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Research article

Antioxidant activity of *Hedera helix* L. extracts and the main Phytoconstituents

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ABSTRACT
The present study was carried out to evaluate antioxidant activity of *Hedera helix* stems extracts and also to investigate the main phyto constituents in the bio-active extract. N-hexane, dichloromethane, ethyl acetate and methanol 80% extract were tested for free radical scavenging activity on model reaction with stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The results showed that ethyl acetate was the most active one as antioxidant agent and phytochemical analysis of that extract revealed the presence of triterpenes, saponins, flavonoids, tannins and carbohydrates. The results suggest new chemical classes of natural antioxidant substances that could serve as selective agents for infectious diseases.
Key words: *Hedera helix*, stems, antioxidant activity

INTRODUCTION
Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical induced oxidative stress. A variety of free radical scavenging antioxidants are found in plants [1]. Therefore, there is an increasing interest amongst scientific communities in identifying natural sources of antioxidants. Traditionally practiced natural antioxidants are already exploited commercially, but still there is demand to find more plant species concerning the antioxidant potential. *Hedera* is a genus of 15 species belong to the family Araliaceae, which has about 70 genera and 700 species of flowering plants**.** *Hedera helix* L. is an evergreen climbing plant, where suitable surfaces are available, and also growing as ground cover where there are no vertical surfaces. It is native to Spain, Turkey and also Asia [2]. In traditional medicine; the leaves were used as analgesic and anti-inflammatory agents. The leaves and berries were taken orally as an expectorant to treat cough and bronchitis. The leaves can cause severe contact dermatitis in some people [3]. Previous pharmacological studies proves that *H. helix* leaf has analgesic and anti-inflammatory activities [4]. The main objectives of the study are to evaluate antioxidant activity of *Hedera helix* stems extracts and also to investigate the main phytoconstituents in the bio-active extract.

MATERIALS AND METHODS
Plant identification and collection
The stems of *Hedera helix* were collected from Al-Zohiriya garden, Giza, Egypt in May 2011. The plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereez Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen is deposited in the herbarium of Al-Zohiriya garden, Giza, Egypt.

Preparation of the extracts
Air dried stems of *Hedera helix* (200 g) were extracted with n-hexane, dichloromethane, ethyl acetate and methanol 80% solvents at room temperature by maceration method. Each extract was concentrated to dryness in vacuo to give 7.8 g,

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5 g, 3.7 g and 22 g of n-hexane, dichloromethane, ethyl acetate and methanol 80% extracts, respectively.

DPPH assay

The scavenging reaction between (DPPH•) and an antioxidant (H-A) can be written as: DPPH • + H - A→ DPPH - H + A• [5]. Antioxidants react with DPPH•, which is a stable free radical and is reduced to the DPPH-H and as consequence the absorbance decreased from the DPPH• radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant extract in terms of hydrogen donating ability. DPPH radical scavenging activity from the plant extract was measured by taking 100µg/ml of extract, 900µl of acetate buffer and 3 ml freshly prepared 100µM DPPH solution in methanol. Reagent blank was 1 ml buffer and 3 ml DPPH solution. The absorbance was measured after 90 min of incubation in dark at 517 nm. DPPH radical scavenging activity (%) was determined by following equation: DPPH radical scavenging: Activity (%) = A_b-A_s /A_b x 100. (A_s - absorbance of the test sample, A_b - absorbance control reaction).

RESULTS AND DISCUSSION

Antioxidant activity of *Hedera helix* stems extracts

The DPPH radical scavenging activity of *Hedera helix* stems extracts were compared with that of known natural green tea (table 1) where ethyl acetate extract showed a significant antioxidant potential (84.95%) and the other extracts were less active as antioxidant agents. As revealed by Ahmadi et al., 2007 [6], DPPH method measures the ability of antioxidants present in scavenging the hydrophilic free radicals. In line to this theory, ethyl acetate extract has better ability in scavenge hydrophilic free radicals as compared to other *Hedera helix* extracts that might due to the presence of hydrophilic antioxidants. Furthermore, the high antioxidant activity could be due to the increased in hydroxyl groups or antioxidant compounds found particularly in the *Hedera helix* ethyl acetate extract. Ethyl acetate extract is very rich with phenolic compounds (tannins and flavonoids). Flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [7]. The highest level of radical scavenging properties at low concentrations of flavonoids exhibits quercetin and in the following order luteolin, rhamnetin, isorhamnetin and apigenin [7]. Tannins are the most abundant antioxidants in the human diet and they exhibit many biologically important functions which include protection against oxidative stress and degenerative diseases, gallic acid showed strong antioxidant activity by preventing lipid per-oxidation [8].

Phytochemical analysis of ethyl acetate extract of *Hedera helix* stems

Phytochemical analysis of ethyl acetate extract has shown that the extract contained triterpenes, flavonoids, tannins and carbohydrates (table 2).

Table 1: Antioxidant activity of *Hedera helix* stems extracts

Extracts	Concentration (%)	DPPH free radical scavenging effect (%)
Green tea extract	1%	96.41%
N-hexane extract	0.1%	44.75%
Dichloromethane extract	0.1%	52.35%
Ethyl acetate extract	0.1%	84.95%
Methanol extract	0.1%	68.24%

Table 2: Phytochemical analysis from *Hedera helix* stems extracts

Constituent	N-hexane	Dichloromethane	Ethyl acetate	Methanol 80%
Triterpenes and /or Sterols	+	+	+	+
Carbohydrates and/or glycosides	-	-	+	+
Flavonoids	-	-	+	+
Coumarins	-	-	-	-
Alkaloids and/or nitrogenous compounds	-	-	-	-
Tannins	-	-	+	+
Saponins	-	-	-	+

(+) presence of constituents, (-) absence of constituents

CONCLUSION

The results indicate that antioxidant potential of *Hedera helix* stems ethyl acetate extract is due the presence of bio-active phytoconstituents as phenolic compounds (tannins and flavonoids) and triterpenes and these results also endorsed the ethno botanical use of this plant from the collected territory due to presence of various chemicals.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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