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Extraction and Identification of β-carotene and Fatty Acid from *Sesamum indicum*

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Abstract: **Beta-carotene is a terpenoid pigment that is highly valuable due to its nutritional benefit as a precursor of vitamin A and its antioxidant properties. Carotenoids forms one of the most important classes of plant pigments and play a crucial role in defining the quality parameters of fruit and vegetables. Fatty acids, the main components of edible oil, are usually converted to fatty acid methyl esters. β-carotene and fatty acids were extracted and identified from *Sesamum indicum*. UV-visible spectra analysis by observing the Plasmon peak. Paper chromatography and thin layer chromatography was carried out to confirm the presence of bioactive compounds. HPLC, Saponification test and iodine test was performed to identify the presence of fatty acid and the degree of unsaturation respectively. The present study concludes that the presence of beta carotene and fatty acids in the *Sesamum indicum.***

Keywords: **β-carotene, fatty acid, *Sesamum indicum*, Saponification, Iodine test, HPLC.**

I. INTRODUCTION

Carotenoids are a group of bioactive compounds responsible for the yellow-red pigmentation of many plant organs, plant derived raw and processed foods, known to improve food healthiness and stability because of their pro-vitamin A activity, antioxidant power, boost of cell-mediated and humoral immune response. More than 700 natural carotenoids identified about 50 could potentially be used by the human body out of which α-carotene, β-carotene, lycopene, lutein, zeaxanthin and β-cryptoxanthin are effectively detected in blood plasma and tissues which are associated with some health benefits [1]. A fatty acid has a carboxylic acid at one end and a methyl group at the other end. Unsaturated fatty acids are fatty acids in which one or more than one double bond exists within the molecule. Essential fatty acids are fats that are extremely important nutrients for health but cannot be made in our body and must be obtained from foods such as nuts, oil seeds and their products and oil rich fish[2].

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. They are widely used in the human therapy, veterinary, agriculture and scientific research [3]

Sesamum indicum is a flowering plant in the genus Sesamum. The flowers are yellow, tubular, 3 to 5 cm (1.2 to 2.0 in) long, with a four-lobed mouth. The flowers may vary in colour, with some being white, blue, or purple. Sesame fruit is a capsule, normally rectangular in section and typically grooved with a short, triangular beak. Sesame seeds are small. The weight of the seeds is between 20 and 40 mg [4]. Sesame seed and its oil have been utilized as an important food components due to the beneficial effects to human health such as antioxidants [5] a lowering platelet aggregation, preventing atherosclerosis, and reducing serum cholesterol [6] .

The present study is to identify the presence of β-carotene by UV-visible spectra analysis, to confirm the presence of β-carotene using Paper and thin layer chromatography, to perform chemical analysis such as Iodine value, saponification test and fatty acid test, to carry out HPLC study.

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II. MATERIALS AND METHODS

1. Collection of plant samples:

Seeds of *Sesamum indicum* were collected, dried and powdered. Aqueous extract was prepared by mixing 1g of dried powder in 20ml of distilled water, incubated in shaking mixer for about 24 hours, filtered and filterate was used for the study. Organic extract was prepared with 20ml of methanol.

2. UV-Vis spectra Analysis:

Analysis was carried out to identify the compound present in the sample. The wavelength and absorbance is identified from the Plasmon resonance peak.

3. Paper Chromatography:

Obtain a rectangular shape chromatography paper . Use a pencil to make a straight line at a distance of 1cm of above the bottom edge. For each sample make a small light circle in a pencil. Next to each circle put an identification mark or code for correct identification . A tiny drop of each extract is kept on the appropriate spot on the line. This can be done repeatedly by dipping a toothpick into the liquid and touching it to the paper. The paper is let to dry. The solvent is prepared in the ratio 4:1:4 (ie) 4ml glacial acetic acid, 1ml distilled water and 4ml of methanol is used for preparation of solvent. Chromatography paper is carefully placed inside the beaker so that it will wet the bottom edge of the paper. The paper is left undisturbed. The chromatography is stopped when the solvent is raised three-fourth towards the top of paper. The height reached by the solvent is marked. Let the paper dry.RF value is calculated.

$$RF = \frac{\text{Distance(cm) moved by solute from the origin}}{\text{Distance(cm) moved by solvent from the origin}}$$

4. Thin layer chromatography:

Chromatography was performed to identify the presence or absence of active compounds. TLC silica gel Plate (Merck) was used . Around 20 µl of the extract was placed on the plate . The plate is let to dry. The solvent is prepared in the ratio 4:1:4 (ie) 4ml glacial acetic acid, 1ml distilled water and 4ml of methanol is used for preparation of solvent. Thin layer plate was carefully placed inside the beaker so that it will wet the bottom edge of the plate. The plate is left undisturbed. The chromatography is stopped when the solvent is raised three-fourth towards the top of the plate. The height reached by the solvent is marked. Let the plate dry. RF value is calculated

$$RF = \frac{\text{Distance(cm) moved by solute from the origin}}{\text{Distance(cm) moved by solvent from the origin}}$$

5. Saponification Test:

Saponification value is the amount (mg) of an alkali required to saponify a definite quantity (1g) of an oil or fat. Saponification test was performed to identify fat content in the sample. 1ml of the aqueous extract of *Sesamum indicum* was mixed with 25ml of 0.5N potassium hydroxide solution in a conical flask. Heated in a boiling water bath for 30 minutes, cooled and added 1to 2 drops of phenolphthalein indicator . It was titrated against 0.5N hydrochloric acid till the disappearance of pale pink colour. The same procedure was repeated for blank test. Saponification value(SV) was calculated from the equation:

SV = (Sample-Blank) x Normality of HCl x 28/Sample weight (g)

6. Iodine Test:

Iodine value is a measure of the degree of unsaturation in an oil. Iodine value or number is the ‘g’ of iodine absorbed by 100g of the oil. 1ml of aqueous extract of *sesamum indicum* was mixed with 5 ml chloroform and 7 ml iodine solution in a conical flask. To this added 5 ml of 10% potassium iodide and 90ml distilled water. It was titrated against 0.1N sodium thiosulphate till disappearance of yellow colour. The same procedure was repeated for blank test. Iodine value (IV) was calculated from the equation,

IV=(Blank-Sample)×Normality of sodium thiosulphate×12.69/sample weight(g)

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7. Free Fatty acid Test:

The acid number is defined as the mg of KOH required to neutralize the free fatty acid present in 1g of oil sample. 25 ml of neutral ethyl alcohol was mixed with 1ml aqueous extract of *Sesamum indicum* in a beaker, allow the mixture to boil, cooled and titrated against 0.1N sodium hydroxide solution, using two drops of phenolphthalein as indicator with consistent shaking for which a permanent pink colour as the end point. The Acid value (AV) was calculated using the expression;

AV = 56.1× Titre value× Normality of KOH/sample weight(g)

8. High Performance Liquid Chromatography:

100 mg of the sample (oil) was dissolved in N-hexane in a test tube. 0.15ml of Sodium Methoxide was added and shaken for 2 minutes in a closed 20 ml vial. The resultant mixture was centrifuged and a clear solution was obtained. The N-hexane was evaporated, the resultant residue was dissolved in 1.0ml acetonitrile and 20 ml of the supernatant was injected into HPLC for fatty acid analysis. The mobile phase was methanol: water (60:40).The instrument used for the analysis was Acme 9000 ISOCRATIC HPLC SYSTEM

III. RESULTS AND DISCUSSION

1. UV –Visible spectra analysis:

UV –visible spectra analysis was performed to identify the compound present in the *Sesamum indicum* . The wavelength and absorbance is identified from the Plasmon resonance peak.

a) In organic extract:

The Plasmon resonance peak of methanol extract was observed at wavelength of 370nm shown in the Fig 1

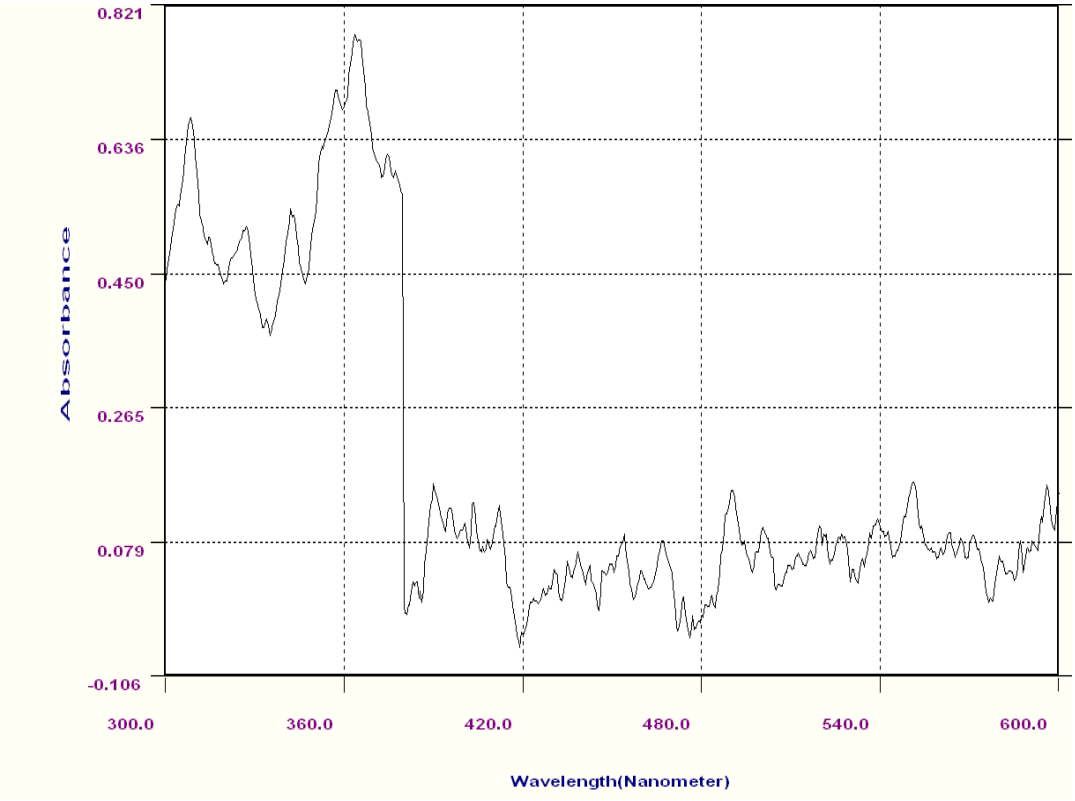

Fig 1. UV-Visible spectra analysis of organic extract

b) In aqueous extract:

The Plasmon resonance peak of aqueous extract was observed at wavelength of 320nm shown in the Fig 2

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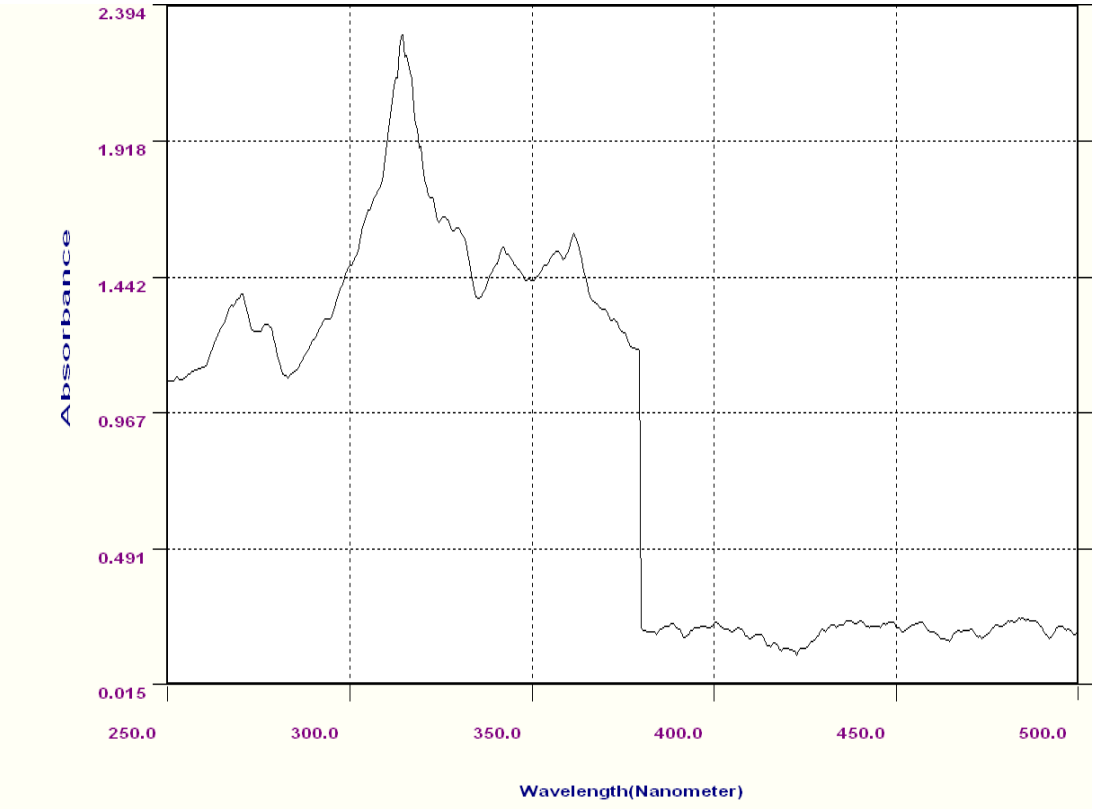

Fig 2.UV- visible spectra of aqueous extract

Sesamum indicum is found to exhibit absorbance at the wavelength of 370nm in the organic extract and 320nm in the aqueous extract which confirms the presence of β- carotene.

Most carotenoids absorb maximally at three wavelengths, (300,370 &420) resulting in a three-peak spectrum [7].

2. Paper Chromatography:

Paper chromatography was carried out in the organic and aqueous extracts of *Sesamum indicum* and the result is shown in Table I

Table I: RF values of *Sesamum indicum*

Extract	RF value
Organic	0.86
Aqueous	0.92

3. Thin layer Chomatography(TLC):

Thin layer chromatography was performed to confirm the presence or absence of active compounds. Aqueous extract of *Sesamum indicum* was used for TLC.. RF value is given in Table II

Table II RF values of *Sesamum indicum*

Extract	RF value
Organic	0.59
Aqueous	0.95

The above RF value confirms the presence of β- carotene in aqueous extract of the sample. Standard RF value of β-carotene is 0.99 [8]

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4. Saponification Test:

Saponification test was performed in the aqueous extract to identify the fat content in the *Sesamum indicum* . The presence of fat was confirmed by the disappearance of pale pink colour as the end point. Saponification value ranged at 190 mg indicates the presence of free fatty acid .The saponification values of the Sesame seeds were found to be within the range of 189 to 190 mg KOH/g. According to (Ziegler *et al.*,1996) a saponification value of 200 mg KOH/g indicates high proportion of fatty acids of low molecular weight[9].

5. Iodine Test:

Iodine value is a measure of the degree of unsaturation in an oil. Iodine value or number is the 'g' of iodine absorbed by 100g of the oil. Iodine test was performed in aqueous extract and iodine value ranged at 104.1mg indicates the presence of unsaturation in oil. According to work of (Namiki et al.,2007) the oil had a clear yellow colour free of haziness. The iodine value (105mg) obtained is high which suggests the presence of unsaturated fatty acid. It indicates the degree of unsaturation in the fatty acids of triacyglycerol. This value could be used to quantify the amount of double bonds present in the oil, which signifies the susceptibility of oil to oxidation [10].

6. Free Fatty Acid Test:

The test was performed to confirm the presence or absence of fatty acid in the sample. The appearance of pale pink is the end point of fatty acid test. The acid value was 53.2 indicates the oil is stable . The oil was stable when the fatty acid value is on lower side of 60.2 [11]. This value shows that this oil is stable.

8. High performance Liquid chromatography:

High performance liquid chromatography was performed to identify the fatty acids present in the oil. The identified fatty acids was olieic acid, cis fatty acid, decanoic acid and palmitic acid.

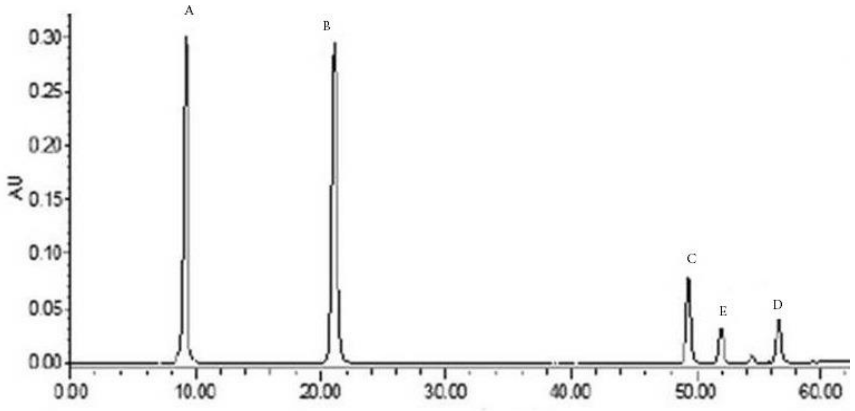

Fig 3 High performance liquid chromatography

High-performance liquid chromatography (HPLC) plays an important role in applications such as the geometrical isomer separation.It shows the presence of oleic acid, Cis fatty acids,Decanoic acid and palmitic acid at a retention time of 10,20,49 and 57 respectively.

IV. CONCLUSION

In the present study β-carotene and fatty acid were extracted and identified from *Sesamum indicum*. Aqueous and organic extracts were prepared and used to analyse β-carotene and fatty acid. UV-visible spectra analysis was carried out and the β-carotene was confirmed by observing the Plasmon peak. Paper chromatography and Thin layer chromatography confirms the presence of bioactive compounds. The result obtained in Saponification test indicates the presence of high proportion of fatty acids. Iodine test shows the degree of unsaturation in aqueous extract of *Sesamum indicum.* Fatty acid test indicates the sample was found to be stable. The presence of fatty acid was confirmed by using HPLC study.

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