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Estimation of total tannin and total phenolic content in plant (*Crataegus azarolus* L) by orbital shaker technique

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Abstract

The plant of *Crataegus Azarolus L*, genus that belongs to the Rosaceae family and is a low, dense, spiny tree with a beautiful inflorescence, the phytochemical investigations on genus Crataegus were mainly performed on the leaves, flowers and berries. The objective of this experiment was to determine the ratio of tannin and phenol compound by method orbital shaker extraction. The plant of *Crataegus azarolus L* was collected in hasanbag mountain soran, Kurdistan. The parts of seed, leaf and steam of *Crataegus Azarolus L* was extract with water solvent and orbital shaker and assessed yield extraction, total tannin and total phenol. The highest significant value (p<0.05) of yield extraction was observed from leaf (71.56%), compared with other part of *Crataegus Azarolus L*. On the other hand, the total tannin was showed uppermost in leaf (3.03 kg/mg), and the steam (2.87 kg/mg). Whereas, the smallest total tannin was observed from the seed (1.10%). Furthermore, the total phenolic contents of the plant were observed the lowest value in seed as (0.98 mg GAE/g). Whereas, the total phenol in leaf was observed a higher significantly value (3.68 mg GAE/g) and in steam (1.92 mg GAE/g), with all significant value (p<0.05). This study showed that the leaf and other part of *Crataegus Azarolus L*, rich in total phenolic and total tannin after orbital shaker method was used.

Keywords: Crataegus Azarolus, Tannin, Orbital shaker, Total Phenolic, Leaf

Introduction

Crataegus L. genus that belongs to the Rosaceae family is one of the most important genera concerning the number of species. *Crataegus azarolus L*. The plant is widely distributed in North Europe, temperate regions of Asia, Africa and North America. Eastern North America and europe were proposition to be the most recent common areas for crataegus L. In Tunisia, the Crataegus' fruits are known by their famous name "Zaarou-ra", while in Spain "Azerolier" and in the anglophones' coun-tries by "Azerole Hawthorne". Crataegus azarolus L. is vastly

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distributed in the Northern West, the Cap Bon (in the centre), and the Dorsal Mountain of Tunisian in bioclimatic regions extending from the upper semi-arid to the decrease humid (Khiari et al., 2015).

Crataegus azarolus var. is a low, dense, spiny tree with a beautiful inflorescence up to six m tall and with orange fruit(Christensen, 1992). Phytochemical investigations on genus Crataegus were fundamentally performed on the berries,-flowers and leaves (T Bahorun et al., 1994; Kao et al., 2005; Kumar et al., 2012).The insulated compounds were:bioflavonoid, oligomeric procyanidins, polysaccharides, catecholamines, vitamin C, saponins, cardiotonic amines, tannins,ursolic acid and purine derivatives (Sokół et al., 2007; Hamahameen and Jamal, 2013; Duke, 1992).

The potential of plant *Crataegus azarolu* a source of antioxidants (Th Bahorun et al., 1996; Ljubuncic et al., 2005). Hawthorn flowers and fruit act as diuretics, and can be used to treat kidney problems and "dropsy" (Twaij *et al.* 1987), the dietary and medicinal cost of C. azarolus fruit to assist the exploitation of azarole according to a Few research (Bignami et al., 2001; Koyuncu et al., 2007).

In Palestine, morocco and Tunisia (Ali-Shtayeh et al., 2000), the hawthorn fruit and vegetation are used to deal with cardiovascular disease, sexual weakness, diabetes and cancer(Bignami et al., 2001; Koyuncu et al., 2007).

The tannins (is a tannic acid) are water-soluble polyphenols that are current in many plant foods. Tannins are a various type of compounds and have a number of effects on health. The antimicrobial and antioxidant activities of tannin are well authenticated. They are additionally used as antiseptics and astringents, antioxidant things to do confer upon the anti-mutagenic and anti-carcinogenic properties of tannins. (Chung et al., 1998).

The aim of his study was to evaluate of total tannin and total phenolic in the plant of *Crataegus azarolus L* by a technique of orbital shaker extraction

Materials and Methods

Plant Collection and Preparation of extraction methods

The plant of *Crataegus azarolus* L was collected from 6/ September to 17 September. 2019. In Hasanbag mountain. after that the plant sample was ground by grinder (Model GI, Capacity/hour 10 Kg, Capacity 4 letter, Speed 13000 rpm, and Cycle 500 gr), has been done at the home. after that prepared of powder plant was soaked in a solvent (distilled water) for 24 h. using the Orbital shaker extraction method. After that filtered and evaporated using Fume Hood.

Yield determination

The yield percentage of the extract was determined by using the following formula for each one of the extraction techniques which was given below: (Murugan and Parimelazhagan 2014)(Zhang *et al.* 2009).

Equation 1: extract percentage yield

Where,

X is the oven dry weight of extract (g),

Y is the oven dry weight of the sample (g).

Determination of total condensed tannin

This assay was carried out by Shimadzu UV-vis spectrophotometer. The extraction solution was prepared by mixing 0.05 g of Fe2SO4, 95 ml *N*-butanol and 5 ml HCl (35%). For determining the condensed tannin, 0.01 g of crude plant in a test tube and 10 ml of extraction solution was added and placed in a water bath for heating 1 h. The absorbance was measured at 580 nm wavelength (Karaogul *et al.* 2017),(Makkar and Singh 1995).

Determination of total phenolic compounds

The total phenolic content was estimated by the Folin Ciocalteu method as described by Dewantoet al. An aliquot of the diluted extract was added to 180 mL of distilled water and 20 mL of Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 5 min, before the addition of 1.60 mL of a 7% sodium carbonate solution were added. The solution was then adjusted with distilled water to a final volume of 3 mL and mixed thoroughly. After incubation in the dark, absorbance at 760 nm was read versus a prepared blank. The total phenol content of plant parts was expressed mg of Gallic acid/g (GAE/g), from a calibration curve with Gallic acid. All samples were analyzed in three replicates (Dewanto *et al.* 2002).

The TPC was measured using a gallic acid standard and expressed as mg of gallic acid/g (GAE/g). All the experiments were carried out in triplicate.

Statistical Analysis

The plant of Crataegus azarolus L. was analyzed and expressed as values of means ± S.E (standard errors) of triplicate calculated all parameter. The results of the three groups were compared using the analysis of One-way ANOVA-samples F-test with significantly different ($p<0.05$), by (IBM SPSS for Windows (version 20.).

Results and Discussion

In general, the results of the yield extraction, total tannin and total phenol of *Crataegus azarolus L* extract to be prepared by orbital shaker technique and with distilled water solvent. In this study, the yield extraction of plants was observed a highest significant value ($p<0.05$) in leaf (71.56%), compared with

steam (53.41%), and seed (35.64%) respectively, table 1 and figure 2. The effects of solvents polarity on extraction yield both qualitatively and quantitatively was confirmed by (Franco1 *et al.* 2008).

The tannin was calculated by the n-butanol- HCl- iron way to the use of this assessment for the amount of tannin was quantitatively released from the sample. The greatest commonly used standard for n-butanol /HCl assay is mimosa-tannin under normal reaction/condition which calculated using the regression equation (y = 151,96x - 6,9042), R^2 = 0.9978 previously earlier from the linear calibration curve (Figure 1). Comparison of the high amount of tannins found in all root plants species by the percentage of tannin was determined, Calculation: % = A/3m, where A = absorbance value, m = mass weight (Karaogul *et al.* 2017).

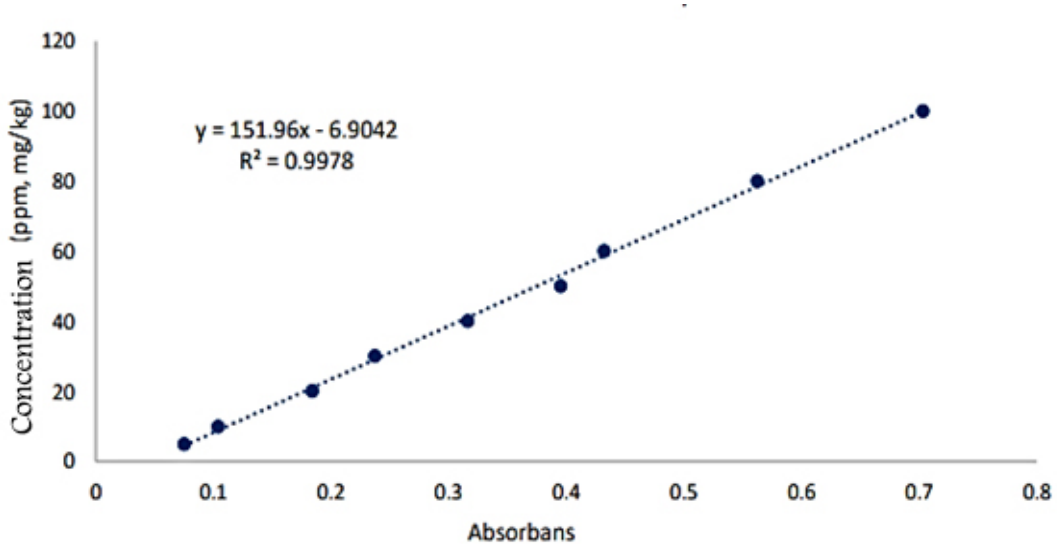

Figure 1. Calibration chart from mimosa tannins

Also, the tannin content determined based on the procedure of (Makkar and Singh 1995), (Karaogul *et al.* 2017), so that the result was expressed as absorbance unit at 580 nm per 1 mg of extract (A580/mg) In this study, the total tannin content was Illustrated the uppermost in leaf (3.03%) mg/kg, and the steam (2.87%) mg/kg. Whereas, the smallest total tannin was observed from the seed (1.10%) mg/kg. Respectively, with all significant value ($p<0.05$), that is shown in (table 1, and figure 3). Many types of research had been studied and reported the importance of tannin and its variation. Their activity is possible because of their capability to connect with extracellular and soluble proteins or combine with the cell wall of fungi. The character of these compounds may disrupt fungal membranes(-Franco1 *et al.* 2008).

Table 1. Extraction yield, total tannin and total phenolic of *Crataegus azarolus L* solvent extracts

	Orbital Shaker Extraction/ D.W solvent		
	Yield extraction	Total tannin(mg/kg)	Total Phenol(mg GAE/g)
plant	Std. Error of Mean	Std. Error of Mean	Std. Error of Mean
seed	35.64±0.881	1.10±0.008	0.98±0.005
Steam	53.41±0.577	2.87±0.008	1.92±0.005
Leaf	71.56±0.881	3.03±0.003	3.68±0.005
F-test	512.324	20724.26	56356.00
(P-value)	0.000	0.000	0.000

Values are Mean ± SE of Triplicate Samples, one-way ANOVA -samples F-test significantly different ($p<0.05$)
OSE: Orbital Shaker Extraction, DW: Distilled Water

Furthermore, the total phenolic of the plant was presented in, table 1 and figure 4. The total phenolic content was observed the lowest value in seed as (0.98 mg GAE/g). Whereas, the total phenol in leaf was observed a higher significantly value (3.68 mg GAE/g) and in steam (1.92 mg GAE/g), respectively ($p<0.05$). Because depend on abilities extract with the plant were found. Indeed, it could be due to the polyphenolic content of the plant being greatly affected by environmental factors as well as edaphic factors like soil type, sun exposure, rainfall, altitude and high tide, soil nutrients. Etc (Manach *et al.* 2004). Our findings are in similar with (Balaky *et al.* 2020; Ismael et al., 2019; Hamahameen and Jamal, 2013; Deliorman Orhan et al., 2012), our results are disagreement with studies by (Rebaya *et al.* 2015)(Kumar *et al.* 2012), because of the method technique and solvents difference.

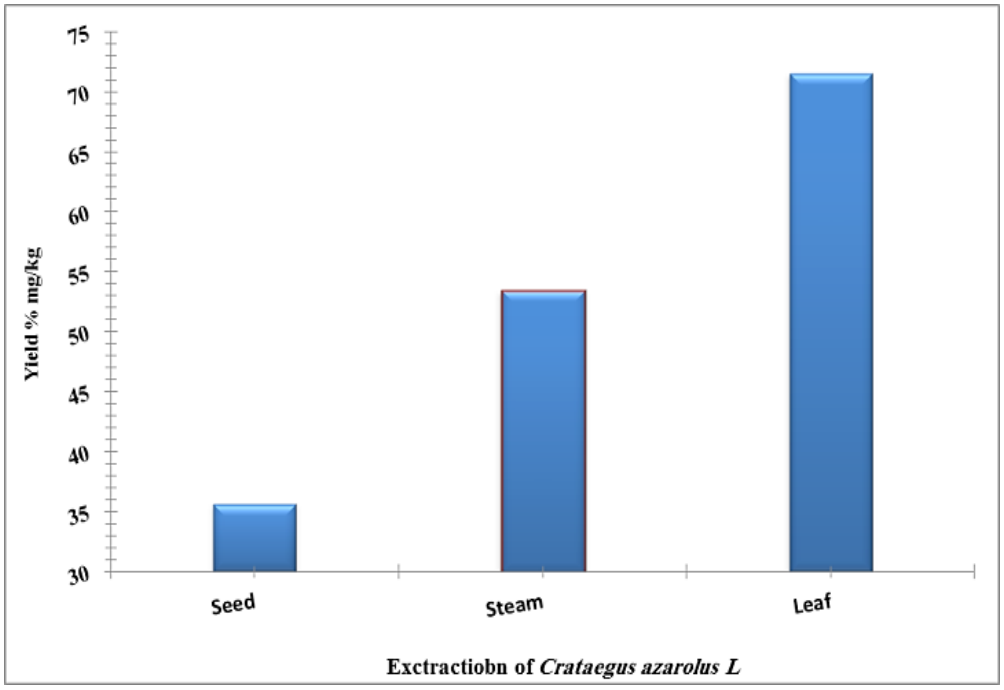

Figure 2. Extraction yield percentage of plant *Crataegus azarolus L*

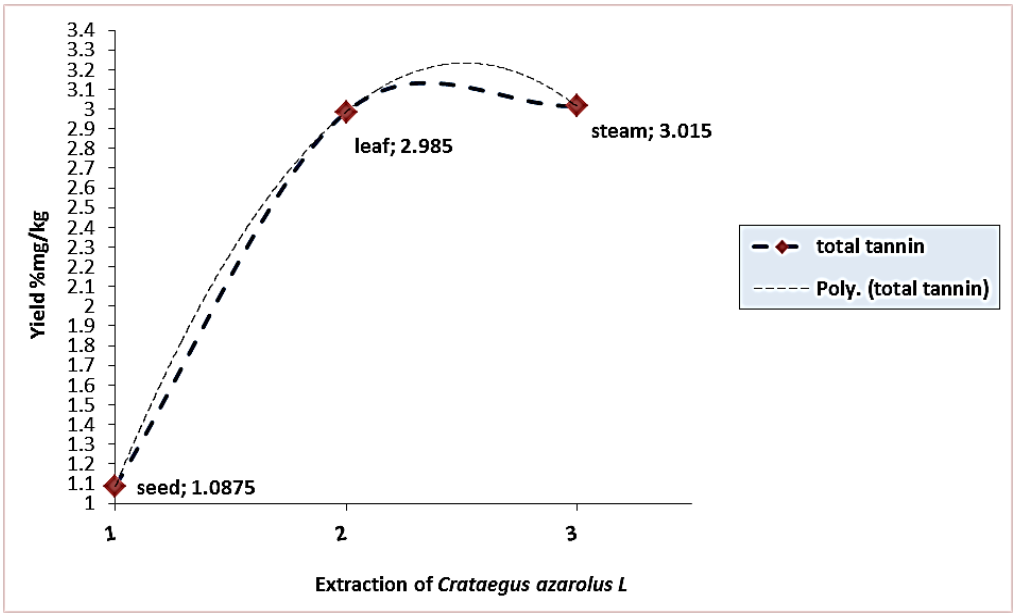

Figure 3. Total Tannin content of *Crataegus Azarolus L* solvent extract

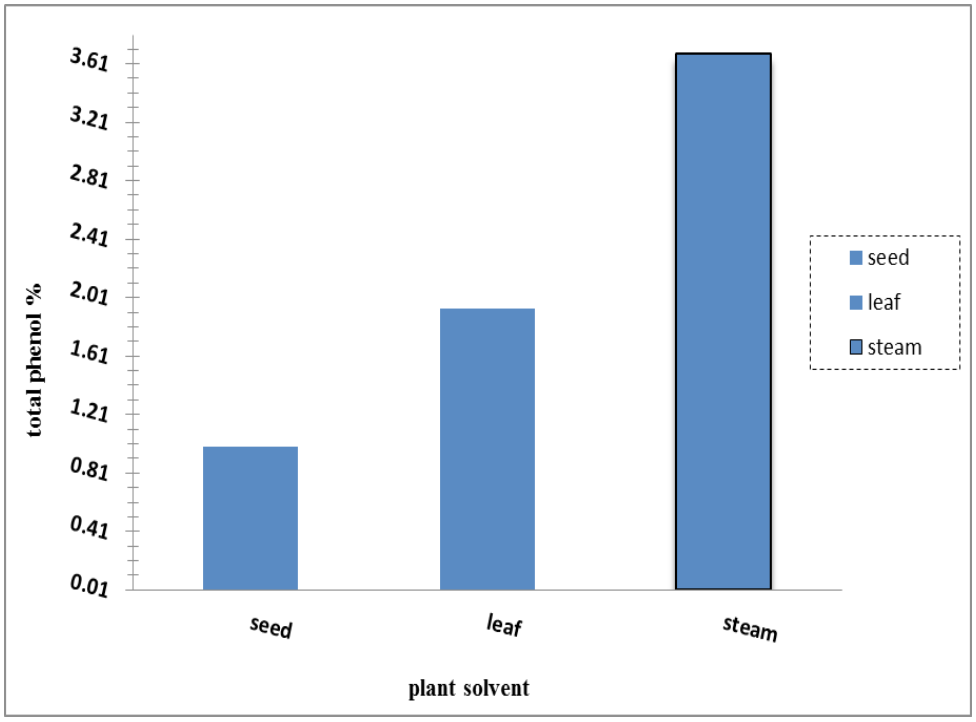

Figure 4. Total Tannin Phenolic content of *Crataegus Azarolus L* solvent extract

Conclusion

This study shows an overview analysis of the solvent of distilled water with the method of orbital shaker extraction in a plant. Those shown all results of the analysis were found as limited value and high significant value ($p<0.05$) of yield extraction (71.56%), total tannin (3.03%), and total phenol (3.68) respectively. Furthermore, the part leaf with all analysis was observed a significantly high value. Our study will be useful to researchers and others and, suggest to researchers who interested in our plant. This study showed that the leaf and other part of plant *Crataegus Azarolus L* that is a good natural edible plant and rich in antioxidant for human consumption after orbital shaker method was used.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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