Chemical Composition and Antioxidant Capacity of Eggplant Parts during Vegetative and Flowering Stage

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Abstract: The parts of eggplant Solanum melongena L. (Solanaceae) (leaf, stem, root, flower, and fruit) were taking in two stages, vegetative and flowering stage (Iraq), to determine the influence of total content of phenolic compounds, (as phenol, flavonoid, tannin, and anthocyanin), alkaloids compound and antioxidant capacity of crude extract of these parts. Ultrasonic extracted by ethyl-acectate solvent, qualitative assay by deferent reagent. The flowers showed high content of phenolic compound as phenol, tannin, anthocyanin, and flavonoids, and antioxidant capacity, while seeds showed high content of alkaloids with significant value, p<0.05. Stage of growth of roots, stems, and leave showed no significant differences effect in phenol content, otherwise, the flowering stage of roots, stems, and leave showed high content of flavonoids with significant value compared with vegetative stage. Flowering stage of roots and stems showed high content of tannin with significant value compared with vegetative and flowering stage otherwise in leave. Vegetative stage of roots showed high value of anthocyanin content with significant value, compared with flowering stage. Otherwise, the vegetative stage of roots, stems, and leave showed high content of alkaloids with significant value high value of flowering stage. Antioxidant capacity were the best in vegetative stage of roots, stems, and leave compared with the flowering stage in conjunction with alkaloids.

Keywords: Phenol, flavonoids, tannin, anthocyanin, alkaloids, antioxidant, vegetative and flowering stage.

Introduction
Eggplant Solanum melongena L. (Solanaceae) is an economic plant in the world and has several forms, shapes and colors (Uthumporn et al., 2015; San José et al., 2016) (S. melongena var. esculentum) (Maroto, 2002; Rajam and Kumar, 2007), is one of most common vegetables consumed all around the world (Boulekbache-Makhlfouf, et al., 2013). The deep-colored fruits and vegetables represent as a good sources of phenolics, including flavonoids, anthocyanin and carotenoids and recognized as more healthy to human body, especially in the oriental countries (Lin and Tang, 2007), and as an antioxidant capacity (Vinson et al., 1998).
Absorbance at 765

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. The natural antioxidants protect food from oxidation, and provide health benefits associated with preventing damages due to biological degeneration (Hu and Kitts, 2005). In the last years this plant has a great interest as functional food, and classified as a top ten vegetables with antioxidant capacity due to the high content of phenol and anthocyanins, (Niño-Medina, et al., 2017).

✅ Material and methods

Eggplant (Solanum melongena L.) used in this study was grown at the Iraq (Najaf), vegetated and flowering parts were collected, dried by oven (50°C). Chemical Reagents, Folin-Ciocalteu phenol, DPPH (2, 2-Diphenyl picrylhydrazyl) reagent, Sodium nitrite (≥ 99.0 % purity), Gallic acid and Catechin reagents were purchased from Sigma Aldrich (St. Louis, MO, USA). Sodium carbonates is from Fluka Biochemika (Switzerland), concentrated hydrochloric acid (37%) and absolute methanol was purchased from Panereac Quimica S.a (Barcelone). Absolute ethanol (≥ 99.8 % purity) is from Scharlab S.L (European Union).

Sample Preparation

The vegetative growth stage parts include leave, stems, and roots. The flowering growth stage parts include leave, stems, roots, seeds, and flowers, all washed and dried in an oven at 50°C overnight to be ready for grinding by homogenizer until fine powder.

Preparation of plant extract

The Samples extracted according to standard methods, 0.5 gram of ground-dried sample extracted with 50 ml ethyl- acetate by ultrasonic bath (Unisonics, Australia) 50 C° for 24 min., An aliquot of extract was centrifugation by ultracentrifugated for 5 min at 14000 rpm, the supernatant was collected and complete up to 50 ml. The extraction was repeated triplicate, the final extractions were dried the re dissolve by methanol 100%, stored at -4 C° in a refrigerator until use for phenol, flavonoids, tannin, anthocyanin, alkaloids and antioxidant activity assay.

Total phenolic compounds assay

The total phenolic compounds of extracts was determined with the folin-ciocalteu reagent (Singleton et al.1999). 100 µL of a crude extract (10 mg/ml) was mixed with 0.2 ml folin-ciocalteu reagent and 2 ml purified water and 1ml of 15% Na₂CO₃. The mixture measured at 765 nm after 2 hours at room temperature. Gallic acid used as a standard (100, 200, 300, 400, 500, 600,700, and 800 µg/l), the total phenolic compounds were calculated and expressed as mg Gallic acid equivalent (mg GAE/100g) sample. Since the assay measures all phenolic compounds. All analyses performed in triplicate.

Preparation of standard curve by measured absorbance of a different concentrations of standard gallic acid, plot the concentration against the absorbance figure (1).

Figure (1) Variation of the absorbance with Gallic acid concentrations
Total flavonoid assay

Spectrophotometric assays based on the reaction of flavonoids with the chromogenic system NaNO$_2$-Al (III)-NaOH have been extensively used in determination of total flavonoids. The mechanism involves the reaction of any aromatic ring bearing a quercetin group. The addition of Al (III) formed a yellow solution of complex, turned immediately to red by NaOH addition, the absorbance is measured at 510 nm (Zhu et al., 2010; Magalhães et al., 2012; Pękal and Pyrzynska, 2014). The total flavonoid content measured with an aluminum chloride colorimetric assay. An aliquot (1ml) of extract (10mg/ml) or a standard solution of (Quercetin) (20, 40, 60, 80, and 100 mg/l) added to a10 ml volumetric flask, containing 4ml of distilled deionized water (dd H$_2$O). To the flask was added 0.3 ml 5% NaNO$_2$. After 5 min 0.3 ml 10% AlCl$_3$ was added. At the sixth minute, 2ml 1 MNaOH added and the total volume made up to 10 ml with dd H$_2$O. The solution mixed well and the absorbance measured against a prepared reagent blank at 510 nm with an UV-VIS Spectrophotometer. The total flavonoid content of the dry herbs expressed as milligrams of Quercetin equivalents per 100 gram dry mass (mg QE/100). All sample were analysis in triplicates. (Atanassova et al., 2011).

\[ TFC = \text{Concentration mg/l} \times 10 \]

Concentration mg/l: of total flavonoids obtained from standard curve of quercetin from line equation (y=0.0016x+0.1965).

![Graph of absorbance vs. quercetin concentration](image)

**Figure (2)** Variation of the absorbance with quercetin concentrations

Total anthocyanin assay

Total anthocyanin contents in the extracts were determined according to the procedure described by Taha et al., (2010) with slight modified as follows:

The absorbance of the extracts was measured using spectrophotometer (Apel, Japan) at 530 and 657 nm. Using the formula (A =A$_{530}$ - 0.25 A$_{657}$) was employed to compensate for the contribution of chlorophyll and its degraded products to the absorption at 530 nm. The anthocyanin content expressed as milligram of Cyanidin-3-glucoside equivalent per 100g of dry sample weight. The anthocyanin content calculated as a follows (Rabino, 1986):

\[ \text{Total content} = \text{Absorbance (A)} \times 449.2 \times \text{Dilution Factor} / 29600 \times \text{Sample Weight (g)} \]

Where 29600 = molar extinction coefficient
449.2 = molecular weight of Cyanidin-3-glucoside
Dilution Factor = final volume/initial volume.
Total tannin assay

Tannin content of the test sample determined by the Folin-Dainas spectrophotometric method according to (Stankovic, 2011) with slight modification. 10mg/ml of each eggplant parts extract was prepared, 5ml of each solution putted in separate 50 ml volume flask. One ml of Folin-Dainas reagent was added to each, then added 2.5ml of saturated Na₂CO₃ solution. The mixture makeup to 50 ml with distilled water and the absorbance of the dark color, which developed, the mixture was incubated in dark space at room temperature for 90 minutes, measured in a spectrophotometer after incubation, the absorbance read at 760nm with a reagent blank at zero. The standard curve prepared by measured absorbance of a different concentration of standard tannic acid versus their concentrations, figure (3).

![Graph showing absorbance variation with tannin concentrations at 760 nm](image)

**Figure (3)** Variation of the absorbance with Tannin concentrations at 760 nm

Antioxidant activity assay

The antioxidant activity occurred according to Taha et al., (2010) with slight modified. determination of antioxidant capacity occurred by using DPPH free radical reagent, one method that is currently popular is based upon the use of the stable free radical diphenylpicrylhydrazyl (DPPH). (Philip, 2004)

In the DPPH radical scavenging test, the scavenging of DPPH is followed by monitoring the decrease in absorbance at 515 nm that occurs due to the reduction by the antioxidant or reaction with a radical species. DPPH is widely used to test the ability of compounds to act as hydrogen donors or free radical scavengers and to evaluate antioxidant activity of foods. 3.8ml of different levels of resolve in methanol only (0.01, 0.02, 0.04, 0.08, and 0.1 mg/mL), the concentration which scavenge (reducing) 50 percent (IC50) of each plant were reacted with 0.2 mL of DPPH solution (50 mg of DPPH in 100 mL of methanol) (0.5mg/ml). The mixture was allowed to settle in the dark for 30 min. Absorbance (A) then was read at 515 nm, against the blank (methanol); a blank was used to remove the influence of the color of the samples. The radical-scavenging activity expressed as percentage of inhibition according to the following formula (Brand-Williams and others 1995):

\[
\text{Inhibition\% (mg/ml)} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100
\]

Control; (0.2 ml of DPPH + 3.8 mL methanol)
Sample; (0.2ml of DPPH +3.8ml of extract)

Standard curve prepared by linear plotting the concentrations of sample versus their DPPH inhibition capacity, IC50 (the concentration of extract in mg/mL needed to scavenge 50% of the DPPH radical mg/ml) calculated from their concentration-response curve by linear equation.

Furthermore, this study was depend on weight unit (mg) of sample that scavenging 50 percent of DPPH (0.1mg) instead of concentration, according to following formula:

\[
\text{IC50\% (mg)} = 3.8\text{ml} \times \text{IC50\% (mg/ml)}
\]
Statistical analysis: In this study excel version 2016 to design the standards curves, Genstat 2nd edition program to do anova testing.

The results and discussion

The flowers showed high content of phenolic compound as phenol, tannin, anthocyanin, and flavonoids, and antioxidant capacity, while seeds showed high content of alkaloids with significant value, p<0.05. The phenol content of roots, stems, and leave showed no significant differences between vegetative and flowering stage. Flowering stage of roots and stems showed high content of tannin with significant value compared with vegetative and flowering stage otherwise in leave. Vegetative stage of roots showed high value of anthocyanin content with significant value, compared with flowering stage. The flowering stage of roots, stems, and leave showed high content of flavonoids with significant value compared with vegetative stage. Otherwise, the vegetative stage of roots, stems, and leave showed high content of alkaloids with significant value compared with flowering stage. Antioxidant capacity were the best in vegetative stage of roots, stems, and leave compared with the flowering stage in conjunction with alkaloids. Table (1).

The almost secondary compound content influence according to the plant parts and growth stage, and the reasons of that may be occur depending to demand of metabolism and protective system of plant, where the alkaloids were high content in vegetative stage in all part, and decrease in flowering stage spontaneously, because using the alkaloids as a nitrogen source that important to growth fruit. Otherwise the phenol may be used as an antioxidant instead of alkaloids in flowering stage, therefore, the almost phenolic compound were high in flowering stage.

Phenolics are secondary metabolites synthesized by the plant during growth and reproduction and also are produced as a response to environment stress conditions, defense against infection by pathogens and UV radiation (Karakaya, 2004). The phenolic metabolites in fruits and vegetables varies depending on the plant origins (Robards and Antolovich, 1997). Therefore, the extracts from different kinds of fruits and vegetables exhibited the different antioxidant capacity.

Table (1) phenolic compounds and alkaloids content mg/100g, and antioxidant capacity (mg) to scavenge 50% of 0.1mg DPPH

| Plant parts | Phenol   | Tannin    | Anthocyanin | flavonoids | Alkaloids | Crude antioxidant |
|-------------|----------|-----------|-------------|------------|-----------|-------------------|
| Roots       |          |           |             |            |           |                   |
| F**         | 0.66±0.01| 2545.83±25| 11.0±1      | 50.56±1.9  | 601.79±6  | 0.535±0.01        |
| V           | 0.89±0.02| 2992.77±18| 7.66±0.2    | 85.97±2    | 517.33±3  | 0.869±0.03        |
| Stems       |          |           |             |            |           |                   |
| V           | 1.01±0.05| 2035.41±23| 3.2±0.9     | 80.14±9    | 336.0±7   | 0.610±0.01        |
| F           | 0.55±0.08| 3518.75±12| 3.26±0.5    | 173.33±9   | 226.66±9  | 0.741±0.03        |
| Leaf        |          |           |             |            |           |                   |
| V           | 0.68±0.03| 2087.49±32| 6.7±0.7     | 44.72±5    | 589.33±12 | 3.184±0.4         |
| F           | 0.51±0.06| 1724.99±17| 6.20±0.9    | 174.17±8   | 552.00±14 | 3.703±0.03        |
| Flowers     |          |           |             |            |           |                   |
| V           | 2.36±0.13| 5389.58±21| 25.46±1.2   | 336.39±9   | 798.66±17 | 0.563±0.04        |
| seeds       |          |           |             |            |           |                   |
| V           | 1.67±0.17| 3919.44±32| 14.46±0.8   | 276.39±9   | 986.00±7  | 0.934±0.03        |
| Average     |          |           |             |            |           |                   |
| L.S.D. 0.05 | 1.04     | 3026.78   | 9.74        | 152.70     | 575.97    | 1.39              |
| L.S.D. 0.05 | 1.28     | 62.592    | 2.638       | 26.3       | 25.632    | 0.12              |

*: vegetative stage
**: flowering stage
Eggplant is characterized for its high content of phenolic compounds with antioxidant properties and according to Cao et al. (1996) is among top ten vegetables with higher antioxidant activity. In addition, eggplant is distinguished for its content of flavonoids in peel with high amounts of the anthocyanin nasunin (Koponen et al., 2007), and also is recognized for high levels of phenolic acids in the flesh, especially chlorogenic acid (Whitaker and Stommel, 2003). Studies of the anthocyanins and phenolic acids present in eggplant demonstrates that they are well metabolized and absorbed (Ichiyanagi et al., 2004), have a good antioxidant capacity (Hanson et al., 2006), a potential beneficial action on hyperlipidemia in animals (Sudheesh et al., 1997) and a modest effect on hypercholesterolemia in humans (Guimaraães et al., 2000).

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