

Antioxidant Activity and the Laxative Agent of Red Hawthorn (*Crataegus Sumbollis*) in Jam Industry

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Abstract

The aim of the study is to use hawthorn as a gel-forming material in the jam industry and as natural plant substitutes in food manufacturing, due to the emergence of many negative effects of industrial food additives, and that the natural active compounds in hawthorn allow to improve to the taste and enhance the texture and nutritional value of the product. The antioxidant activity and the amount of flavonoids present were estimated, The chemical and mineral structure of the aqueous and ethanolic extract of hawthorn were determined. Hawthorn contains many active compounds when diagnosed with GC-MS (Gas Chromatography Mass Spectrometer) such as catechol, catechin, gamma-sitosterol, hexadecanoic acid in ethanolic extract, also present catechol and catechin in aqueous extract, In addition to a wide range of active compounds. The total content of flavonoids was estimated for the ethanolic and aqueous extracts, as the aqueous extract recorded the highest concentration of 149 mg / ml at a concentration of 25 mg/ml compared to the ethanolic extract, which recorded 95 mg/ml at the same concentration. Antioxidant activity were studied by DPPH radical, The study found that hawthorn extracts had a high ability to stop DPPH free radicals, especially at a concentration of 25 mg / ml, as the inhibition ratio was 93% and 97% for the ethanolic and aqueous extracts, respectively. The findings revealed the presence of elements such as calcium, magnesium and potassium in high concentrations. Sensory characteristics such as Color, Texture, Texture, Appearance, Odor and Flavor were also studied. The study showed that hawthorn has excellent potential when used as an effective component in jam manufacture.

Keywords: *Red Hawthorn Jam, Antioxidants, Effective compounds.*

INTRODUCTION

The genus (*Crataegus sumbollis*) harbors the red hawthorn plant, and this genus includes hundreds of species belonging to the Rosaceae family, widespread widely in Asia and Europe (Alirezalu et al 2018). It has recently proven its medical importance through its positive effects

on the heart and blood vessels, as an antimicrobial, and antioxidant activities. The high percentage of phenolic compounds contained in hawthorn made it a valuable plant with an aura of wide biological activities as anti-inflammatory (Li and Wang, 2011). lowering blood pressure, diuretic and anti-

atherosclerotic (Nabavi et al ., 2015) . The results of the antioxidant activity and the amount of phenolic compounds showed great differences in the different types of hawthorn, and accordingly, the evaluation of the genetic sources of hawthorn provides us with valuable data about the genotypes rich in the content of the antioxidant compounds that possess distinctive importance in the food and pharmaceutical industries (Alirezalu et al .,2020) . Hawthorn fruits are a rich source of phenolic acids, flavonoids, procyanidins, vitamins, glycosides, and tannins (Hellenbrand et al .,2015). It is also a great source of antioxidant compounds due to its high content of phenols such as Rutin, Quercetin, Isoquercetin, Protocatechin, and Epicatechin (Žugić et al ., 2014) . The amount of these compounds is affected by genetic diversity and the degree of maturity at harvest)Wang et al .,2009(.The plausible reason to use antioxidants as substitutes for synthetic antioxidants is that they are safe and inexpensive, due to the increased health risks associated with synthetic antioxidants and their potential toxicity (Munekata et al ., 2020). There are many studies that evaluated the performance of these natural compounds, for instance essential oils, as distinct alternates to industrial antioxidants (Pateiro et al., 2018 ; Aljazy and abdulstar,2021). The genetic diversity of hawthorn flourishes in Iran, and it is one of the main countries famous for cultivating this plant. However, few studies have research in this regard, the most important of which is the estimation of phenolic compounds in 15 different types of hawthorn fruits collected from several regions of Iran (Alirezalu et al .,2020). The studies revealed a statistically proven relationship between the amount of phenolic compounds in the plant and the antioxidant activity (Agregan et al., 2019 ; Roselló-Soto et al.,2019). These compounds minimize oxidation processes, as in beef with compounds derived from *Allium sativum* extracts (Yin et al., 2003). The sauce contains mixed extracts of nettle, olive leaves

and green tea (Alirezalu et al., 2019). The results of studies showed that hawthorn fruits contain a variety of active compounds such as Hyperoside, isoquercetin and chlorogenic acid are among the most common phenolic compounds in these fruits (Alirezalu et al .,2020). Some hawthorn genotypes contain a higher amount of phenols than other fruits such as peaches and strawberries, and thus provide an added nutritional value (García-Mateos et al 2013). Several classes of phenols have been identified in hawthorn flowers and they can be considered as an important source of bioactive compounds. Hydroxycinnamic acids, flavonols and vitexin derivatives were also diagnosed at higher levels compared to the fruit, while anthocyanins were present in the fruit only. The phenolic content was associated with antioxidant activity, although The difference in the amount of phenols in fruits and flowers, but their antioxidant activity was not statistically affected (Issaadi et al.,2020). Numerous studies have examined medicinal plants and their various parts and components that resist oxidation to treat or alleviate many chronic diseases, including viral infections. Hawthorn is a medicinal plant in many parts of the world (Chang et al .,2001). Hawthorn is used as a laxative or gel component in the food industry, in addition to enhancing texture, improving taste and nutritional value, and as natural alternatives to food additives, due to the presence of pectin that widely used in the manufacture of jelly, jam and yoghurt . Also , It is characterized by exceptional virtues in condensation, formation of gelatinous material and emulsification. (Sun et al .,2020). In addition, pectin has various vital activities, most notably antioxidants (Hammi et al .,2016). In addition to improving digestion and combating atherosclerosis, hawthorn pectin has attracted more attention due to its multiple functions and the ability to extract it better than other fruits such as apples and lemons. Most of the studies of hawthorn are summarized about phenolic compounds and their limitations on

pectin (Zhu et al., 2015). Hawthorn fruit and waste showed high quality rheological properties when used as emulsifying agents compared to those made from citrus pectin (Dickinson 2003; Lozano–Grande et al., 2016). Hawthorn pectin forms a gelatinous substance in the presence of sucrose and under acidic conditions (Linares–García et al., 2015). The histological properties of this substance were studied and compared with the commercial gelatinous substance of lemon. It was found that its hardness is ten times higher than that of the gelatinous substance of lemon. In addition, the chewing of this gel material was 46 times higher than that of the substance. Gelatinized in lemon, it was also found that hawthorn pectin affects the physical and sensory properties of hawthorn yogurt (Lu et al., 2007). It is worth noting that the hawthorn pectin is very suitable to achieve the required thickness in hawthorn tea, which negates the need to add thickeners as carboxymethyl cellulose, agar or xanthan gum (Xu et al., 1998). There is still more to be done, such as optimal extraction methods for hawthorn pectin, which has various properties, the most important of which are antioxidants, lipid reduction and cholesterol regulation, in addition to its unique role in the food industry such as emulsifiers, thickeners and gelling agents. The difference in extraction methods leads to a diversity of functional, chemical and physical properties and contributes to the development of new products from hawthorn (Li et al., 2021). This study came with the aim of using natural plant alternatives that contribute to the introduction of the hawthorn plant in the jam industry as a laxative and gel-forming substance, in addition to improving the texture, taste, taste and nutritional value of the product.

Material and Methods

1- Material of Red Hawthorn (*Crataegus sambollis*)

Red Hawthorn (*Crataegus sambollis*) obtained from the Dhi Qar Governorate's marketplaces

in Iraq. After that, the nuclei were removed manually and disposed of, and then the hawthorn was dried in an oven at a temperature of 40 °C, crushed into a fine powder and kept in airtight glass containers until use.

Figure 1: Red Hawthorn



2-Preparation of Red Hawthorn extracts

The aqueous and alcoholic extract of Red Hawthorn powder was prepared according to (de Florio Almeida et al., 2017), 20 g of Red Hawthorn powder was mixed with 200 ml of ethanol solution (500 ml / l of purified water), after which the mix was added in an incubator vibration at 40 °C for 60 minutes at a vibration rate of 150 rpm., a high quality filter paper was used to filter the extract from impurities. Then the extract was left to evaporate in a vacuum until completely dry.

3- Detection of Bioactive ingredients utilizing mass spectrometry and gas chromatography

The bioactive molecules in Hawthorn extracts were defined using a gas chromatograph attached to a mass spectrometer, GC-MS assessment has been conducted at the Basrah oil company Lab, by using a Diagnostic Technologies, 7890B GC mechanism combined to a Diagnostic Technologies Techniques 5977A MSD with EI Transmission sensor, using HP-5ms 5% phenyl, 95% methyl siloxane (30m*250um*0.25), the furnace temperature was The injecting method was

pulse Splitting fewer, the injection temperature was 290 C, and the injected sample size was 1 µl. Ion Beam Temperature was used in the mass spectrometer.

4-Metal detection using ICP-OES OPTIMA 8300 Perkinelmer (Inductively Coupled Plasma Optical Emission Spectroscopy) :

The metallic elements of Red Hawthorn flour were assessed by ICP-OES OPTIMA 8300 DV in according to what (Al-Ghazi et al.,2021) reported on (ASTM, 1976).

5- Estimation of the flavonoid content:

A procedure was used (El-Baky et al , 2009 : Djeridane et al .,2006) order to determine the amount of flavonoids in aqueous, alcoholic hawthorn extracts by diluted. 5, 10,15 ,20 ,25 mg/ml. A standard rutin solution was prepared at a concentration of 10 to 160 mg/ml, and through the relation between rutin concentration and absorbance at a wavelength of 430 nm, the amount of flavonoids in hawthorn extracts was estimated based on the straight line equation.

-6Assay for DPPH radical Scavenging

The reaction of 0.5 ml of hawthorn extract (5, 10, 15, 20, and 25 mg/ml) with 0.3 ml of ethanol and 0.3 ml (0.5 mM DPPH) was used to determine the red hawthorn's capacity to neutralize the free radical DPPH (2,2 Diphenyl-1-picryl-hydrazyl). The mixture was incubated for 45 minutes at room temperature before the absorbance was measured at 517 nm (Brand-Williams et al., 1995).and the following equation was used in the measurement :

$$\{1- [\text{Abs sample} / \text{Abs control}] \} \times 100$$

7-Manufacture of Red hawthorn jam :

A mixture of hawthorn fruit jam and apple jam was prepared as a control, after obtaining ripe hawthorn and apple fruits, all the preliminary preparations were made for it from washing, removing seeds and cutting the fruit into small

pieces, after that sugar was added at a ratio of 55 parts to 45 parts of the fruit. Sour at a rate of 2-3 gm per 1 kg of sugar and pectin at a rate of 0.7-0.8% of the weight of the sugar, then the cooking process was carried out at a temperature of 80 C for 15 minutes under normal pressure until the concentration was reached 65-68%, then it was packed in tight containers The voluptuous valuation of jam was conducted with the participation of 50 tasters thru the voluptuous assent form, that includes the five quality aspects of color, texture, appearance, Odor and flavor, (Al-Ghazi et al., 2021)

9- Statistical analysis

All scientific experiment data were analyzed in 3 replications that use the Statistical Package for Social Sciences (SPSS) software package (version 26.0), as well as the information were analyzed utilising ANOVA, with findings deemed statistically meaningful at ($p < 0.05$).

Results

1. Detection of Bioactive ingredients utilizing mass spectrometry and gas chromatography

A group of biologically active compounds were determined in the aqueous and ethanolic extracts by GC-MS technique, and these compounds vary in their proportions according to the type of solvent used. The aqueous and ethanolic extract also share some active compounds most notably: Catechol , which was appeared 43.54 % at peak 18 and retention time13.675 For the aqueous extract of hawthorn fruit and with a percentage of 6.6552% and a retention time of 13.704, in peak 18 for the ethanolic extract. The results revealed the presence of 30 peaks representing the bioactive substances in the aqueous extract of hawthorn fruit such as catechol, followed by the compound dimethyl ether with a percentage of 16.5027% in peak 1 and a retention time of 4.367.. The findings showed the predominance of the following compounds in the ethanolic extract of hawthorn fruit: 5-

Hydroxymethylfurfural with a ratio of 38.1398% in peak 19 and retention time of 14.169 followed by 1,3-Dioxane, 4-methyl with a rate of 7.4972% in a peak of 15 and a rate of detention 12,775 then the compound catechol.

Table (1, 2) and Figure (2, 3) show the biologically active compounds in the aqueous and ethanolic extracts of hawthorn fruit that were diagnosed by GC-MS. These compounds differ in their proportions and according to the type of solvent used.

The aqueous and ethanolic extract also share some biologically active compounds, most

notably catechol, which appeared at a rate of 43.54% at peak 18 and a retention time of 13.675 for the aqueous extract of hawthorn fruit, at a rate of 6.6552% and a retention period of 13.704, at peak 18 for the ethanolic extract as well as catechin, which appeared at a rate of 2.2306% at the peak 22 and a retention time of 15.32 for the aqueous extract of hawthorn fruit, with a percentage of 2.289% and a retention period of 22.82, at a peak of 25 for the ethanolic extract. The results also showed the presence of 30 peaks representing the compounds present in the aqueous extract of hawthorn fruit, such as dimethyl ether compound, with a percentage of 16.5027% at peak 1 and a retention time of 4.367. The

TABLE 1: Effective chemical compounds of the aqueous extract of hawthorn fruits determined by the GC –Mass technique

PEAK	R.T.	AREA	AREA PCT	LIBRARY/ID
1	4.367	15024710	16.5027	Dimethyl ether
2	4.463	2304206	2.5309	Formamide, N-methoxy
3	5.953	1296284	1.4238	Ethyl aminomethylformimidate
4	6.189	1263951	1.3883	Furfural
5	6.801	331078	0.3636	Propanoic acid
6	7.981	407574	0.4477	2-Methyl-3-(methylthio)-1-propene
7	8.593	319295	0.3507	2(5H)-Furanone
8	8.815	981278	1.0778	2(3H)-Furanone, 5-methyl-
9	9.788	549649	0.6037	1,3-Dioxane, 2,4-dimethyl-
10	10.12	1521310	1.671	2H-Pyran, 3,4-dihydro-
11	10.467	708009	0.7777	Pyrazole-5-carboxylic acid
12	10.71	276276	0.3035	2-Cyclopentene -1-1, 2-hydroxy-3-methyl
13	11.197	466843	0.5128	1,3-Diethoxy-2-propanol
14	11.484	1523799	1.6737	6-Ethyl -5 , 6 – dihydro - 2H-pyran - 2- one
15	12.664	524996	0.5766	4-Pyridinol
16	12.782	2150510	2.3621	1,3-Dioxane, 4-methyl
17	13.446	683721	0.751	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-
18	13.675	39640429	43.54	Catechol
19	14.11	12909455	14.1794	5-Hydroxymethylfurfural
20	14.973	1328854	1.4596	1-Propyne, 1-(methylthio)-
21	15.179	275416	0.3025	sec-Butyl nitrite
22	15.32	2030807	2.2306	catechin
23	16.758	346768	0.3809	Benzeneethanol, 4-hydroxy
24	17.355	353065	0.3878	1,3-Butadiene-1-carboxylic acid
25	17.591	342400	0.3761	1,4-Anhydro-d-galactitol

26	18.469	486824	0.5347	1-(4-Methoxyphenyl)propane-1,2-diol
27	18.55	327844	0.3601	1-(4-Methoxyphenyl)propane-1,2-diol
28	20.349	1542950	1.6947	1-Deoxy-d-mannitol
29	20.718	578351	0.6352	Phenol, 3-methyl-4-nitroso
30	21.965	547036	0.6009	Sorbitol

Figure 2: Active compounds identified in the aqueous extract of hawthorn fruit powder

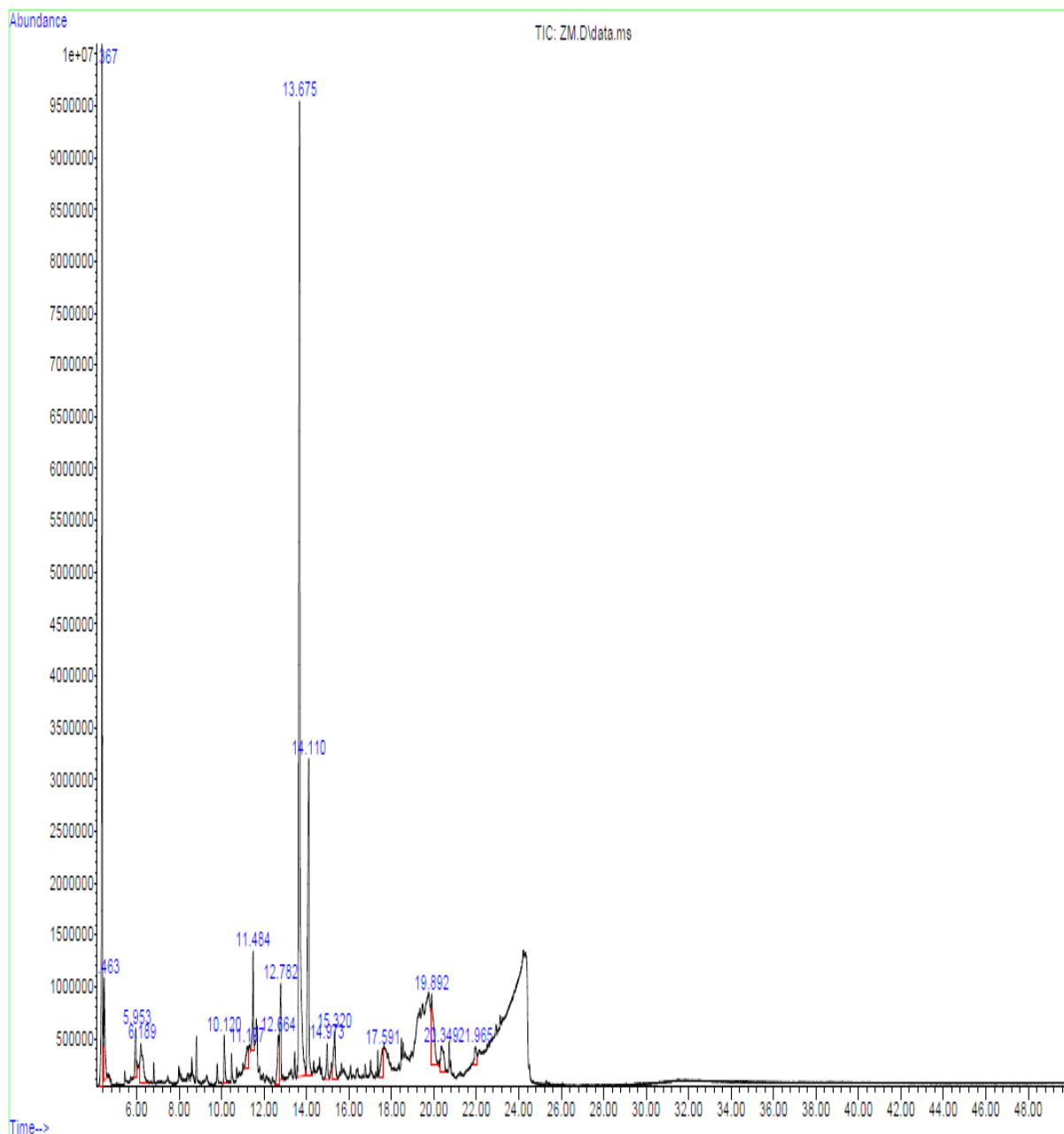
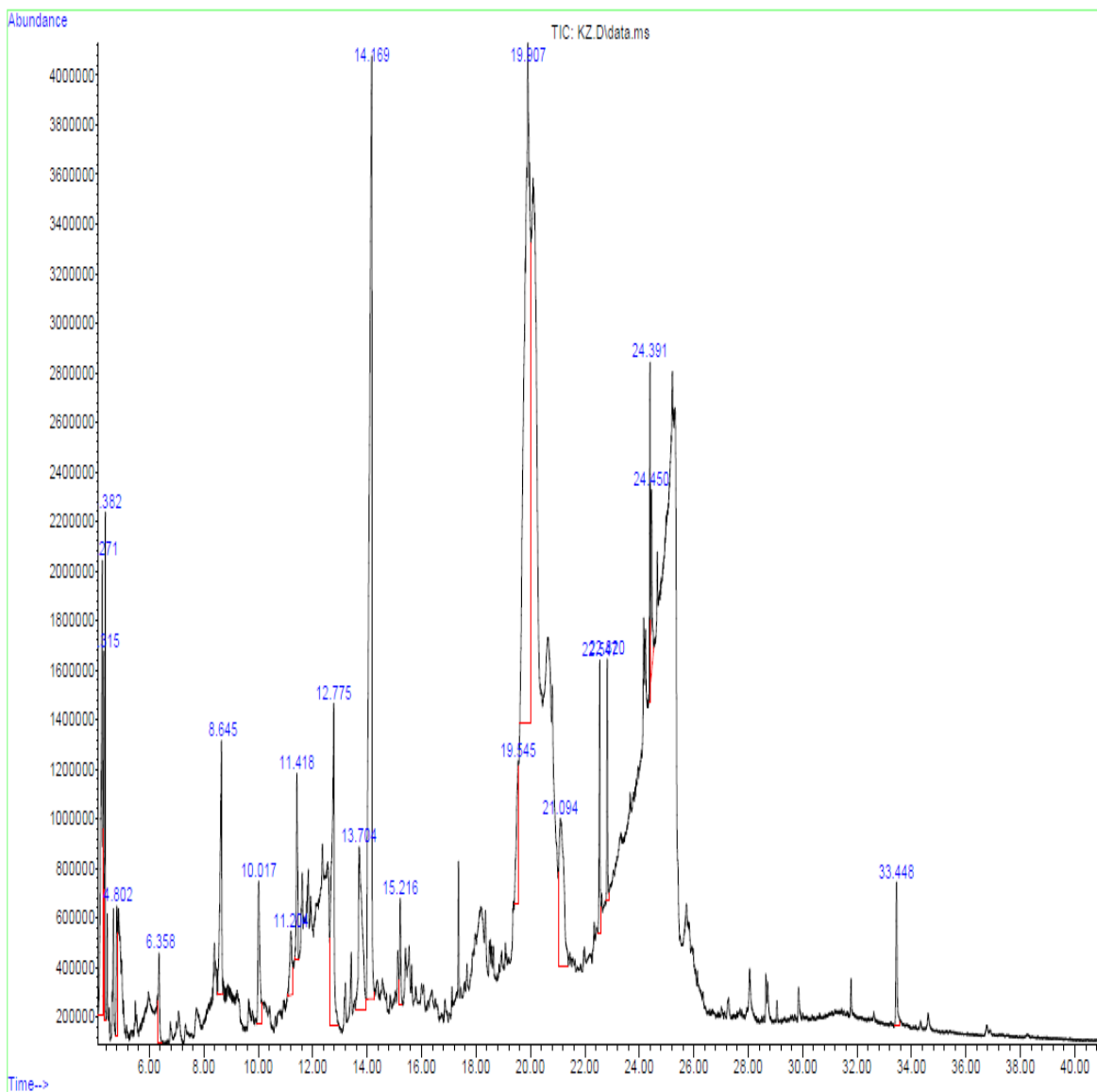


Table 2: Effective chemical compounds of the ethanolic extract of hawthorn fruits determined by the GC –Mass technique

PEAK	R.T.	AREA	AREA PCT	LIBRARY/ID
1	4.271	3489278	5.194	Ethanol
2	4.315	1089809	1.6222	Formic acid
3	4.382	1306427	1.9447	Acetic acid
4	4.456	498450	0.742	Butane, 1-methoxy-2-methyl-
5	4.677	619712	0.9225	Propargyl alcohol
6	4.802	579301	0.8623	2-Formylhistamine
7	6.358	462922	0.6891	3-Furanmethanol
8	7.723	431440	0.6422	2-Methyl-3-(methylthio)-1-propene
9	8.387	464351	0.6912	Hexaethylene glycol
10	8.645	4315650	6.424	2(5H)-Furanone, 5-methyl-
11	10.017	2214742	3.2967	2H-Pyran, 3,4-dihydro-
12	11.204	436914	0.6504	3-Hexenoic acid, (E)-
13	11.418	2227134	3.3152	Ethyl 3-methylbut-3-enyl carbonate
14	11.617	764110	1.1374	Erythritol
15	12.775	5036600	7.4972	1,3-Dioxane, 4-methyl-
16	13.203	550403	0.8193	1-Butyne, 3-chloro-
17	13.417	738526	1.0993	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-
18	13.704	4470939	6.6552	Catechol
19	14.169	25622139	38.1398	5-Hydroxymethylfurfural
20	15.128	434285	0.6465	Hydroquinone
21	15.216	766148	1.1404	Isosorbide
22	15.408	435138	0.6477	3-Ethyl-3-heptanol
23	17.355	796676	1.1859	1,3-Butadiene-1-carboxylic acid
24	22.547	2206448	3.2844	n-Hexadecanoic acid
25	22.82	1537747	2.289	catechin
26	24.391	1813682	2.6998	Sorbitol
27	24.45	1035772	1.5418	1-Propyne, 1-(methylthio)
28	28.057	717062	1.0674	5-Methyl-1-nitropyrazole
29	29.849	415162	0.618	1-Bromoeicosane
30	33.448	1702627	2.5344	.gamma.-Sitosterol

Figure 3: Active compounds identified in the ethanolic extract of hawthorn fruit powder



2. Metal Estimation

Red hawthorn fruit powder is unique in the diversity of its important mineral components. The results in Table 3 showed that dried hawthorn powder contained high concentrations of mineral elements. Ca and Mg recorded the highest percentages (620.53, 311.5) ppm respectively, followed by Fe, Zn, K and P (84.77, 31.535 , 9.2 and 0.25) ppm respectively .

Table: 3 The content of red hawthorn powder of mineral elements

metallic elements	Amount ppm
Ca	620.53
Fe	84.77
Zn	31.535
Mg	311.5
Na	N.D
K	9.2

$$\frac{P}{0.25}$$

3. Finding the quantity of total flavonoids

By using the standard curve of the routine (Fig. 4), the amount of flavonoids present in the extract of hawthorn fruits (aqueous and ethanol) was calculated, as shown in (Fig. 5). The findings revealed that the aqueous extract was superior as the total flavonoid content ranged from (21-149) mg/ml compared to the ethanolic extract as the total flavonoid content ranged from (6-95) mg/ml. The aqueous extract was given at a concentration of (149, 95) mg/ml at a concentration of (25, 20) mg / ml, respectively, compared to the ethanolic extract, as the highest total flavonoid content reached (95, 81) mg / ml at a concentration of (25, 20). mg/ml.

Figure 4: Standard curve of Rutin

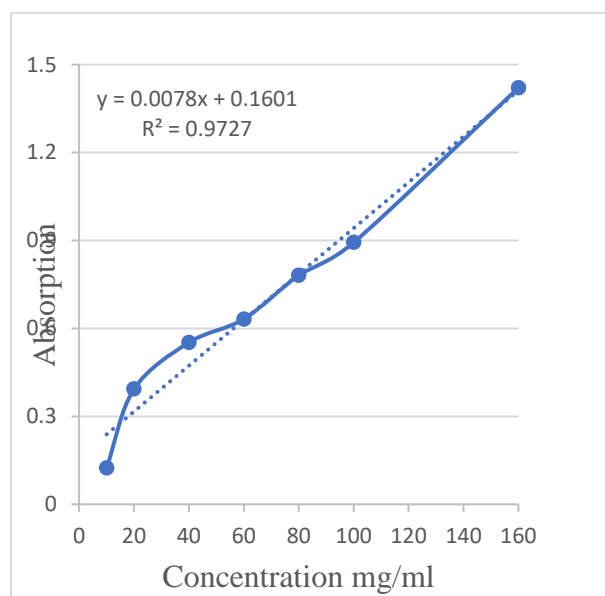
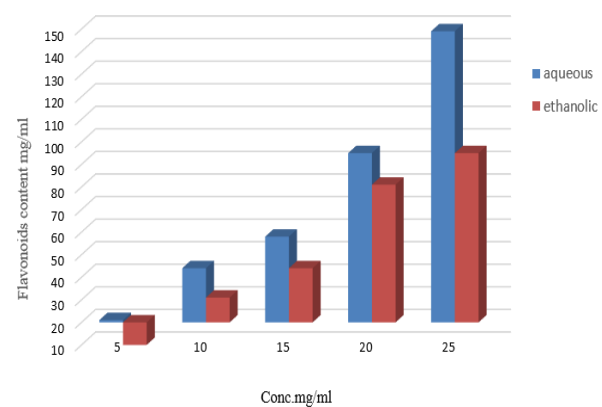


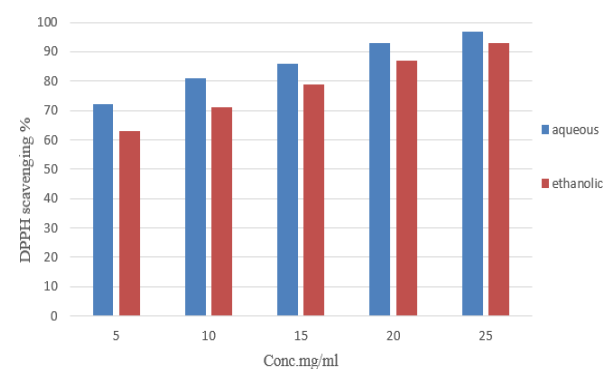
Figure 5: Total flavonoid content in hawthorn fruit extracts



4. DPPH radical Scavenging assay

It is evident from (Fig. 6) the antioxidant effectiveness of the aqueous and alcoholic extract of hawthorn fruit using DPPH free radical. Hawthorn extracts demonstrated excellent inhibition of DPPH roots. The percentage of inhibition of DPPH ranged between 97% for the aqueous extract at a concentration of 25 mg/ml and 93% for the ethanolic at the same concentration, while the percentage of inhibition for the aqueous and ethanolic extracts at a concentration of 5 mg/ml was 72% , 63% respectively.

Figure 6: DPPH Scavenging by Red hawthorn fruit extracts



Manufacture of jam:

Hawthorn fruit jam (sample A) was prepared and compared with sample B of apple jam.

Samples A and B had common characteristics with unique color and delicious and wonderful aroma. On the other hand, the amount of pectin in hawthorn is comparable to what is found in apples, the findings showed that there were highly significant differences between sample

A and B in terms of sensory characteristics such as Color, Texture, Texture, Appearance and Flavor, where the residents praised the quality of the characteristics of the jam made of hawthorn fruits, as shown in Table 4

Figure 8: Hawthorn and apple jam



Table: 4 Sensory evaluation of red hawthorn and apple jam

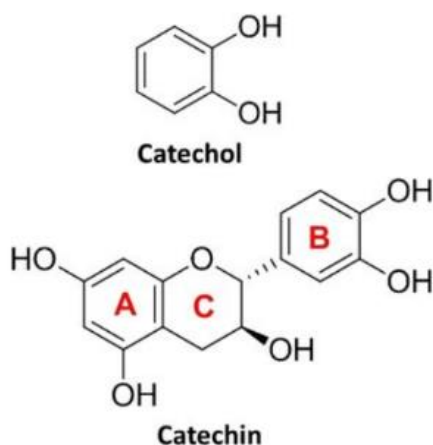
Sample	Color (20)	Texture(20)	Appearance(20)	Odor(20)	Flavor(20)	Total
A	16.3±1.76 b	15.7±1.56 b	15.5± 1.43 b	15.8 ±1.549a	16 ±1.69b	79± 3.74b
B	18.7±0.82 a	17.3±1.63 a	18.5 ±1.58 a	17.1±1.44 a	18.4±1.17a	90±2.44a

Discussion

A group of biologically active compounds were identified in the aqueous and ethanolic extract of hawthorn fruit by GC-MS technique. One of the most important compounds is Catechol C₆H₆O₂ or ortho-dihydroxy benzene, They are phenolic compounds that possess two hydrogen bonds linked to two oxygen atoms, which makes the radical forms more stable and stable as they enter into strong competition with the chain reaction of lipid peroxide, which is responsible for a wide range of harmful effects. Ortho-dihydroxy benzene is primarily responsible for the antioxidant power of phenols)Andreasen et al .,2001(. The results also indicated that the aqueous and ethanolic extracts contained catechin C₁₅H₁₄, which is a polyphenolic chemical. Naturally found in many fruits and plant foods, this substance is

characterized as an antioxidant and thus plays an important role in reducing oxidative stress (Fan et al.,2017). This result was less than what was found by (Zayzafoon et al., 2010), who indicated that the percentage of catechin in *C. sinaica* hawthorn fruit extract was about 8.31%, respectively.

Figure 9: The chemical composition of Catechol and catechin



The hawthorn fruit is characterized by its good content of nutrients necessary for the growth of the body. The results showed in (Table 3) that the hawthorn fruit contains high levels of calcium, phosphorus, potassium and magnesium, respectively. These results were similar to what was reached (Mironeasa et al, 2017), which indicated that hawthorn fruits contain (580.43, 278.31) ppm calcium and magnesium, respectively. While these results were less than what was found by (Demir and Özcan, 2001) when they estimated the mineral elements K, Mg, Na, Ni, P and Zn for hawthorn fruit.

Shows (Fig. 4) the total flavonoid content of hawthorn fruit extracts.. The results showed that the aqueous extract was significantly superior in flavonoid content compared to the ethanolic extract. These results were higher than those of (Froehlicher et al., 2009), who indicated that the total concentration of flavonoids in hawthorn fruit extract was about 23.68 mg/g dw. While (Alirezalu et al., 2020) found that the total flavonoid content of hawthorn fruit extracts ranged between (2.44 - 6.08) mg QUE/g DW).

Valuation of the anti-oxidative activity of hawthorn extracts showed that it has strong

activity thanks to the presence of active compounds such as catechol and hydroquinone and some secondary compounds that act synergistically as strong free radical scavenging agents in these extracts (Bahri-Sahloul et al 2009). These results were consistent with what was indicated by (Ljubuncic et al.,2005 ; Kostić et al.,2012) that the fruits of hawthorn possess high potential as antioxidant compounds, and this may be due to its polyphenolic compounds.

Hawthorn fruit is characterized by its high content of pectin, which makes it a good fruit in making jam, in addition to its high content of antioxidants and vitamins that are equivalent to those found in red apples and peaches. The results in (Table 4) showed a significant superiority in the sensory characteristics of hawthorn fruit jam compared to apple fruit, as the raters praised all the sensory qualities of hawthorn jam compared to apple jam.

Conclusions

30 compounds were identified in both aqueous and ethanolic extract of hawthorn by gas-liquid chromatography, the most important of these compounds were the catechol, catechin, dimethyl ether and 1,3-Dioxane, 4-methyl ,Hydroxymethylfurfural, 1,3-Dioxane, 4-methyl . hawthorn fruits showed antioxidant activity using DPPH assays, which was stronger mainly for the aqueous extract compared to the ethanolic extract, which was less active, and this may be due to the presence of a phenolic catechol group in a high percentage in the aqueous extract, and the results also showed that the powder contained Dried hawthorn fruits contain high concentrations of mineral elements such as calcium and magnesium, and new concentrations of iron, zinc, potassium and phosphorus. The study also proved that hawthorn fruit has a high functional potential when used in the manufacture of jam, in addition to its high ability to improve the

texture, taste and nutritional value of the product.

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