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STUDY OF INVITRO ANTIOXIDANT ACTIVITY AND GCMS ANALYSIS OF SEEDS OF *CUCUMIS MELO*

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ABSTRACT

This study investigates with the Phytochemical analysis and antioxidant activity of methanolic extract of *Cucumis melo* belongs to cucurbitaceae family. The seed extract of this fruit exhibit Tannins, Steroids, Terpenoids, Coumarins, Leucoanthocyanins, Phytosterols, Proteins and amino acids as phytochemicals during analysis. The bionutrients were qualitatively analysed and their antioxidant activity was estimated using Hydrogen peroxide. GC-MS (Gas Chromotography-Mass Spectrometry) analysis of the seed extract exposed presence of compounds such as 2,6,10-trimethyl, 14-ethylene-14-pen, 9,12,15-Octadecatrienoic acid, methyl ester, 2-hexadecen-1-ol, 3,7,11,15-tetramethyl, 9-Octadecen-1-ol, 9,12,15-Octadecatrienoic acid, (Z,Z,Z), Octadecanoic acid and their biological activity quantitatively. Isolation of bioactive compounds could help to find new drugs to treat various diseases.

KEYWORDS: *Cucumis melo*, Phytocomponents, Antioxidant activity, GCMS.

INTRODUCTION

Cucumis melo has 5-15mm long seeds. Fruit vary in size and shape, green or yellow is their natural color. This fruit is available all over India. People consume the raw fruit pulp with or without sugars and as a beverage after intermingling it with other ingredients. The muskmelon based processed products are attracting and becoming popular in the market because of its rich nutrient profile. Jam, fruit bar, wine, chutneys or sherbet, pulp powder, juice, ice like products are made from the pulp of the muskmelon. It also involved in the

prevention of atherosclerosis and Hypothyroidism. The bioactive compounds are used as source for medicine against toxins. More than 4000 phytochemicals have been classified by their chemical and physical characteristics.[16] Phytocomponents are not a substance which are necessarily required for human but they have potentiality to act against diseases which affect human health. Phenolics, Saponins, Tannins, Glycosides, Tannins, Alkaloids and Terpenoids are the phytochemicals which are commonly present in medicinal plant and fruit to provide health benefits as macronutrients and micronutrients. An individual component may have more than one biological activity such as Antioxidant activity, Anti-inflammatory activity, Anti-diuretic activity, Anti-histimic activity, Antimicrobial activity, Antifungal activity, Hepato protective activity. Free radicals contribute to more than one hundred disorders in human due to environmental pollutants, deep fried and spicy foods as well as physical stress is one of the reasons for the change in gene expression and induce abnormal proteins. Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) have been suspected to prompt negative health effects.[7] There is a trend to substitute them with naturally occurring antioxidants which have more solubility than synthetic antioxidants. The GC-MS study also carried out to ensure the presence of compounds such as 2,6,10-trimethyl,14-ethylene-14-pen, 9,12,15-Octadecatrienoic acid, methyl ester, 2-hexadecen-1-ol, 3,7,11,15-tetra methyl, which are responsible for the antioxidant activity against free radicals.

MATERIALS AND METHODS

Extraction

Fresh ripened fruits of muskmelon were purchased from the local market Dindigul. The seeds of fruit were separated manually and shade dried for overnight. The dried materials were powdered using a blender. For the extraction, 10g of sample was tighten to a lint free cloth and soaked in 100ml of methanol for 48 hours at 37℃ was kept in shaker. After that, the sample was poured on to petridishes for evaporation. The extract was collected and stored at 4℃.

Phytochemical analysis

Table 2.2(a). Table for Methods of Phytochemicals

S.NO	PHYTOCHEMICALS	METHODS
1	Tannins	Extracts were treated with 1% gelatin solution that have sodium chloride. After few minutes the existence of tannins identified by the formation of white precipitate. This is known as gelatin test.
2	Protein and amino acids	Few drops of concentrated nitric acid was added to the extract. The presence of proteins are indicated by the yellow color formation.
3	Steroids	1ml of extracts were treated with 10ml of chloroform. 10ml of concentrated sulphuric acid was added side by side. The red color of the upper layer and the green fluorescence color of another layer indicates the presence of steroids.
4	Phytosterols	The few drops of concentrated sulphuric acid was added to the aqueous extract. Appearance of yellow color denotes its attendance.
5	Coumarins	2ml of extracts were treated with 3ml of 10% NaOH. The appearance of yellow color indicates the presence of coumarins.
6	Terpenoids	1ml of extracts were treated with 2ml of chloroform and 3ml of concentrated sulphuric acid. The formation of reddish brown color indicates the presence of terpenoids.
7	Leucoanthocyanins	1ml of extracts were treated with 1ml of iso amyl alcohol. The appearance of red color layer indicates its presence.

Free radical scavenging activity assay

The hydroxyl radical scavenging activity assay was based on the methodology with fenton reaction.[4] Different concentration of extracts(20,40,60 and 80μ g/ml) were chosen for in vitro antioxidant activity. L-Ascorbic acid was used as the standard. 60μl of 1.0mM $FeCl_3$, 90μl of 1mM 1,10-phenanthroline, 2.4ml of 0.2M phosphate buffer(pH 7.8), 150lμl of 0.17M H_2O_2 are present in reaction mixture and 1.5ml of extract at various concentrations. Adding H_2O_2 started the reaction. After incubation at room temperature for 5min, the absorbance of the mixture at 560nm was measured with a spectrophotometer. The following equation was used to calculate the radial scavenging activity.

$$\%\text{Inhibition} = ((A_0 - A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without extract) known as reference and A_1 was the absorbance in the presence of the extract under various concentration.

GC MS Analysis

Shimadzu 2010 plus comprising a AOC-20i auto sampler used to done GCMS and the following conditions were utilized by gas chromatograph interfaced to a mass spectrometer: column RTX 5Ms (Column diameter is 0..32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.99%) was used as carrier gas. The constant flow of gas 1.73 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 543K; ion-source temperature 473K. The oven temperature was programmed from 313K (isothermal for 2 min), with an increase of 281K/min to 423K,then 281K/min to 523K ending with a 20min isothermal at 553K. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. 51.25min is a total running time of GC. By comparing its average peak area to the total areas, the relative percentage amount of each component was calculated. Software adopted to observe mass spectra and chromatograms was a Turbo Mass Ver 5.2.0[17]

RESULT AND DISCUSSION

The seeds of *Cucumis melo* were extracted with methanol solvent. The extract shows the considerable phytochemicals which are used as a raw material for medicine. The presence and absence of the phytochemicals are tabulated below.

Table 3(a): Presence and absence of Phytochemicals.

Phytochemical	Methanolic extract
Tannins	+
Phytosterols	+
Terpenoids	+
Coumarins	+
Protein and aminoacids	+
Steroids	+
Leucoanthocyanins	+

+indicates presence; - indicates absence

Statistical analysis

Tests were carried out in triplicates. The amount of extract needed to inhibit free radicals concentration by 50%, A non-linear regression algorithm was used to estimate IC_{50} graphically. This result shows that the yellow color extract of *cucumis melo* seeds have high inhibition than the standard. It scavenges the free radicals more than standard L-Ascorbic acid.

Table 3(b): Hydroxyl radical scavenging activity of *Cucumis melo* extract and Standard.

S.NO	Concentrations (µg/ml)	*Cucumis melo* extract	Ascorbic acid (Standard)
1	20	18.33 ±1.45	32.21± 2.51
2	40	37.50±2.61	56.45± 4.40
3	60	58.75±3.81	78.65±6.13
4	80	78.75±5.12	92.75±7.2
	IC_{50}	51.64	35.26

Values are expressed as Mean ± SD for triplicates

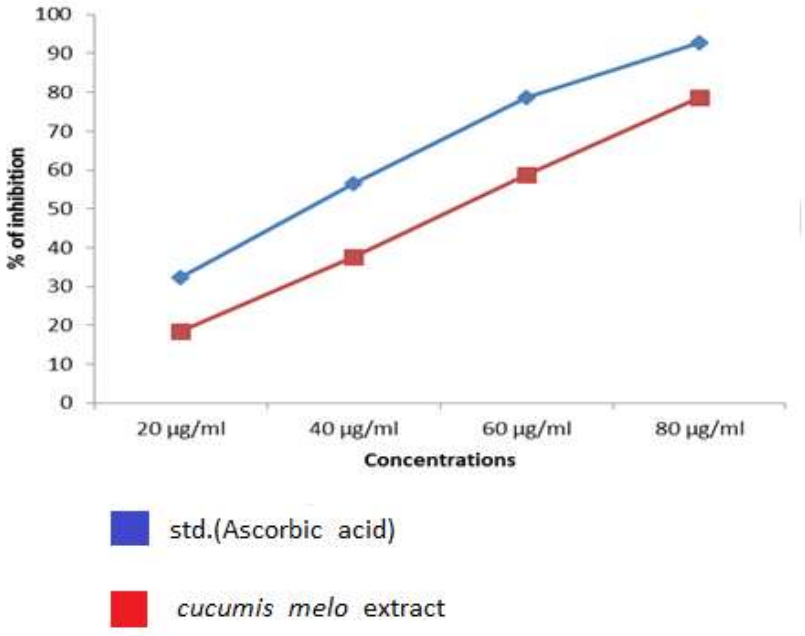

Fig 3(a): Hydroxy radical scavenging activity of *Cucumis melo* extract and Standard.

Identification of components

Interpretation on GCMS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components and the spectrum of the known components were compared and stored in the NIST library. The components of the test materials were assured by name, molecular weight and structure.[18]

Table 3(c): Identification Table: Identification of active compounds in *Cucumis melo* using GCMS.

Peak	R.Time	Area %	Height %	Molecular Formula	Molecular Weight	Name of the compounds
1	13.479	2.24	3.29	$C_{15}H_{24}$	204	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-
2	15.637	1.86	2.25	$C_{12}H_{14}O_4$	222	Diethyl phthalate
3	17.961	0.97	1.21	$C_{11}H_{16}O_3$	196	2(4h)-benzofuranone, 5,6,7,7a-tetra
4	18.328	15.94	25.29	$C_{20}H_{38}$	278	2,6,10-trimethyl,14-ethylene-14-pen
5	18.400	0.82	1.19	$C_{11}H_{20}O_2$	184	2-Ethylhexyl acrylate
6	18.599	2.91	4.32	$C_{20}H_{40}O$	296	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
7	18.809	7.34	9.83	$C_{20}H_{40}O$	296	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-,
8	19.269	0.54	0.92	$C_{17}H_{34}O_2$	270	Hexadecanoic acid, methyl ester
9	19.641	24.53	20.72	$C_{16}H_{32}O_2$	256	N-Hexadecanoic acid
10	20.934	2.02	2.34	$C_{19}H_{40}O$	284	N-Nonadecanol-1
11	21.167	0.91	0.85	$C_{19}H_{32}O_2$	292	9,12,15-Octadecatrienoic acid, methyl ester
12	21.290	8.51	8.93	$C_{20}H_{40}O$	296	2-hexadecen-1-ol, 3,7,11,15-tetramethyl
13	21.567	5.60	4.87	$C_{18}H_{36}O$	268	9-Octadecen-1-ol
14	21.639	20.05	9.90	$C_{18}H_{30}O_2$	278	9,12,15-Octadecatrienoic acid, (Z,Z,Z)
15	21.796	5.76	4.09	$C_{18}H_{36}O_2$	284	Octadecanoic acid

Table 3(d): Biological activity of compounds identified in *Cucumismelo* using GCMS.

S.No	R.Time	Name of the compounds	Biological activity**
1	18.599	3,7, 11,15-Tetramethyl-2-hexadecen-1-ol.	Cancer-Preventive, Antimicrobial, Anti-Inflammatory, Anti-Diuretic Antioxidants
2	18.809	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-,	Precursor for the manufacture of Synthetic forms of vitamin E and Vitamin k1. Used in the fragrance Industry and used in cosmetics, Shampoos, toilet soaps, household Cleaners, and detergents
3	19.269	Hexadecanoic acid methyl ester	Antioxidant, Flavor, Antifibrinolytic, Hypocholesterolemic, Antiandrogenic, Lubricant, Hemolytic, 5-Alpha Reductase Inhibitor, Nematicide, Antialopecic.
4	20.934	N-Nonadecanol-1	Anti-microbial and cytotoxic properties
5	21.167	9,12,15-Octadecatrienoic acid, methyl ester	Antimicrobial, Antioxidant, Hypocholesterolemic, Anti-Inflammatory, Cancer Preventive, Hepatoprotective, Anti-Arthritic, Anti-Histimic, Anti-Enzemic And Anti-Coronary. Methyl Octadecanoate Exhibits Antifungal And Anti-Cancer Activities
6	21.567	9-Octadecen-1-ol	Antimicrobial Activity
7	21.796	Octadecanoic acid	Lower LDL Cholesterol Level, Antiviral, Antiinflammatory, Hypocholesterolemic,

**Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].

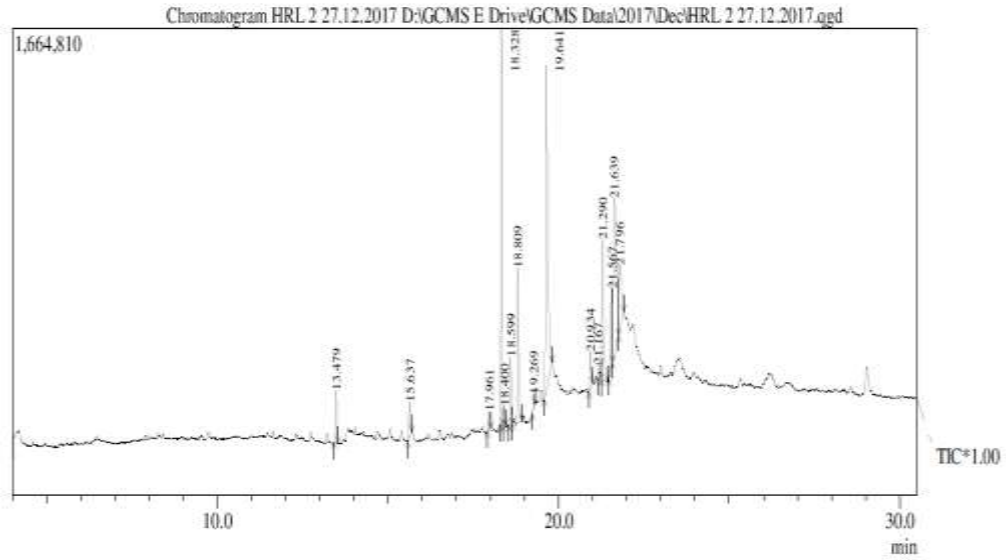

Fig 2: Graphical representation of *Cucumis melo* extract.

Cucurbitaceae plants are very useful as they have large potential against many health ailments.[13] In this study the phytochemical analysis of the methanolic extract naked the presence of Phytosterols, Tannins, Steroids, Terpenoids, Coumarins, Leucoanthocyanins, Proteins and amino acids. Due to the attendance of bionutrients the seeds offer chance to expand as medicines, cosmetics, value added products and dietary supplements.[14] Antioxidants are work by preventing the formation of new radical species and converting the existing free radicals into less harmful molecules.[15] Here the presence antioxidant activity ensured by invitro free radical scavenging assay using Hydrogen peroxide. The bioactive components and their biological activity was identified quantitatively by GCMS. 9-Octadecen-1-01, Octadecanoic acid, 9,12,15-octa decatrienoic acid (Z,Z,Z) are the components present in this sample more than others. These are all inexpensive components and add texture to processed food. Since, the seeds were used as a flavouring agent in foods like halwa and kesari.

CONCLUSION

The graphical representation of antioxidant activity of seed extract confirms the property of *Cucumis melo* which has the capacity to act as a precursor for the manufacture of the synthetic forms of Vitamin E and Vitamin K1. GC MS is a fast analytical method with excellent precision and accuracy. It estimates the bioactive components of *Cucumis melo* with retention time by graph and their biological activity was tabulated at Table3(d).

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