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Research Article

In Vitro Pharmacognostical Studies and Evaluation of Bioactive Constituents from the Fruits of *Cucumis melo* L. (Muskmelon)

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ABSTRACT
The objective of the present study is to evaluate the presence of bioactive constituents and antioxidant, antimicrobial activity of solvent extract of fruit of *Cucumis Melo* Linn. The extraction was done with Ethanol using reflux apparatus. The GC-MS analysis was done by using the standard procedure. Antimicrobial activity was evaluated by Agar well diffusion method against five human pathogens. DPPH radical-scavenging activity was done by using standard procedure. Ten compounds in ethanolic extract were identified. 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-4H-chromen-4-one (21.04%) was the major proportion. The extract and fractionates of fresh fruits of *Cucumis Melo* showed a significant and remarkable activity against all the microorganisms. Based on the antioxidant analysis obtained, it was showed that the extract of *Cucumis melo* fruits exhibits the greatest antioxidant activity through the DPPH radicals scavenging activity. The present study concludes that the ethanol extract of fruit of *Cucumis melo* contains broad spectrum of bioactive compounds and also exhibit antimicrobial activity against all the tested microorganisms. It indicates the presence of these biologically active chemical in *Cucumis Melo* may justify their wide usage in traditional medicine.

Keywords: *Cucumis melo* Linn, Ethanol extract, Pharmacognostical studies, *Salmonella typhi*, Antioxidant, Antimicrobial activity.

INTRODUCTION

Natural products play an important roles of drug discovery process include provide basic compounds affording less toxic and more effective drug molecules, serve as extremely useful natural drugs, exploration of biologically active prototypes towards newer and better synthetic drugs and modification of inactive natural products by suitable biological or chemical means into potent drugs.

Muskmelons are monoecious plants. They do not cross with watermelon, cucumber, pubpkin, or squash but varieties within the species intercross frequently. The genome of *Cucumis melo* L. was first sequenced in 2012. Muskmelons are an excellent source of vitamin A and vitamin C, and a good source of potassium. In addition to their consumption when fresh, melons are sometimes dried. Other varieties are cooked, or grown for their seeds, which are processed to produce melon oil.

The word "phyto" is the Greek word for plant. Phytochemicals which not only that they are non nutritive plants chemicals they have protective or disease preventive properties but also protect human from a host of disease. Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins. Alkaloids and flavonoids have been used as antiviral, antibacterial, antimicrobial and anticancer agents. Phenolic and polyphenolic are the other group of secondary metabolites. The uses of plant – derived products as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance.

Antioxidants are substances that are used to fight against the free radicals which has involved in food and chemical material degradation and oxidize nucleic acids, proteins and lipids which will cause oxidative stress and initiating degenerative diseases such as cancer, Alzheimer's disease, Parkinson's diseases and some cardiovascular diseases Over the years, prevalence of the diseases result from oxidative stress has been increasing over the year.

Hence the present paper reports the antimicrobial, antioxidant properties and Gas Chromatography - Mass Spectrometry (GC – MS) analysis on the isolated essential oil of *Cucumis melo* Linn fruits.

MATERIALS AND METHODS

Plant Collection: The Fruits of *Cucumis melo* were purchased from Reliance daily fresh, Villapuram, Madurai, District, Tamil Nadu, India. The fruits were washed several times with distilled water to remove soil particles and impurities. The whole fruits were dried for 24 hours under low sun intensity. The dried materials were powdered mechanically and they were stored in polythene bag to be used as samples for the extraction.

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Table 1: Secondary Metabolites detected in the isolated essential oil from the fruits of *Cucumis Melo*

S.No	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak area %
1	13.68	Anhalinine	$C_{12}H_{17}NO_3$	222	9.63
2	18.03	Estra-1,3,5(10)-trien-17-beta-ol	$C_{19}H_{26}O_2$	256	10.73
3	18.63	Hexadeca-2,4,15-trienoic acid, ethyl ester	$C_{16}H_{26}O_2$	278	6.31
4	18.85	7,10-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	12.63
5	18.9	16-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	10.11
6	19.98	5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-4H-chromen-4-one	$C_{15}H_{18}O_5$	280	21.04
7	20.73	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	10.11
8	21.97	1,3,12-Nonadecatriene	$C_{19}H_{34}$	262	6.63
9	22.42	Methyl (5α,12β,19α)-2,3-didehydroaspidospermidine-3-carboxylate	$C_{21}H_{26}N_2O_2$	338	5.68
10	23.1	Hexadecanoic acid, 2-hydroxy ethyl ester	$C_{18}H_{36}O_3$	300	6.95

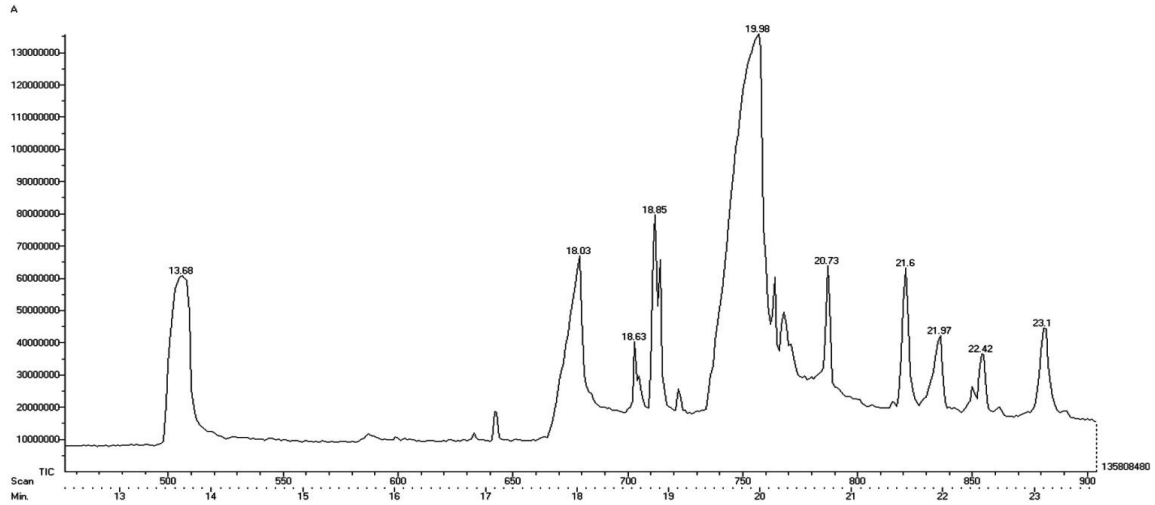

Fig. 1: GC – MS Chromatogram of the isolated essential oil from the fruits of Cucumis Melo

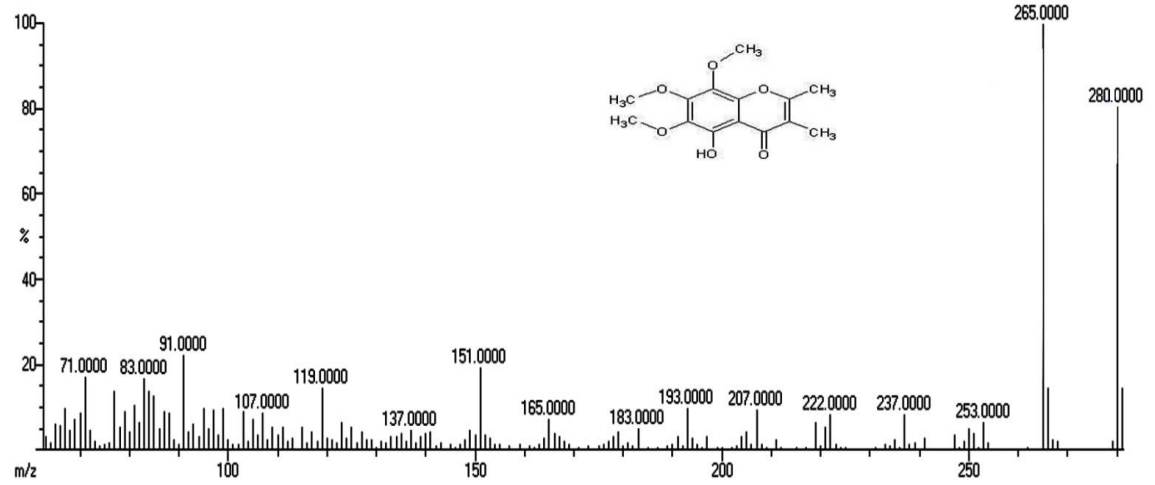

Fig. 2: Mass Spectrum of 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-4H-chromen-4-one

Preparation of Extracts: 100 g of the *Cucumis melo* fruit was extracted with 250 ml of Ethanol in a round bottom flask using reflux condenser apparatus. The reaction was carried out for 24 hours and the extract was collected the excess ethanol was removed by using a distillation process. The extract was stored in refrigerator until used. The extract contained both polar and non-polar phyto components of the plant material used. (R. Sasi Kumar et al, 2014).

GC – MS Analysis: The aqueous alcohol extract was examined in GC-MS for its chemical composition by GC-MS engine model GCClarus 500 Perkin Elmer and Computer Mass Library with a GC column Elite -1 (100% Methyl Poly Siloxane). The other conditions were as follows: Injector: GC-Clarus -500; Perkin Elmer; Carrier gas flow Helium 1ml/min; Split ratio – 1:25; Sample injected 1μL; Oven temperature- 50ºC upto 270ºC at the ratio of 5ºC/min – 4min hold; Injector temperature 250ºC; Total GC-time 30 min; MS inlet line temperature 200ºC;

Table 2: Antimicrobial activity of *Cucumis Melo* L. fruit ethanol extract against bacteria pathogens with reference to Amikacin

Compound	Weight of the Compound (µg/ml)	Zone of inhibition (in mm) *Salmonella Typhi*	*Staphylococcus aureus*	*Bacillus cereus*	*Klebsiella*	*Escherichia coli*
Cucumis Melo	30	6	11	8	13	6
Cucumis Melo	50	12	16	15	21	15
Amikacin (Standard)	30	17	18	18	17	17

Table 3: DPPH radical scavenging activity of plant extract and standard (Ascorbic acid)

Concentrations (µg/ml)	Plant extract (% of inhibition)	Ascorbic acid (% of inhibition)
20	15.23±1.27	41.00 ± 9.4
40	36.23±2.54	68.10 ± 8.6
60	52.23±3.78	84.64 ± 7.9
80	85.23±5.40	98.23 ± 5.4
IC_{50} (µg/ml)	52.45	25.57

Values are expressed as Mean ± Standard deviation for triplicates

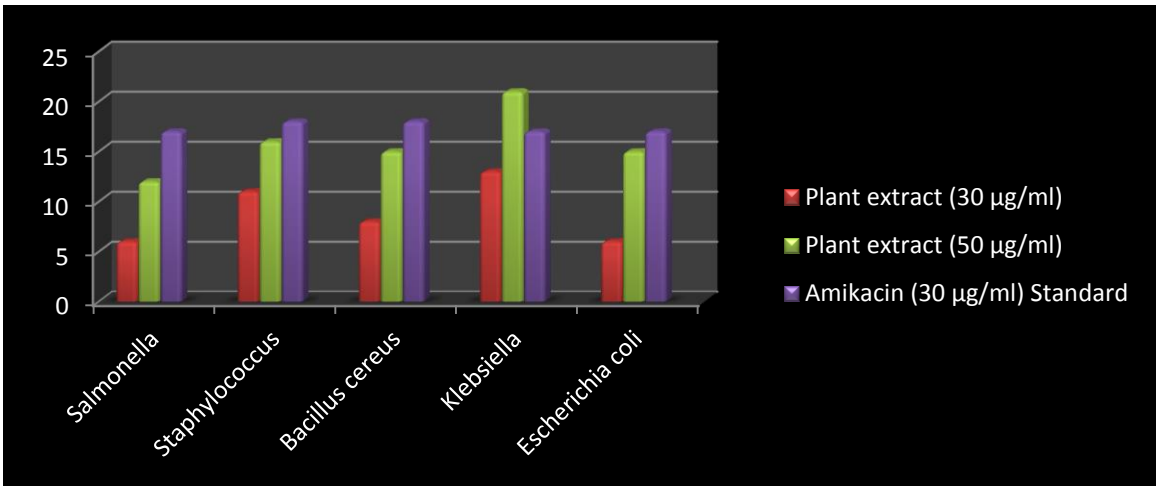

Fig. 3: Comparison of Antimicrobial activity of Cucumis Melo L. fruit and Standard Amikacin

Source temperature 200ºC; Electron energy 70eV; Mass Scan 25-400; MS Time 31 min.

Identification of Bioactive compounds: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The 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 components of the test materials were ascertained.

Antimicrobial Activity: The anti bacterial activity for the isolated essential oil from the fruits of *Cucumis Melo* was determined by Disc-Diffusion method. The antibacterial activity studies in nutrient agar medium for the following organisms *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus cereus*, and *Staphylococcus aureus* were carried out.

Preparation of Nutrient Agar Medium: Exactly 1g of peptone 0.5g of Beef extract and 0.5 g of sodium chloride were weighed and transferred into conical flask and dissolved in 100 ml of distilled water after the pH range was checked for 7.0 – 7.2 finally added 1.5 g of agar into the conical flask. It was closely packed with cotton plug and placed in an autoclave for 15 minutes for sterilization. The antimicrobial assay was carried out using Agar well diffusion method. Amikacin is used as reference drug and corresponding solvent (Ethanol) is used as positive controls. About 20 ml of nutrient agar medium for bacteria poured sterilized Petri dishes and allow solidifying. The agar medium was spread was 24 hrs cultured 108 CFU/ml microbial sterilized rod. Wells of 6 mm diameter were made in the culture medium using sterile cork borers. About 30 µg and 50 µg of the plant extracts (1 µg/ml) was added the wells. Plates were than incubated at 37 ºC for 24 h. Antimicrobial activity was evaluated by measuring the inhibition zone diameters in mm formed around the well. The assay was carried out in triplicates and the results thus obtained is taken as the mean of the three readings for each concentration and no statistical tools were used calculate the standard deviation. The Antimicrobial activity of *Cucumis Melo* L. fruits ethanol extract against bacteria pathogens with reference to Amikacin is reported in table 3 and also represented as Figure 3.

Screening for Antioxidant assay

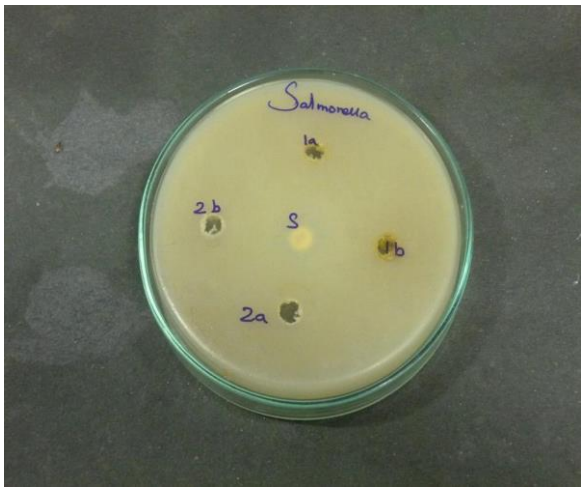

Fig. 4: Zone of inhibition – bacteria Salmonella typhi

Fig. 5: Zone of inhibition – bacteria Staphylococcus aureus

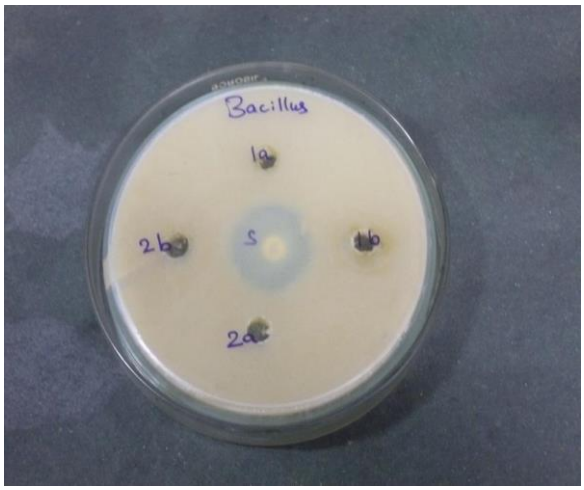

Fig. 6: Zone of inhibition – bacteria Bacillus cereus

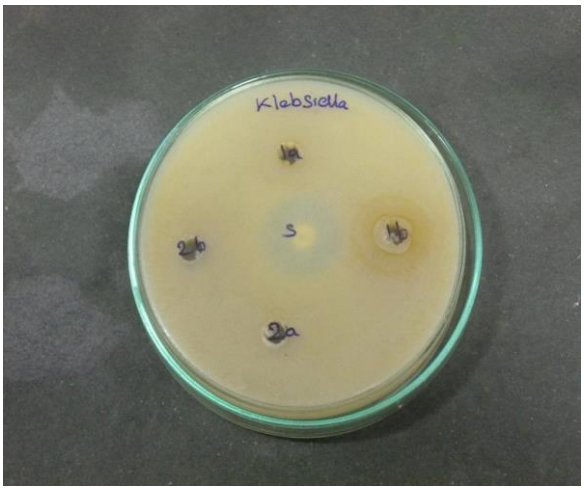

Fig. 7: Zone of inhibition – bacteria Klebsiella

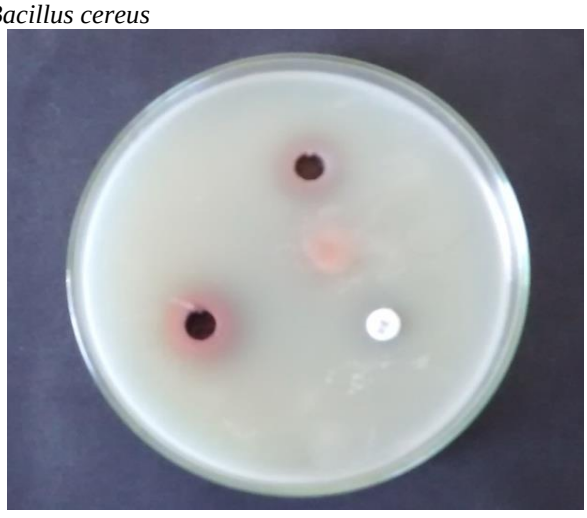

Fig. 8: Zone of inhibition – bacteria Escherichia coli

Preparation of sample: The different concentrations of plant extract (20, 40, 60 and 80 µg/ml) were chosen for *in vitro* antioxidant activity. L-Ascorbic acid was used as the standard.

DPPH radical-scavenging activity**:** DPPH radical-scavenging activity was determined by the method of Shimada, *et al.,* (1992) [14]. To added 2 ml aliquot of DPPH solution (25µg/ml) to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

Radical scavenging activity (%) =

$$100 - \left(\frac{A_C - A_S}{A_C} \times 100 \right)$$

Where A_C = control is the absorbance and A_S = sample is the absorbance of reaction mixture (in the presence of sample).

Statistical analysis: Tests were carried out in triplicate for 3 separate experiments. The scavenging activity of sample

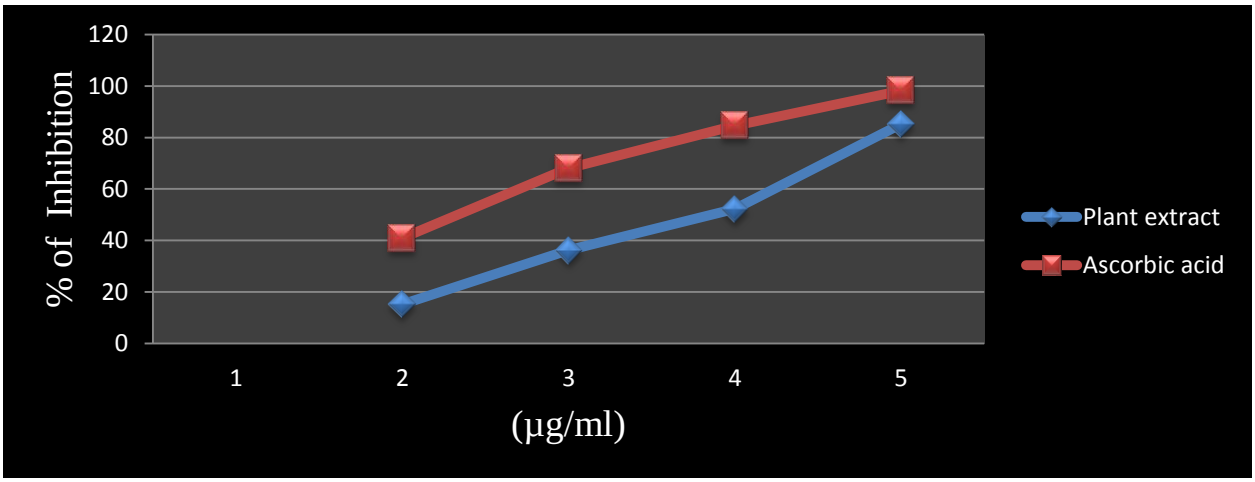

Fig. 9: DPPH radical scavenging activity of plant extract and standard (Ascorbic acid)

was expressed as 50% inhibition concentration (IC_{50}), which represented the concentration of sample having 50% of radical scavenging effect. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically determined by a linear regression method using Ms - Windows based graph pad Instant (version 3) software. Results were expressed as graphically / Mean ± standard deviation.

RESULTS AND DISCUSSION

GC - MS Analysis: The secondary metabolites present in the ethanolic extracts of *Cucumis Melo* were identified by GC-MS analysis (Figure 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanolic extracts of *Cucumis Melo* are presented in Table 1. Ten compounds were identified in ethanol extract by GC-MS. The major secondary metabolites present in the fruit of *Cucumis Melo* were Anhalinine, Estra-1,3,5(10)-trien-17-beta-ol, Hexadeca-2,4,15-trienoic acid, ethyl ester, 7,10-Octadecadienoic acid, methyl ester, 16-Octadecenoic acid, methyl ester, 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl chromone, 9,12-Octadecadienoic acid, 1,3,12-Nonadecatriene, Aspidospermidine-3-carboxylic acid 2,3-didehydro, methyl ester and Hexadecanoic acid, 2-hydroxy ethyl ester. Figure 2 shows the mass spectrum and structure of medicinally important secondary metabolite which contribute to the medicinal activity of the ethanolic extracts of *Cucumis Melo*. 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-4H-chromen-4-one is the major component found in the fruits of *Cucumis Melo* which is being used for the pharmacological work.

DPPH radical scavenging activity of sample: DPPH radical scavenging activity (%) can be calculated by using the formula as mentioned previously. The values obtained are tabulated in Table 4 and it is expressed as mean ± standard deviation.

CONCLUSION

In the present study, ten compounds have been identified from ethanolic extract of the fruits of *Cucumis Melo* by Gas chromatography – Mass spectrometry (GC-MS) analysis. Secondary metabolites such as alkaloids, flavonoids, terpenoids, sterols, carbohydrates, saponins and phenolic compounds were detected to be present in the fruits of *Cucumis Melo* plant. The present study portrays that the secondary metabolites in fresh *Cucumis Melo* fruit may contribute in many significant ways for various studies in a truthful manner to the pharmaceutical activity of the plant. The antioxidant results showed that the extract of *Cucumis melo* fruits exhibits the greatest antioxidant activity through the DPPH radicals scavenging activity. Since this plant had been used in the treatment of different ailment such as malaria, cancer and skin burn etc., the medicinal roles of these plants could be related to such identify bioactive compounds.

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