



Study of the Effect of *Epipremnum Aureum* Extracts and *Tribulus Terrestris L.* as a Natural Alternative for the use of Industrial Antioxidants

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Article information

Article history:

Received: July, 01, 2022

Accepted: August, 11, 2022

Available online: October, 20, 2022

Keywords:

Epipremnum aureum,
Tribulus terrestris,
Antioxidant

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DOI:

<https://doi.org/10.53523/ijoirVol9I2ID253>

Abstract

In this study, two types of local plants were chosen, the first is the plant golden pothos *Epipremnum aureum* and the second is the Iraqi Sheikh's chin plant *Tribulus terrestris L.*, for the purpose of making a comparison between them in terms of their possession of chemical groups with antioxidant activity in order to use them as a natural alternative to using antioxidants Industrial that cause negative effects on human health, the samples were prepared using the method of water and alcohol extraction (ethanol 70%) for both plants. It revealed the presence of a number of chemical groups (tannins, carbohydrates, phenols, flavonoids, alkaloids) for both plants, the aqueous and alcoholic extracts. Coumarins are only found in the sheikh's chin plant, while steroids are only found in the pothos plant. It was found that the alcoholic extract of the Sheikh's chin plant was best than aqueous extract in terms of antioxidant activity using the DPPH method, as the concentration of 20 mg / ml achieved an effectiveness of 91.2, which is close to control (BHT) 92.3, while the opposite was recorded in the pothos plant as the aqueous extract was better than the alcoholic extract. As the concentration of 20 mg / ml recorded an activity of 89.22, while the alcoholic extract had a significant difference of 65.9, with a significant difference on the level of probability $P < 0.01$, and for the purpose of demonstrating the efficiency of the process of capturing free radicals of plant extracts, the effective concentration was determined (EC50) and found that the best concentration was achieved to capture 50% of The DPPH complex had an aqueous extract of pothos at 0.5 mg / mL which is very close to the EC50 value for control (vitamin C) followed by the alcoholic extract at 7.75 mg / mL. The Folin-Ciocalteu method was used to find the total content of phenols, it was found that the aqueous extract of the pothos plant had the highest content of phenols as it recorded the highest concentration of 49.33 compared to the alcohol extract 38.05. In contrast to the result of the Sheikh' chin plant, the alcoholic extract recorded the most phenol content at a concentration of 65.11 compared to the aqueous extract which recorded a concentration of 42.15.

1. Introduction

Plants are the main source of many basic compounds of medicinal value and have been used since ancient times in the treatment of many diseases. Diseases resulting from bacterial infections cause a large percentage of deaths every year and come in second place [1]. With the exacerbation of the problem of germs resistance to antibiotics and the high cost of manufacturing new generations of antibiotics, Scientists have recently focused and increased their interest in using plants and their derivatives as alternatives to many chemical medicines that have negative harmful effects. Two types of plants spread in different parts of Iraq were chosen in this paper. The first is the pothos plant and the second is the Iraqi Sheikh chin plant. The Araceae family is one of the plant families known at the medical and therapeutic level and has many vital active substances and one of the members of this family is the pothos plant *Epipremnum aureum*, which is known in different countries of the world and by different names pothos and sometimes known as (money plant) and it is one of the plants that are widely cultivated indoors due to its possession of the superior ability to remove internal pollutants such as formaldehyde, xylene, benzene. pothos is known for scrambler shrub events and has the ability to attach and climb trees by extending its aerial roots on them [2,3]. pothos leaves are distinguished by their brightness and take the shape of the heart and long, thin stems, which can reach a height of between 5-9 meters, the flowers of the plant are small green, this plant is known in some countries as an anti-cancer as well as in the treatment of some skin diseases [4]. The aerial roots and leaves have efficacy. Strong as an anti-bacterial [5]. Many medicinal plants have antioxidant properties, including pothos, as natural antioxidants in the form of raw extract or in the form of chemical components have great efficiency to prevent the destructive processes of free radicals of reactive oxygen species (ROS). Iraqi Sheik Chin plant, or what is known as Hasakah, Qutba, Shaqq, or old molar, *Tribulus terrestris L.*, of the Leguminosae family. Zygophyllaceae includes about 25 genera and 240 different species around the world in semi-desert regions and in the Arab region, including Iraq, and on a large scale in the western parts of it [8] from the annual natural herbs, which are small shrubs with a height of about 10-60 cm. The leaves are often opposite. Asymmetric, the flowers are yellow in color. The fruits and roots are widely used as a popular medicine for treating various ailments, including antiseptic, impotence, stomach ailments, antihypertensive, diuretic, and stone removal [6]. The sheikh's chin plant, as well as the pothos plant, has antioxidant properties, as it has many chemical compounds that work to capture free radicals resulting from the biological and metabolic processes inside the living cell or produced by immune cells and thus work to restore balance when any imbalance occurs as a result of any symptom of the body [7]. The importance of antioxidants on human health is getting clearer as a result of the tremendous progress in understanding the mechanism of their action with oxidizing substances. Moreover, some epidemiological studies have indicated that natural antioxidants have an effective positive effect on human health [9]. The aim of the research is to extract antioxidants from two local plants, pothos and el-sheikh plant, as well as alternative natural sources of industrial antioxidants that have harmful health effects that may infect humans with many diseases and serious complications such as cancer and others.

2. Experimental Procedure

The Experimental Collecting and proving the authenticity of plants: pothos plant: The leaves and fresh aerial roots of the pothos plant cultivated in public gardens were collected from the various regions of Baghdad. The type required to be studied and classified in the College of Science, University of Baghdad, was ascertained and proved that the plant belongs to the genus and type *Epipremnum aureum*. Wash the plant with water to remove dust and dirt and leave it to dry in the shade at room temperature for 7 days, after which the leaves and other parts of the plant are ground in a ceramic mortar and put in an airtight box until use. Alcohol extraction method: Weighing 50 grams of the powder prepared in paragraph 1 and placing in a glass beaker containing 500 milliliters of ethanol alcohol at a concentration of 70% for a period of 24 hours, after which the form was filtered using whatman No.1 filter paper. Concentrate the form using a rotary evaporator device, then pour the remaining In glass dishes and placed inside the electric oven at 40 degrees Celsius to dry, the dry form was collected and ground using a ceramic mortar and placed in an opaque sealed box until further tests were carried out

The aqueous extraction method: The same method used for alcoholic extraction was used with the same weight and volume except for using water as a solvent.

The sheikh's chin plant: Various parts of the leaves, fruits and roots of the Iraqi sheikh's chin plant were collected from the local markets, and it was confirmed that it was classified as the herbarium College of Science / University

of Baghdad under the genus *Tribulus terrestris*. Tightly closed box until use.

Alcohol extraction method: Weighing 250 grams of the form prepared in paragraph 2 and submerging it in a glass beaker containing methanol absolute alcohol in a volume of 1.25 liters and leaving for 7 days, after which the form was filtered using medical gauze cloth in the first stage and then filtered using whatman NO.1 filter papers. With a funnel under pressure, the form was then dried using a spray dryer at a temperature of 40 °C.

Aqueous extraction method: Weighing 100 grams of the sample prepared in paragraph 2 and submerging it with distilled water in a volume of 1400 milliliters for an hour, then filtering the form with medical gauze cloth, then filtering it using whatman No.1 filter paper, then drying the form with a spray dryer.

Detection of active compounds:

1. Detection of proteins: Use a biuret solution to detect the presence of proteins. Mix (1) mL of the reagent concentration of 10% with aqueous copper sulfate at a concentration of 80%. The appearance of a violet color indicates the presence of protein.
2. Detection of tannins: (1) milliliter of aqueous lead acetate at a concentration of 1% was added to (1) milliliter of extract. The result is considered positive when a yellow deposit appears.
3. Detection of glycosides: Fehlings' reagent was used for this purpose and the formation of a red deposit indicates the presence of glycosides.
4. Detecting phenols: Prepared by mixing (0.1) grams of extract in (1) milliliter of distilled water, then adding a drop of ferric chloride solution to it. The appearance of a green or blue color indicates the presence of phenols
5. Detection of flavonoids: prepare by dissolving (1) milliliter of alcoholic potassium hydroxide at a concentration of (5) normality to (1) milliliter of extract. The appearance of a yellow precipitate indicates the presence of flavonoids.
6. Detection of saponins: prepare by adding (1) milliliter of aqueous mercury chloride reagent at a concentration of (5%) to (1) milliliter of the extract, and when a white precipitate is formed, the result is considered positive and indicates the presence of saponins.
7. Detection of alkaloids: - Detection of alkaloids using the Wagners' reagent by adding several drops of the reagent to (1) milliliter of the extract and the appearance of a brown precipitate indicates that the result is positive.
8. Resins test: - Detection was done by adding (1) milliliter of lead acetate (1%) lead acetate to (1) milliliter of extract, and when a white precipitate was formed, the result would be positive and indicate the presence of resins.

Measurement of antioxidant potency with DPPH: The antioxidant activity of samples prepared from alcoholic and aqueous pothos and the sheikh's chin plant extracts depends on the efficacy of scavenging free radicals of the compound DPPH (1, 1-diphenyl-2-picrylhydrazyl) and was determined according to the method of Lee et al [6] following: Use a known volume of the prepared model (0.625, 1.25, 2.5, 5, 10, 20) mg / ml and put into test tubes and complete the volume with distilled water to (1 ml). (1 mL) of DPPH solution at a concentration of (0.2 mM in ethanol) was added to each test tube, mixed well and incubated at room temperature for (30 minutes). Prepare the control model by the same method without the plant extract model and use a solution of ascorbic acid (0.03% w / v) as a positive control model from a natural source and a compound BHT as an industrial source. The absorbance was controlled at a wavelength (517 nm) by a Spectrophotometer. The percentage of free radical inhibition of DPPH was calculated at according to the following equation: % of inhibition of DPPH radical = $[(A\text{-control} - A\text{-sample}) / A\text{-control}] \times 100$.

The total phenolic content: Thee total phenolic contentof the plant extracts prepared by the Folin – Ciocalteu method [10] was determined. Briefly apply 200 µl of the crude extract (1 mg / ml) to 3 ml with distilled water, thoroughly mixed with 0.5 ml of Folin-ciocalteus' phenol reagent for 3 minutes, followed by an addition of 2 ml of 20% (w / v) sodium carbonate. The mixture was then left for another 60 minutes in the dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from the titration curve using gallic acid (Fig. 1), and the results were expressed as mg of gallic acid equivalent per gram of dry weight.

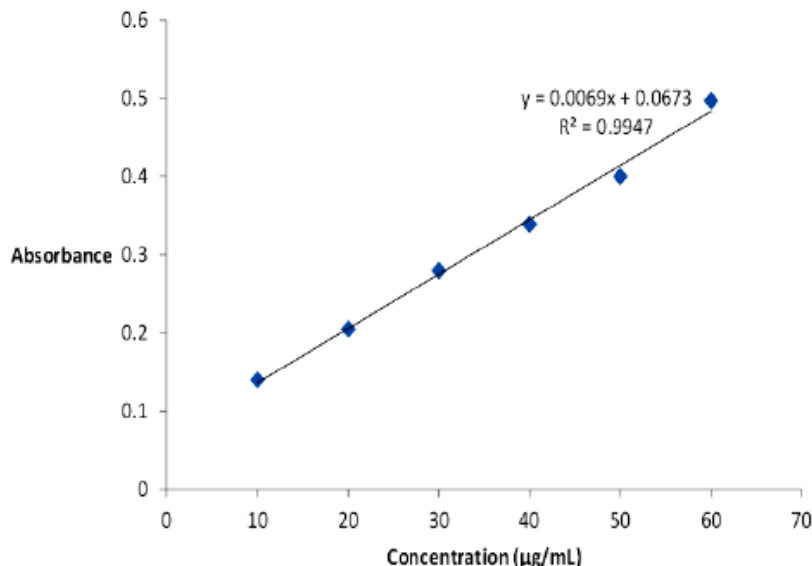


Figure (1). Calibration curve for standard gallic acid.

Odumosu, et al (2015) [11] section must precede the Results and discussion. A concise and accurate description of methods enabling their reproduction by others is necessary. Sections can be divided into subsections in a sensible way so that the text would not be fragmented into many small paragraphs having a few lines. Experimental part should be Times New Roman, justified, regular; font size: 11 single. If you have any figures or tables in this section, please use the same format that will be mentioned in the “Results and discussion” part. If you have equations, please use the format and manner mentioned in the “Theoretical Part” above.

3. Results and Discussion

The results of the analysis of the chemical findings of the aqueous and alcoholic extracts of Sheikh Chin plant, as shown in Table (1), showed the presence of various chemical compounds, and the results were positive with the presence of groups of tannins, carbohydrates, phenols, flavonoids, alkaloids and coumarins, except for both forms of steroids, terpenes and proteins. The presence of glycosides in the alcoholic extract only and the appearance of resins and saponins in the aqueous extract only. In the pothos plant, it revealed the presence of most of the aforementioned groups except for the resins and proteins and the absence of the aqueous extract of terpenes and the absence of the steroids group in the alcoholic extract. This result is consistent with some studies and research in the presence of active groups in plants and sometimes varies according to many factors, including that the concentration of secondary metabolites varies greatly from one plant to another due to the same genus and even within the same plant in different parts of it and this is due to the different environmental factors and the different nature, Soil composition, texture, depth, humidity and ventilation all this can create a highly specialized diversity of different chemical compounds even within a single country [10].

DPPH Test Results: From the observation of Table (2), it was found that the alcoholic extract of the Sheikh Chin plant was better than the aqueous extract in terms of effectiveness in capturing free radicals and by almost twice the amount and the concentration of 20 mg / ml was the best as it achieved an effectiveness of (91.2) for the alcoholic extract, which is close to the effectiveness of the antioxidant From the industrial source BHT, which recorded an effectiveness of (92.32), as well as for an antioxidant from a natural source Vit C, which recorded an effectiveness of (97.19).

Table (1). The chemical disclosures of the extracts of sheikh chin, pothos alcoholic and aquatic.

No.	Detection type	Aquas ex.	coholic ex.	Aques ex	Lcoholic ex
		Alshek		Pothos	
1	Tannins Test	+	+	+	+
2	Carbohydrate Test	+	+	+	+
3	Glycosides Test	-	++	+	+
4	Phenols Test	+	+	+	+
5	Resins Test	+	-	-	-
6	Flavonoids Test	+	+	+	+
7	Saponin Test	+	-	+	+
8	Alkaloid Test	+	+	+	+
9	Protein Test	-	-	-	-
10	Coumarins Test	+	+	-	-
11	Terpenes Test	-	-	-	+
12	Steroids Test	-	-	+	+

Table (2). the efficacy of scavenging the free radicals of Tribulus terrestris L extract. It shows that the effect is highly significant in terms of concentrations as well as in terms of the type of extract.

Concentration mg/ml	Water extract	Ethanolic extract	BHT	Vit. C	LSD value
0.625	5.49 ±0.21	10.20 ±0.12	38.23 ±0.13	67.13 ±0.18	0.556 **
1.25	8.76 ±0.03	16.07 ±0.07	56.08 ±0.05	89.87 ±0.10	0.239 **
2.5	11.58 ±0.12	21.90 ±0.11	75.01 ±0.08	92.77 ±0.19	0.436 **
5	17.96 ±0.08	35.50 ±0.28	86.71 ±0.36	94.03 ±0.22	0.840 **
10	26.61 ±0.23	52.49 ±0.29	88.35 ±0.25	96.68 ±0.25	0.846 **
20	78.68 ±0.20	91.20 ±0.13	92.38 ±0.08	97.19 ±0.09	0.448 **
LSD value	0.514 **	0.588 **	0.600 **	0.571 **	---
** (P<0.01).					

As for the second model of pothos plant extract, the aqueous extract showed clear superiority and significant differences in the level of probability of $p < 0.01$ over the alcoholic extract in terms of its capture of free radicals, and by almost three times the effectiveness, Table (3). The concentration of 20 mg / ml of aqueous extract recorded the highest efficacy of (89.22), which is close to the effectiveness of natural and synthetic antioxidants (Vit C, BHT), with an effect of (97.13, 92.11), respectively.

Table (3). the efficacy of scavenging free radicals of the pothos leaf extract, *Epipremnum aureum*, showing the significant effect on two sides in terms of concentrations and on the type of extract.

Concentration mg/ml	Water extract	Ethanolic extract	BHT	Vit. C	LSD value
0.625	50.94±0.06	10.03±0.05	38.10±0.02	67.47±0.02	0.113**
1.25	84.65±0.15	11.69±0.04	56.33±0.02	89.51±0.19	0.336**
2.5	85.03±0.05	17.82±0.33	75.32±0.02	92.4±0.14	0.494**
5	86.12±0.01	34.68±0.49	86.57±0.37	94.14±0.02	0.834**
10	86.46±0.01	58.68±0.08	88.13±0.06	96.79±0.04	0.149**
20	89.15±0.15	66.12±0.15	92.11±0.01	97±13±0.01	0.299**
LSD value	0.232**	0.626**	0.379**	0.246**	---

** (p<0.01)

For the purpose of determining the efficiency of plant extracts in the reduction process of DPPH, the value of EC50 was determined, which is the value of the Effect Concentration in which the extract or compound reduces 50% of DPPH. The value of the amount of efficiency of antioxidants has an inverse relationship with the value of EC50. If the EC50 value of the extract is less than 10 mg/ml, this means that the extract is efficient and sufficient in terms of antioxidant activity. We note through Figure (2) that the value of EC50 for Al Sheikh's chin extract is 9.5 mg / ml, and it is within the limit that proves the efficiency of this extract as an antioxidant substance, while the value of EC50 for the aqueous extract is 14.75 mg / ml, which is more than the required limit and thus the aqueous extract Less efficient in this area.

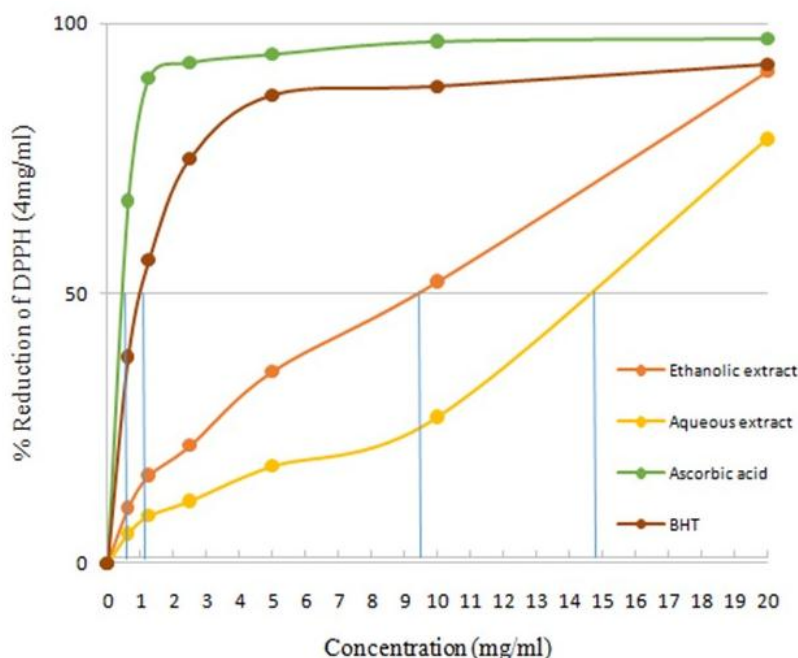


Figure (2). EC50 value for *Tribulus terrestris* extract.

From Figure (3) we notice that the value of EC50 for aqueous extract of pothos is 0.5 mg / ml and very close to the value of EC50 for vitamin C (ascorbic acid) from the natural source and superior to the industrial source BHT as the value of EC50 was 1 mg / ml, The EC50 value of the alcoholic extract was 7.75 mg/ml, which is within the limit that qualifies it as an efficient material for scavenging free radicals.

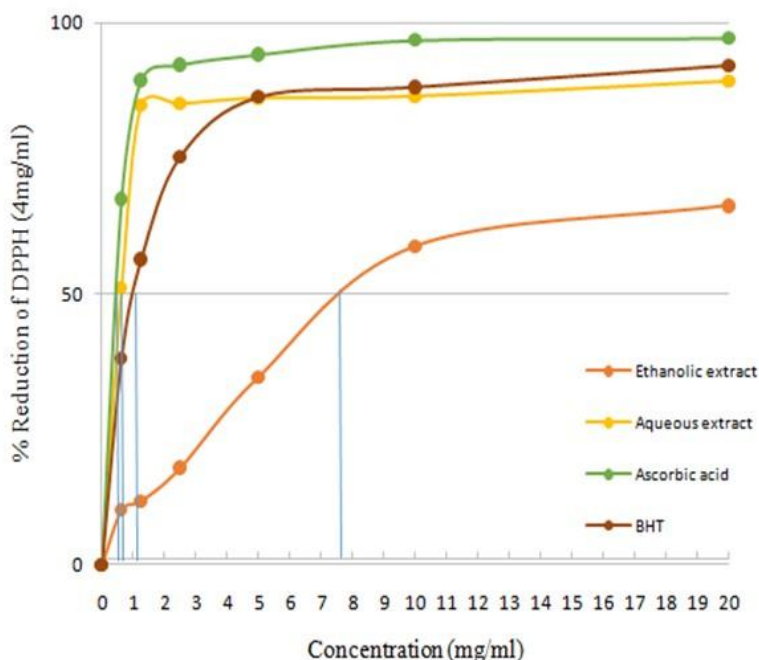


Figure (3). EC50 value for *Epipremnum aureum* pothos extract

Results of an experiment on the total content of phenolic substances: It is known that phenolic substances play a major and important role as antioxidants, so the total content of them was determined for plant extracts, from table (4) we note that the total content of aqueous pothos extract recorded a higher concentration compared to the alcoholic extract as the concentration of the total content of the phenolic substances of the aqueous extract increased with increasing The concentration of the extract reached the amount of (49.33) mg / g, while its concentration was (38.05) mg / g for the alcoholic extract, so we see that the aqueous extract was the most efficient as an antioxidant substance from the alcoholic extract, as it was shown from the previous experiment. as for the extracts of the sheikh’s chin, it was found, as we note in table (5), that the alcoholic extract of the sheikh’s chin contains more phenolic substances than the aqueous extract. The concentration of 50 mg ml of alcoholic extract was recorded as a phenolic content of (65.11) mg/g, superior to the water extract, which recorded a concentration of 42.15 mg/g, and this proves the efficiency and adequacy of the alcoholic extract of the sheikh’s chin as an antioxidant, which was demonstrated through the previous experiment.

Table (4). Calculation of the total phenolic content of the plant extracts of *Epipremnum aureum*, showing that the effect is significant on one side, i.e. in terms of concentrations only

Solvent	12.5 (mg/ml)	25 (mg/ml)	50 (mg/ml)	LSD value
	Concentration (mg/g)			
Ethanol extract	14.10± 0.01	24.25± 0.47	38.02±0.04	0.921**
Aqueous extract	17.31± 0.03	26.68± 0.03	49.31± 0.02	0.068**
LSD value	0.069**	1.261**	0.102**	-----
** (P<0.01).				

Table (5). the total content of the phenolic substances of the extracts of Sheikh's chin and it shows that the effect is significant on two sides in terms of the concentrations of the extracts and also in terms of the type of extract.

Concentration (mg/ml)	Aqueous extract (mg/g)	Ethanolic extract (mg/g)	LSD value
12.5	22.48 ±0.01	30.67 ±0.20	0.563 **
25	36.01 ±0.02	50.99 ±0.04	0.154 **
50	42.15 ±0.02	65.11 ±0.02	0.079 **
LSD value	0.070 **	0.418 **	---
** (P≤0.01).			

4. Conclusions

1. The pothos plant extracts are more efficient and more effective as antioxidant agents and from natural sources than the extracts of the Iraqi Sheikh chin plant.
2. The aqueous extract of pothos is more efficient than the alcoholic extract.
3. The alcoholic extract of the sheikh's chin plant is more efficient than the aqueous extract
4. The extract of Al-Sheikh Al-alcoholic plant was the most abundant of phenolic substances.

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