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PHENOLIC COMPOUNDS AND PHYTOCHEMICAL STUDIES OF THE *TRIBULUS TERRESTRIS* FROM SOUTH ALGERIA

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ABSTRACT
For the valorization of medicinal plants in the El Oued region, we conducted a phytochemical study and estimated the antioxidant capacity of the plant Tribulus terrestris L. After the extraction processes, the yield of the aqueous extract obtained is 12.53% and the ethanolic extract is 5.2%. The results show that the total phenols were estimated to be 117.61 ± 0.102 mg AGE / g and 71.05 ± 0.063 mg AGE / g for the ethanol extract and the aqueous extract successively. For flavonoids, the ethanolic extract contains 81.19 ± 0.085 mg QU E / g and the aqueous extract is 26.18 ± 0.083 mg QU E / g. The result of the antioxidant effect show a preferable efficacy of the ethanol extract IC50 is 0.019 mg / ml and for the aqueous extract IC_{50} is 0.023 mg / ml. Concerning the qualitative analysis of phenolic compounds for both extracts using high performance liquid chromatography (HPLC) has been demonstrated that the presence of gallic acid, chlorogenic acid, caffeic acid, quercetin, vanillin, Rutin, naringin, vanillic acid and comarin in both extracts with different concentrations.

Keywords: *Tribulus terrestris L, ethanolic extract, aqueous extract, phenol , flavonoids, antioxidant activity, HPLC.*

1. INTRODUCTION
The *tribulus terrestris* (Zigophyllaceae family) commonly known as devil's thorn, cat head, puncture vine, goat head and caltrop, is a genus of dry and compact soil, found in many warm areas. It is an annual or perennial plant of warm regions with very hairy, creeping stem, with tiny silvery hairs and very little branching; its length can reach 90 cm. The leaves are composed comprising 8 to 16 oval leaflets, opposite to short petiole. Its small yellow flowers, borne by short peduncles, are often located in the axils of the leaves. Pollinated, they give fruits in the shape of a star more or less elongated, green color, are enveloped by very solid spines, including 2 to 3 seeds embedded [1].

The *Tribulus Terrestris* plant especially its fruits; its root and its leaves were then recommended for all problems of kidneys, prostate and physical faintness. It was also used in the treatment of diseases related to food poisoning, snake bite and digestion. Traditionally, it has been used to fight diseases of the liver, kidneys and urinary diseases. Currently, the plant is mainly used for its aphrodisiac property, but also as anabolic in sportsmen and bodybuilders who want to increase their muscle mass [2, 3].

Some species are cultivated as ornamental plants in warm regions [3]. Thirteen species of Tribulus are identified, especially *T. cistoides, T. longipetalus, T. terrestris, and T. zeyheri*, are believed weeds [4].

The purpose of the present work is to determine the antioxidant activities of the ethanolic extracts from aerial parts of *T. terrestris*

2. MATERIALS AND METHODS
2.1. Plant material
The plant was collected from south Algeria (Oued souf region), in Mars 2017, plant material was shade dried at room temperature. The dried stems with leaves were ground in commercial coffee mill.

2.2. Phytochemical screening

The phytochemical screening was carried out according to standard procedures [5].

2.3. Determinations of the total extract phenol content

The content of total contents was measured using Folin-Ciocaltau assay [6]. 7 ml of distilled water, 0.5 ml of sample and 0.5 ml Folin-Ciocaltau reagent were added to 25 ml volumetric flask , the content were mixed and allowed to stand for 3 min at room temperature , next 2ml of a 20 % sodium carbonate solution was added. The samples were vortexed and then left to stand at room temperature during 30 min. Absorbance of the clear supernatants was measured at 750 nm using a spectrophotometer; gainst a blank consisting of all reagents and solvents without extracts. The content of total phenolic was calculated using a standard curve as above prepared using gallic acid and expressed as micrograms of gallic acid equivalents (GAE).

2.3. Estimation of total flavonoid content

Total flavonoids were measured by a colorimetric assay according to Dewanto and Coll (2002). An aliquot of diluted sample or standard solution was added to a 75 µl of $NaNO_2$ solution (5%), and mixed for 6 min before adding 0.15 ml $AlCl_3$ (10%). After 5 min, 0.5 ml of NaOH was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed.

Absorbance of the mixture was determined at 510 nm against the blank where the sample was omitted. Total flavonoid content was expressed as mg per gram of DW. All samples were analysed in triplicate.

2.4. Free radical scavenging capacity in DPPH radical assay

The free radical scavenging capacity of extract solution was evaluated with DPPH assay assessed by the method of Tagashiba [8]. 0.1ml of test sample variable concentration was added to 3.9ml of DPPH solution, after vortexing, the mixture was incubated for 10 min room temperature in the darkness and the absorbance at 517 nm was measured (UV 2550 shimadzu). Triplicate measurements were carried out. The DPPH radical scavenging activity was calculated using following formula. BHA, BHT, Trolox and Rutine were the reagent used as standards.

DPPH radical scavenging activity % = $[(1-A_1/A_0) \times 100]$

Were A_0 is the absorbance of the control, A_1 is the absorbance of the sample or standard

2.5. HPLC analysis

High performance liquid chromatography was carried out for the detection of Phenolic compounds in the extract. A liquid chromatography system with high performance, Shimadzu LC 20 U equipped with universal injector (Hamilton 25 ul) SPD 20A UV-VIS detector SPD 20A (Shimadzu) was used. The effluent was detected at 300 nm. The column temperature was maintained at room temperature and the injection volume was 10µl. Before injection substance to be analyzed, the column was equilibrated for 40 to 50 min with the mobile phase [9].

3. RESULTS AND DISCUSSION

3.1. Preliminary Phytochemical screening

The Aqueous extract has shown the presence of alkaloids, flavonoids, Saponins and tannins with absence of sterols, saponins and tannins [10].The ethanolic extract has shown the presence of Flavonoids and Sterols with absence of sterols, saponins and tannins (Table 1).

Table 1. Phytochemical screening of ethanolic and Aqueous extract of *T. terrestris*

Phytochemical Tests	Ethanolic extract	Aqueous extract
Alkaloids	-	+
Flavonoids	++	+++
Sterols	+	/

Saponins	+	+++
Tannins	-	+

+ Existence - Absence

3.2 Total phenolic and flavonoid content

The total polyphenols and flavonoid contents based on the absorbance values. The amount of total polyphenols was 117.61±0.102mgAG/g and 71.05±0.0.063mgAG/g for the ethanolic and aqueous extracts respectively. The flavonoid was 81.19±0.085 mgQUE/g and 26.18±0.083 mgQUE/gfor the ethanolic and aqueous extracts respectively. Therefore, the high content of total phenols of the extract might explain the strong antioxidant properties of this plant.

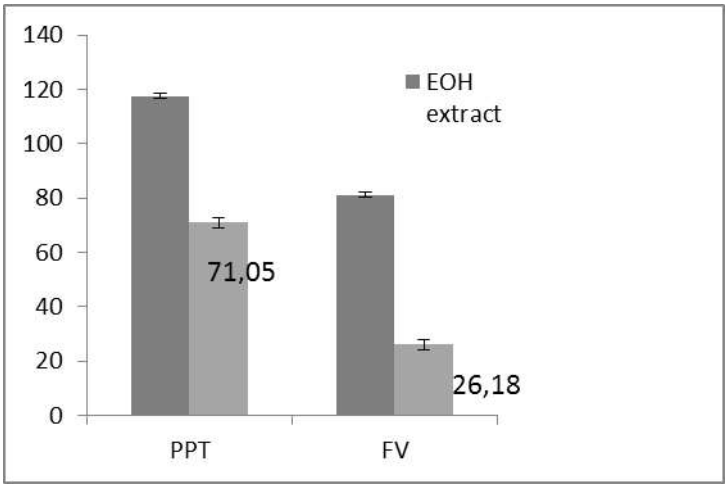

Figure 1. PPT an FV of Ethanolic and aquouse extract.

3.2 DPPH radical-scavenging activity

Diverse methods are currently used to assess the antioxidant activity of plant phenolic compounds. DPPH method is based on the reduction of DPPH in ethanol solution in the presence of a hydrogen-donating antioxidant due to the formation of the nonradical form DPPH-H in the reaction [11,12]. The reduction capacity of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants [13].

The obtained two extract of Terrestris shows a great effect on the DPPH (Figure1). This result represents the high correlation between the antioxidant activity and the concentration of polyphenols content. These results suggest that the physico-chemical nature of the individual phenolic including flavonoid glycosides in the extracts [14], may be the major contributor in the antioxidant activity [15,16] .

The results of DPPH radical scavenging capacity (Figure 02) show that both extracts have the ability to scavenge free radicals as a function of the concentration of the aqueous extract and the ethanol extract. There is a positive correlation between the concentration of the extracts and the inhibition rate of free radicals. For the ethanolic extract at the concentration (0.05 mg / ml) the best antioxidant activity was (88.20%) compared with the aqueous extract was 82.16%.

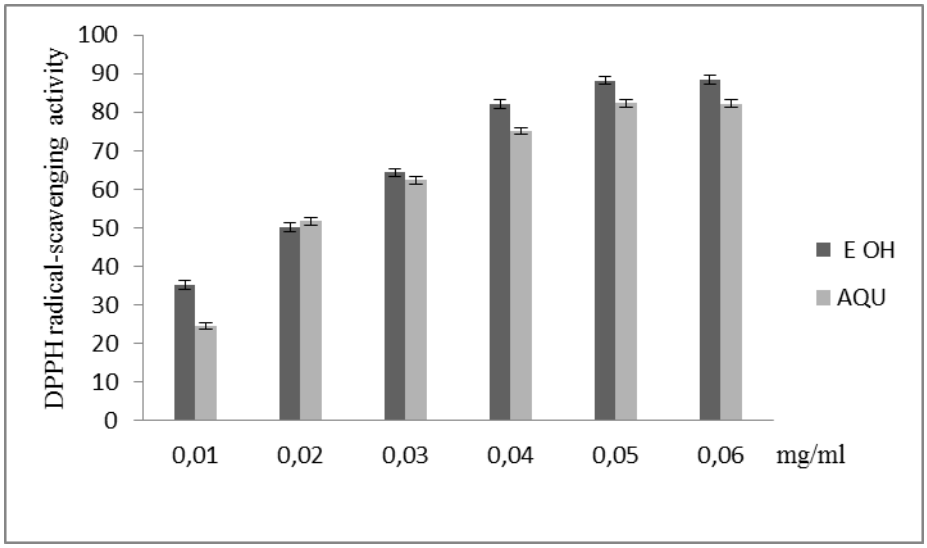

Figure 2. DPPH radical-scavenging activity.

Ethanolic extract showed a highest scavenging capacity the IC50 equal to 0.019 mg / ml while the aqueous extract is lowest was 0.023 mg / ml and IC50 of ascorbic acid was estimated to be 0.014 mg /ml. the antioxidant capacity of the Ethanolic extract and the aqueous extract is close to the capacity of ascorbic acid.

These results indicate that the antioxidant capacity of the aqueous and ethanolic extracts of the herb is average. This antioxidant effect is attributed to the amounts of phenolic compounds and flavonoids contained in these extracts. Several studies have confirmed that there is a close association between phenolic content and flavonoids and the exogenous effect of plant extracts [17]. The differences in antioxidant activity between the same plants can be attributed to environmental factors such as climate, geographical location and the temperature which can significantly affect the accumulation of antioxidant components in the plant material [18]. Also, showed that IC50 for the methanolic extract of this commercial plant was estimated at 0.65 mg / ml [19].

3.2 Determination of some phenolic compounds for plant extracts by high performance liquid chromatography (HPLC)

HPLC analysis was carried out with the Ethanolic and Aqueous extract of *T. terrestris* to detect the polyphenolic compounds present in the sample. Nine poly phenols detected by the HPLC analysis, which were identified by comparing the sample retention times with reference retention times of the phenolic compounds library at different wavelengths ranging (Table 2) and (Figure3) [20].

Table 2. HPLC based phenolic compounds identification of ethanolic and Aqu extract of *T. terrestris*

Compounds name	EOH retention time (min)	[C] µg/mg	Aqu retention time (min)	[C] µg/mg
galic	5.03	0.16	5.41	0.3
chlorogenic	13.37	8.77	13.70	0.31
vanilic a	15.35	0.15	15.60	0.11
caffiac	16.03	0.05	16.41	0.03
vanilin	21.39	0.1	21.41	0.009
coumarin	24.01	1.1	23.50	0.009
rutin	28	/	/	/
narginig	34.37	2.9	34.47	0.23
quercetin	44.89	5.05	44.89	4.54

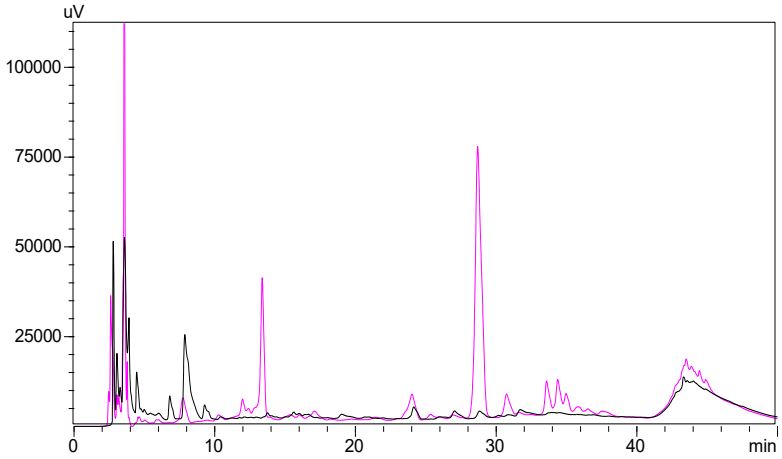

Figure 2. HPLC based phenolic compounds identification of ethanolic and aqueous extract.

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