Chemical Engineering Department

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Graduation Project II

GC-MS Analysis and Antioxidant Activity of Arum Palaestinum

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Abstract

Medicinal plants are important sources of novel pharmacological compounds; Arum palestinum, which belongs to the Araceae family is used as a cancer remedy. Arum palestinum have played a major role in treating infectious diseases in recent decades as well. Natural products have emerged as promising tools for drug development despite advances in synthetic chemistry.

The objectives of studying this medicinal plant are carrying out qualitative tests for phytochemicals, identification of chemical substances by MS-GC device and conducting of DPPH antioxidant test.

The qualitative chemical tests that are carried out previously have given a good picture on the fact that Arum leaves contain different antioxidant phytochemicals.

While the GC-MS phytochemical identification test has presented lists of chemical substances that are probably found in this medicinal plant. While the DPPH antioxidant test that applied successfully on Arum Plant has highlighted the facts of the present of antioxidant phytochemicals on this plant.

The main finding of this work shows that the arum palestinum plant contain many compounds like: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Epoxyoctane, Citronellyl propionate, 1-Pentadecyne, 1-Decanecarboxylic, 9,12,15-Octadecatrienal, 10-Undecyn-1-ol, allylpentadecyl ester and Sulfurous acid, 2-ethylhexyl isohexyl ester.

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Disclaimer statement:

This report was written by students (Alaa, Ahmad, Anwar, and Mike) at the Engineering Department, Faculty of Engineering, An-Najah National University. It has not been altered or corrected, other than editorial corrections, as a result of assessment and it may contain language as well as content errors. The views expressed in it together with any outcomes and recommendations are solely those of the student (Alaa, Ahmad, Anwar, and mike). An-Najah National University accepts no responsibility or liability for the consequences of this report being used for a purpose other than the purpose for which it was commissioned.

Contents

List of Tables:

List of Figures:

Chapter 1: Introduction

1.1 Background:

Many plants and medicinal herbal extracts are used in the treatment of various ailments. Herbal medicine (botanical medicine or phytomedicine) is a concept used to describe the usage of plant's roots, seeds, bark, stems, flowers, or leaves for medicinal purposes. Herbal remedies can be utilized to manage many health problems such as facilitating digestion, decreasing pain sensation, stimulants, increasing libido, cleansing the bowel and many other purposes. [1] In addition, natural remedies are preferred over synthetic drugs, which can be harmful or cause undesirable side effects. Arum palaestinum as shown in Figure 1, is one of these medicinal plants that have been frequently used in traditional medicine. [1][2] It is widely used in traditional Arabic Palestinian herbal medicine for the treatment of diverse disease conditions such as stomach acidity, atherosclerosis, cancer, and diabetes and food toxicity. [3]

Arum palaestinum Boiss (family: Araceae, Figure 1), is a low-growing tuberous perennial plants, 20-60 cm tall with heart or arrow shaped leaves having bitter and burning taste, Arum genus has different species that are generally recognized and it is native to southern Europe, northern Africa and has very high species diversity in the Mediterranean region. [1]

Figure 1: Arum palaestinum Plant

Several studies have investigated the inhibitory effect of Arum palaestinum on certain types of cancer cells and different types of fungal and bacterial isolates.

Extraction as part of phytochemical or biological investigations presents specific challenges that must be addressed throughout the solvent extraction process. Successful extraction begins with careful selection and preparation of plant samples, and thorough review of the appropriate literature for indications of which protocols are suitable for a particular class of compounds or plant species. [4]

The different species of Arum Palaestinum plant can be found in all over the world, and a wide range of medicinal applications has been mentioned for them. Thus, it can also be valued as a source of natural compounds with antioxidant and antimicrobial activities. In this study. The antioxidant capacity of Arum Palaestinum leaves extracts was investigated by using DPPH assay. DPPH assay is a rapid, simple and widely used method to measure the ability of compounds to act as free radical scavengers, and to evaluate antioxidant activity of foods. It can also be used to quantify antioxidants in complex biological systems, for solid or liquid samples [5].

Different analytical chemical methods used for examining the existence of anti-oxidants in any herb or medicinal plant. They are the DPPH method, FRAP method, and ABTS method. The researchers use one method or more to justify the presence of anti-oxidant materials especially on Arum plants [6].

When compared to other methods for evaluating antioxidant activity, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity method has gained popularity due to its rapid and easy application. Due to its colorful character, DPPH is utilized to assess the radical scavenging properties of an electron or hydrogen atom donating components. [6]

A GC-MS experiment begins with sample preparation, injection and separation on a GC column. Since the operation of a mass spectrometer requires a high vacuum system, an interface is necessary to direct the molecules from the GC to the mass spectrometer. In the most common type of instrument, the molecules leaving the column enter an ionization chamber where they are bombarded with a stream of energetic electrons, which ionizes and fragments some of the molecules [7].

Preliminary compound identification and peak annotation in gas chromatography–mass spectrometry is usually made using mass spectral databases. There are a few algorithms that enable performing a search of a spectrum in a large mass spectral library. In many cases, a library search procedure returns a wrong answer even if a correct compound is contained in a library.

GC-MS was used in our experiment because Image result GC–FID can only identify a compound based on its retention time, whereas GC–MS also provides mass spectral information for each peak. When you get to the trace levels of drugs and metabolites in biological fluids, you need sensitivity and specificity; these are readily provided by an MS detector [8].

1.2 Project statement:

The aim of the project is to study and identify the phyto-chemical compounds existed at leaves of Arum Palestinum, and to determine the antioxidant activity of plant extract. The healing effect of plant is measured.

1.3 Objective:

The objectives of this study were to investigate the antioxidative effects of Arum Palaestinum through the use of the most simple and well-known DPPH test and to assess the DPPH radical scavenging activity, to investigate and identify the phytochemical compounds of Arum and its quantity by using GC-MS analysis.

1.4 Significant of the work:

In traditional Palestinian medicine, Arum Palaestinum extracts have been used to treat cancer, intestinal worms, open wound infections, urinary tract obstruction and kidney stones, and are believed to strengthen bones. Arum contains antioxidants that help defend your cells from damage caused by potentially harmful molecules known as free radicals. So, this plant worth to deal with.

1.5: Organization of the Report

This report consists of six main chapters. The first one introduces the arum plant and the analytical tests that have been done. The second chapter covers shortly the previous studies. The next chapter includes a brief methodology of our work, and summarizes what has been accomplished. The fourth chapter shows the results and analysis. Finally, the fifth and sixth chapters show the conclusion and recommendations.

1.6 Limitation and Restriction:

There were some limitations in the providing of some materials and equipment needed to do experiments like DPPH and quartz cuvette because they are expensive. The DPPH test

is done with accordance with well-known Protocols. The extract sample must be dissolved in acetonitrile as eluent used in GC-MS test.

1.7: Source of data

All the information was supplied by the electronic search (Google Scholar, Science Direct, Research Gate, etc.).

Chapter 2: Literature review:

2.1 Medical uses:

Arum Palaestinum. Is a time-honored botanical in Traditional Arabic Palestinian herbal medicine, where it has been used to strengthen bones and treat cancer, parasites, infections, and many other maladies. Recent work demonstrates anticarcinogenic action, Arum Palaestinum is revered as a treatment for skin sores, syphilis, rheumatism, tuberculosis, diarrhea, and stomach worms.[9]

In folk medicine Arum It is proven to be used in diseases of kidney stones, colitis, liver diseases and hyperacidity. Furthermore, the plant has been reported as a highly effective treatment against hemorrhoids medical condition for which there is hardly any other effective alternative treatment except surgery.[10]

Cancer is a severe health concern that affects people all over the world. It is the second leading cause of death in the world, after heart disease. [11] Chemotherapeutic drugs radiation therapy or surgery, are the only anticancer treatments available today. However, Chemotherapy has a number of drawbacks, including a lack of selectivity, which means it kills both normal and abnormal cells.[12] Cancerous cells Long-term treatment may also result in medication resistance. As a result, there is many individuals are turning to complementary and alternative medicine. As a result, the search for potential anti-cancer drugs from natural sources becomes more rational. Several studies have been conducted. It is found that herbs such as Arum Palaestinum have anti-cancer properties. [13]

2.2 Antioxidant:

There are multiple methods for evaluating the antioxidant properties using different methods such as 1,1-diphenyl-2-picrylhydrazine (DPPH) and 2,2-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) radical scanning method (ABTS). Two methods are found to be the most popular and reliable as it is important to use a consistent and rapid method as this has been improved in recent years. Other methods used, oxygen radical absorption capacity (ORAC) assay and reduce energy assay

Antioxidants, including phenolic compounds (e.g., flavonoids, phenolic acids and tannins), have diverse biological effects, such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic effects, as a result of their antioxidant activity.[14]

Control of oxidation of fats/oils and lipid-containing products may be achieved by excluding the initiator and promoter elements during processing and storage. Thus, packaging of highly sensitive products must be properly stored under vacuum or an inert atmosphere. When used as nutraceuticals or pharmaceuticals, the highly unstable polyunsaturated lipid components are protected by addition of antioxidants or by encapsulation, micro- and nano-encapsulation. The membrane of the capsule protects the core material from undesirable effects of light, moisture, and oxygen, thus increasing the shelf-life of the final product Among the many methods employed for controlling lipid oxidation, use of antioxidants, mainly phenolic and polyphenolic compounds or extracts of plant material, is the most effective, convenient, and economical means. Antioxidants are also used in health-related areas for disease risk reduction and health promotion due to their ability to protect the body against oxidative damage. Antioxidants are substances that, when present in food or in the body at very low concentrations, delay, control, or prevent oxidative processes leading to food quality deterioration or initiation and propagation of degenerative diseases in the body. Antioxidants that fit in this definition include free radical scavengers, singlet oxygen quenchers, inactivators of peroxides, and other reactive oxygen species (ROS), metal ion chelators, quenchers of secondary oxidation products, and inhibitors of prooxidative enzymes, among others. These substances exert their inhibitory effect against oxidation processes through different mechanisms and with varied activities.

An in-depth discussion of antioxidant mechanisms has already been provided elsewhere [17].

❖ DPPH Test:

The 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging assay is one of the most extensively used antioxidant assays for plant samples. DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom. This method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolourizes the DPPH solution, the absorbance was measured at 517 nm. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound. and the percentage inhibition activity was calculated from the formula:[15]

$$\frac{A_o - A_1}{A_o} * 100$$

Where:

A_o= Absorbance of the control (DPPH solution)

A_1= Absorbance of the extract

❖ ABTS method:

The ABTS radical scavenging method modification is based on the activation of metmyoglobin with hydrogen peroxide in the presence of $ABTS^{\bullet+}$ to produce a radical cation. This improved method generates a blue/green $ABTS^{\bullet+}$chromophore via the reaction of ABTS and potassium persulfate and is now widely used. Along with the DPPH method, the ABTS radical scavenging method is one of the most extensively used antioxidant assays for plant samples.

The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, and its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically at 734 nm. This decolourisation assay measures the total antioxidant capacity in both lipophilic and hydrophilic substances. The effect of the antioxidant concentration and the duration of the inhibition of the radical cation's absorption are taken into account when the antioxidant activity is determined. Trolox, a water-soluble analog of Vitamin E, is used as a positive control. The activity is expressed in terms of the Trolox-equivalent antioxidant capacity of the extract.

- **ORAC assay:**

The ORAC assay uses beta-phycoerythrin (PE) as an oxidizable protein substrate and 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH) as a peroxyl radical generator or a Cu_2+-H_2O_2 system as a hydroxyl radical generator. To date, it is the only method that takes the free radical reaction to completion and uses an area-under-the-curve (AUC) technique for quantification, thereby combining both the inhibition percentage and the length of the inhibition time of the free radical's action into a single quantity. The assay has been widely used in many recent studies of plants.[16]

Chapter 3: Methodology and experimental part

- **Preparation of Arum extract**

The arum collated in February 2021, then arum was dried in air for 3 days in air, the fresh dried Arum leaves were grinded by Pilot scale laboratory milling machine. About 25 g of grinded Arum was weighted and placed in conical flask, 150 ml of diethyl ether was added because its good solvent to dissolve active ingredients compared to others. The flask was placed on hot plate at a temperature of about 35-40℃ for 3 hours and closed by foil [12].

The solution was filtered twice under vacuum to ensure that the extract is very clean, then evaporated on Rotary vacuum evaporator. Oily material was obtained.

Preparation of Arum aqueous extract

Fresh Arum green leaves was pressed by special grass green juicer. The obtained juice was filtered three times. Part of it is treated further by centrifugal machine. The samples were sent for anticancer test (colon cancer cells) to cancer laboratory at medicinal department.

GC-MS test preparation.

Known amount of Arum sample that obtained from solvent extraction as discussed above have been dissolved in acetonitrile solvent because it is the same solvent used in the GC-MS analysis device. The acetonitrile fraction was filtered, and then it centrifuged for 5 minutes at 1000 rpm. Then it was filtered again then sent for GC-MS test.

GC-MS test.

Micrograms of acetonitrile Arum sample was injected to Gas chromatography-Mass spectroscopy device for identification of phyto-chemical that are included in the sample.

- **DPPH anti-oxidant assay test**

Preparation of extract sample

First, 1g of arum plant powder was weighted and put in flask, 25 ml of methanol was added to the sample, flask was sealed by using a foil, after that the sample placed in water bath at room temperature for 2.5 hours.

Preparation of DPPH and Arum extract

DPPH solution was prepared (4 mg DPPH in 100 ml methanol). Cover the DPPH solution by using foil and put in cool place. 2.5 hours later the sample was placed it the centrifuge for 15 minutes at 1000 rpm. A solution series of sample extract and methanol was prepared (40, 80, 120, 160 and 200µg/ml), 1ml of each sample was taken, and 3ml of DPPH solution was add to each, methanol was added to each sample until reach 10 ml. The samples were placed in the dark for 30 minutes, use spectrophotometer to scan the DPPH solution as to know the wavelength should use for samples prepared, after that the absorbance was measured for all samples.

The blank was methanol the wavelength was 517 nm of control sample (DPPH solution).

Preparation of ascorbic acid

The ascorbic acid prepared as reference to compare it with our arum extract, 2mg of ascorbic acid was weighted, 10ml of methanol was added. Solution series of ascorbic acid and methanol was prepared (50, 25, 12.5, 6.25 and 3.152 µg/ml). 1 ml of each solution was taken, and 3ml of DPPH solution was add to each, the solutions put in the dark for 30 minutes. After that the absorbance was measured for all solutions by using spectrophotometer.

The blank was methanol and the wavelength was 517 nm.

Chapter 4: Results and Discussion

GC- MS analysis:

Arum was extracted by using diethyl ether and then applied to a vacuum filtration as to remove all solid materials, after completing the filtration. The ether solvent was easily recovered by vacuum Rotary device. No solvent traces were remained in the sample. The dried sample was placed in the refrigerator and then the sample was dissolved in Acetonitrile, this solvent was added because it is the same solvent used in the GC- MS analysis device.

During Arum sample extraction care must be taken so the temperature should not be higher than 50 ºC because diethyl ether is low boiling point a flammable material.

Acetonitrile is a chemical compound with the formula (CH_3CN) it is colorless liquid and it is used as a polar aprotic solvent in organic synthesis.

The result obtained shows in the figures 2 below:

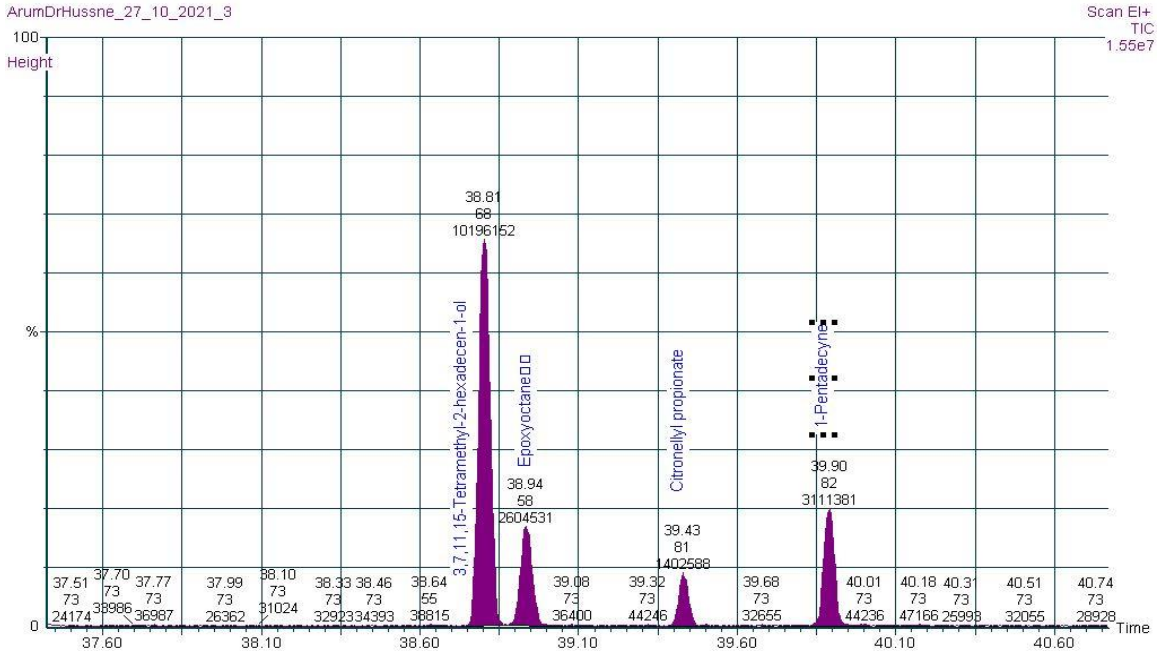

Figure 2a: GC-MS results of arum extract.

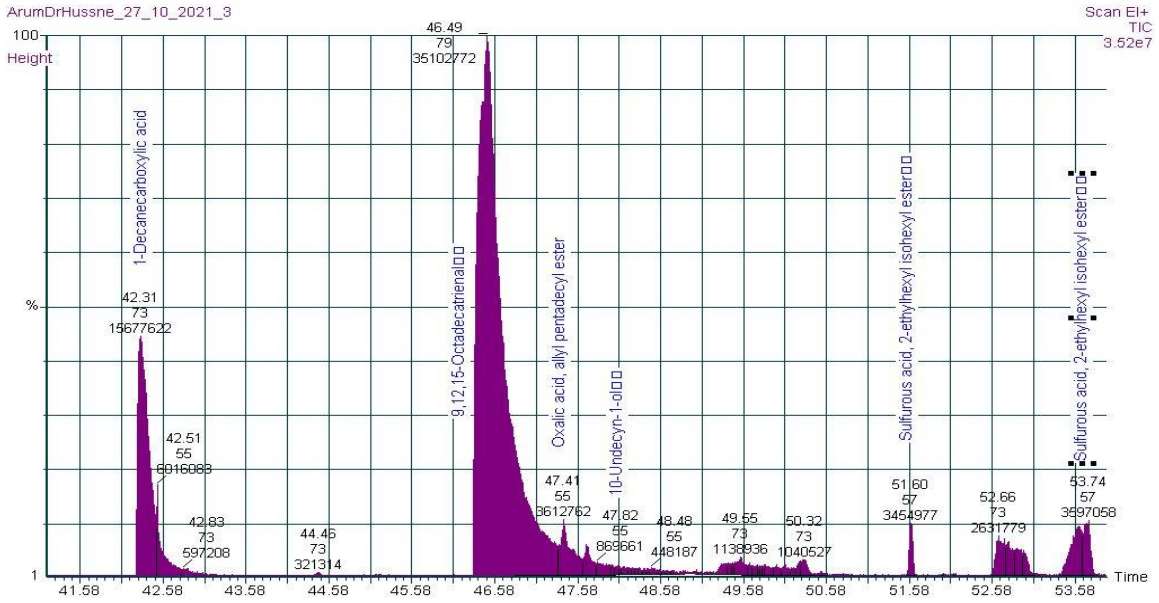

Figure 2b: GC-MS results of arum extract.

As shown in the above figures, the compounds in the arum plant are represented in the form of peaks as presented in Table 1, the higher peak means a higher concentration of the compound, the arum extract pass through a column that breaks the compound into functional groups, each compound needs a specific time to disintegrate according to its boiling point, and the compound known from a library that compares all similar functional groups. the percentage of each compound was measured by divide the area to the summation of all areas.

Table 1: GC-MS analysis for arum plant.

Name	Retention time	Area	%Area
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	38.81	10196152	0.128045
Epoxyoctane	38.94	2604531	0.032708
Citronellyl propionate	39.43	1402588	0.017614
1-Pentadecyne	39.9	3111381	0.039073
1-Decanecarboxylic	42.31	15677622	0.196882
9,12,15-Octadecatrienal	46.49	35102772	0.440826
Oxalic acid , allylpentadecyl ester	47.41	3612762	0.04537
10-Undecyn-1-ol	47.82	869661	0.010921
Sulfurous acid , 2-ethylhexyl isohexyl ester	51.6	3454977	0.043388
Sulfurous acid , 2-ethylhexyl isohexyl ester	53.74	3597058	0.045172

In the table above, these compounds are found in the arum plant with the area of each compound, where each compound has been separated according to its boiling point.

- **DPPH assay:**

❖ **Arum extract:**

Arum was extracted using 99% methanol, the sample was placed in the centrifuge to get rid of sediments that settle down, after that, filter was done to the top solution part through a filter paper, the DPPH and the samples with different concentrations were prepared and DPPH was added to each of them.

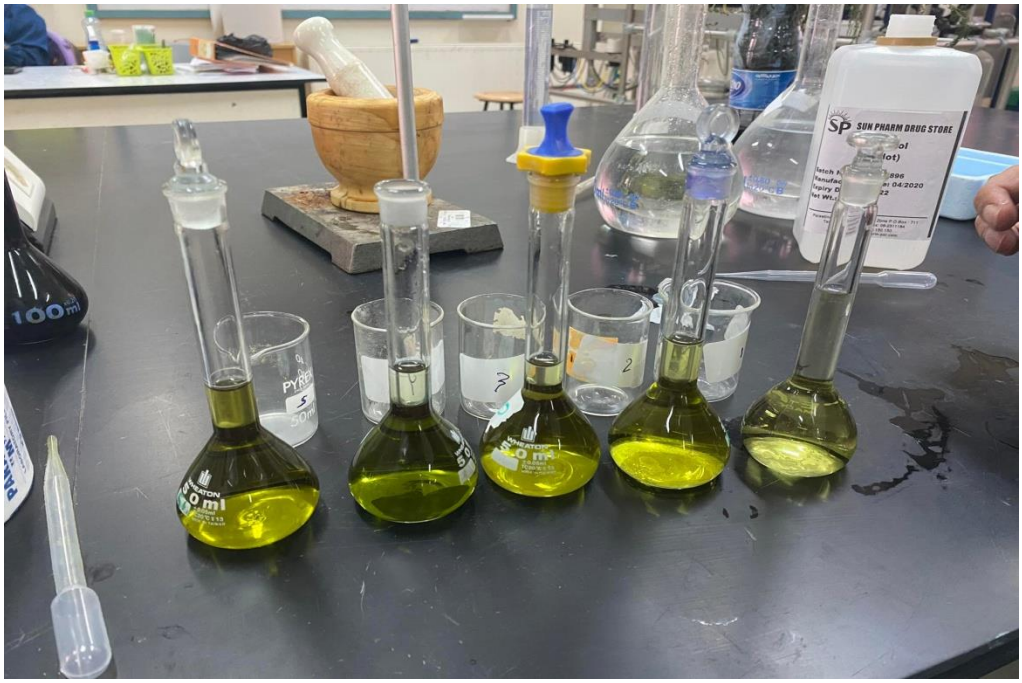

Figure 3: Different concentration of arum extract.

A sample of each concentration was taken and placed in cuvettes in spectrophotometer at wavelength 517 nm to measure the absorbance, and methanol was the blank.

The result obtained from spectrophotometer was as follows:

Table 2: The absorbance of arum extract for different concentrations.

Concentration (µg/ml)	Absorbance (517nm)
0	0.254
40	0.150
80	0.073
120	0.053
160	0.048

❖ Ascorbic acid:

The test was carried out for ascorbic acid which is the reference for our plant, ascorbic acid was made with different concentrations, and then DPPH was added to each concentration, the intensity of the color obtained shown in the figure.

Figure 4: Different concentrations of ascorbic acid after added DPPH.

Then these samples were taken to a spectrophotometer to measure the absorbance at 517 nm wavelength, and the blank was methanol

The result obtained from spectrophotometer was as follows:

Table 3: The absorbance of ascorbic acid for different concentrations.

Concentration (µg/ml)	Absorbance (517nm)
0	1.403
6.25	0.939
12.5	0.893
25	0.786
50	0.744

From the tables 2 and 3, The result shows that the increase of concentration of arum extract and ascorbic acid the absorbance decrease and the inhabitation increase.

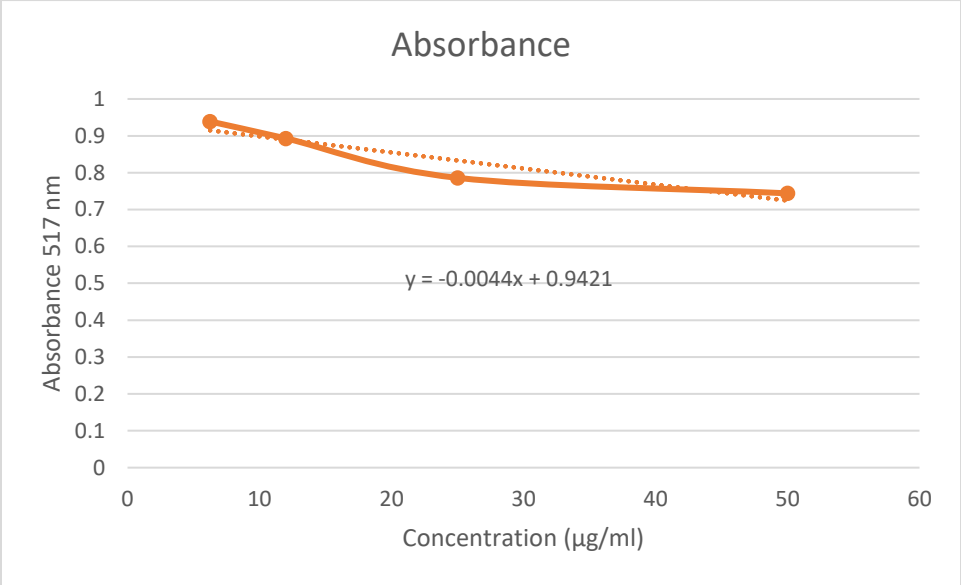

Figure 5: Relation between the concentration and absorbance for ascorbic acid.

From figure 5 the equation was used to measure absorbance to the same concentration of our extract for easily compare the inhabitation and to calculate the IC50.

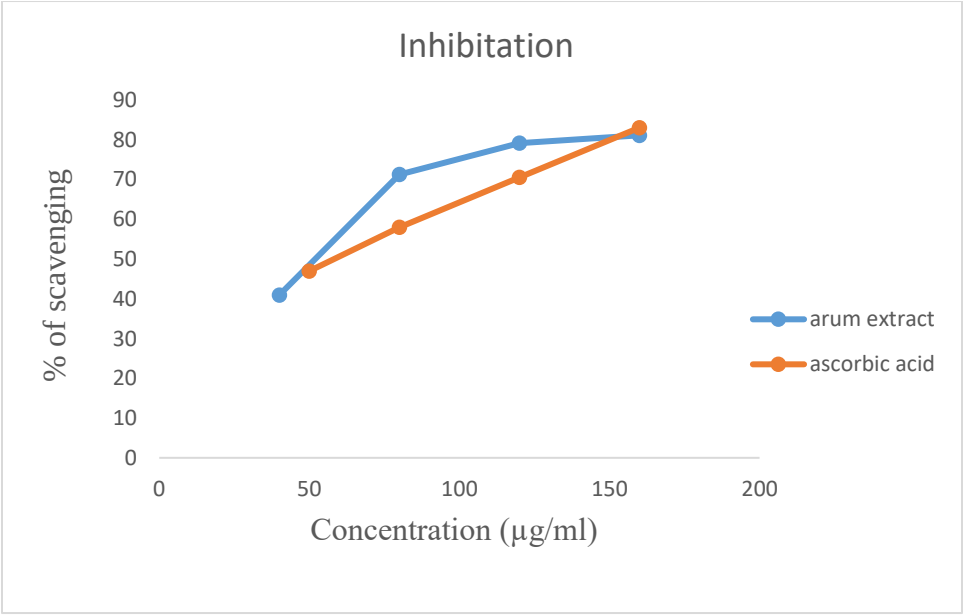

Figure 6: Comparison the inhabitation between the arum extract and ascorbic acid

From the figure 6 shows that the ascorbic acid has lower antioxidant than arum extract, and to calculate the IC50 the logarithm of the figure 6 taken.

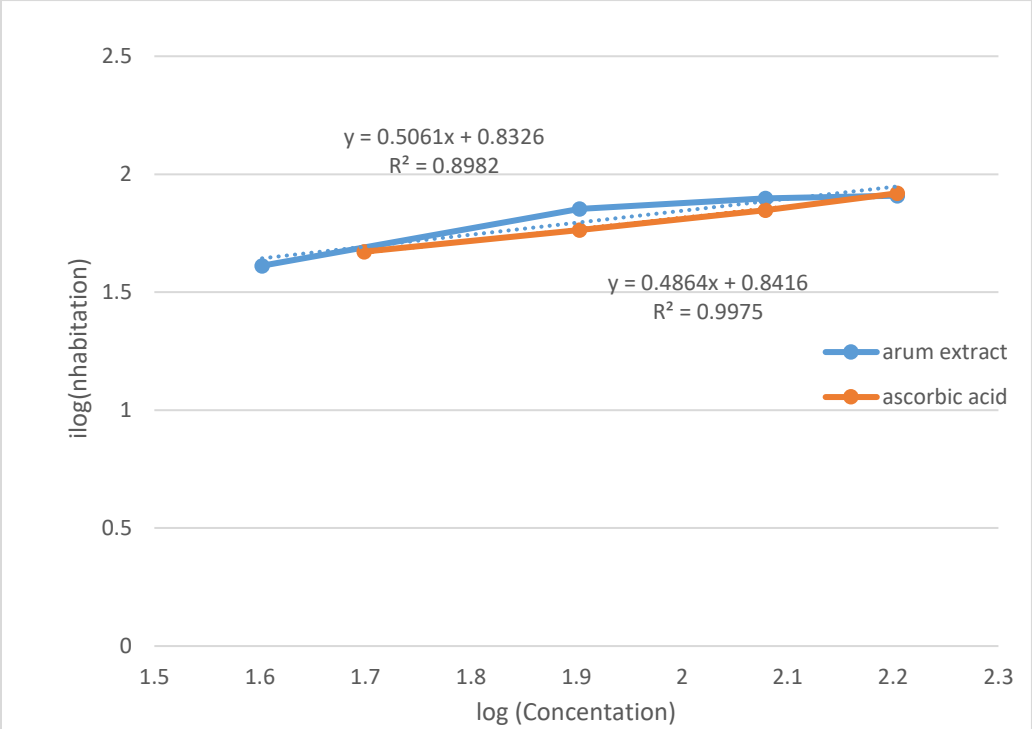

Figure 7: the logarithm of inhabitation for arum extract and ascorbic acid.

After linearization the IC50 calculated from the equations from figure 7 and the results was taken as follows:

Table 4: the results of IC50 for arum extract and ascorbic acid.

Sample	IC50 (µg/ml)
Arum sample	51.5
Ascorbic acid	57.9

One of the most important results that have been studied in this project is the determination of the compounds inside the plant using the GC-MS device and compare it with literature in table 4, and the results were as follows:

Table 5: Comparison between GC-MS Test Result and Literature Review.

No.	Arum compounds	%	Arum compounds in Literature Review [12]	%
1	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol)	12.8	phytol	25.29
2	Epoxyoctane	3.27	Dihydroactinidiolide	0.76
3	Citronellyl propionate	1.76	Loliolide	0.79
4	1-Pentadecyne	3.91	Pluchidiol	1.03
5	1-Decanecarboxylic	19.69	Hexahydrofarnesyl acetone	4.5
6	9,12,15-Octadecatrienal	44.08	Hexadecanoic acid, methyl ester	21.89
7	Oxalic acid , allylpentadecyl ester	4.53	Linoleic acid, methyl ester	6.54
8	10-Undecyn-1-ol	1.09	Linolenic acid, methyl ester	22.37
9	Sulfurous acid , 2-ethylhexyl isohexyl ester	4.34	Butylated hydroxytoluene	1.61
10	Sulfurous acid , 2-ethylhexyl isohexyl ester	4.52	Phytol acetate	5.05

in the table above, the reason that not all compounds are similar is due to the type of extraction and the climatic conditions surrounding the plant, on the other hand, there are similar compounds like phytol.

Conclusion:

Our results showed that Arum can be used as an easily accessible source of natural bioactive compounds with antioxidant properties that can be a proper substitute for synthetic antioxidants to use in various food products.

It was found through the use of the DPPH assay that the arum plant contains antioxidants, and this was demonstrated through the low absorption, and this is evidence of the disappearance of free radicals and its containment of antioxidants.

The cancer test results are not so clear because the liquid sample effects colon cancer on high concentrations on microgram while the anticancer concentration should be in nano gram, so our anticancer results are not promising.

Recommendation

First, general safety conditions such as lab coat and safety shoes must be taken, make sure that you use dried leaves for the GC-MS analysis and DPPH assay to ensure that you got correct result, care must be taken when you deal with diethyl ether because its flammable, small quantities of ascorbic acid should be used and if the amount exceed than allowable dilution should be used.

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