Investigation of photosynthetically active pigments in tomato (*Solanum lycopersicum*) crop leaves per growth stage for an optimum indoor crop cultivation

ABSTRACT

Photosynthesis is a necessary process for plants during which light energy is absorbed via pigments presents in leaves for essential processes and needs of the particular plant.This work identified and studied the active photosynthetic pigments in tomato crop(*Solanum lycopersicum*) at each growth stage. With phytochemical screening giving an insight about triterpenoids and carotenoids presents, UV spectra combined with FTIR and thin layer chromatography showed that chlorophylls (a and b), xanthophyll (lutein) and carotene (mostly β) are being synthesized at different proportions during all growth stages.More pigments are being used by plants during the flowering stage than during the other growth stages. The various action spectrashowed intense absorption within the intervals 400 nm – 510 nm and 560 nm – 710 nm which seem to be vital radiation ranges for photosynthesis. With an optimum eluent, photosynthetic pigments were clearly identified. Knowing these optimum plant's needs can help in a choice of better outdoor and indoor cultivation conditions that can assure necessary radiation for optimum plant growth.

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Keywords: Spectro-photochemical monitoring, Thin Layer chromatography, eluent optimization,action spectrum

1 Introduction

Pigments are compounds that impart color to materials around us. This color is as a result of light absorption and its transmission making it possible for the naked eye to appreciate colors. In plants, pigments known as photosynthetic pigments help in the process of photosynthesis by absorbing sunlight and through chemical processes this absorbed energy is transmitted to plant tissues for internal useful and vital processes(Simkin et al., 2021). All this process assuring plant's growth is conditioned under adapted environmental and climatic conditions. Unfortunately, with climate changing due to anthropogenic activities, most conducive ecosystems are being destroyed leading to less productivity and,at worst case, disappearance of plant varieties(Raymond et al., 2020). In order to conserve plant species and also agricultural productivity, crop cultivation indoors under controlled microclimate such as greenhouse cultivation attracted many researchers interest(Djibrilla et al., 2023; Kumar et al., 2009). However, in order to assure conducive indoor microclimate for crop cultivation, it is important to understand photosynthetic pigments activity in order to assure optimum healthy

growth of the plants(Bacon, 1967; Pharmawati & Wrasiati, 2020). It is in that regards that this study tried to understand photosynthetic pigments acting at each growth stage of tomato crop for its optimum growth as a way to better suggest designs for indoor tomato(*Solanum lycopersicum*) cultivation.

2 Materials and methods

2.1 Plant's part sampling

Samples of tomato plant leaves were collected in a systematic random manner and were put in an ice chest for transportation till Chemistry laboratory of the University Abdou Moumouni where analyses were conducted..

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Lifting stage Flowering stage Fruiting stage

Figure 1 Tomato crop at different growth stages

2.2 Sample preparation

Leaves samples were washed and rinsed with distilled water before being partially dried for removing water. 5 g of the partially dried leaves was weighed using an electronic balance and were finely cut into small pieces using a pair of scissors. This process was repeated each time an analysis was needed..

2.3 Photosynthetic pigments extraction

2.3.1 Solvent optimization for extraction

With 5 g mass of vegetable leaves, volumes of 5, 10 and 20 mL of ethanol were used in order to find the appropriate volume for better extraction of photosynthetic pigments.

2.3.2 Pigments extraction through cold maceration process

Ethanol (98% concentration from VWR ProLabo Chemicals (UK)), an organic polar solvent, was used during the extraction process. The various plant materials were placed in a mortar and ground with 2.5 g of fine sand and 5 mL of ethanol until the solvent takes on a marked green tint. Then, the crushed was filtered using a filter paper, and the filtrate was used for further analyses such asphytochemical screening test, thin layer chromatographic characterization process and UV-Vis spectrophotometric reading.

2.3.3 Phytochemical screening

About 5 mL of the crude extract ethanolic solution was used for checking the presence of phytoconstituents such as saponins (Froth test), anthocyanins, flavonoids (alkaline reagent test) and carotenoids(Delgado-vargas, 2000; Sanda et al., 2021).

2.3.4 Eluent optimization for TLC

In order to determine the best eluent, six compositions were prepared with petroleum ether and acetone in different proportions:

i. Eluent 1: 40% petroleum ether and 60% acetone.
ii. Eluent 2: 50% petroleum ether and 50% acetone.
iii. Eluent 3: 60% petroleum ether and 40% acetone.
iv. Eluent 4: 70% petroleum ether and 30% acetone.
v. Eluent 5: 75% petroleum ether and 25% acetone.
vi. Eluent 6: 80% petroleum ether and 20% acetone.

2.3.5 Fourier Transform Infrared spectroscopy (FTIR)

FTIR test was done on leaves' dry powder samples in order to confirm the expected main functional groups present.

2.3.6 Thin Layer Chromatography (TLC)

Photosynthetic pigments are sought after at all growth stages of the plant. The frontal ratio (Rf) was determined for the identification of these pigments.

$$Rf = \frac{\text{Distance travelled by pigment}}{\text{Distance travelled by eluent}}$$

A TLC plate (Merck) was labeled with a cross using a pencil 1 cm from the lower and upper edge, taking care not to damage the silica layer. At this cross, using a capillary tube, a few drops (0.5 mL) of the extracted solution was placed, leaving one drop drying before dropping another. The plates were then gently deposited in a glass container, closed with a lid and placed in a dark place away from the light. When the eluent rises to within 1 cm away from the top edge of the plate, the plate was removed and the observation of the different photosynthetic pigments of interest was made (figure 2). The same method was applied for all plant samples.

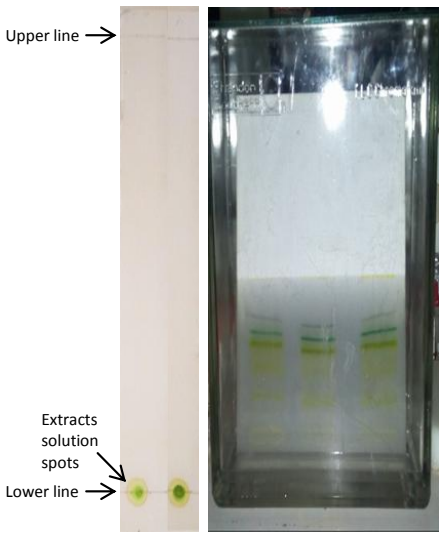

Figure 2 TLC test set up

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2.3.7 UV spectroscopy

Absorption spectrophotometry is a physical method of chemical analysis which measures the proportion of light absorbed by a colored species in solution.

2.3.7.1 Analysis of raw samples

Using the UV-Visible Evolution 300 dual-beam spectrophotometer, the measurements of the absorbance (A) and the transmittance (T) of the extracts containing the photosynthetic pigments were performed against ethanol as a reference, scanning between 190 and 1100 nm. This scan covered the three most important regions of the solar spectrum (Ultra-Violet, Visible light and Infra-Red) for the absorption of light by plants. At necessary, dilutions were carried out to make the spectra of the raw extracts.

2.3.7.2 Analysis of separated samples

The clearly separated pigments with the best eluent were recovered by scraping the colored part at the TLC plate's level. Dissolved into ethanol, separated samples or pigments were scanned through the spectrophotometer in order to obtain the characteristic peaks of each of them.

3 Results and discussion

3.1 Optimum solvent

On one hand, with 5 to 10 mL volume of ethanol, the extract obtained was insignificant and the expected greenish coloration was hardly observable. On the other hand, the quantity of the extract and the greenish coloring became very significant with a volume of 20 mL of ethanol. Traditionally a ratio (m:v) of 1:1 is used between the mass of the plant material and the volume of the solvent extracting. These results obtained showed that this report was not up to a good extraction. Using the volume of 20 mL, a ratio of 1:4, although resulting in 4 times more ethanol, gave better extraction result. The nature of the filter plays an important role in filtration. Using n^{o}1 filter paper, filtration took more time. However, the use of the rapid filter such as cotton wool allowed rapid filtration with a better result. This type of filter in addition to allowing good filtration is also cheaper and readily available. Usually, the origin of the slowness noted in the filtration with the filter paper could cause the retention of

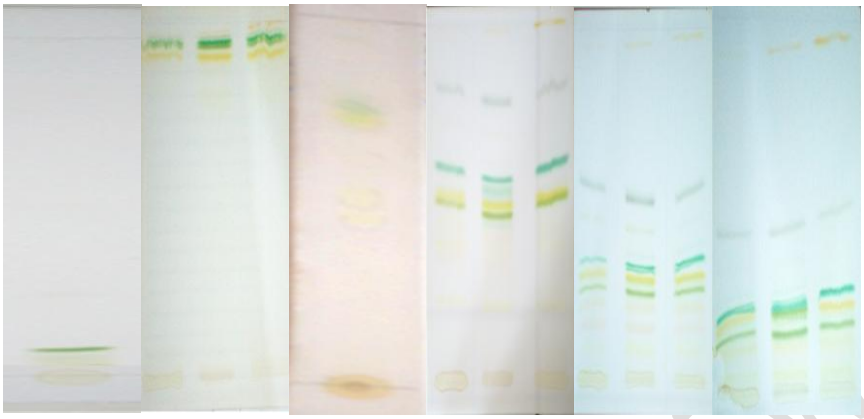
some pigments which was a big drawback. At the end of this optimization, the best extraction conditions were a volume of 20 mL of ethanol and the use of the cotton wool filter..

3.2 Optimum eluent

Figure3shows the chromatograms of extracts of photosynthetic pigments identifiedusing different eluent compositions in terms of petroleum ether and acetone. The analysis of the chromatograms highlights five kinds of pigments:

a) Carotene (yellow-orange):non polar,
b) Pheophytin (grey): very little polar,
c) Chlorophyll a (blue green): little polar,
d) Lutein (a xanthophyll) (yellow): polar,
e) Chlorophyll b (green-yellow): more polar.

The test with eluent 1 (40% petroleum ether and 60% acetone) did not give any stain on the plate at the level of its upper terminal. This means that no pigment could migrate. The fact that no elution was observed could also come from the pigments that were not much soluble in this eluent. Besides, the molecules of the desired pigments interacted strongly with those of the supporting plate. When the proportions in the eluent passed to 50% petroleum ether and 50% of acetone (eluent 2), the pigments started to appear clearly. Yet, separation was not good because the pigments began to separate after passing the middle of the plate. The eluent 2 was, therefore, very polar, which explains why the molecules were easily entrained. Passing to eluent 3 where petroleum ether and acetone were in proportion of 60 and 40% respectively, spots were observed on the plate. Therefore, this composition allowed a relative migration of the desired pigments. The fact that petroleum ether was in higher proportion than acetone, made the medium less polar favoring the migration of non-polar molecules and hardly entraining polar pigments. However, these spots obtained were not explicit to allow a possible identification of the pigments sought. Spots were observed clearly on the TLC plate with eluent 4 (70% petroleum ether and 30% acetone), eluent 5 (75% petroleum ether and 25% acetone) and eluent 6 (80% petroleum ether and 20% acetone). Although the good separation of pigments, chlorophyll b – lutein pigments and lutein - chlorophyll apigments were not well-separated in the cases of eluent 4 and 6 respectively. That situation would make recovery of individual pigment difficult. The results obtained with the proportions of 75% petroleum ether and 25% acetone (eluent 5) solved that issue by allowing good pigments recovery while separating them well. The pigments were well separated by a distance allowing their recovery without any difficulty.



Eluent 1 Eluent 2 Eluent 3 Eluent 4 Eluent 5 Eluent 6

Figure 3Eluent of optimization for Thin layer Chromatography

3.3 Tomato crop leaves characterization

The radiation needs of plants during their growth could be estimated by knowing the activity of photosynthetic pigments present in their leaves' extracts. Indeed, those pigments help plants capture sun energy and convert it into chemical energy for their needs via photosynthesis.

3.3.1 Phytoconstituents

A quick phytochemical screening test of the leaves extracts showed a presence of triterpenoids and carotenoids whereas an absence of anthocyanin and flavonoids were observed (table 1). This result was observed in all growth stages of the tomato crop.

Table 1 Phytochemical screening result

Chemical constituents	Leaves extracts
Saponins	+
Triterpenoids	+
Flavonoids	-
Anthocyanins	-
Carotenoids	+

+ indicates present, - indicates absent

The presence of saponins and triterpenoids showed the presence of compounds composed of isoprene units (pentacyclic structures) which are biosynthesized by plants. Such classes of compounds possess a rich chemistry with pharmacologic properties (especially cholesterols)(Pharmawati & Wrasiati, 2020). Triterpenoid saponins are usually synthesized by plants for their defensive mechanism (Pharmawati & Wrasiati, 2020; Simkin et al., 2021). Carotenoids are tetraterpenes (8 isoprene). Theyhelp leaves in the capturing of light energy by broadening the absorption spectrum by completing chlorophylls(Simkin et al., 2021).

3.3.2 FTIR analysis of tomato leaves

FTIR analysis allowed the prediction of the types of chemical bonds or functional groups present in dried leaf powder of *Solanum lycopersicum*(tomato). Similar graphs were obtained irrespective of the growth stage of tomato crop.

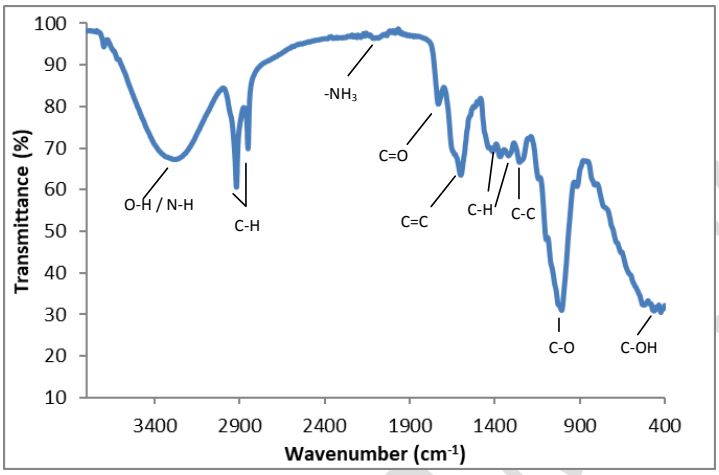


Figure 4 FTIR spectrum of Tomato crop leaves powder

Figure 4 presents an FTIR spectrum of the leaves'powder of tomato crop which results complete the data obtained from phytoconstituents found in those leaves.From the FTIR test results, the following assignments could be done: a broad peak signal at 3272.91 cm^{-1} corresponded to O–H stretching vibration due to hydroxyl groupor N–H stretching.Two sharp peaks followed up at 2917.14 $cm^{-1}$and 2849.80 cm^{-1}respectively which are characteristics of C–H stretch due to methyl (CH_3) and methylene (CH_2)groups' asymmetry stretching from lipids and alkane respectively. The peak at 2104.36 cm^{-1} represents the stretching vibration of $–NH_3^+$ as in free amino acids and their halides. At 1732.67 cm^{-1} the C=O vibration was seen which could also be due to esters group of lipid membrane and fatty acid. This bond wasconjugated with the alkene C=C stretch appearing at 1600.98 cm^{-1}. Many studies demonstrated that peaks around 1600 cm^{-1} are due to the presence of chlorophylls and proteins (Kishimoto et al., 2005; Lichtenthaler & Buschmann, 2001).The two close peaks at 1369.83 cm^{-1} and 1320.19 cm^{-1} could be assigned to C-H deformation vibration. The peak at 1256.30 cm^{-1} corresponded to C-C skeletal vibration from –C $(CH_3)_3$ of alkane.A sharp peak at 1008.94cm^{-1} showed the C–O stretching in carbohydrates. Anaromatic C–OH in plane bending vibration was seen at around 426.64 cm^{-1}.Alkanes, amines, long chain conjugated structures are considered as the major functional groups of bioactive compounds (Pharmawati & Wrasiati, 2020).

3.3.3 UV analysis of crude extract of tomato leaves

The spectra presented in figure 5gave an overview of the different photosynthetic pigments present in tomato crop leaves.During all three main growth stages (figure 1), the same characteristic photosynthetic pigments were present as confirmed in figure 5. Besides, thespectra obtained via UV spectroscopywere similar and characteristic of the action spectrum of most plants. The spectrum at flowering stage was presenting higher and more intense peaks followed by the spectrum of fruiting stage and finally, the spectrum of lifting stage (figure 5). This shows that more pigments were being used during the flowering stage probably for harnessing much more sun energy in order for the plant to prepare for development of fruits(Grossiord et al., 2020; Guichard et al., 2005).

From the absorption spectra, it could be observed that distinct peaks appeared within the yellow-red region (560 nm – 710 nm) whichwas due to chlorophylls, and overlapped peaks within violet-green region (400 nm – 510 nm) which confirmed presence of chlorophylls previously seen in FTIR spectrum (nitrogenous ring compound in figure 4) and other photosynthetic pigments such as xanthophyll, carotenes that were detected via FTIR spectrum through long chain conjugated double bonds. Peaks were observed within the ultraviolet region between 320 nm and 400 nm as well.This absorption within the ultraviolet and infrared regions pushed researchers to investigate plants behavior considering rays from such part of the spectrum(Acock et al., 1978; Iraqi et al., 1997; Kalaitzoglou et al., 2019). On the other hand, most photosynthetic pigments absorb within the visible light region are more synthesized during plant's full growth time or at the moments it needed more energy towards fruit creation(Grossiord et al., 2020; Guichard et al., 2005).

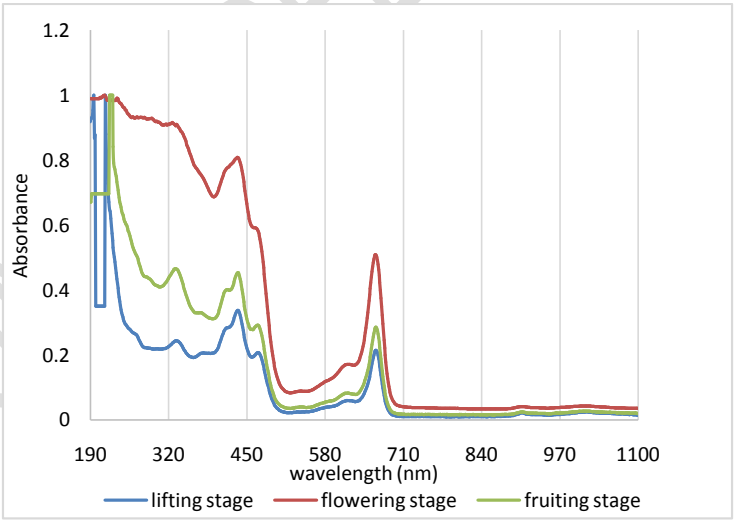
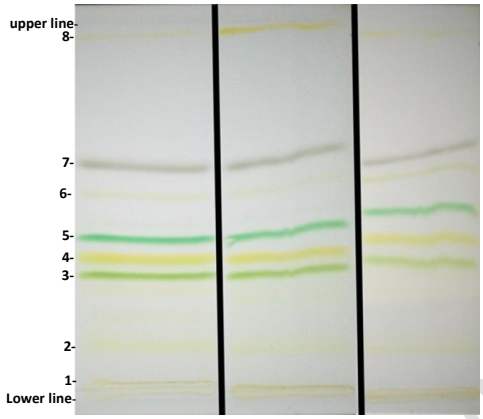


Figure 5 UV graphs of crude extract from tomato crop leaves at various growth stages

3.3.4 Analysis of separated pigments of tomato leaves through Thin Layer Chromatography (TLC) and UV spectroscopy



Lifting stageFlowering stage Fruiting stage

Figure 6TLC chromatographofcrude extract from tomato crop leaves at different growth stages**.**

Figure 6presents the results of the TLC tests of the extract of the tomato leaves at different growth stages of the plant. The TLC chromatograph displayed 8 colored lines representatives of 8 separated pigments. Each pigment after separation was analyzed and results were presented in Table 2 completed by details of individual UV spectrum in figure 7, 9, 11, 13 and 15.

Starting from the starting line 1cm away from the TLC bottom edge, a rapid separation of pigments was observed. This migration of pigments was made according to polarity and molecular weight. As soon as the migration started, a first fraction (Fraction 1) was observed which was a thin green line with Rf of 0.13. This line was similar to the line of fraction 3 which was a thicker green line with clearer peaks at 463 and 648 nm (figure 11) and Rf of 0.37. Fraction 3 (figure 6) was assigned to be chlorophyll b (structure in figure 12) due to its characteristics which conducts to assume fraction 1 to be a form of chlorophyll b that got separated during the spot making.In fact, during the spots making, each spot was allowed to dry before putting into the cuvette for elution and separation. Since then, separations could be observed.Extracts were dissolved into ethanol which is an organic solvent. As mentioned by Strouse (1973), if chlorophyll dissolved in an organic solvent is allowed to spread without restriction on a polar surface such as water, evaporation of that solvent leaves an amorphous layer with peak absorption of 675 nm. If further crystallization occurs, the absorption shits to 735 nm. Strouse (1973) found that crystalline monolayers of chlorophyll a and ethyl chlorophyllide a all show maximum absorption peaks between 675 and 735 nm (Strouse, 1973). Fraction 1 being the same color as Fraction 3 but with different Rf (lower Rf), it could be assigned to be ethyl chlorophyllide b(Bacon, 1967). Therefore, Chlorophyll b corresponding to fraction 3 wasclearly distinct with a green color band in conformity with many authors (Pharmawati & Wrasiati, 2020; Tomkins & Miller, 1993).

Fraction 2 displayed a light green color and was assigned to be ethyl chlorophyllide a with Rf of 0.18. This corresponded to similar results obtained by Pharmawati and Wrasiati (2020) and Bacon (1967)that got its peaks to be 434 and 654 nm. On the other hand, crystalline form of ethyl chlorophyllide a was found to absorb in the range of 730 to 735 nm(Strouse, 1973).

Fraction 4 was a yellow distinct line with spectrum peak at 445 and 475 nm (figure 13) and Rf of 0.40. This was assigned to be Lutein (structure in figure 14).Pharmawati & Wrasiati (2020) and Tomkins (1993) both found this yellow fraction to be lutein as well.

Fraction 5 was assigned to be chlorophyll a due to its characteristics (maximum absorbance at 414 and 666 nm (figure 9), Dark/ blue green color and Rf 0.50) and fraction 6 (Rf around 0.58, peaks 630 and 656 nm) was its form that started degrading by losing magnesium atom as also found by Pharmawati & Wrasiati (2020). Using Thricholoethane-propanone-petroleum ether eluent, Tomkins and Miller (1993) found chlorophyll a (structure in figure 10) to be blue-green color (Tomkins & Miller, 1993). Similar results were found by Pharmawati & Wrasiati (2020) using petroleum ether, acetone and n-propanol as solvent for elution.

Fraction 7 had a grey color with Rf of 0.66. This was assigned to be pheophytin, a degraded form of chlorophyll. Its peaks were at 420 and 665 nm (figure 15) similar to those of chlorophyll a which pushed thinking about the degradation of chlorophyll a as fraction 5 was already an intermediate degradation of the same chlorophyll a. Hence, Fraction 7 could be pheophytin a.

The fraction 8 on the chromatographs (figure 6) was of yellowish orange color whose spectrum peak appeared around 460 nm (figure 7) with Rf of about 0.98. This peak is characteristic of either α or β carotene yet looking at the color (yellowish orange) and referring to literature (Pharmawati & Wrasiati, 2020), it was more likely to be β carotene. β carotene (figure 8) help in the light harvesting by allowing the absorption of the part of the spectrum not covered by chlorophylls.

The molecular structures (figure 8, 10, 12 and 14) of the various identified fractions (fraction 1 to 8) using TLC tests complied with the FTIR results via the predicted functional groups and phytochemical screening results via families of compound found. Indeed, α carotene, β carotene and lutein are all carotenoids.

Table 2Identification of photosynthetic pigments of Tomato crop based on frontal ratio (Rf) and TLC color profile

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Fraction No.	Pigments' color	Photosynthetic pigments	Maximum spectra (nm)	Rf value by stages of growth

				Stage 1	Stage 2	Stage 3
1	Green	Ethyl chlorophyllide b	between 450 and 650	0.13	0.12	0.11
2	Light Green	Ethyl chlorophyllide a	434 and 654 (Pharmawati & Wrasiati, 2020)	0.18	0.18	0.19
3	Green	Chlorophyll b	463 and 648	0.37	0.37	0.38
4	Yellow	Lutein (a xanthophyll)	445 and 475	0.40	0.40	0.41
5	Dark/Blue Green	Chlorophyll a	414 and 666	0.50	0.50	0.51
6	Greenish Grey	Mg-free chlorophyll a	630 and 656 (Pharmawati & Wrasiati, 2020)	0.58	0.58	0.59
7	Grey	Pheophytin a (a breakdown product)	420 and 665	0.66	0.67	0.66
8	Yellowish Orange	β Carotene	460	0.98	0.99	0.98

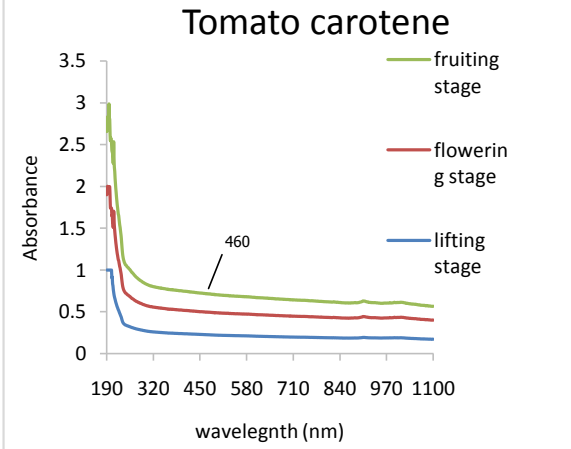

Figure 7 UV spectra of β Carotene from tomato leaves' extract

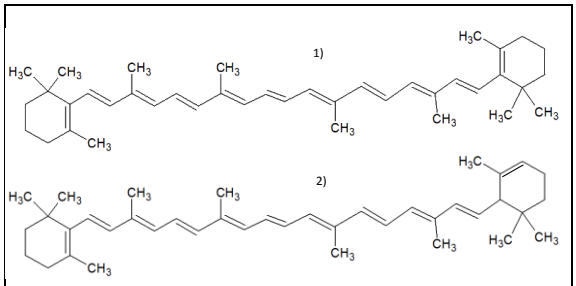

Figure8 1) α-carotene and 2) β-carotene ($C_{40}H_{56}$)

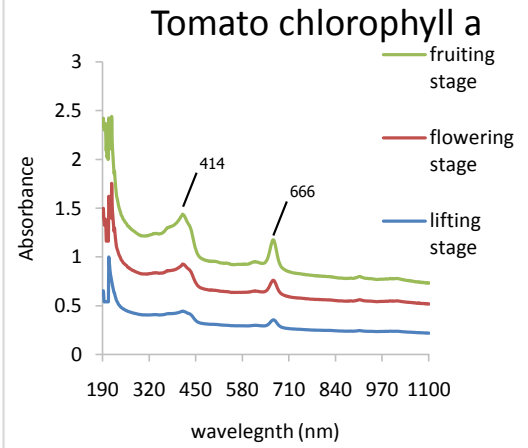

Figure 9 UV spectra of Chlorophyll a from tomato leaves' extract

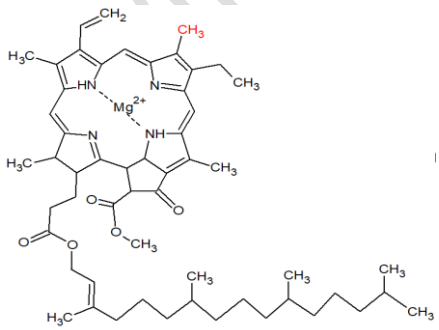

Figure 10 Chlorophyll a (C_{55} H_{72} O_5 N_4 Mg)

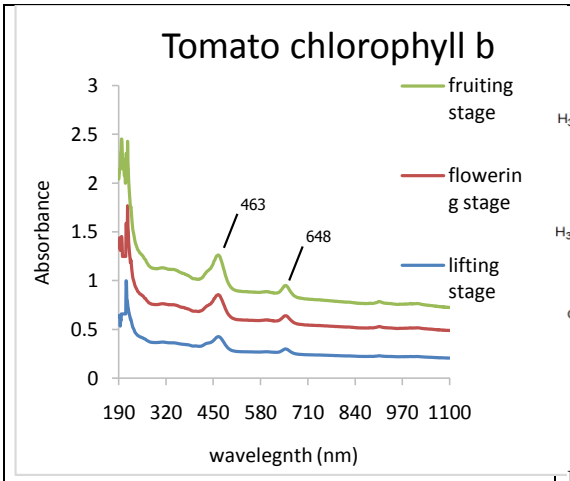

Figure11UV spectra of Chlorophyll b from tomato leaves' extract

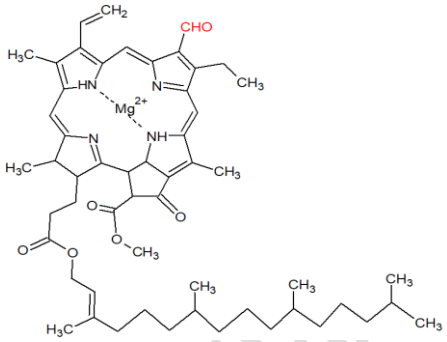

Figure 12 Chlorophyll b (C_{55} H_{70} O_6 N_4 Mg)

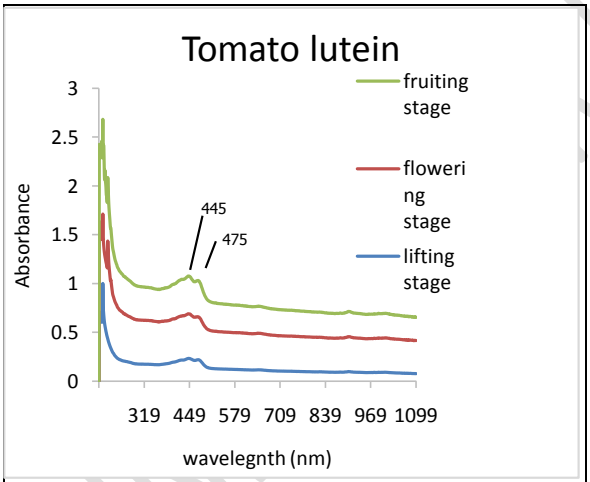

Figure 13 UV spectra of Lutein from tomato leaves' extract

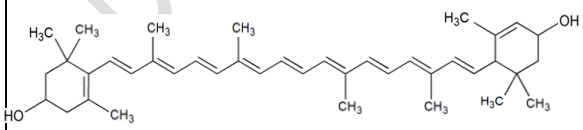

Figure 14 Lutein (C_{40} H_{56} O_2)

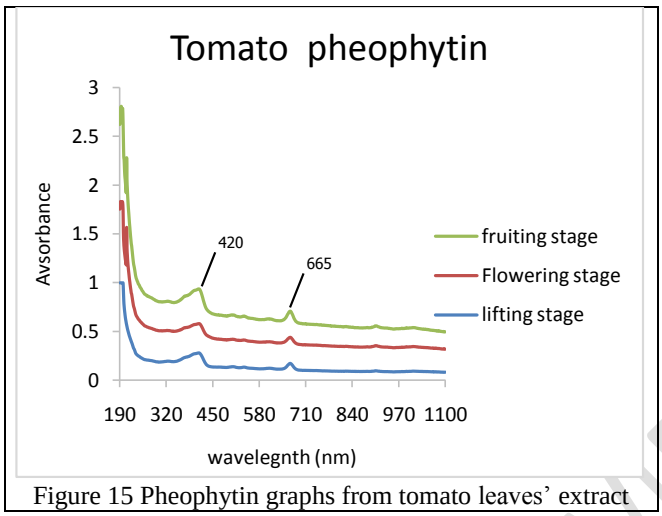

Figure 15 Pheophytin graphs from tomato leaves' extract

4 Conclusion

In this work, the active photosynthetic pigments were successfully identified via phytochemical screening that help obtain a quick overview on the main families of compounds and FTIR test which allowed functional group identification. With those two tests, long chain of carbon with conjugation containing nitrogen and hydroxyl groups such as carotenoids and triterpenoids were identified. The UV spectroscopy results allowed further identification of subcomponents of various leaves at different growth stages even though some shifts in the readings of the maxima were noticed due to the type of solvent and the spectrophotometer used. In fact, the type of eluents and the volume ratio of their constituentsaffect the Rf and separation efficiency. Nonetheless, the action spectrum of tomato crop was successfully determined as well as individual pigments acting in the photosynthesis process. Indeed, chlorophylls and their aggregates as well as their degraded form were identified as pheophytin a and b during all main growth stages of tomato crop. In addition, the presence of pigments such as chlorophylls, lutein and carotenesverified their needs by the plantinmain metabolism of the plant such as chemical reactions, defensive systems or enhancement of light harnessing ability of leaves. In whole, these pigments are vital for plant's photosynthesis.

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