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UV- VIS, GC- MS AND FT-IR ANALYSIS AND DETERMINATION OF *IN-VITRO* ANTIOXIDANT ACTIVITY OF LYCOPENE FROM *CITRULLUS LANATUS*

A. Vidhya * and S. Arunadevi

Department of Microbiology, Department of Microbiology DKM College for Women, Vellore, Tamilnadu, India.

Keywords:

Carotenoid, Lycopene, *Citrullus lanatus*, antioxidant, GC-MS, FT-IR spectroscopy

Correspondence to Author:
A. Vidhya

Head, Department of Microbiology, DKM College for Women, Vellore – 632 001, Tamilnadu, India.

E-mail : vidhyasur76@gmail.com

ABSTRACT: Lycopene makes up the majority of carotenoids in watermelon. Carotenoids are thought to be responsible for the beneficial properties of fruits and vegetables in preventing human diseases including cardiovascular diseases, cancer and other chronic diseases. They are important dietary sources of vitamin A. Lycopene extraction from the fruit juice was done individually by using various organic solvents such as hexane, acetone and ethanol. The chemical compound was identified by UV Vis, GC- MS and FT-IR, totally nine chemical compounds were identified by GC-MS and different peaks were observed by FTIR. The major chemical constituents were identified as N,N'- Ethylenebis [2-[2-hydroxyphenylglycine, Cyclopropanebutanoic acid, n- Hexadecanoic acid, 10-Octadecenoic acid, oleic acid, Heptadecanoic acid, Hexadecanoic acid, 9-octadecenoic acid. The chemical compounds found in groups were methyl branched fatty acid, ethylester based fatty acid, glycine based amino acid, palmitic acid and oleic acid. The results showed that lycopene content of watermelon juice was approximately 13mg/Kg. Antioxidant testing assays 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assay, hydroxyl radical scavenging assay, superoxide anion radical scavenging assay and reducing capacity assessment. The red fleshed watermelon used for lycopene extraction had higher lycopene content and also had higher primary antioxidant activity free radical scavenging, hydrogen peroxide scavenging and reducing power activity. Thus, it was evaluated that the watermelon fresh juice contain effective bioactive compound responsible for antioxidant activity.

INTRODUCTION: Fruits and vegetables constitute the major sources of carotenoid in the human diet. They are important dietary sources of Vitamin A. Carotenoids are a family of pigmented compounds that are synthesized by plants and microorganisms but not animals [1]. Lycopene, intake has been particularly associated with protection from prostate cancer, lung cancer and lower the risk of developing coronary heart disease [2].

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Watermelon is under genera of citrullus, which rank among top ten in economic importance among vegetable crops globally [3]. Different carotenoid patterns were found in red fleshed and yellow fleshed watermelon. Red fleshed watermelon contains high levels of lycopene and varying amount of β carotene [4].

In recent years the antioxidant properties of carotenoids have been the major focus of research. Although best known as an antioxidant, both oxidative and non-oxidative mechanisms are involved in lycopene's bioprotective activity. Lycopene's configuration enables it to inactivate free radicals. As free radicals are electrochemically imbalanced molecules, they are highly aggressive,

and are always ready to react with cell components and cause permanent damage. Oxygen-derived free radicals are the most reactive species. These toxic chemicals are formed naturally as byproducts during oxidative cellular metabolism. As an antioxidant, lycopene has a singlet oxygen-quenching ability twice as high as that of beta-carotene (vitamin A relative) and ten times higher than that of alpha-tocopherol (vitamin E relative). The objective of this study was to develop methodology for the rapid, accurate, and sensitive extraction and determination of lycopene in watermelon using GC-MS and FT-IR spectroscopy and to evaluate the in-vitro antioxidant activity.

MATERIALS AND METHODS:

Red flesh watermelons were purchased from a local supermarket. The fruits were washed, drained and wiped - dry. It was cut into a few small portions and then blended to paste-like state for approximately 2 minutes using a blender. During the blending process, intermittent stops were required to minimize heating effects on the watermelons. The homogenized sample was centrifuged at 1000 g for 30 minutes and at 4^{o}C being filtered under suction. The sample was stored at-20^{o}C until use within a week.

Extraction of Lycopene and UV- Vis analysis:

Approximately 0.6 g of sample was weighed and added to 5 ml of 0.05 % (W/V) BHT in acetone, 5 ml of 95 % ethanol and 10mL of hexane. The homogenate was centrifuged at 400g for 15 minutes at 4^{o}C. After that, 3mL of distilled water was added. The vials were agitated for 5 minutes and left at room temperature to allow phase separation. The absorbance of upper hexane layer was measured in a 1cm-pathlength quartz cuvette at 503 nm using using ELCO, SL 164 UV- VIS spectrophotometer. Hexane was used as blank. The lycopene content in the sample was estimated according to the equation [5].

$$\text{Lycopene (mg/kg tissue)} = \frac{\text{A 503 X 31.2}}{\text{Mass of tissue (g)}}$$

Where A 503 is the absorbance of upper hexane layer [6]. UV-VIS light absorption pattern of lycopene was kinetically monitored in the range of 503 nm using ELCO, SL 164 UV- VIS spectrophotometer.

Gas chromatography- mass spectrometry:

GC-MS technique was used in this study to identify the phytocomponents present in the extract [7]. An analysis was performed on a JEOL GCMATE II GC-MS with Data system is a high resolution, double focusing instrument, maximum resolution. The electron impact ionisation method was analysed. The linear velocity of the high pure helium carrier gas was 30 cm/s. The injector temperatures were 220º C. The oven temperature was programmed from 50 to 25º C at 10º C/ min and held for 50 min.

Fourier transform infrared spectroscopy:

The spectra or fingerprints of the selected samples were obtained using FT-Raman and/or FT-IR spectroscopy. The samples of FT-IR were prepared by using potassium bromide disks. FT-Raman spectra were obtained using a PERKIN ELMER SPECTRUM ONE at the resolution 4 cm $^{-1}$ with the spectral range of 4000 – 450 cm-^{1}.

In- vitro antioxidant assay:

Determination of free radical scavenging activity:

Lycopene extract (1 ml) were prepared and then added to 2 ml DPPH solutions (0.05 M) in ethanol, respectively. The reduction of DPPH in the samples was measured at 517 nm after 30 minutes against a blank assay (samples added to 2 ml of ethanol, respectively) [8].

The percentage of remaining radical was calculated by using this formula:

Percentage inhibition=

(Abs control- Abs sample) /Abs control x 100

Where, Abs control was the absorbance of solution without extract and Abs sample was the absorbance of lycopene extract. The amount of sample required to decrease the initial DPPH concentration by 50%, $EC_{50,}$ was calculated. The anti-radical power is calculated by using equation:

$$\text{Anti- radical power (ARP)} = 1/ EC_{50}$$

Scavenging of hydrogen peroxide:
A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS, pH 7.4). One ml of lycopene extracts were added to 2 ml of hydrogen peroxide solution in PBS. The absorbance was measured at 230 nm after 10 minutes against a blank solution that contained extract or standard in PBS without hydrogen peroxide.

Reducing power assay:
One ml of Lycopene extracts were mixed with phosphate buffer (2.5ml, 0.2M, pH 6.8) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50^{o}C for 20 minutes. To this mixture, 2.5 ml of 10% trichloroacetic acids (TCA) was added and then centrifuged at 3000rpm for 10 minutes. The upper layer of the solution (2,5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%) was added and the absorbance was measured at 700 nm.

The percentage of reducing power was calculated by using the formula:

Reducing power (%) =

Abs control- Abs sample/Abs control

Where, Abs control was the absorbance of solution without extract and Abs sample was the absorbance of lycopene extracts.

RESULTS AND DISCUSSION: Solutions of lycopene in n-hexane at different concentrations were prepared and absorbance was measured at 503 nm. It was found that lycopene were the most important contribution in the absorption spectrum bands at 503 nm. The content of lycopene in the samples can be estimated by the molar extinction coefficient. By properly substituting the molar extinction coefficient of lycopene in hexane (17.2 X 10^4 M^{-1} cm^{-1}) as well as the molecular weight (536.9 g) and by changing the units,

Lycopene content (mg/kg) = A503 X 31.2/g tissue

the lycopene content of red fleshed watermelon juice showed higher yield was approximately 13mg/Kg.

The lycopene content of the red fleshed watermelon was higher than those reported by Isabelle et *al*.,[9] (10.95 mg/Kg), and Liana Maria Alda *et al.*,[10] (12 mg/Kg). This difference was due to red fleshed watermelons varied in their lycopene content depending on genotype and environmental conditions.

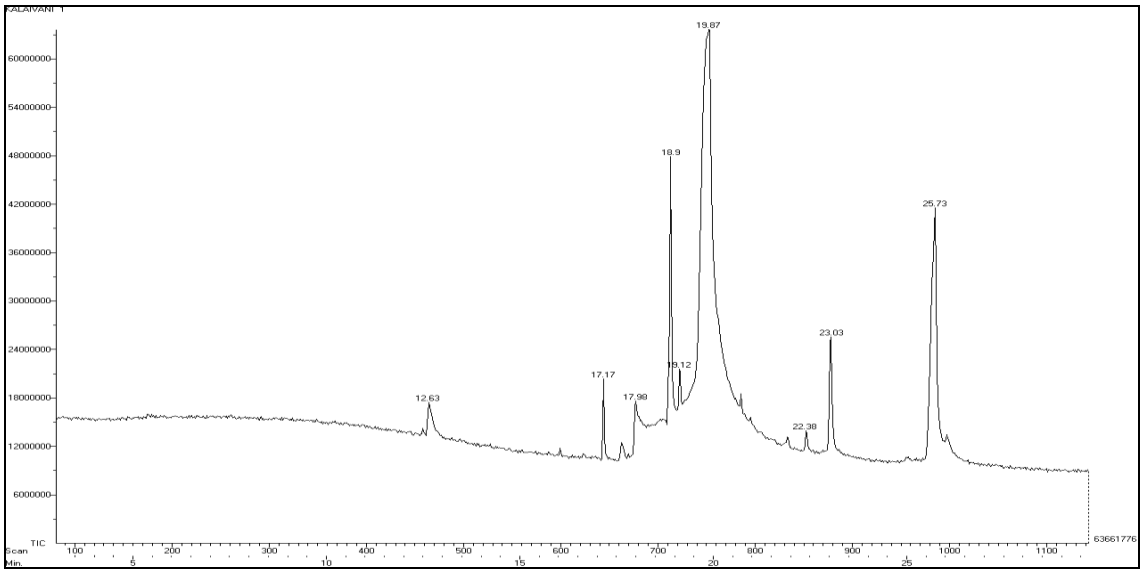

FIG.2: GC-MS SPECTRA OF LYCOPENE

The lycopene extracts of watermelon has been subjected to GC-MS analysis. Nine chemical constituents have been identified. The major chemical constituents are N, N'- Ethylenebis [2-[2-hydroxyphenylglycine, Cyclopropanebutanoic acid, n- Hexadecanoic acid, 10- Octadecenoic acid, oleic acid, Heptadecanoic acid, Hexadecanoic acid, 9-octadecenoic acid]. The chemical compound in

groups were also identified, to be the Methyl branched fatty acid, ethyl ester based fatty acid, Glycine based amino acids, palmitic acid and oleic acid was found (**Table 1**).

TABLE 1: GC-MS ANALYSIS OF LYCOPENE COMPOUND

Peak No	Compounds	Retention Time	Molecular Formula	Molecular Weight
1	N, N'-Ethylenebis [2-[2-Hydroxyphenylglycine]	12.78	$C_{18}H_{20}N_2O_6$	360.36
2	Cyclopropanebutanoic acid	17.22	$C_{25}H_{42}O_2$	374.59
3	n- Hexadecanoic acid	17.98	$C_{16}H_{32}O_2$	256.42
4	10- Octadecenoic acid	18.9	$C_{19}H_{36}O_2$	296.48
5	Oleic acid	18.96	$C_{18}H_{34}O_2$	282.46
6	Heptadecanoic acid	19.15	$C_{19}H_{38}O_2$	298.50
7	Oleic acid	20.17	$C_{18}H_{34}O_2$	282.46
8	Hexadecanoic acid	23.07	$C_{35}H_{68}O_5$	568.91
9	9-Octadecenoic acid	25.83	$C_{21}H_{40}O_4$	356.53

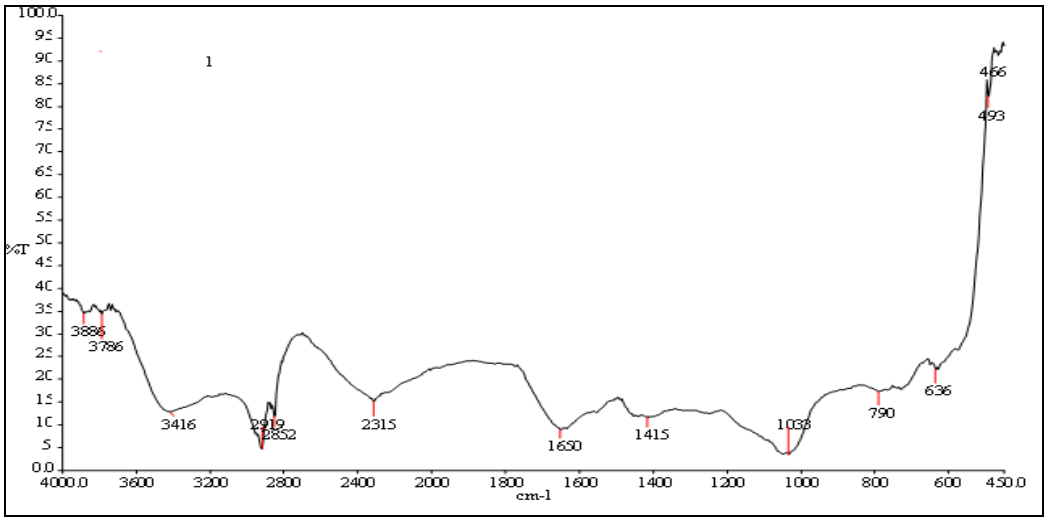

FIG -1 FT-IR SPECTRUM OF LYCOPENE

In this study the changes of chemical compositions and volatile components of lycopene was identified by using Fourier transform infrared spectroscopy technique. The spectral Peaks of lycopene showed C-H symmetrical stretch alone and C-H asymmetrical stretch methyl at (2960- 2850), C-H stretch of alkane at (3000- 2850), other bands occurs at (1620- 1680) C-H alkene, C-H bends alkene, C-H bends stretch alkene at (900-670) simpler forms of lipids were identified (Figure 1).

DPPH radical was used in the evaluation of free radical scavenging activity of watermelons. 1, 1-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay is the most widely used methods for screening antioxidant activity. DPPH assay was used to determine the scavenging potential of antioxidant extract based on its capability as hydrogen donator and electron transfer. The reaction between antioxidant compounds with the stable DPPH radical cause reduction in absorbance. EC50 is defined as the concentration of antioxidant necessary to scavenge 50% of DPPH radicals.

TABLE 2: DPPH ACTIVITY

DPPH radical scavenging activity	Activity at 517 nm
Test	3.000
Control	1.077

The percentage of remaining radical was calculated by dividing the absorbance of the sample with that DPPH control and multiplied by 100. The inhibition of radical was expressed in terms of the EC50 278.55 and ARP values. Higher ARP value of 0.0035 was observed in the lycopene extract, which reflects higher efficiency of antioxidants in the fruit (**Table 2**).

The effect of lycopene extract on hydrogen peroxide radical scavenging activity is shown in **Table 3.** The lycopene extract showed significant

antioxidant activity against hydrogen peroxide radical. The IC50 value of the extract was 10.636µg/ml.

TABLE 3: HYDROGEN PEROXIDE RADICAL SCAVENGING ACTIVITY

Hydrogen peroxide radical scavenging activity	Activity at 230 nm
Test	3.000
Control	3.281

Fe (III) reduction is used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3-} to Fe^{2-} by donating an electron. Amount of Fe^{2-} complex can be monitored at 700 nm indicates an increase in reductive ability. The extracts had shown good reducing power (**Table 4**). The IC50 value of the extract was 0.227µg/ml.

TABLE 4: TOTAL REDUCING POWER ACTIVITY

Total reducing power activity	Activity at 700 nm
Test	0.614
Control	0.265

In this study primary antioxidant property was analysed through in- vitro test using three different methods such as DPPH free radical scavenging activity, hydrogen peroxide scavenging activity and reducing power method with significant IC50 and ARP value respectively. The red fleshed watermelon used for lycopene extraction had higher lycopene content and also had higher primary antioxidant activity free radical scavenging, hydrogen peroxide scavenging and reducing power activity.

CONCLUSION: UV- Vis, GC-MS and FTIR fingerprinting was the best method for chemical characterization and compound identification and this present study determined the fingerprints of the active lycopene compounds. The present study revealed that the extract of watermelon has *in- vtiro* antioxidant activity. This study had significantly identified the properties of compound present in the lycopene extract, which act as an antioxidant compound. The chemical fingerprint profiles of antioxidant compounds of watermelon identified in the present study may enable drug manufacturers to adjust the proportion of herbs and prepare a standardized product with consistent activity.

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