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تأثير نوع ونسبة المذيب والوقت على استخلاص المركبات الفعالة من أوراق نبات الفيزاليس انجولاتا للأغراض التجريبية

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المستخلص:

نبات *Physalis angulata AH-ZE* ينتمي إلى عائلة Solanaceae (أو الباذنجانية) ومن المعروف أنه يوجد في المناطق الاستوائية وشبه الاستوائية. وقد تم تحديد هذا النبات على أنه غني بالمركبات الفينولية لخصائصه المضادة للالتهابات والبكتيريا ومضادات الأكسدة ومضادات السرطان. في هذا البحث تم استخلاص الفلافونويدات والفينولات الكلية من أوراق نبات الفيزاليس انجولاتا ودراسة عوامل الاستخلاص متمثلة بنوع المذيب (الايثانول، الميثانول، الاسيتون، الماء البارد، الماء المغلي) بتركيز (١٠،٣٠،٥٠،٧٠،٨٠)% للمذيبات الثلاثة الاولى، ومدة زمنية (٩٦.٢٤ ساعة): افضل تركيز لاستخلاص الفلافونويدات والفينولات الكلية من الايثانول هو(٧٠ و٨٠)% على التوالي، في حين كان الميثانول (٧٠%) هو التركيز الافضل لاستخلاص الفلافونويدات والفينولات الكلية، بينما كان الاسيتون بتركيز (٨٠%) هو الافضل لاستخلاص الفلافونويدات والفينولات الكلية، وكان افضل مدة استخلاص اعطت اعلى نسبة من الفلافونويدات والفينولات الكلية كانت ٤٨ ساعة عند درجة حرارة ٣٧ مئوية.

الكلمات المفتاحية: المركبات الفينولية، النباتات الطبية، *Physalis angulata* .

Effects of solvent type and ratio, and time on the extraction of active compounds from *Physalis angulata AH-ZE* leaves for Experimental

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Purposes

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Abstract:

Physalis angulata AH-ZE belongs to the Solanaceae (or Nightshade) family and is known to be found in tropical and subtropical regions. This plant has been identified as rich in phenolic compounds for its anti-inflammatory, anti-bacterial, antioxidant, and anti-cancer characteristics. In this research, flavonoids and the total phenols are extracted from *Physalis angulata* leaves by investigating of the extraction factors represented by the type of solvent (Ethanol, Methanol, Acetone, cold water, boiling water), in 10,30,50,70, and 80 concentrations % for the first three solvents for 24–96 hour. Out of the flavonoids and total phenols extracted from ethanol with a (70 and 80) % concentration respectively, methanol (70%) was the best solvent for flavonoids and total phenols extraction, while the acetone in concentrations (80%) was the best for total flavonoids and total phenols extraction. The best extraction time that gave the highest flavonoids and total phenols was 48 hours at 37 °C.

Key words: phenolic compounds, Medicinal Plants, *Physalis angulata.*

Introduction:

Multidrug Resistance will likely to increase by 10 million deaths annually by 2050 (O'Neill *et al*,.and De Kraker *et al*,.2٠١٦). As biological detection

has evolved, isolation of the phytochemicals, and therapeutic experiments of medicinal plants have advanced over years ago which clarified the secrets of traditional herbal treatments (Bibi *et al*,.٢٠١٧). Over a period of more than 10 years, *Physalis angulata* plant was included among the medicinal plants as a result of the scientific research findings that included anti-inflammatory, antibacterial, anti-parasitic, anticancer, and antidiabetic agents (Ariyani,2024) .Extracts of different parts of plants contain bioactive compounds that fight against diseases such as phenols, flavonoids, alkaloids, tannins, steroids, glycosides, volatile oils, fixed oils, resins, and terpenoids Kutama *et al*,.٢٠١٨). Extraction of medicinal plants is a process of separating active plant materials or secondary metabolites such as alkaloids, flavonoids, steroids, terpenes, saponins, and glycosides from inert or inactive material using an appropriate solvent and standard extraction procedure (Azwanida,٢٠١٥ &Sasidharan *et al*,.٢٠١١). Many factors must be considered when performing extraction techniques, the most important are the solvent ratio, its type, extraction temperatures, and extraction period in order to ensure the extraction of all important compounds from the plant of interest, while avoiding chemical modification (Altemimi *et al*,٢٠١٧ &Majeed *et al*,& .٢٠١٦Lee *et al*,.٢٠١٦). In practice, ethanol is widely preferred over other solvents for food and pharmaceutical processing due to its safety and affordability(Hemwimon *et al*, .٢٠٠٧& Guo *et al*,. ٢٠٠1). This could be explained by the inability of ethanol to extract 100% of the phenolic compounds, some of which are more water-soluble (hydrophilic). Therefore, the presence of water in the extraction eases the release of hydrophilic antioxidants(Thoo *et al*,.٢٠١٠). The study aims to find the optimal conditions of extraction (solvent type, extraction time, and optimum solvent concentration) to extract the phenolic components from *Physalis angulata AH-ZE* leaves and determine the optimal concentration of the optimal extract to be used to inhibit bacterial activity.

Materials and Methodology:

Folin–Ciocalteu phenol reagents were obtained from GCC - UK, Absolute Ethanol C2H5OH, Absolute Methanol CH3OH and Absolute Aston C3H6O were obtained from Sigma/ USA, Sodium carbonate

(Na2CO3) and potassium hydroxide KOH were purchased from Merck (Darmstadt, Germany), Quercetin C12H10O7.2H2O and AlCl3Aluminum chloride were purchased from (Himedia, India), Sodium acetate from (BDH, England), CH3COOH Glacial acetic acid was obtained from (GCC, UK), Potassium dihydrogen phosphate from (Fluka, Switzerland), deionized water was available in the lab.

Physalis angulata AH-ZE were collected from various agricultural areas in Karbala province in October. After collection, the plant leaves used in this study were cleaned with distilled with water to remove dust, and sun-dried in the air several times until a fine powder was obtained.

Five solvents were used to extract phenolic substances from *Physalis angulata AH-ZE* leaves. They included methanol, ethanol, acetone, boiling water and cold water. The first three solvents were used at concentrations of (10, 30, 50, 70, 80) % by placing 10 g of plant leaf powder with 100 ml of the solvent prepared at the concentrations described above. This was followed by placing the extract in a shaking incubator at a speed of 100 rpm for 72 hours at a temperature of 37 °C. After the incubation period, the extract was filtered with medical gauze first and then centrifuged at a speed of 5000 rpm for 15 minutes. After obtaining the extract, it was dried at room temperature until it completely dried. As for water extraction, it was done by placing 10 grams of the plant powder with 90 ml of distilled water. In the case of extraction with boiling water, this mixture is placed on a heat source until it boils for 3 minutes and then left at room temperature. After it cools, it was filtered, centrifuged and dried as well as for extraction with cold water, the same steps as extraction with boiling water are followed, except that it was not heated.

Extraction of phenolic substances: The method described by (Ahmed *et al*. 1998) was used to extract phenolic substances from the plant under study.

Estimation of flavonoids: The amount of flavonoids was estimated during the study stages following the method described by(Kosalec *et al*. 2005) and based on the standard curve of quercetin.

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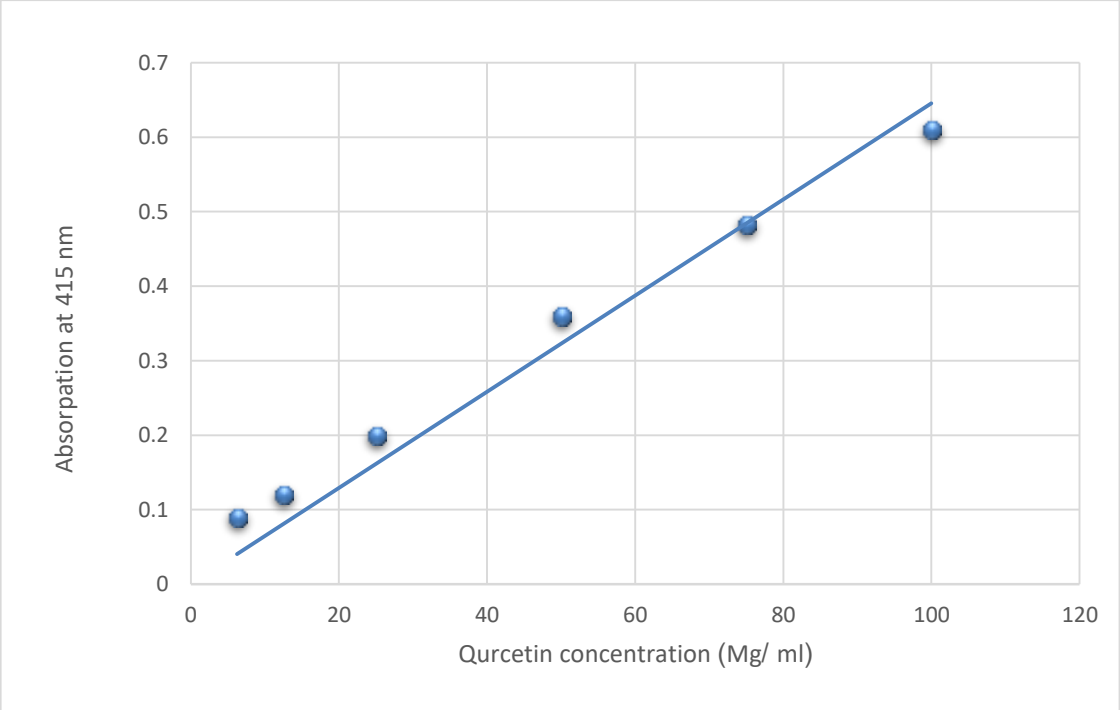

Figure (1): Standard curve for estimation of flavonoids using quercetin.

Estimation of total phenolic content:

The total phenolic content of the plant extract was estimated according to the method described by (Budrat and Shotipruk2008). Standard curve for gallic acid:

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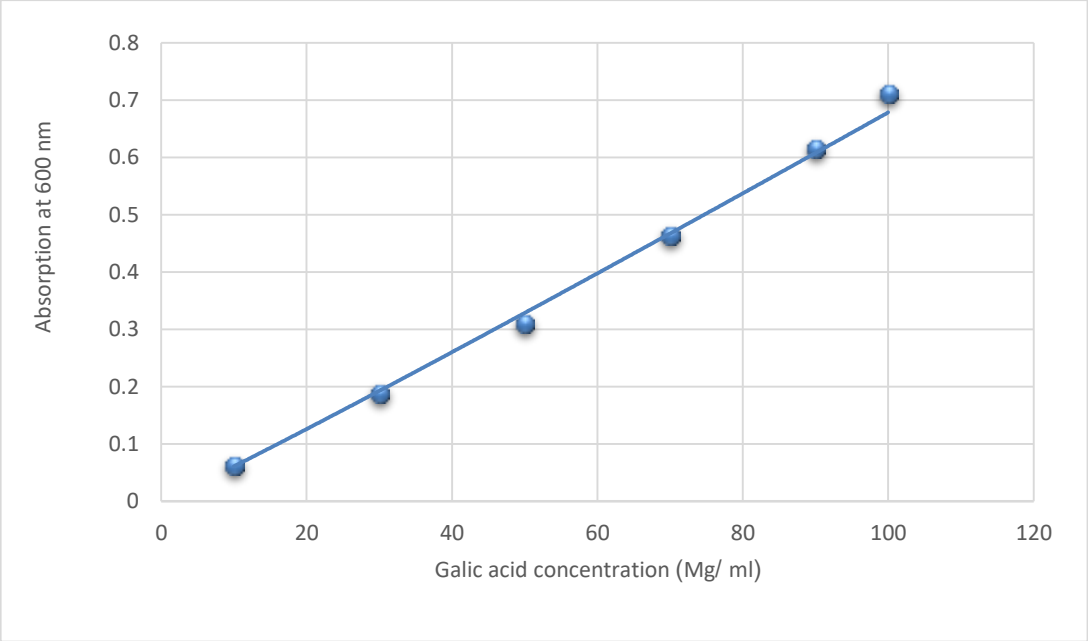

Figure (2): Standard curve of gallic acid by Folin Ciocalteu method

Determine the optimal extraction time:

The effect of the optimal extraction period on the extraction of flavonoids from the selected plant was studied. The extraction process was followed during 24, 48, 72 and 96 hours.

Results and Discussion:

Determining the optimal conditions for extracting flavonoids and total phenols from *Physalis angulate* leaves:

Solvent type and concentration:

Five solvents were used to extract flavonoids and total phenols from *Physalis angulate* leaves. The accuracy of the extraction method depends on the consistency and water content of the plant material and the type of material to be separated.

Effect of solvent concentration: -

1- Effect of ethyl alcohol concentration: It was clear from Figure (٣) that the best concentration for extracting flavonoids from *Physalis angulate* is 70% ethanol and decreases with a concentration of 80%, as it reached 1.433 mg/ml at a concentration of 70% ethanol, while it reached 1.072 mg/ml at a concentration of 80% ethanol.

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As for the total phenols, their concentration increased with the concentration of ethyl alcohol, reaching 1.072 mg/ml at a concentration of 80% ethanol. As in Figure (٤).

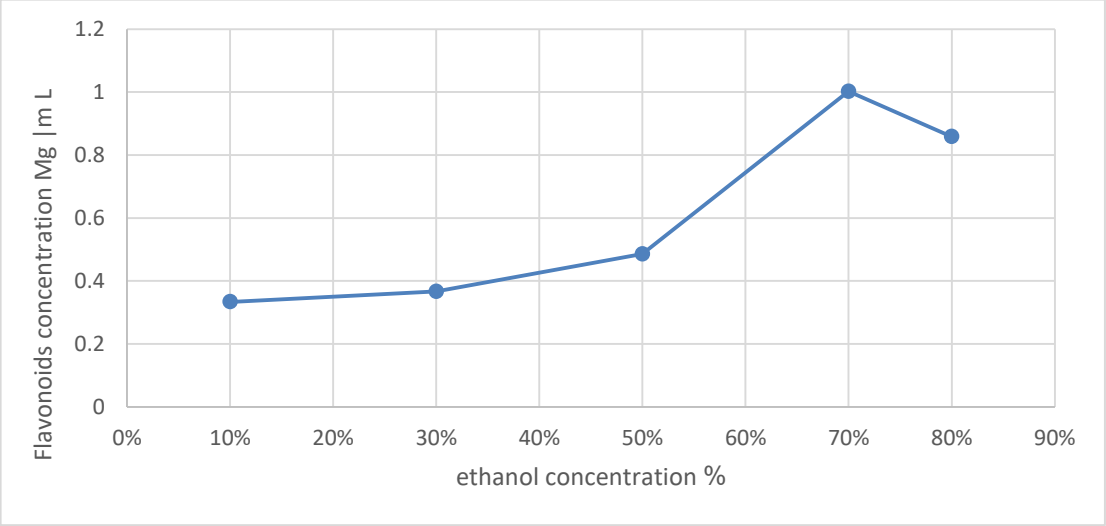

Figure 3: Extraction of flavonoids from *Physalis angulate* leaves using ethyl alcohol.

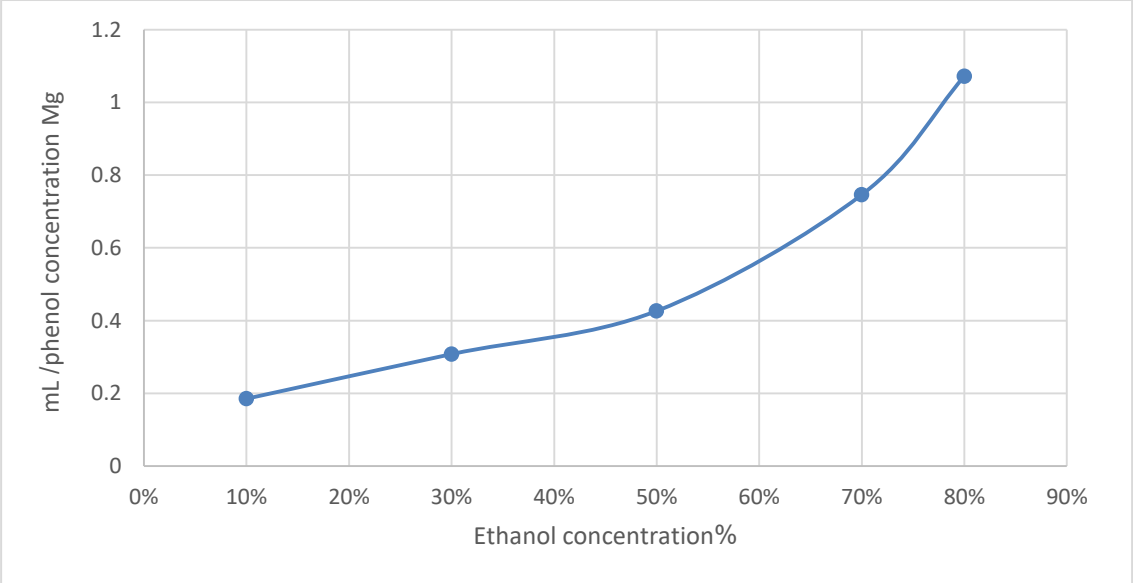

Figure 4: Extraction of phenols from *Physalis angulate* leaves using ethyl alcohol.

The extraction yield of flavonoids from *Physalis angulate* leaves is greatly affected by the concentration of ethanol, as it increases with increasing concentration of this solvent up to a concentration of 70%. This is due to the

solubility of flavonoids in ethanol solutions, but at concentrations higher than 70%, the yield of flavonoids going down. This may be because rise concentrations of ethanol affect quality and composition of flavonoids (Shouqin *et al*,.2005). The above results show the high efficiency of extraction with hydro alcoholics, which can be attributed to the water-soluble nature of phenols, whose solubility increases in the presence of organic solvents, as these solvents facilitate the dissolution of phenols by penetrating the cell structure (Moure *et al*,.2001). The use of alcoholic solutions gives acceptable results for the extraction process (Perva *et al*,.2006). Plants also contain phenols, which are a mixture of several types of phenols, whose solubility varies according to solvents. Therefore, a mixture of alcohol and water was useful in changing the polarity of alcoholic solvents, noting that the solubility of phenols depends on hydroxyl groups, molecular size, and hydrocarbon length (Mohammedi *et al*,2011). As for pure ethyl alcohol, its use leads to a reduction in the efficiency of the phenol extraction process due to the presence of hydroxyl groups (such as flavonoids, especially those containing sugars in the molecule), i.e. they were hydrophilic, and for this reason they dissolve when water is added to ethyl alcohol [Jokić *et al*,.2010). In addition to the above, phenolic compounds in the extract are often associated with other molecules such as (proteins, fats and other inorganic compounds) [Koffi *et al*,.2010).

٢-Effect of methyl alcohol concentration: The choice of solvent depends on the nature of the material to be extracted and the most important requirement for this type of extraction is to obtain a high percentage of the material in a small volume of solvent (Al-Haidari *et al*,.1989).

In Figures (5, 6) it was clear that the best concentration for extracting flavonoids and total phenols from *Physalis angulate* is methanol concentration of 70%, as it reached (1.0, 0.605) mg/ml respectively.

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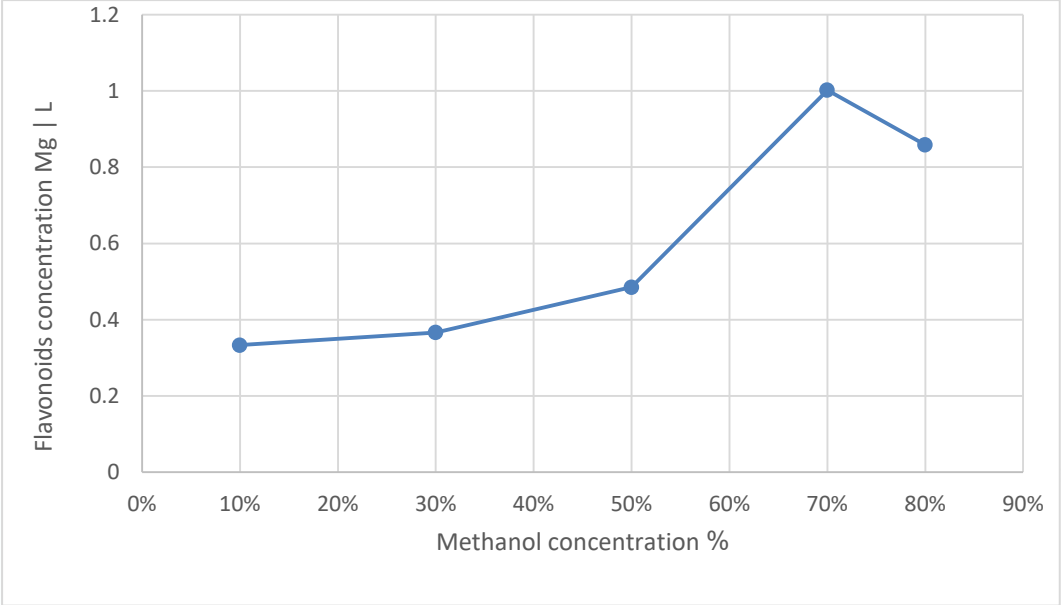

Figure 5: Extraction of flavonoids from *Physalis angulate* leaves using methyl alcohol.

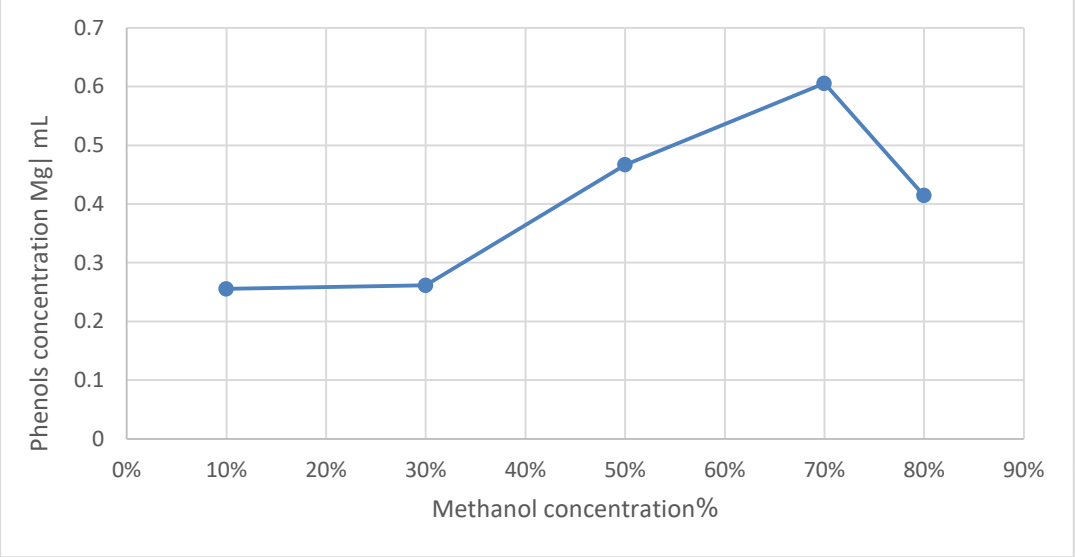

Figure 6: Extraction of phenols from *Physalis angulate* leaves using methyl alcohol.

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In many studies related to the extraction of phenols from plants, methyl alcohol was used. In a study that included sixteen plants, it was shown that methyl alcohol extract was better than ethyl alcohol in extracting phenols (Akroum *et al*,.2009).

٣-Effect of acetone concentration: In Figure (٧)، it was clear that the highest concentration of flavonoids obtained from extracting the leaves of *Physalis angulate* using acetone solvent was 80% concentration, reaching (1.847) mg/ml

Also, the highest phenolic content was obtained from extracting the leaves of this plant using acetone solvent also at a concentration of 80%, reaching (1.536) mg/ml as in Figure (٨). Therefore, it is noted that the concentration of flavonoids and total phenols increases with the increase in acetone concentration.

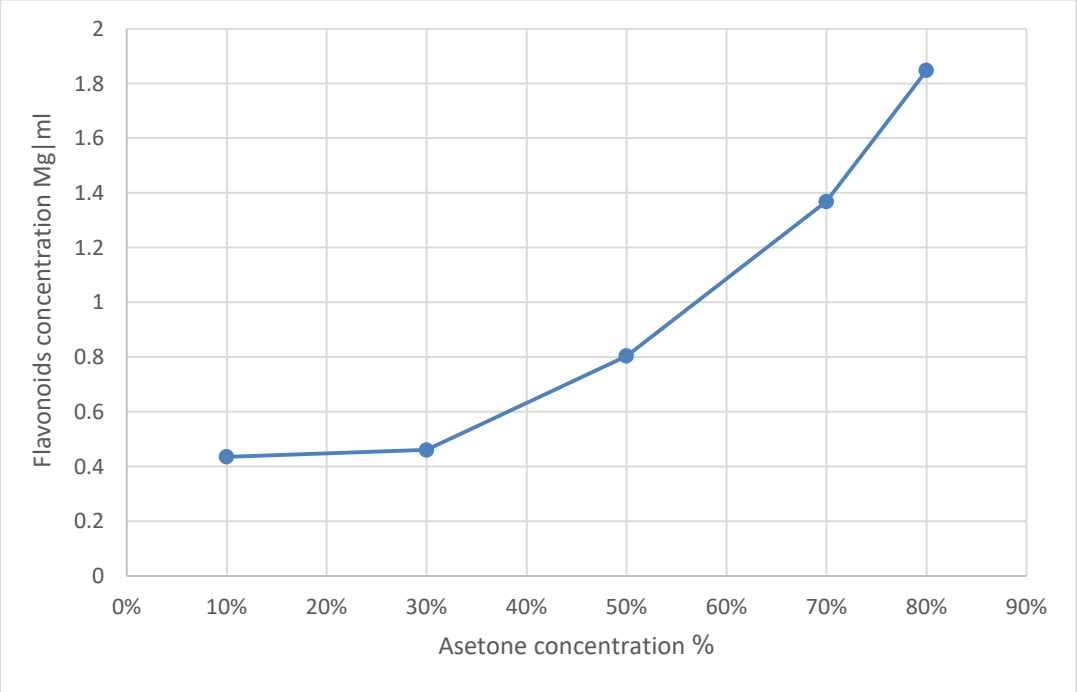

Figure 7: Extraction of flavonoids from *Physalis angulate* leaves using acetone.

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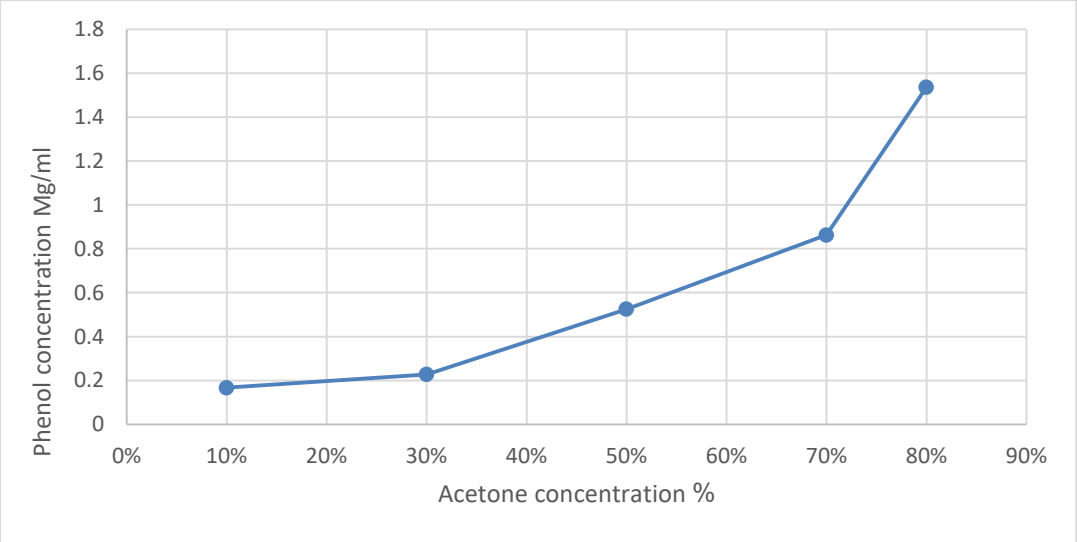

Figure 8: Extraction of phenols from *Physalis angulate* leaves using acetone.

A number of studies have indicated the use of acetone in extracting phenols. ([Chan *et al*,.2009). demonstrated the superiority of aqueous acetone over aqueous ethyl alcohol and methyl alcohol in extracting phenols from Citrus hystrix peels. (Turkmen *et al*,.2007). also confirmed the superiority of acetone over dimethyl formamide, ethyl alcohol and methyl alcohol in extracting total phenols from black tea (Uma *et al*,.2010).

Effect of extraction by distilled water and boiling water:

The results obtained from the study showed that the values of total flavonoids and phenolics reached (0.458, 0.138) mg/ml respectively when using deionized water at room temperature when extracting them from *Physalis angulate* leaves. While the values of total flavonoids and phenolics were obtained using boiling water (0.516, 0.160) mg/ml respectively in the extraction process.

Referring to the previous results obtained using different concentrations of ethyl alcohol, methyl alcohol and acetone in extracting flavonoids and total phenolics, it was noted that water was inefficient in extracting the mentioned materials.

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The effect of extraction duration on the extraction of flavonoids and total phenols from *Physalis angulate* leaves:

The results shown in Figures (9, 10) showed that the best duration for extracting flavonoids and total phenols was the same, reaching (48) hours, with a concentration of (0.766, 0.545), respectively.

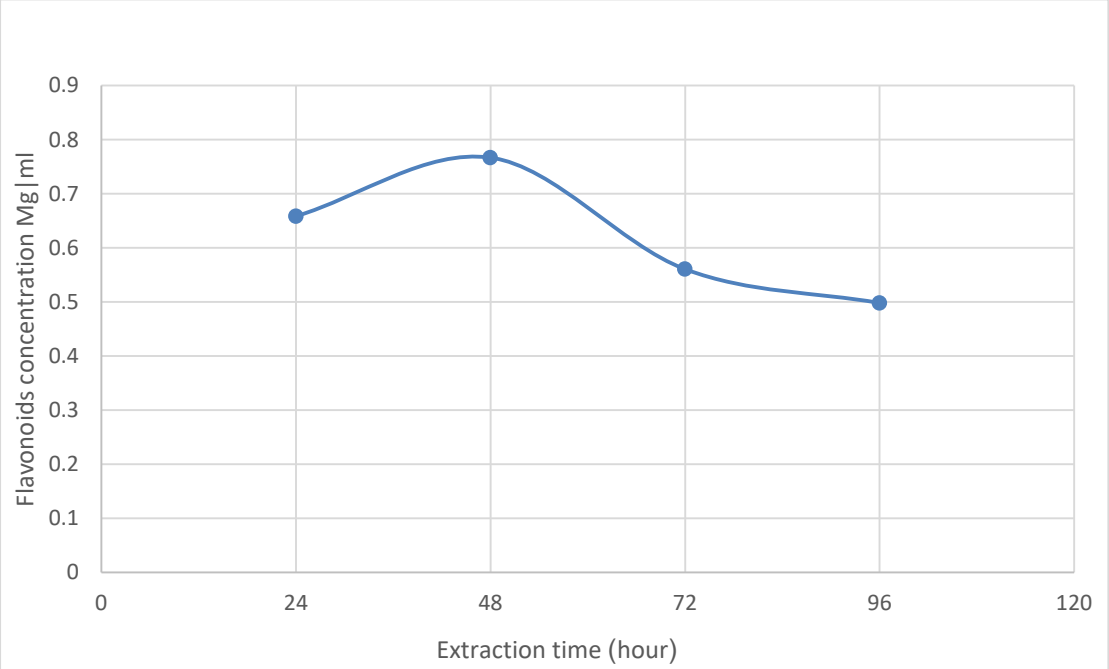

Figure 9: Effect of extraction duration on the concentration of flavonoids from *Physalis angulate* leaves.

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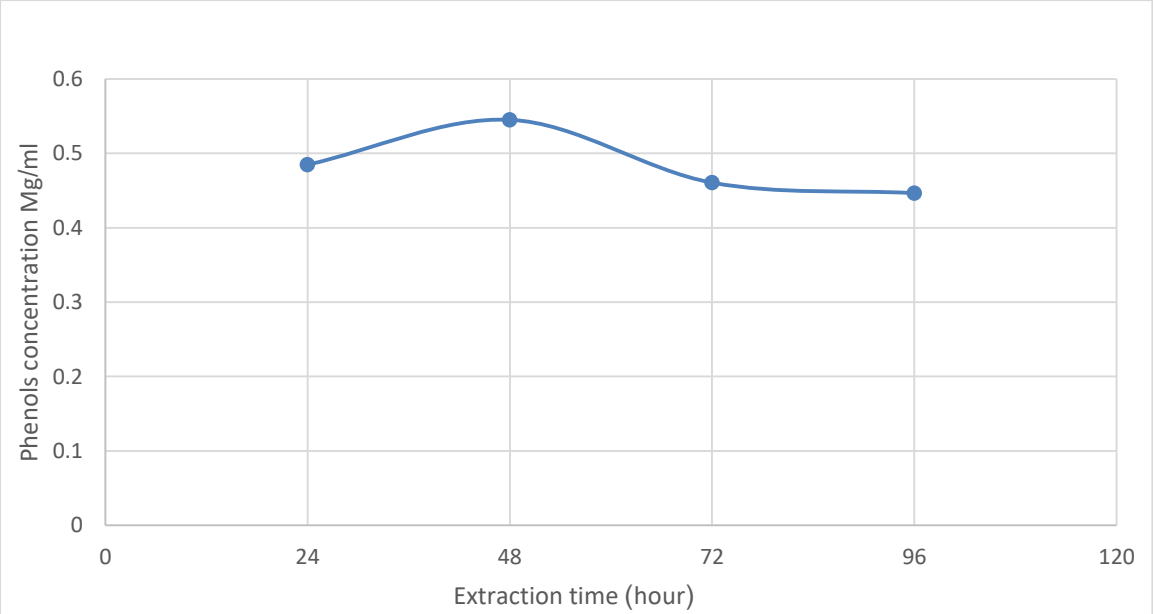

Figure 10: Effect of extraction duration on the concentration of phenolics from *Physalis angulate* leaves.

Studies have varied widely in determining the optimal conditions for extracting flavonoids and total phenols for different plants, from hours to days (Cai *et al*,.2010). concluded that the best time for extracting flavonoids from *Opuntia milpa alta* was 6 hours, while the results of (Ciou *et al*,.2008). and [Loganayaki *et al*,.2011). agreed, indicating that 24 hours was the best time for extracting flavonoids and total phenols from *Trapa taiwanensis nakai* and *Helicteres isora L*., respectively. While the results of our current study agreed with the results of two studies, where where 48hr. was used to extract total phenols from five medicinal plants in Burkina Faso (Konaté *et al*,.2011). and from *Tamarix aphylla L*. [Mohammedi *et al*,.2011).

Ethical Approval to Research

achieved an ethical certificate to complete the search from the relevant committees in the Karbala University.

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