Studies on Phytochemical screening , Proximate analysis and Antioxidant activity of *Cucumis melo* seed extract

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Introduction

Cucumis melo **– "Muskmelon "**

Family : Cucurbitaceae

Distribution : *Cucumis melo* is a native of Iran, Anatolia and Armenia and also widely cultivated many places of India. In India have been used economically important species

Scientific classification

- Kingdom : Plantae
- Order : Cucurbitales
- Family : Cucurbitaceae
- Genus : Cucumis
- Species : *C. melo*

- Binomial name : *Cucumis melo*

Medicinal properties of *Cucumis melo*

- **Obesity**
- **Heart diseases**
- **Skin diseases**
- **Anti- inflammatory**
- **Liver tonic**
- **Gastrointestinal disorder**

Objective of the present study

1. **The healthy fruit of *Cucumis melo* collected from Ponniyammen medu, Chennai, Tamil Nadu.**
2. **To study the phytochemical analysis of *Cucumis melo* seed extract.**
3. **To study the Quantification of Proximate analysis and phytochemical from *Cucumis melo* seed extract**
4. **To study the antioxidant activity of *Cucumis melo* seed extract.**

    a) Qualitative analysis

    b) Quantitative analysis

Objective of the present study (Continue)

5. To extract the active component by Column Chromatography of *Cucumis melo* seed extract.
6. To study the Anti – bacterial activity of *Cucumis melo* seed extract from the Best Fraction from Column Chromatography.
7. To identify the bioactive components present in the Best Fraction from Column Chromatography.

Collection of *Cucumis melo* seed

The healthy fruit of *Cucumis melo* collected from Ponniyammen medu, Chennai, Tamil Nadu and dehulled the seeds and Established at Poonga Biotech research Centre, Chennai .

Phytochemical Screening of *Cucumis melo* :

- **The phytochemical screening of seed extracts were assessed by standard method as described by Brinda *et al.*, (1981); Siddiqui and Ali (1997) and Savithramma *et al.*, (2011).**

- **Phytochemical screening was carried out on the seed extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, alkaloids, terpenoids, glycosides, cardiac glycosides, coumarins and steroids.**

- **General reactions in these analyses revealed the presence or absence of these compounds in the seed extracts tested.**

Phytochemical screening from seed extracts of *Cucumis melo*

Phytochemicals Tested	Seed extracts of *Cucumis melo*				
	Aqueous	Ethanol	Chloroform	Petroleum ether	Acetone
Tannins	+	-	-	-	-
Saponins	+	-	-	-	+
Quinones	+	+	-	-	-
Terpenoids	+	+	-	+	-
Steroids	+	+	-	+	-
Flavonoids	+	+	+	+	-
Phenol	+	+	+	+	+
Alkaloids	+	-	-	-	-
Glycosides	-	-	-	-	-
Cardiac glycosides	+	-	-	-	-
Coumarins	-	-	-	-	-
Antho cyanin	-	-	-	-	-
Beta cyanin	+	+	-	+	-

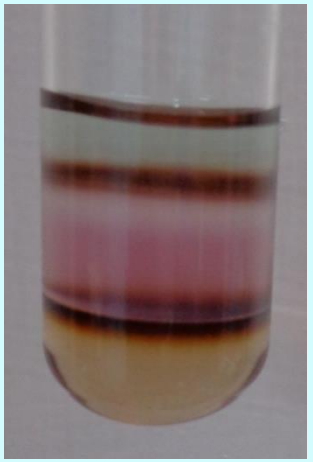
+ = positive; - = negative

Phytochemical Screening of *Cucumis melo*

tannins

saponins

Quinones



steroids

Terpenoids

phenols

alkaloids

flavonoids

Conclude the phytochemical results

- **In the present study, phytochemical screening was performed with five different solvents such as chloroform, petroleum ether, acetone, ethanol and aqueous seed extract of *Cucumis melo* .**

- **Among the five different extracts of *Cucumis melo* , the aqueous seed extract of *Cucumis melo* - terpenoids, quinones, Cardiac glycosides, flavonoids, steroids, phenols, tannins and saponins followed by other accessions.**

- **The maximum amount of phytochemicals was screened in Aqueous**

Qualitative analysis of Antioxidant activity of *Cucumis melo*

- **The antioxidant activity of seed extracts of *Cucumis melo* was determined by following the method as described by Selvaraj *et al.*, (2014).**

- **50µL of seed extracts of *Cucumis melo* were taken in the microtiter plate. 100µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition.**

- **The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively.**

- **The antioxidant positive samples were subjected for further quantitative analysis.**

Quantitative analysis of Antioxidantactivity of *Cucumis melo*

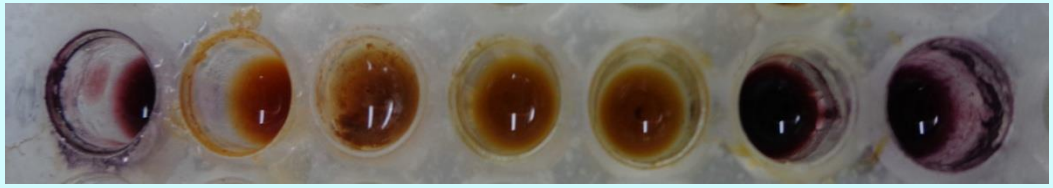
- **The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical.**

- **Seed extract of 100µl were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition.**

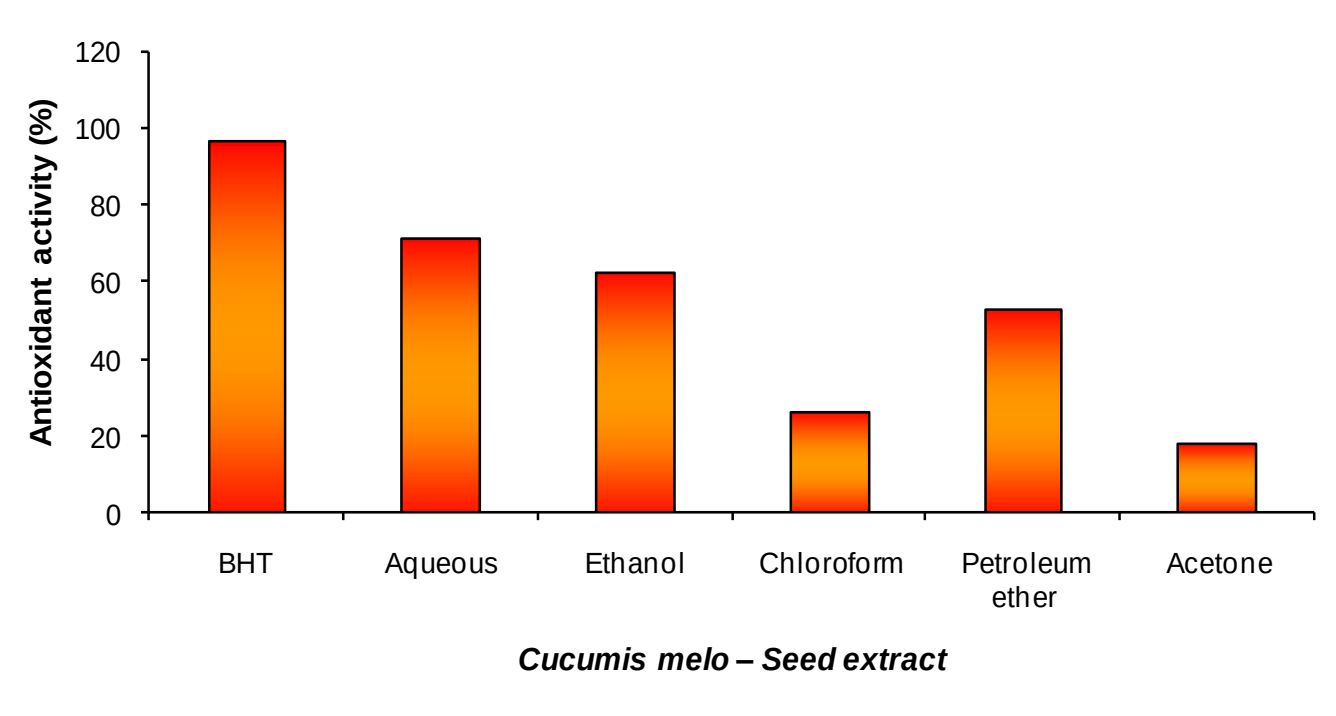
- **Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al.*, 2005). Subsequently, at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm.**

- **The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicate.**

- **Free radical scavenging activity was calculated by the following formula: % DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance Of control)] x 100.**



Qualitative analysis of Antioxidant activity of *Cucumis melo*



Quantitative analysis of Antioxidantactivity of *Cucumis melo*

Antioxidant activity results

- ✓**In DPPH assay - the percentage of antioxidant activity of *Cucumis melo* Seed extracts values recorded at 30 minutes were analyzed.**

- ✓***Cucumis melo* seed extracts were analysed, samples collected from Ponniyammen medu, Chennai had the highest radical scavenging activity (73.2 %) and followed by others.**

- ✓**Among five different solvent extractions used, Aqueous extract was found to be good than other extractions.**

Estimation of total phenol and flavonoid content of *Cucumis melo*

- **Total phenol content in the seed extracts of Cucumis melo was determined by the Folin Ciocalteau colorimetric method (Slinkard and Singleton, 1977).**

- **Total flavonoid content in the seed extracts was determined by the aluminium chloride colorimetric method (Mervat et al., 2009).**

Quantitative estimation of

- ❖Total phenol
- ❖Total flavonoid,

S.No	Plant sample	Total phenol content (mg GAE/g)	Total flavonoid content (mg QE/g)
1	*Cucumis melo*	23.32	63.1

Proximate Analysis

- **The protein and carbohydrate content in seed extracts of *Cucumis melo* was estimated by following the method as described by Lowry *et al.*, (1951) and Dubois *et al.*, (1956)**

- **The moisture and Ash Content in seed extracts of *Cucumis melo* was estimated by following the method as described by (Pedavaoh *et al.*, 2014)**

Proximate analysis from seed extract of *Cucumis melo*

- **Carbohydrates – 154.8 mg/g**
- **Protein – 73.5 mg/g**
- **Ash Content – 7.62%**
- **Moisture Content – 28.7%**

Extraction of active compounds from seed extract of *Cucumis melo*(Shimizu et al., 1997).

- **The seed extract in ethanol was analysed by Column chromatography technique as per standard methods.**

Column Chromatography

- **Silica gel (100 - 200 mesh - Fisher Scientific – India) were washed thoroughly using methanol solvent for 3 times.**

- **The cleaned silica gel, 10gm of silica gel was dissolved with 20 ml of Double distilled water; the slurry of semisolid / liquid silica gel carefully poured to column without any air bubbles.**

- **Concentrated seed extract of *Cucumis melo* (10mg /ml) was carefully transferred on to the upper surface of silica gel.**

- **Mobile phase methanol: chloroform (2 :1) ratio; Eluent is slowly passed through the column.**

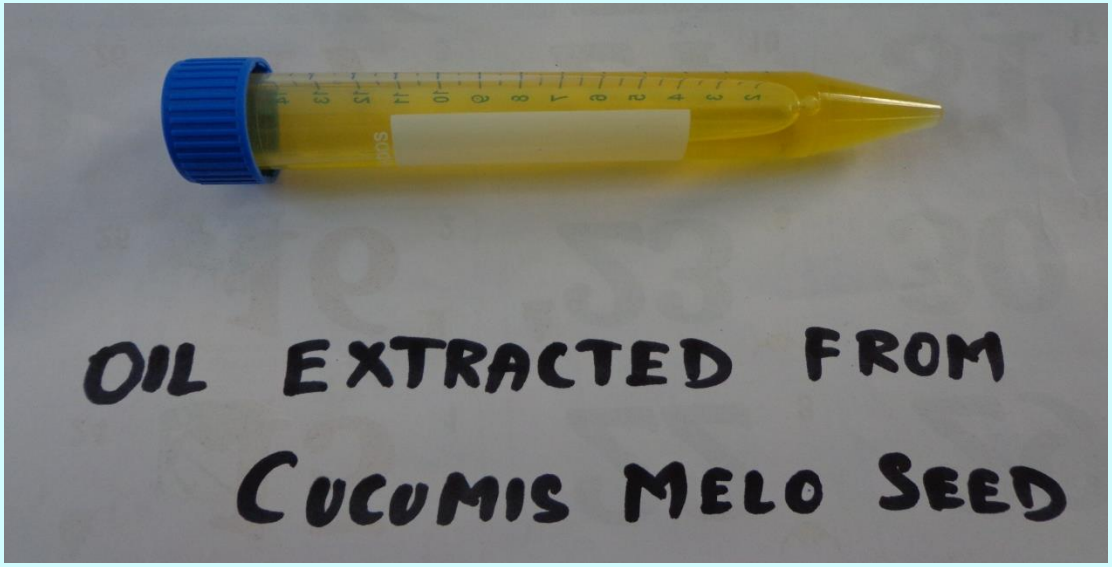
Fraction I : (0 – 5 mins)	**Fraction V : (20 -25 mins)**
Fraction II : (5 – 10 mins)	**Fraction VI : (25-30 mins)**
Fraction III : (10 - 15 mins)	**Fraction VII : (30 -35 mins)**
Fraction IV : (15 -20 mins)	**Fraction VIII : (35 - 40 mins)**

Antioxidant activity results for Fractions

- ✓**In DPPH assay - the percentage of antioxidant activity of *Cucumis melo* Seed extracts(Fractions) values recorded at 30 minutes were analyzed.**

- ✓***Cucumis melo* seed extracts were analysed, samples collected from Ponniyammen medu, Chennai had the highest radical scavenging activity (94.49%) in Fraction V.**

- ✓**Among eight different fractions obtained, Fraction V was found to be good than other extractions.**


OIL EXTRACTED FROM
CUCUMIS MELO SEED

Anti-bacterial activity of seed extract of *Cucumis melo*

- The best fraction (V) from seed extract of *Cucumis melo* was used for antibacterial study (Ozkan *et al.*, 2004; Janarthanam and Sumathi 2010).
- Different concentrations (10mg, 20mg and 30mg /ml) of the concentrated seed extract was tested for its antimicrobial activity against pathogenic bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli and Pseudomonas aeruginosa*.

- The bacterial cultures were grown in Mueller Hinton Agar and Mueller Hinton Broth (Himedia) (Lopez *et al.*, 2001).

Antibacterial activity assays

✓Antibacterial activity was measured using the standard method of diffusion disc plates on agar (Erturk *et al.*, 2003).

✓For antimicrobial assay, all bacterial strains were grown in Mueller Hinton Broth Medium (Himedia) for 24 hours at 37° C and plated on Mueller Hinton Agar (Himedia) for agar diffusion experiments.

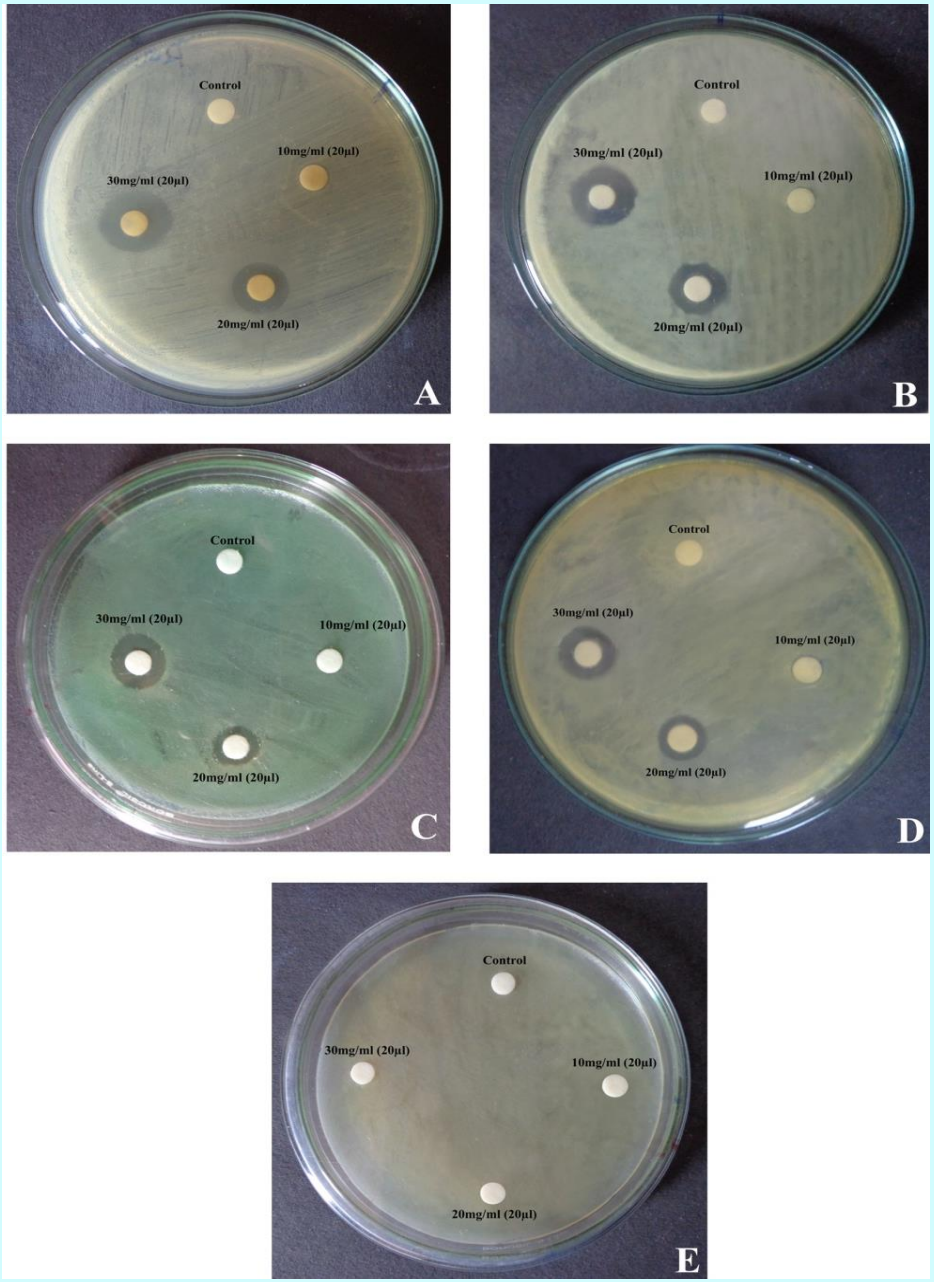
✓Then 0.1ml of each culture of bacteria was spread on agar plate surfaces. Sterile disc (Hi Media, 6mm in diameter) were placed on the agar medium to load 20µl of different concentration (10 -30mg /ml) of best fraction (V) seed extract of *Cucumis melo* were tested.

✓Inhibition diameters were measured after incubation for 24 hours at 37° C. Blanks of solvent only (processed in the same way), were also tested for antibacterial activity.

Antibacterial activity of *Cucumis melo* seed extract

Inhibition Zone in diameter (mm)*			
Micro-organisms Tested	**Concentrations of extract**		
Seed extract (V fraction)	10mg/ml	20mg/ml	30mg/ml
Bacillus subtilis•	-	12	15
Bacillus cereus•	-	11	14
Pseudomonas aeruginosa•	-	12	12
Staphylococcus aureus•	-	10	12
Escherichia coli•	-	-	-

Antibacterial activity of *Cucumis melo* seed extract



A. Bacillus subtilis.
B. Bacillus cereus.
C. Pseudomonas aeruginosa.
D. Staphylococcus aureus.
E. Escherichia coli.

GC-MS analysis

- For the Identification of bioactive components in extract with greater antioxidant activity, the extract was subjected to GC-MS analysis.
- GC-MS analysis was carried out on a GC-MS -5975C agilent system comprising an auto sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument, employing the following conditions: column Elite-1 fused silica capillary column (30×0.25 mm ID × 1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1.51 ml/min and an injection volume of 1µl was employed (split ratio of 10:1) injector temperature 2400C; ion-source temperature 2000C.
- The oven temperature was programmed from 700C (isothermal for 2 min), with an increase of 100C/min, to 3000C/min, ending with a 9 min isothermal at 3000C.
- Mass spectra were taken at 70eV; with a scan range 40-1000 m/z. Solvent cut time was 5 min; MS start time being 5 min; MS end time being 35 min; Ion source temperature set to 2000C and interface temperature being 2400C.

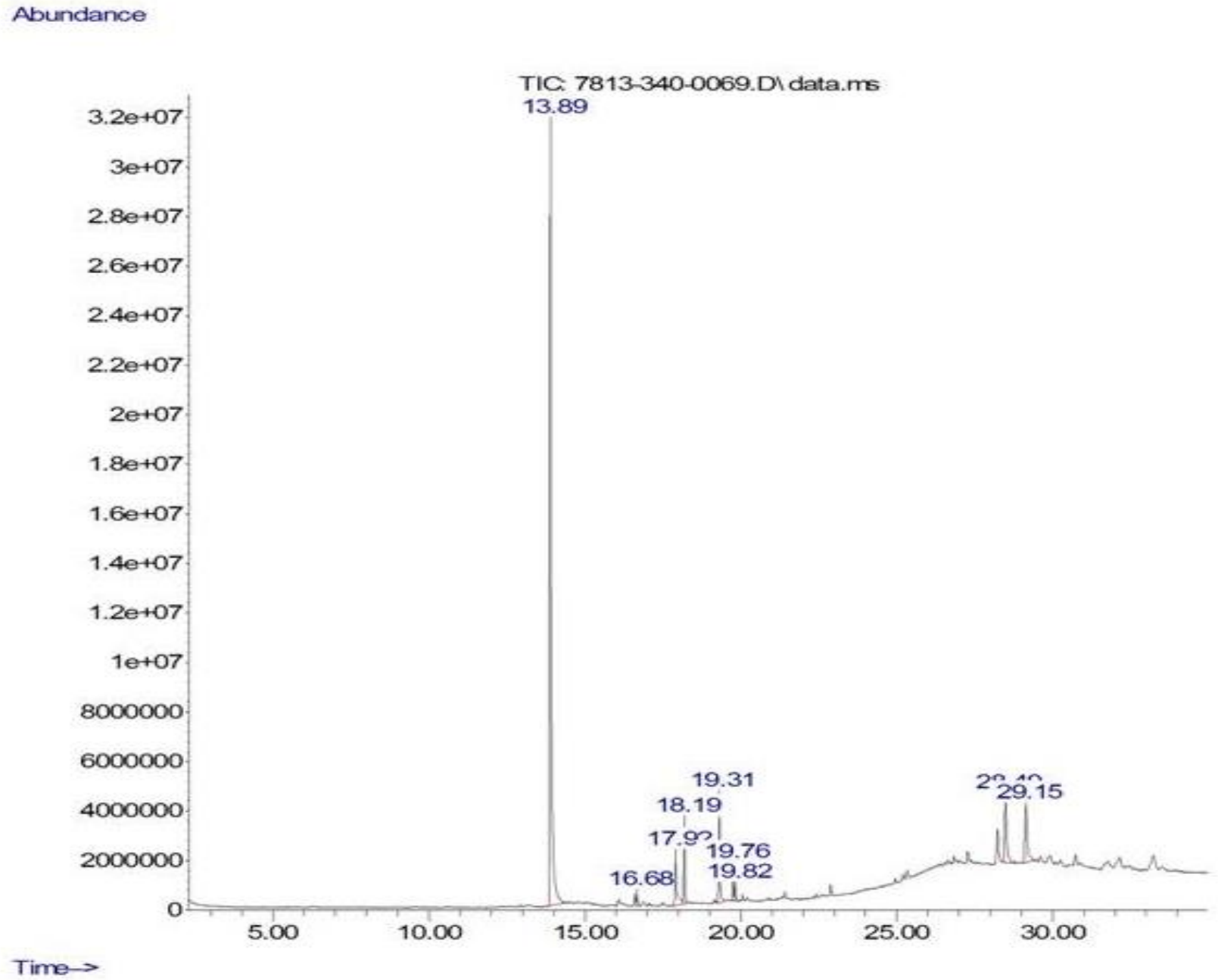
Identification of Components

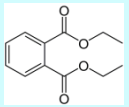
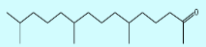
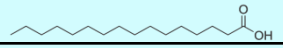
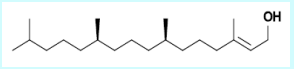
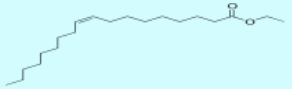
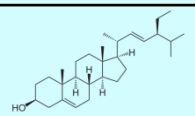
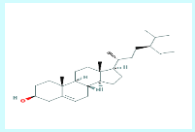
- Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62000 patterns.

- The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

- The name, molecular weight and structure of the component of the test materials were identified.

GC- MS Chromatogram of *Cucumis melo* seed extract



No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %	Structure
1	13.891	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.089	62.37	
2	16.679	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	268.27	1.09	
3	17.914	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.24	5.00	
4	18.190	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.27	4.60	
5	19.308	Phytol	$C_{20}H_{40}O$	296.308	8.30	
6	19.758	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.272	1.74	
7	19.816	Ethyl Oleate	$C_{20}H_{38}O_2$	310.287	1.02	
8	28.486	Stigmasterol	$C_{29}H_{48}O$	412.371	8.22	
9	29.140	gamma.-Sitosterol	$C_{29}H_{50}O$	414.386	7.67	

n-hexadecanoic acid (Palmitic acid)

- Used to produce soaps and cosmetics.
- Inexpensive and adds texture to processed food.
- Sodium palmitate is permitted as a natural additive in organic products
- Hydrogenation -> Palmitic acid -> Cetyl alcohol -> Used to produce detergents.

Phytol

- Phytol is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1.

- Used in
  - Fragnance industry
  - Cosmetics
  - Shampoos
  - Detergents

Ethyl oleate

- Fatty acid ester.
- Food Additive (FDA approved under "Food Additives Permitted for Direct Addition to Food for Human Consumption")
- Used as
  - Lubricant
  - Plasticizer

Conclusion

- Since it has a good nutrition value, phytochemical as well as antioxidant value and antibacterial activity it can be used as a **supplement oil** in **cooking oil**.

- Since the seeds were not utilised by the people, it can be added as a **flavouring agent** in foods like **kesari, halwa**.

- For children, those who don't eat such seeds, it can be added as a **substitute** for almonds.


THANK YOU
for your time