexane) to more polar (methanol) solvents to properly complete the extraction of all types of compounds with different polarities.

At first, 1000 mL of *n*-hexane was transferred into the conical flask. It was kept for three days with occasional stirring. After that duration, the mixture in the flask was filtered, and the filtrate was collected to be processed into the concentrated extract. The residue was preserved to repeat this process a further two times, and the filtrate was collected in the same manner. Afterwards, the filtrate was evaporated using a rotary evaporator and solid *n*-hexane extract was collected and weighed. The exact process was followed for the other two solvents. After the extraction process, 4.02 g *n*-hexane, 3.1 g DCM and 64.01 g methanol extracts were obtained.

A glass column (59 cm × 4 cm) was packed with the slurry of column-grade silica gel (Silica gel 60 (0.063-0.200 mm), MERCK: 120 g taken) in *n*-hexane, and a column of bed length 20 cm was prepared. 2.50 g of DCM extract was dissolved in DCM and mixed with column-grade silica gel (5 g). The mixture was then evaporated to dryness and made into a finely flowing powder. On top of the packed column bed, the powdered sample was then applied. After that, a cotton plug was placed on the top to avoid any disturbance to the sample layer and the column bed.

The column was first eluted with 100% *n*-hexane with a flow rate of 50 to 60 drops per minute. Gradient elution of a solvent system with a varying composition according to increasing polarity (DCM followed by MeOH) was used to elute different fractions.

Based on the TLC patterns, similar fractions were combined to obtain nine fractions. From fraction Fr-4, compound **1** was obtained. Fraction Fr-7 was subjected to column chromatography, and five subfractions were collected. From the subfraction SF-3, compound **2** was obtained. From fraction Fr-3, compound **3** was obtained. Fraction Fr-6 was purified by Pasteur pipette column chromatography. From the subfractions SC-1 and SC-2, compound **4** was obtained. The structures of these compounds were elucidated by comparing ^{1}H and ^{13}C spectral data with literature values.

Properties of compound **1**: White crystalline (10 mg), R_f value= 0.39 (DCM: *n*-hexane = 70:30).

FT-IR (KBr pellets): λ_{max} 3430.75 (O-H stretching), 2923.07 (C-H symmetric stretching), 2859.43 (C-H asymmetric stretching), 1653.88 (C=C stretching), 1455.82 (C-H stretching of cycloalkanes), 1093.29 cm^{-1} (=C-H out-of-plane bending of alkene) (Kwaji et al., 2019).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 5.18 (1H, *s*, H-7); 5.14 (1H, *s*, H-22); 5.05 (1H, *dd*, H-23); 3.59 (1H, *t*, H-3); 0.53 (3H, *s*, H-18); 0.79 (3H, *s*, H-19); 1.03 (3H, *d*, H-21); 0.97 (3H, *d*, H-26); 0.92 (3H, *d*, H-27); 0.81 (3H, *d*, H-29).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 37.17 (C-1); 31.51 (C-2); 71.09 (C-3); 38.03 (C-4); 40.29 (C-5); 29.67 (C-6); 117.48 (C-7); 139.64 (C-8); 49.49 (C-9); 34.24 (C-10); 21.58 (C-11); 39.60 (C-12); 43.31 (C-13); 55.15 (C-14); 22.99 (C-15); 28.51 (C-16); 55.94 (C-17); 12.07 (C-18); 13.05 (C-19); 40.82 (C-20); 21.38 (C-21); 138.17 (C-22); 129.52 (C-23); 51.27 (C-24); 31.89 (C-25); 21.09 (C-26); 18.96 (C-27); 25.40 (C-28); 12.25 (C-29).

Properties of compound **2**: Off-white semi-solid (7 mg), R_f value= 0.51 (DCM: MeOH = 94:6).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 5.35 (1H, *m*, H-7); 5.14 (1H, *s*, H-22); 4.71(1H, *dd*, H-23); 4.37 (1H, *dd*, H-3); 2.04 (3H, *s*, H-2'); 0.53 (3H, *s*, H-18); 0.79 (3H, *s*, H-19); 1.02 (3H, *d*, H-21); 0.83 (3H, *d*, H-26); 0.78 (3H, *d*, H-27); 0.81 (3H, *d*, H-29).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 37.21 (C-1); 174.17 (C-1'); 27.98 (C-2); 21.54 (C-2'); 73.70 (C-3); 33.88 (C-4); 40.29 (C-5); 29.52 (C-6); 117.31 (C-7); 139.53 (C-8); 46.06 (C-9); 34.33 (C-10); 21.54 (C-11); 39.58 (C-12); 43.35 (C-13); 55.03 (C-14); 23.02 (C-15); 28.99 (C-16); 56.22 (C-17); 12.05 (C-18); 13.03 (C-19); 40.82 (C-20); 20.55 (C-21); 138.11 (C-22); 129.55 (C-23); 49.42 (C-24); 31.94 (C-25); 22.66 (C-26); 18.98 (C-27); 25.53 (C-28); 12.31 (C-29).

Properties of compound **3**: Pale-yellow liquid (6 mg), R_f value = 0.43 (DCM: *n*-hexane = 50:50).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 0.85-0.88 (–CH_3 (saturated linoleic acyl group), *t*, H-18); 1.24-1.29 (–$(CH_2)_n$- (acyl group), *m*, H-4 to H-7, and H-15 to H-17); 1.59-1.67 (–OCO-CH_2-CH_2- (acyl group), *m*, H-3); 1.99-2.08 (–CH2-CH=CH- (acyl groups), *m*, H-8, H-14); 2.28-2.32 (–OCO-CH_2- (acyl group), *t*, H-2); 2.74-2.79 (=HC-CH_2-CH= (acyl groups), *m*, H-11); 4.11-4.15 (>CH_2OCOR (glyceryl group), d, H-1'); 4.27-4.29 (>CH_2OCOR (glyceryl group), *d*, H-3'); 5.25-5.26 (-CHOCOR (glyceryl group), *m*, H-2'); 5.33-5.34 (–CH=CH- (acyl group), *d*, H-9, H-10, H-12, H-13).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 62.12 (Glyceryl CH_2); 68.91 (Glyceryl CH); 173.26 (C=O α); 172.85 (C=O β); 34.06 (C-2 α); 34.21 (C-2 β); 24.85 (C-3 α); 24.88 (C-3 β); 29.10 (C-4 α); 29.06 (C-4 β); 29.28 (C-5 α); 29.37 (C-5 β); 29.13 (C-6 α); 29.19 (C-6 β); 29.63 (C-7 α); 29.67 (C-7 β); 27.19 (C-8 α,C-8 β); 129.95 (C-9 α); 129.72 (C-9 β); 128.09 (C-10 α); 128.25 (C-10 β); 25.64 (C-11 α, C-11 β); 127.91 (C-12 α); 127.77 (C-12 β); 130.02 (C-13 α); 130.24 (C-13 β); 27.22 (C-14 α, C-14 β); 29.46 (C-15 α, C-15 β); 31.54 (C-16 α, C-16 β); 22.58 (C-17 α, C-17 β); 14.07 (C-18 α, C-18 β).

Properties of compound **4**: A pale-yellow liquid (8 mg). R_f value= 0.80 (DCM: MeOH = 98:2).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 5.32-5.34 (-CH=CH-, *m*, H-9, H-10); 2.35 (-CH_2COOH, *t*, H-2); 1.99-2.04 (=CH-CH_2-, *m*, H-8, H-11); 1.58-1.63 (-CH_2CH_2COOH, *m*, H-3); 1.24-1.29 (-$(CH_2)_n$-, *m*, H-4 to H-7, and H-12 to H-17); 0.88 (-CH_3, *m*, H-18).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm): 179.90 (C-1); 129.85 (C-9, C-10); 33.98 (C-2); 28.81-31.54 (C-4 to C-7, and C-12 to C-16); 27.16 (C-8, C-11); 24.58 (C-3); 22.57 (C-17); 14.11 (C-18).

2.3. Total Phenolic Content

According to the modified version of the procedure described in (Alhakmani et al., 2013), the total phenolic content of a plant extract can be assessed using the Folin-Ciocalteau reagent and gallic acid as a standard. Separate test tubes were filled with 0.5 mL (1 mg/mL) of a methanolic solution of *n*-hexane, DCM, and methanolic extracts of *C. melo* fruit. To each of these solutions, 2.5 mL of Folin-Ciocalteu reagent (10-fold diluted with distilled water) followed by 4 mL (7.5 % w/v) of Na_2CO_3 solution were added. Two minutes of vortexing and thirty minutes of incubation were performed on the solutions. Using a double-beam UV-Visible spectrophotometer (UV-1800), the absorbance at 765 nm was measured against a blank. Instead of extracts, gallic acid was used to prepare standard gallic acid solutions with concentrations of 6.25, 12.5, 25, 50, 100, 200 and 400 μg/mL. Absorbance of these solutions is measured at 765 nm. Then the total phenolic content was determined using the equation obtained from the standard gallic acid calibration curve ($y = 0.009x - 0.0336$, $R^2 = 0.995$) and expressed as mg GAE/g of dry extract.

2.4. Total Flavonoid Content

Modifications were made to the aluminum chloride colorimetric method (Bhaigyabati et al., 2014) to assess the total flavonoid content. By dissolving each extract in 20 mL of methanol, the stock solutions of *n*-hexane, DCM, and methanolic extracts were prepared. 2.5 mL of $AlCl_3$ reagent (2% $AlCl_3$ + 1M CH_3COONa) was transferred to the 5 mL of each stock solution. After incubating the

mixtures for 30 minutes at room temperature, the absorbance was measured at 430 nm using a double-beam UV-Visible spectrophotometer against a blank. Standard quercetin solutions with concentrations of 2, 5, 10, 20, 40, 50 and 100 µg/mL were used to make a calibration curve. Then the total flavonoid content was determined using the equation obtained from the standard gallic acid calibration curve ($y = 0.0082x - 0.0173$, $R^2 = 0.9908$) and expressed as mg QE/g of dry extractives.

2.5. Total Antioxidant Capacity

Total antioxidant capacity assay was performed by the formation of phosphomolybdenum complex described by Prieto et al., 1999, with modifications. The solutions of *n*-hexane, DCM, and methanolic extracts were prepared, each with a concentration of 1 mg/mL. 3 mL of reagent (mixture of 28 mM Na_3PO_4, 0.6 M H_2SO_4, and 4 mM ammonium molybdate in a 4:2:4 ratio) solution was added to 0.3 mL of each extract solution. After 90 minutes of incubation at 95 °C, it was left to cool down at room temperature. The absorbance of each solution was then measured against a blank using a UV-Visible spectrometer at 695 nm. Standard ascorbic acid solutions of concentration 200, 100, 50, 25, 12.5, 6.25 and 3.125 µg/mL were prepared by the same process as the extract to draw a calibration curve ($y = 0.0016x - 0.0103$, $R^2 = 0.9935$). Total antioxidant capacity was then determined using the equation obtained from the calibration curve, and the results were represented as mg AAE/g of dry extract.

2.6. DPPH radical scavenging activity

DPPH radical scavenging activity assay was conducted using an altered version of the procedure presented by Rahman et al., 2015. The antioxidant potential was assessed by comparing the bleaching of the violet-colored DPPH radical solution by the sample extracts to that of the standard butylated hydroxytoluene (BHT). A series of solutions of *n*-hexane, DCM and MeOH extracts of different concentrations (500, 250, 100, 80, 60, 40, 20, 10, 5 µg/mL) were prepared by serial dilutions from stock solutions (1 mg/mL) of different solvent fractions. A 2 mL methanolic solution of each extract was mixed with 2 mL of 0.1 mM DPPH solution in methanol. The mixtures were vortexed for two minutes and then incubated in the dark for thirty minutes. Using a double-beam UV-Vis spectrophotometer, the absorbance of each solution was measured against a blank at 517 nm. IC_{50} value for each extract was calculated using the formula (% Inhibition= $(A_0 - A_s/A_0) \times 100$); A_0= Absorbance of control, A_s= Absorbance of the sample).

3. Results and discussion

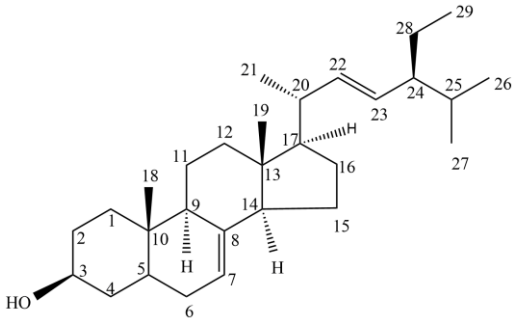
3.1. Compound isolation and structure elucidation

A total of four compounds could be isolated by repeated silica gel column chromatography from the DCM extract of *C. melo* L. fruit. The structures of these compounds were established by comparing the ^1H and ^{13}C NMR data with the reported data.

The ^1H NMR (400 MHz in $CDCl_3$) spectrum of compound **1** exhibited a triplet at δ 3.19 ppm, which confirmed the presence of an oxygenated methine proton (H-3) in the structure of the compound. The olefinic protons adjacent to C-7, C-22, and C-23 appeared as downfield (deshielded) signals at δ 5.18, 5.14, and 5.05 ppm, respectively. Signals of six methyl groups were exhibited at δ 0.53, 0.79, 1.03, 0.97, 0.92, and 0.81 ppm, which corresponded to H-18, H-19, H-21, H-26, H-27, and H-29, respectively.

The ^{13}C NMR spectrum of compound **1** produced signals at δ 138.17, 129.52, 117.48, and 139.64 ppm, which were due to four olefinic carbons. The signal at δ 71.03 ppm appeared due to oxygenated methine carbon. Signals at δ 34.24 (C-10), and 43.31 (C-13) ppm corresponded to quaternary carbons to which two angular methyl groups are attached. These data are typical of a sterol-type compound with two double bonds in the structure. Methyl carbons showed signals at δ 12.07, 13.05, 21.38, 21.09, 18.96, 12.25 ppm. The rest of the signals were exhibited either due to the nine methylene or seven methine groups.

The ^1H and ^{13}C NMR spectral data of compound **1** were in good agreement with reported NMR data of Stigmasta-7,22-dien-3β-ol (α-Spinasterol) (Yuan-Chuen et al., 2009; Meneses-Sagrero et al., 2017). Thus, compound **1** was established as α-Spinasterol.

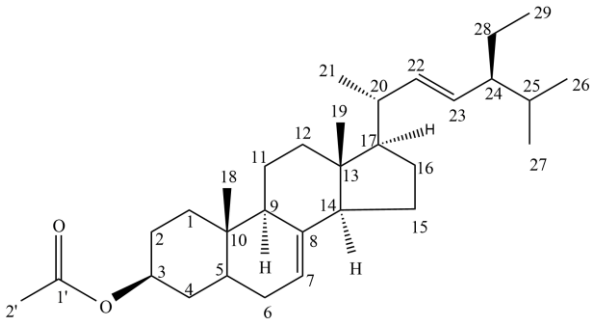


Compound **1**: Sitgmasta-7,22-dien-3β-ol (α-Spinasterol)

The ^1H NMR (400 MHz in $CDCl_3$) spectrum of compound **2** produced a doublet of doublet at δ 4.37 ppm, which demonstrated the presence of an oxygenated methine proton (H-3) in the structure of the compound. The olefinic protons attached to C-7, C-22, and C-23 appeared as downfield signals at δ 5.35, 5.14, and 4.71 ppm, respectively. The methyl group protons were exhibited at δ 0.53, 0.79, 1.02, 0.83, 0.78, 0.81, and 2.04 ppm, which corresponded to C-18, C-19, C-21, C-26, C-27, C-29, and C-2', respectively.

The ^{13}C NMR spectrum (100 MHz in $CDCl_3$) of compound **2** produced a signal at δ 174.17 ppm, which represented the carbon (sp^2 hybridized) of the ester carbonyl group (C-1'). Carbon (C-2') of the methyl group from the acetate side chain gave a signal at δ 21.54 ppm. The signal at δ 73.70 ppm appeared due to the oxygenated methine carbon. Signals at δ 34.33 (C-10) and 43.35 (C-13) ppm correspond to quaternary carbons to which two angular methyl groups are attached. Signals at δ 138.11, 129.55, 117.31, and 139.53 ppm were due to four olefinic carbons. The rest of the methyl carbons (except C-2') showed signals at δ 12.05, 13.03, 20.55, 22.66, 18.98, 12.31 ppm. The rest of the signals were exhibited either due to the nine methylene or seven methine groups. These data are typical of a sterol-type compound with two double bonds along with an acetate group in the structure.

^1H and ^{13}C NMR spectral data of compound **2** are in good agreement with reported NMR data of α-Spinasteryl acetate (Yuan-Chuen et al., 2009; Gomes and Alegrio, 1998; Henry and Chantalat-Dublanche, 2017; Raga et al., 2011). Thus, compound **2** was established as α-Spinasteryl acetate.

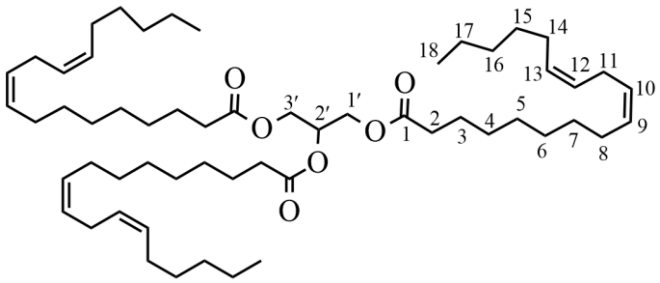


Compound **2**: α-Spinasteryl acetate

The ^{1}H NMR (400 MHz in $CDCl_3$) spectrum of compound **3** produced an overlapping triplet centered at δ 0.87 ppm due to the presence of methyl protons of saturated, linoleic acyl groups. Multiplet in the range δ 1.24-1.29 ppm was exhibited due to the methylene protons either in position β or γ, or away from olefinic groups or carbonylic groups inside the triglyceride molecule. Multiplet at δ 1.59-1.67 ppm and triplet at δ 2.28-2.32 ppm demonstrated the existence of methylene protons in β and α positions with respect to the carbonyl group, respectively. Allylic hydrogen atoms (α methylene protons) generated a multiplet at δ 1.99-2.08 ppm. Overlapping signals at δ 2.74-2.79 ppm are assignable to bis-allylic protons. Two doublets at δ 4.11-4.15 ppm and δ 4.27-4.29 ppm correspond to the hydrogen atoms joined with C-1' and C-3', respectively. Multiplet at δ 5.25-5.26 ppm appeared due to the hydrogen atom on C-2' of the glyceryl group. This signal overlapped slightly with doublet at δ 5.33-5.34 ppm, which occurred due to olefinic hydrogen atoms of acyl groups.

The ^{13}C NMR spectrum (100 MHz in $CDCl_3$) of compound **3** produced signals at δ 62.12 and 68.91 ppm, which are characteristic of glyceryl CH_2 (C-1' and C-3') and glyceryl CH (C-2') groups of the triacyl glyceride molecule. The carbonyl carbons of acyl groups produced signals at δ 173.26, and 172.85 for α and β positions respectively. The olefinic carbons (C-9, C-10, C-12, and C-13) produced signals at δ 129.95 (C-9 α), 129.72 (C-9 β), 128.09 (C-10 α), 128.25 (C-10 β), 25.64 (C-11 α, C-11 β), 127.91 (C-12 α), 127.77 (C-12 β), 130.02 (C-13 α), 130.24 (C-13 β). The signal at δ 14.07 ppm is assignable to methyl carbons (C-18). The backbone methylene carbons (C-2 to C-8, C-11, and C-14 to C-17) provided signals at various positions in the range δ 22.58-34.21 ppm. These data are typical of a triacyl glyceride molecule with the simultaneous presence of glyceryl, carbonyl, and allylic groups in the structure.

^{1}H and ^{13}C NMR spectral data of compound **3** are in good agreement with reported NMR data of Trilinolein (Guillén and Ruiz, 2003; McKenzie and Koch, 2004; Alemany et al., 2002). Thus, compound **3** was established as Trilinolein.

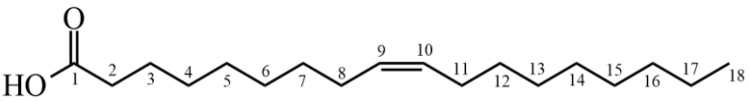


Compound **3**: Trilinolein

The ^{1}H NMR (400 MHz in $CDCl_3$) spectrum of compound **4** produced signal in the spectral region δ 5.32-5.34 ppm, which was indicative of olefinic protons attached to C-9 and C-10. The signal at δ 2.35 ppm was compatible with α-methylene group hydrogens adjacent to -COOH group. Allylic protons (α-methylene groups to one double bond) appeared in the range δ 1.99-2.04 ppm. Signals in the range δ 1.24-1.29 ppm were assignable to hydrogens of backbone methylene groups. The terminal methyl group appeared as multiplet at δ 0.88 ppm.

The ^{13}C NMR spectrum (100 MHz in $CDCl_3$) of compound **4** produced a signal at δ 179.90 ppm that was characteristic of the existence of the carbonyl group carbon (C-1) characteristic of carboxylic acid. The signal at δ 129.85 ppm was due to the presence of olefinic carbon atoms (C-9, and C-10). The methylene group carbon (C-2) adjacent to -COOH group produced signal at δ 33.98 ppm. The signal at δ 27.16 ppm was exhibited due to the presence of allylic carbon atoms. Signals in the range δ 28.18 to 31.54 ppm were assignable to the backbone carbons of methylene groups (C-4 to C-7, and C-12 to C-16). The signal corresponding to terminal methyl group carbon (C-18) appeared at 14.11 ppm. These data are typical of a fatty acid present in edible oil in free form.

^{1}H and ^{13}C NMR spectral data of compound **4** well fitted with reported data of Oleic acid (Satyarthi et al., 2009; Pietro et al., 2020). Thus, compound **4** was elucidated as Oleic acid.



Compound **4**: Oleic acid

3.2. Total phenolic content

Phenolics can play a crucial role in neutralizing free radicals and quenching singlet as well as triplet oxygen (Zheng and Wang, 2001). The MeOH extract showed the highest total phenolic content, which is 14.25 ± 0.83 mg GAE/g of dry extract, followed by DCM extract (12.06 ± 0.98 mg GAE/g of dry extract) and *n*-hexane extract (7.36 ± 0.63 mg GAE/g of dry extract) (Table 1, Fig. 1a). The highest value of TPC in MeOH extract was thought to be due to the greater solubility of phenolic compounds in methanol.

Among the extrinsic factors that influence phenolic concentration in fruits and other plant materials are geographical location, plant origin, cultivation techniques, climate, extraction methods, plant variety, choice of solvents, and the composition of the food matrix (Eseberri et al., 2022).

3.3. Total flavonoid content

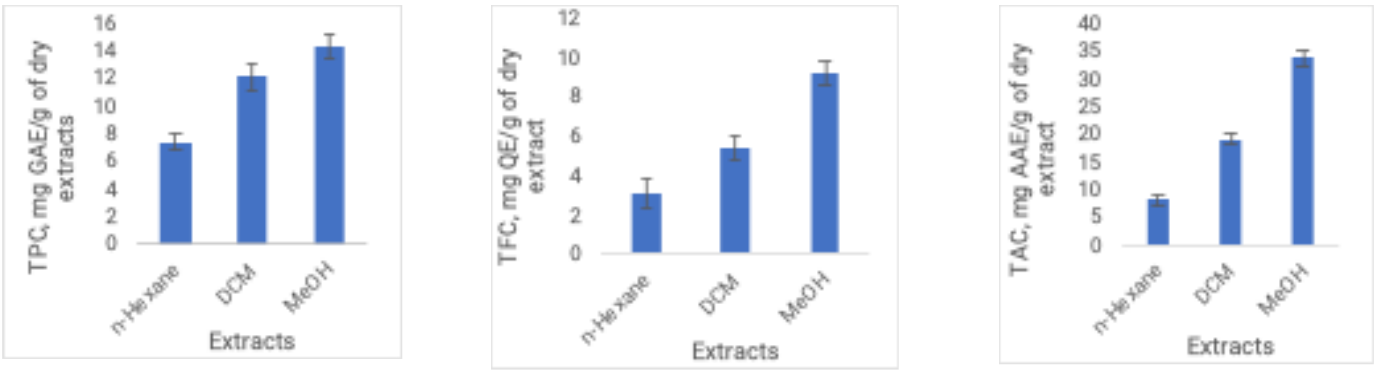
The functional hydroxyl groups in flavonoids mediate their antioxidant effects by removing free radicals from the environment or chelating metal ions (Rice-Evans et al., 1996). The MeOH extract exhibited the maximum flavonoid content (9.20 ± 0.58 mg QE/g of dry extract) compared to *n*-hexane (3.01 ± 0.73 mg QE/g of dry extract) and DCM extracts (5.37 ± 0.67 mg QE/g of dry extract) (Table 1, Fig. 1b). Thus, it was apparent that flavonoid compounds are much more soluble in MeOH than in the other two solvents.

3.4. Total antioxidant capacity

Reactive Oxygen Species that is a latent source of different health disorders in humans. Antioxidants play a role in terminating reactive oxygen species. So, they possess fabulous potential to remove free radicals from the human body, and keep our health robust, as well as disease-free (Saeed et al., 2012). This assay revealed that the total antioxidant capacity of MeOH extract was the highest (33.77 ± 1.58 mg AAE/g of dry extract), followed by DCM (18.98 ± 0.95 mg AAE/g of dry extract) and *n*-hexane extracts (8.15 ± 0.96 mg AAE/g of dry extract) (Table 1, Fig. 1c). The presence of phenolics and flavonoids may enhance the antioxidant activity of the methanolic extract, as these plant-derived metabolites are widely reported to exhibit antioxidant properties.

Table 1. Total phenolic content, total flavonoid content and total antioxidant capacity of different extracts of *C. melo* L.

Extract	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)	Total antioxidant capacity (mg AAE/g)
n-hexane	7.36 ± 0.63	3.01 ± 0.73	8.15 ± 0.96
DCM	12.06 ± 0.98	5.37 ± 0.67	18.98 ± 0.95
Methanol	14.25 ± 0.83	9.20 ± 0.58	33.77 ± 1.58
All results are represented as (Mean ± SD, n = 3)			



(a) (b) (c)

Fig. 1. (a) Total phenolic content, (b) Total flavonoid content, (c) Total antioxidant capacity

3.5. DPPH radical scavenging activity

Antioxidants can act as free radical scavengers that resist cellular damage, different fatal diseases like cancer, neurological disorders etc (Bai et al., 2016; Lobo et al., 2010). The DPPH radical scavenging assay is mainly performed for the evaluation of the antioxidant activity of plant extracts (Sowndhararajan and Kang, 2013). In the research, the DPPH radical scavenging activity of different extracts of *C. melo* was determined in a concentration-dependent manner, and IC_{50} value was calculated (Fig. 2, Fig. 3).

The methanolic extract possessed comparatively stronger radical scavenging activity (IC_{50} value of 11.13 µg/mL) than DCM and *n*-hexane extracts (IC_{50} value of 21.32 and 31.84 µg/mL, respectively). Radical scavenging potential of BHT was the highest with an IC_{50} value of 3.22 µg/mL.

The smaller half-maximum inhibitory concentration (IC_{50}) value suggests more antioxidant activity (Sowndhararajan and Kang, 2013). As the IC_{50} value of MeOH extract was smaller than the other two (DCM and *n*-hexane extracts), it possesses the most potent antioxidant activity. So, the order of radical scavenging activity was found as follows;

BHT > MeOH extract > DCM extract > n-hexane extract

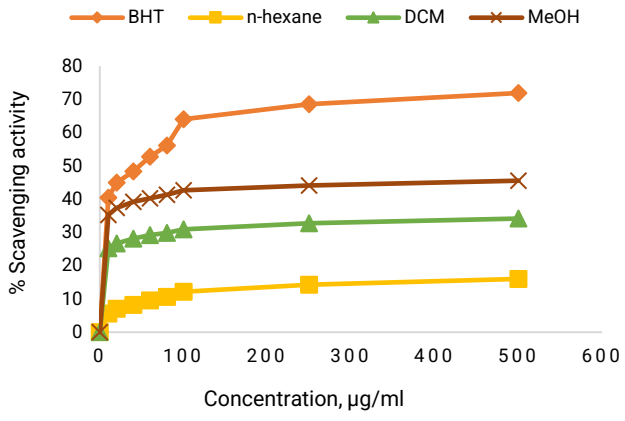


Fig. 2. % Scavenging activity of samples

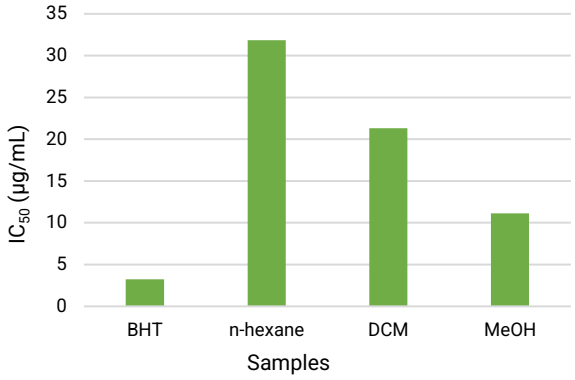


Fig. 3. IC_{50} value of samples

4. Conclusion

Plants with therapeutic properties have long been in high demand due to their accessibility, affordability, and relatively fewer adverse effects. In particular, there has been a growing global interest in the edible parts of plants among scientists. The present study on *Cucumis melo* L. fruit suggests that it is a promising source of natural antioxidants and various bioactive phytochemicals with a broad spectrum of biological activities. Four distinct bioactive compounds were isolated and characterized from the dichloromethane (DCM) extract, each exhibiting potential anti-inflammatory, antidiabetic, antiulcer, and antioxidant properties, with additional wound-healing capabilities in some cases.

Therefore, the fruit could be incorporated into the daily diet to help fulfill the body's regular demand for essential plant-derived nutrients. In conclusion, the findings of this study imply that *C. melo* fruit possesses significant antioxidant potential and may protect the human body from the harmful effects of free radicals. Furthermore, it is important to assess the bioavailability and efficacy of these nutraceuticals to determine whether they can serve as potential lead compounds in the development of therapeutic drugs for treating various currently incurable diseases.

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Conflict of interest

The authors declare no conflict of interest in this research.

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