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Antioxidant Activity and HPLC Screening of Pimpinella Anisum

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Abstract: Background: Arising medications and their complications in recent years, and as a reason using alternative folk medications will be a solution for many diseases. The rule of medicinal plants is to strengthen the defense mechanism of the body with natural products such as polyphenols, which is a way to fight diseases and their complications. ***Pimpinella anisum*** proved to be able to treat many diseases, but its phenolic constituents are not fully identified, also its antioxidant ability needs more studies. **Methodology:** ***Pimpinella anisum*** Libyan endemic plant was extracted by acetone through socking; the solvent was then evaporated and the extract was analyzed for antioxidants using DPPH and phenolic compounds using HPLC. **The Results:** antioxidant activity was proved for acetone extract with IC50 of 7.83 ug/ml, and 15 polyphenolic compounds were identified by HPLC with different concentrations. **Conclusion:** We suggest considering this study as a seek for future studies on ***Pimpinella anisum***.

Keywords: *Pimpinella anisum*; polyphenols; antioxidants; HPLC, DPPH.

1 Introduction

Living creatures have a variety of defense mechanisms that lower the number of reactive oxygen species (ROS) by scavenging free radicals, chelating catalytic metals, and serving as electron donors [1][2]. Many earlier studies have suggested the biological effect of natural antioxidants, including the suppression of reactive oxygen species (ROS). Antioxidants, thereby, shield living organisms from the generation of ROS that leads to lipid peroxidation, and protein and DNA damage [3][4][5].

Star Anise (Illicium verum) also known as ***Pimpinella anisum*** is a dark brown colored fruit originally distributed in tropical areas of Asia [6], southern Europe [7], and the Mediterranean region [8] including Libya [9]. ***P. anisum*** is an aromatic vegetable [10][9] that contains a large quantity of essential oil (EO) whose predominant component is trans-anethole [10], other substance in ***P. anisum*** includes terpenoids that have an insecticidal and acaricidal effect and low toxicity [11]. Furthermore, ***P. anisum*** can scavenge free radicals, and inhibit hydrogen peroxide including lipid peroxidation and activation of lipoxygenase [6]. In addition antimicrobial, antifungal, insecticidal and anticorrosive activities of ***P. anisum*** have been proven [8]. Previous studies on ***P. anisum's*** chemical composition were mainly on lipophilic compounds, especially volatile compounds[12].

The antioxidant activity of natural products is due to the presence of phenolic compounds [13][14][15], and a few studies discussed the polyphenolic profile of ***P. anisum*** [16] [17]. Despite the previous studies' outcomes, many of them were unable to identify the phenolic compounds of the Libyan ***P. anisum*** acetone extract. Therefore, the aim of the present study aimed to identify the antioxidant potential of ***P. anisum*** in terms of phenolic compounds, and correlatphytochemicalsls with the antioxidant activity of tplant'sts' extracts.

2 Experimental Section

2.1 Plant Collection and Identification

The plant was collected from the green mountain in the east of Libya. The botany department at Benghazi University identified the plant.

2.2 Chemicals

Acetone, 1,1-Diphenylpicrylhydrazyl (DPPH), ethanol, trifluoroacetic acid, and acetonitrile obtained from Teiba Company**.**

2.3 Preparation of the leaf extract

250 g of the fine-grained plant was added to 350 ml of acetone in a 500 ml flask, and then socked for 48 h. The plant biomass was separated through Whatman No.1 filter paper and concentrated with a rotary evaporator (RE2000). The extract was kept in a dry clean container until the analysis.

2.4 Evaluation of antioxidant activity by DPPH radical scavenging method:

Free radical scavenging activity of the plant extract was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in ethanol at different concentrations (concentrations 62, 31.25, 62.5, 125, 250, 500, and 1000 µg/ml). Here, only those extracts are used which are solubilized in ethanol and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min, and then the absorbance was measured at 517 nm. By using a

spectrophotometer (UV-VIS miltonroy). Ascorbic acid was the reference standard compound used in the experiment was done in triplicate [18]. The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using the Log dose inhibition curve. The lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using the following equation:

DPPH scavenging effect (%) or

Percent inhibition = A0 - A 1 / A0×100.

Where A0 was the Absorbance of the control reaction and A1 was the Absorbance in presence of a test or standard sample.

2.5 HPLC conditions

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A) and 16-20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl of the sample solutions. The column temperature was maintained at 40 °C. Gallic acid, Chlorogenic acid, Catechin, Methyl gallate, Coffeic acid, Syringic acid, Pyro catechol, Rutin, Ellagic acid, Coumaric acid, Vanillin, Ferulic acid, Naringenin, Daidzein, Quercetin, Cinnamic acid, Apigenin, Kaempferol, and Hesperetin were used as standard solutions for the quantification of phenolic compounds.

3 Results and Discussion

3.1 Antioxidant activity by DPPH

DPPH radical process can detect a minimum amount of antioxidants at low concentrations [12][19] and the results are expressed as IC50 values [20]. The scavenging activities of radicals are curial in preventing the developing effect of free radicals during different diseases like cancer [21][22].

DPPH is a stable free radical with an unpaired electron that is delocalized over the whole molecule, furthermore, DPPH has a purple color that can be absorbed in 519 nm in ethanol [23]. In this study ***P. anisum*** antioxidants can scavenge DPPH and cause a decrease in the absorption reading over time, this decrease is directly proportional to the antioxidant present in the acetone extract.

The data obtained from ***P. anisum*** acetone extract reveals high antioxidant activities with IC50 0f 7.83 ug/ml. The results also revealed that as the concentration of the extract increases, the radical scavenging percent increase may be due to the increase of phenolic content, and the highest scavenging percent was at 1000 ug/ml. These results were lower than the results obtained by [24] who used oil rather than acetone extract, also the method of extraction was different which affect the type and the number of antioxidant compounds. Furthermore, [25] reported IC50 = 98.470 ± 6.341 ug/ml for the water extract, which is higher than our results, and this is due to the change in cultivation conditions.

Table 1: Antioxidant activity assay for *P. anisum*

Sample *P. anisum* (Conc. ug/ml)	DPPH scavenging%	SD	SE
1000	76.0 %	0.002	0.001
500	69.2 %	0.007	0.002
250	62.8 %	0.003	0.001
125	55.0 %	0.006	0.002
62.5	48.4 %	0.005	0.002
31.25	28.5 %	0.008	0.002
15.625	23.4 %	0.002	0.000
7.8125	19.1 %	0.003	0.001
3.9	12.9 %	0.003	0.001
1.95	6.1 %	0.004	0.001
0	0.0 %	0.003	0.001
IC50	101.76 ug/ml		

3.2 HPLC for Pimpinella anisum acetone extract

Phenolic acids and flavonoids are known as polyphenolic compounds [26] and are also known as antioxidants have long been interesting [27], and their identified number is raising every day [26] because of their beneficial effect on health and disease [27].

To obtain a more inclusive profile of these polyphenolic compounds in ***P. anisum***, a simple, rapid, analytical HPLC method was developed, using water and trifluoroacetic acid in acetonitrile as the mobile phase. The HPLC profile was analyzed for nineteen phenolic compounds viz. Gallic acid, Chlorogenic acid, Catechin, Methyl gallate, Coffeic acid, Syringic acid, Pyro catechol, Rutin, Ellagic acid, Coumaric acid, Vanillin, Ferulic acid, Naringenin, Daidzein, Quercetin, Cinnamic acid, Apigenin, Kaempferol, and Hesperetin. Phenolic compounds present in the acetone extract of ***P. anisum*** are shown in Table (2) with peaks showing different retention times (RT). Of the nineteen compounds, only 15 phenolic compounds were present with the highest concentration of Ellagic acid (80.78 µg/21mg)
Followed by Cinnamic acid and Coffeic acid (55.73 µg/21mg) and (50.21 µg/21mg) respectively. Cinnamic acid has many biological activities like antibacterial, and inhibitor for tyrosinase[29]. Also, act as an antioxidant due to the presence of vinyl fragments in its structure [30]. Coffeic acid has enormous biological activities including antioxidant properties [31].

Furthermore, these results partially agreed with HPLC analysis of the EtOH extract of ***P. anisum*** done by [28], in which Chlorogenic acid, Catechin, Syringic acid, Ferulic acid, Quercetin, Cinnamic acid, and Hesperetin were present in different concentrations, whereas Coffeic acid, Apigenin, and Kaempferol were absent of EtOH extract and present in acetone extract, while Vanillin was present in EtOH extract and absent in our extract. This variation may be due to the usage of different polarity solvents, also climate change, and other factors of planting the ***P.***

anisum that may affect the composition.

Previously, there were many reports of the composition of various compounds in this plant species via Gas Chromatography and HPLC, but the present study is the first attempt to develop a method of determination of polyphenolic compounds with antioxidant activity of the acetone extract.

Table 2: HPLC for *P. anisum*

	Area	Conc. (µg/ml=µg/21mg)
Gallic acid	161.66	12.73
Chlorogenic acid	224.63	27.81
Catechin	7.77	1.76
Methyl gallate	23.77	1.35
Coffeic acid	613.86	50.21
Syringic acid	106.54	9.73
Pyro catechol	15.83	2.16
Rutin	103.53	13.20
Ellagic acid	292.27	80.78
Coumaric acid	16.70	0.52
Vanillin	0.00	0.00
Ferulic acid	0.00	0.00
Naringenin	140.19	15.30
Daidzein	348.79	22.56
Quercetin	104.72	12.79
Cinnamic acid	2751.39	55.73
Apigenin	0.00	0.00
Kaempferol	0.00	0.00
Hesperetin	20.33	1.18

4 Conclusions

During the investigation of ***P. anisum*** antioxidant activity for the acetone extract, we identified fifteen phenolic compounds with an antioxidant activity which are Gallic acid, Chlorogenic acid, Catechin, Methyl gallate, Coffeic acid, Syringic acid, Pyro catechol, Rutin, Ellagic acid, Coumaric acid, Naringenin, Daidzein, Quercetin, Cinnamic acid, and Hesperetin. These compounds along with other constituents in ***P. anisum*** were able to prevent disease and enhance the defense system of the body; further studies are needed to examine the function of each ingredient.

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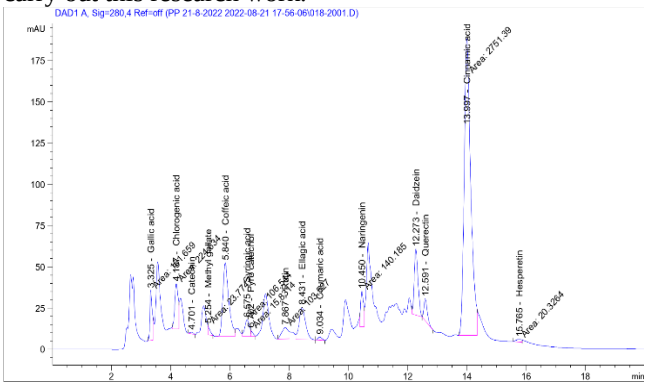

Fig. 1. HPLC for *P. anisum*

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