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Extraction of Phenolic Compounds from the Henna Plant

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Abstract

Phenolic compounds were extracted from the henna plant using hexane, chloroform, ethyl. Ethanol and methanol, the amount of phenols and flavonoids in these extracts has been estimated and their counter effectiveness measured. For oxidation, as it evaluated its effectiveness to bind the iron ion and measured its reductive strength, it studied the inhibitory effectiveness. The methanol impedance extract has oxidized olive oil. Methanol extract out performed phenolic compounds, and flavonoids, its antioxidant effectiveness, its reduction power and its iron binding potential on the rest of the extracts. The phenolic compounds were diagnosed with GC-MS and the compound was found to be (2-hydroxy-1,4-naphthoquinone) Lawsone of Ben. At the higher concentration between 10 diagnosed compound.

Keywords:* *Henna; Phenolic compound; Antioxidant efficacy; GC-MS

Introduction

At present, many industrial fat antioxidants are used on a commercial scale such as, Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT). In the last few years, many doubts have been raised as to how safe these antidotes are for Health. The use of antigen can result in carcinogens or toxic substances.

So many researchers turned to antioxidants. Healthy, I have focused attention on the natural sources of plants and good eat. Phenolic compounds are among the most prominent natural antioxidants that include flavonoids, tannins and carotenes and phenolic acids which are aromatic compounds with one or more sets of aggregates. Hydroxylation substituted and found approximately in all parts of plants such as leaves, flowers and fruits [1]. The substituted hydroxylation is almost there in roots, bark and seeds are secondary metabolites and in addition to acting as antioxidants many have been proven. One study is the role of phenolic compounds as antibacterial, viruses and fungi, where compounds are characteristic. Phenolic in general with its ability to capture free roots and bind henna is a widely grown and used syphilis plant. This plant leaves after grinding to dye hair and skin, which contains high accumulations of phenolic compounds, flavonoids, carbohydrates, proteins, tannins, alkaloids and fatty acids. Globally classified belongs to division *Magnoliopsida*, class *Myrtales*, family *Lythraceae*, genus *Lawsonia*, scientific name *Lawsoniainermis*.

In previous studies of phenolic compounds for henna plants. Acetone extractors of henna paper are grown in Kwalampur/Malaysia has outgrown its compound content [2]. Phenolic on methanol and ethanol extractors and on study planted in the Kerman/Iran region, the water extract was found to be superior in content. It turns out that the hydrophobic extract out performed phenolic compounds. Methanol extract derived outweighs the anti-oxidant and free root discoloration effectiveness. Henna extract grown in New Delhi/India. Water, acetone, and acetyl ethyl are the roots of the henna plant grown in Coimbatore, and extract superiority has been found. The acetone is more effective than the sniper, while the water extract is more effective than the antioxidant and the force of its reduction, p-coumaric acid, Lawson, Cosmosiin,2-methoxy-3-methyl-1, 4-naphthoquinone, apiin, apigenin, luteolin. Antioxidant potency from henna extract grown in Mansoura/Egypt and diagnosed with techniques, IR, NMRTLC. As for the microbial efficacy of the henna plant, methanolytic extract has been demonstrated. Grown in India is a good inhibitor for bacteria *Escherishia coli*, *Staphylococcus aureu*, *Proteus mirablis*, *Pseudomonas aeruginosa*, *Klebsiellapneumoni*.

There are no previous studies relating to the estimation, extraction and diagnosis of phenolic compounds of a plant. Henna planted in Iraq/Basra Governorate/Al-Fao District. Phenolic from the henna plant using different solvents and recognizing its disabling oxidation of linoleic acid, and knowing its mechanics and its impotent ability to oxidize olive oil and diagnose it.

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Materials and Methods

The leaves of the henna plant *Lawsoniainermis* were obtained from the Faufi district farms, Basra Province. The leaves dried with shade in open air and then grined the dry leaves to get in. Homogeneous powder and save until you do and phenolic compounds in extracts were estimated using the folin-ciocalteu method [3] by dissolving 1 gm of plant extracts in 64 ml of distilled water was added 1 ml of folin-Ciocalteu reagent, and the combination was well mixed. And after 3 minutes, add 3ml of sodium carbonate 2% (Na_2Co_3) and leave the mixture for 2 hours with intermittent moist. The suction was measured at 760 nm wavelength. A standard on the graphic relationship between TR. Depending on sour keys and suction at 760 nm wavelength using a standard solution of Gallic acid and with a concentration of 0 mg/ml, 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml, 100 mg/ml.

Estimate of total flavonoids

We use method Aluminum chloride $Alcl_3$. To estimate the total content of flavonoids in plant extracts, melt 1 gm of plant extracts in 1 ml-5 ml ethanol with an equal volume of ($Alcl_3.6H_2O$) 1% recorded at 100 ml methanol. Reset the mixture and then measure absorption at 340 nm wavelength 10 minutes later. The amount of flavonoids in the extracts was calculated by preparing a standard solution from the flavonoid compound rutin from 0 mg/ml-100 mg/ml. Absorption was measured at 367 nm wavelength and the amount of flavonoids was calculated based on the graphic relationship between acid concentration and absorption.

Antioxidant effectiveness measurements

I followed the iron thiocyanate method that he mentioned [4]. To measure the antioxidant effectiveness of linoleic acid for extracts and as it comes: Mixing 1 ml of each model with 4 ml ethanol concentration 95% (4 ml-1 ml linoleic acid) concentration 2.5% ethanol (8 ml solution Phosphate concentration 50 ml mole, pH 7. Save the mixture at 45°C M for 24 hours, the add 0.1 ml of this mixture to 9.7 ml ethanol) concentration 75% and 0.1 ml aluminum thiocyanate its concentration is 30% and then 0.1 ml of iron chloride, its concentration is 20 ml mole transcript in 3.5% hydrochloric acid to the reaction mixture to form a red colored complex with oxidizing peroxides. Attend the compressed sample models in the same manner as above except for mixing 1ml of distilled water instead of the sample [5]. Absorption at wavelength was measured at 500 nm and antioxidant effectiveness was calculated according to the following equation.

$$\%Antioxidant\ effectiveness = 1 - \left[\frac{absorbtion\ of\ the\ sample}{stander\ absorption} \right] \times 100$$

Measurement of reduction power

I followed method Oyaizu which included mixing 2 ml-50 ml of extracts BHT and alpha-tocopherol of 0 mg/ml-100 mg/ml (transcript ethanol 98% concentration with solution phosphate and pH 6.6 and 2.5 mlof solution potassium ferricyanide 1% saved the mixture at 50°C temperature for 20 minutes and then 2.5 ml of trichloroacidic acid was added (chloroacetic acid 1%) The centrifuge of the mixture was performed at a speed of 2000/minute cycles for 10 minutes separated the top layer of the solution and added 5 ml of water distilled by 1 ml of iron chloride 0.1% suction gauge along the 700 nm wavelength [6-7]. Except for the addition of 2.5 ml instead of plant extracts, the following equation has been applied to calculate the amount of reduction force.

$$\%Reduction\ power = 1 - \left[\frac{absorbtion\ of\ the\ sample}{stander\ absorption} \right] \times 100$$

Iron ion binding

The ability of extracts to bind the iron ion was measured by the method [8] which involved mixing 0.4 mL of plant extracts and petroquis ranging from 1 mg/mL-5 mg/mL of iron chloride to 2 ml molar of 8 hydroxyquinoline with a concentration of 5 mlmolar of ethanol 98% Save the mixture for 10 minutes at room temperature in a dark place. The absorption was measured at 562 nm wavelength as well as the binding potential of the iron ion of the Ethylenediamine Tetra-Acetic Acid (EDTA) disodium and citric acid in the same method for comparison. The compressor sample was present the same way in the above except for the addition of extracts. The ability of extracts to bind the iron ion has been calculated according to for the following equation.

$$\%Binding\ capability = 1 - \left[\frac{absorbtion\ of\ the\ sample}{stander\ absorption} \right] \times 100$$

Obstruction of oxidation of oils

I followed the method that he mentioned Tanaka to estimate the potential of henna extract. Methanol for inhibiting the self-oxidation of olive oil, which included melting 1gm of oil in 24 ml of oil Chloroform-methanol mixture (1:2) added to the extract mixture with combinations 2 mg/g, 4 mg/g, 6 mg/g, 8 mg/g and 10 mg/g oil Congenital save mixture at 45 m for time

5 days, 10 days, 15 days, 20 days and 25 days. Peroxide values were estimated by the method mentioned by Pearson. The compressed sample was treated in the same manner as above except for the addition of 1 ml of distilled water to the mixture instead of the extract. The industrial antioxidant Butylated Hydroxytoluene (BHT) was used for comparison at a concentration of 2 mg/g oil.

Diagnosis of ring compounds by GC-MS

The ringed compounds in the local henna leaf extract were diagnosed by the mass spectrometer related gas chromatographic *via* technology (Shimadzu GC-MS-PQ2010 Ultra) [9-12]. In the laboratory of the Faculty of Agriculture/University of Baghdad, depending on the following class conditions: Type of column 30 m x 0.25 mm indicator film thickness 0.25 µmDP-5MS Helium gas was used as an inert gas, with a runoff rate of 1 ml/s. The temperature of the syringe and the interlude was 280°C. The fern program was tuned at an initial temperature of 100°C for 1 minute, after which the furnace was raised to 280°C by 6 temperature/minute and the spectra of the curves were matched.

Results and Discussion

Total content of phenols and flavonoids **TABLE 1** shows the concentration of phenolic and flavones compounds derived from the henna plant with different solvents. It turns out that methanol and ethanol extractors gave the highest concentration with a clear superiority of the first solvent over the second. It may be due to the high polarity of this solvent compared to other solvents and show it. Hexane extract is less concentrated for phenolic compounds because most derived phenolic compounds are highly polar [13]. However, some of these compounds are non-polar and can therefore be derived by hexane. Arunet found henna methanol extract contained 25 mg/g flavones compounds, while khodaparast found that phenolic compounds had a concentration of 145 mg/g. This variation in concentration may be due the circumstances of extraction and the sources of henna vary according to the country of agriculture [14-16].

TABLE 1. **Concentration of phenolic and flavonoid compounds derived from henna plants with different solvents.**

Solvent	Phenols (mg/g)	Flavonoids (mg/g)
Hexane	38	28
Chloroform	46	40
Ethyl acetate	65	39
Ethanol	68	46
Methanol	80	59

Antioxidant effectiveness

TABLE 2 shows the anti-oxidation of linoleic acid of henna extract with different solvents compared to with butylated hydroxyl toluene and tocopherol, the **TABLE** shows the superiority of methanol extract and this is its high phenolic and flavonoid content may be due to a strong correlation between the concentration of derived phenolic compounds and antioxidant efficacy [17]. Indicating that these compounds are primarily responsible for antioxidant efficacy and are polar in nature, this result is consistent with khodaparastal, which found that methanol henna extract exceeds ethanol, acetone, chloroform and hexane extracts. As can be seen from the table, the industrial antioxidant BHT outperformed all extracts and purity of this antioxidant, which contains only one compound, while the rest contains many ineffective compounds. Its need to do additional purification to get high purity for effective compound.

TABLE 2. **Anti-oxidation of linoleic acid of henna extract with different solvents compared with butylated hydroxyl toluene and tocopherol.**

Solvent	Anti-oxidation activeness (%)
Hexane	38
Chloroform	60
Ethyl acetate	64
Ethanol	69
Methanol	77
BHT	96
Tocopherol	83

Reduction power

TABLE 3 shows the potential of henna extracts to reduce the iron ion compared to the industrial antioxidant BHT, tocopherols, exposes the superiority of methanol extract over the rest of the henna extracts, and also notes the evolution of the reduction force associated with the evolution of the antioxidant action. Indicating that the accumulation of reduction compounds is necessary for the development of the antioxidant effect, and therefore that these compounds are responsible for the development of the antioxidant effect of the henna plant, as well as the possibility of assessing the effect. Antioxidant effect based on the measurement of the development of the reduction force.

The reduction power of phenolic cofactors depends on the number of hydroxyl aggregates in them and their potential to reduce the iron ion by giving it the hydrogen atom. This result indicates that phenolic compounds of these extracts contain compounds and hydrogen stand by that are able to interact with free roots to transform them into more stable products and then terminate the free root chain reaction [18]. The reduced strength of phenolic compounds is caused by the presence of retroactivity compounds. It reacts to peroxides and has the potential to reduce the iron ion by giving it the hydrogen atom that turns the iron into iron and the blue color measured along the 700 nm wavelength [19].

TABLE 3.**The potential of henna extracts to reduce the iron ion compared to the industrial antioxidant.**

Solvent	Reduction Power (%)
Hexane	37
Chloroform	36
Ethyl acetate	46
Ethanol	51
Methanol	58
BHT	99
Tocopherol	94

Obstruction of oxidation of oils

TABLE 4 shows the inhibitory effect of methanol henna extract to hinder the oxidation of olive oil in terraces 2 mg/g, 4 mg/g, 6 mg/g, 8 mg/g and 10 mg/g oil, and for reservoir duration 5, 10, 15, 20, 25 days at 45°C compared to the antioxidant BHT (2 mg/g oil concentration) and the controlled sample It turns out that the inhibitory effect of the mixer oxidation impedance was higher at concentration 10 mg/g oil.

Antioxidant compounds destroy the molecular destruction of oil forming peroxides and thus prevent the formation of hydro peroxides to give more stable products [20-22]. This may be due to the low concentration of antioxidant compounds at these concentrations. Concentrations of 6.8 mg/g oil showed an effect in the first storage duration only, after which there was a rapid increase in peroxide values at these two concentrations. This may be due to the breakdown of antioxidant compounds by temperature, and the antioxidant BHT has achieved the highest efficiency of reducing levels of peroxide formed by advanced storage duration. Olive oil contains a high proportion of non-monounsaturated fatty acids. It's 73%, and it's 15% saturated fatty acids that are vulnerable to oxidation.

TABLE 4. **Inhibitory effect of methanol henna extracts to impede the oxidation of olive oil for a different storage period.**

Oil Concentration (mg/g)	Peroxide value of storage time				
	5 days	10 days	15 days	20 days	25 days
2	7.6	10.5	14.5	18	20.2
4	7.8	8.2	11.2	13.6	17.4
6	6.2	7	7.8	11.5	15
8	6.1	6.4	8.2	10.8	13.2
10	5.8	8	7.1	8.3	10.4
BHT (2 mg/g) Oil	4	5	5.2	5.7	6.5
Standard sample	8	12.5	16.3	18.8	21.4

Diagnosis of compounds with a device GC-MS

FIG.1. and **TABLE 5** show phenolic compounds derived from methanol by technique GC-MS and show that the compound 1,4-Naphthoquinone (Lawson) 2-hydroxy is the most concentrated by the value of the top area occupied, which was 68.57% of the total phenolic compound diagnosed [23]. This compound is classified as an antioxidant group of henna plants, which is primarily responsible for the antioxidant effectiveness of henna plants. This compound also has therapeutic and applied effects, as it has the trait properties of the henna plant, because it is a high thousand protein bindings, and it migrates gradually from the pigment paste to the outer layer of the skin to bind to its proteins.

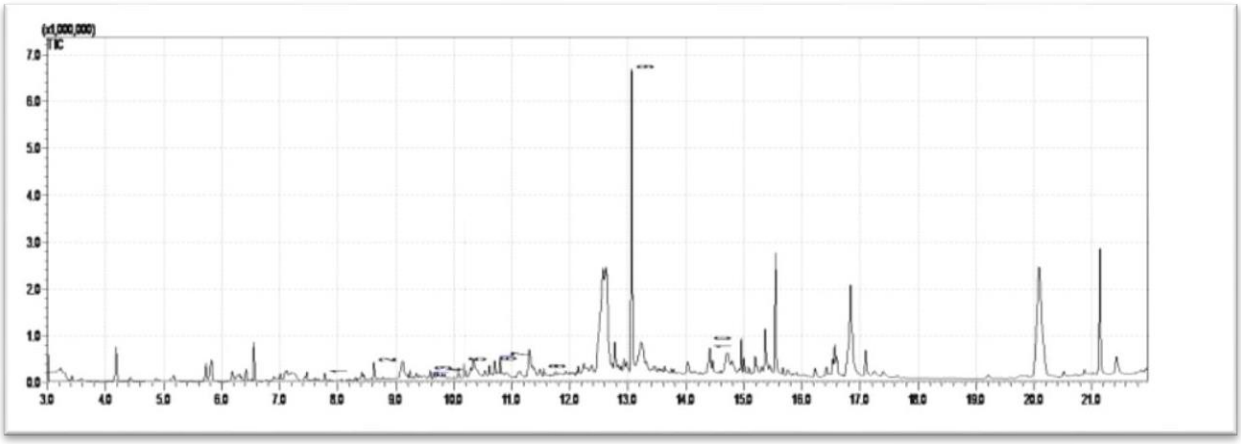
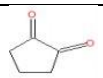
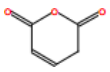
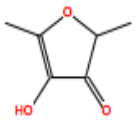
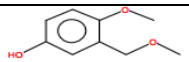
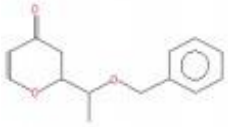
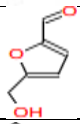
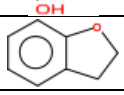
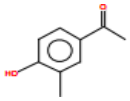
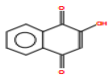
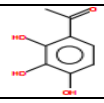


FIG.1. **Diagnosis of ring compounds in local henna leaf methanol extract.**

TABLE 5. **Phenolic compounds derived from methanol by technique GC-MS.**

Compound no.	Compound	Mole weight	R.T	Area%	Similarity%	Structures
1	1,2-Cyclopentanedione	98	7.76	1.98	82	
2	2H-Pyran-2,6(3H)-dione	112	8.65	4.64	86	
3	2,5-Dimethyl-4-hydroxy-3(2h)-furanone	128	9.59	1.16	83	
4	Phenol,4-methoxy-3methyl	138	9.67	0.88	87	
5	H-Pyran-4-one,2,3 dihydro-4-3, 5-dihydroxy-6-methyl	232	10.1	3.05	83	
			7			
6	Furancarboxaldehyde, 5-2-hydroxmethyl	126	11.6	2.58	91	
			4			
7	Benzofuran, 2,3-dihydro-methyl	120	10.7	2.77	91	
			8			
8	4-hydroxy-3-methylacetophenone	150	11.5	1.25	84	

			6			
9	2-hydroxy-1,4-naphthoquinone (Lawsone)	174	13.6 2	68.5 7	95	
10	2',3',4'-trihydroxyacetophenone	168	14.4 5	13.1 3	82	

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