

## Phytochemical Screening, Essential Metals Analysis and *in vitro* Anti-oxidation Activity of *Rhus Tripartita* Plant Roots

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### الملخص:

أظهر المسح الكيمونباتي لمستخلص جذور نبات الجداري وجود الفلافونويدات، الفينولات، الجلايكوسيدات، التربينات الثنائية، الكربوهيدرات، الستيرويدات النباتية، الكوينونات و الانثراكوينونات. كل الاصناف سابقة الذكر معروفة جيدا بفوائدها الطبية وهذا مايفسر شيوع استعمال النبات في الطب الشعبي. كما تم ايضا تحديد تراكيز العناصر الفلزية الأساسية و هي الليثيوم و الصوديوم و البوتاسيوم و الكالسيوم و الباريوم، حيث وجد أن تركيزعنصر البوتاسيوم كان الأعلى (0.789%) بينما كان تركيز الليثيوم هو الاقل (0.003%). تم ايضا تقدير النشاط المضاد للاكسدة للمستخلص الكحولي لجذور النبات المدروس باستخدام طريقة ازالة الجذور الحرة بمركب (1,1- DiPhenyl-2-Ppicryl Hydrazyl, DPPH)، حيث أظهر النبات نشاط عالي في ازالة هذه الجذور و بالتالي يعتبر مضاد قوي للاكسدة مقارنة بحمض الاسكوربيك (مضاد الاكسدة القياسي في هذه الطريقة)، بقيم IC 10.78 ميكروجرام للتر مقارنة بالاسكوربيك 13.49 ميكروجرام للتر. هذه الفعالية الملحوظة المضادة للاكسدة تعود غالبا لوجود المركبات الكحولية سالفة الذكر مثل الفلافونويدات والفينولات المعروفة بهذه الخاصية.

### Abstract

Phytochemical screening of *Rhus Tripartita* roots extract revealed presence of flavonoids, phenols, glycosides, diterpenes, carbohydrates, phytosterols, quinones and anthraquinones. All these organic classes are well known for their biological activity and this explains the use of this plant in folk medicine. Essential metals nutrients (Li, Na, K, Ca and Ba) levels were also determined. Potassium element concentration was the highest (0.789%) while Lithium element level was the lowest (0.003%). Anti-oxidation activity was performed by DPPH (1,1 diphenyl-2-picryl hydrazyl) radical scavenging method, the alcoholic extract of studied plant showed strong anti-oxidation activity comparing to the reference ascorbic acid, with IC<sub>50</sub> values of 10.78 µg/ml and 13.49 µg/ml, respectively. The strong antioxidant activity of ethanol extract may be attributed to the presence of anti-oxidants phytochemicals such as flavonoids and phenols and phytosterols.

**Keywords:** *Rhus Tripartita*, Phytochemical screening, Antioxidants, DPPH, Essential elements.

### Introduction

Despite of the fast development in drugs industry, folk medicine is still favored in most countries around the globe (1). In the last decades, research in natural products growing very fast leading to isolation of many biologically active compound such as, cancer and AIDS drugs and antibiotics agents (2). The Mediterranean region is very rich area with medicinal plants and one species of the widely spread in this area is *Rhus* species, especially in North Africa (3). *Rhus* species is well known for its nutritional and medicinal uses (4). The center of this study, *Rhus Tripartita* plant (Figure 1) is a member of *Rhus* family, grown in North Africa and known locally with Jdari name (5).



Figure 1: Photo of the *Rhus Tripartita* plant

*R. Tripartita* plant was reported to exhibit anti diarrhea, anti-ulcer, hepatoprotective and cardio protective properties (6), in addition to anti tumorigenic, hypoglycemic and antimicrobial activity (7).

Molecular Oxygen ( $O_2$ ) is one of the metabolism process products in living cells, the process also leads to production of very harming oxygenated species such as peroxides and hydroxide radicals (8). However, the cells start using self-defense strategy by producing agents work as antioxidants to tackle this problem, otherwise, damage effect of these reactive radical would lead to chronic diseases such as diabetes and Alzheimer (9).

Finding cheap, safe and sustainable sources of natural anti-oxidation agents became important target of nutrition and drugs researchers. Different protocols were used to evaluate anti-oxidation activity of both pure compounds and plants extracts, using DPPH method in one of the simplest and effective methods and was chosen to be used in this research (10).

During plant growth process, sufficient supplements of basic nutrients has an important role, deficiency of these elements lead to slow growth and poor production (11). Potassium, Sodium, Lithium, Calcium and Barium are important basic nutrients for the plant to perform necessary bioprocesses (11). In the present research, an attempt was made to explore the medicinal value of *Rhus Tripartita* roots by determining the phytochemical and metal composition, and Anti-oxidation activity present in the root.

**Materials and Methods**  
**Plant Material Collection**

Roots of *Rhus Tripartita* Plant were collected from south of Gaser Khiar rocky region (32°46'26"N 13°46'56"E) in summer of 2020. The roots were identified by a specialist taxonomist from Botany department, Arts and Science faculty, Elmergib University.

**Extraction**

The roots were cut to small pieces then grounded to fine powder using domestic blender. 3.0 g of the powder and 100 ml of the solvent was put in 250 ml flask then stirred for 48 hours using Jenway 1002 Stirrer machine at room temperature to keep most of the low boiling point components. Different polarity solvents were used to guarantee extraction of all types of chemical classes, the solvents were; ether, chloroform, ethyl acetate and methanol. Aqueous extract was prepared by heating 2.0 g of the powder in 100 ml of water at 70 °C for 20 min., after cooling the mixture was filtered and kept in fridge. The extracts were filtered then concentrated using water bath then kept until using in phytochemical screening process illustrated by Harborn (12).

**Dry Digestion**

Roots powder was divided to five parts (A1 to A5) for more accurate results. 1.0 g of dry roots dry was turned into ash using furnace at 550 °C for 5 hours, the ash was transferred quantitatively to 250 ml flask using distilled water, 50 ml of HNO<sub>3</sub> 1M was added then the solution was warmed until dissolving all solid material then completed with distilled water till the mark (13). Calculations performed using percentage by sample weight method. Determination of elements concentrations was performed using flam photometer BWB Technologies, UK.

**Antioxidant Activity Method**

Free radical scavenging activity of *Rhus Tripartita*. plant extract was measured by 1, 1-diphenyl-2-picryl hydrazyl (DPPH). 0.1 mM solution of DPPH in ethanol was prepared. This solution (1.0 ml) was added to 3.0 ml of different extracts in ethanol at different concentration (5, 10, 15, 20, 25, 30 µg/ml). Only those extracts are used which are solubilize in ethanol and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm by using UV-VIS spectrophotometer (Shimadzu).

Reference standard compound being used was ascorbic acid and experiment was done in triplicate. The IC<sub>50</sub> value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using following equation:

DPPH scavenging effect (%) or Percent inhibition =  $A_0 - A_1 / A_0 \times 100$ .

Where A<sub>0</sub> was the Absorbance of control reaction, A<sub>1</sub> was the Absorbance in presence of test sample (14).

## Results and Discussion

As shown in Table 1, Phytochemical screening investigation revealed that, the plant is very rich with biologically important chemical classes, the investigation includes different tests for each chemical class to ensure the presence or absence of components. Exceptionally, from all known natural products, nitrogen containing molecules, alkaloids and amino acids were absent as all tests for all extracts were negative.

Table 1: Phytochemical screening *R. Tripartita* roots extracts

Extract		Aqueous	Chloroform	Ethyl acetate	Methanol	Ether
Test						
Alkaloids	Mayer	-	-	-	-	-
	Wagner	-	-	-	-	-
	Dragendorf	-	-	-	-	-
	Hager	-	-	-	-	-
Carbohydrates	Molisch	+++	++	++	+++	+++
	Benedict	+	-	++	+	++
	Fehling	++	-	-	++	+++
Glycosides	Borntrager	+	++	++	-	+
	Keller-Kelaini	++	+	+++	++	+
Saponines		+++	+	+	+++	+++
Phytosterols	Salkowsky	+++	++	++	+	+
	Liebermann	+++	+	+++	+++	+
Phenols		+++	-	+++	+++	+++
Tannins	Lead acetate	+	+	++	++	+
	Gelatin	+	++	++	+	+
Phlopatannins		-	-	-	+	-
Flavonoids	Basic Test	-	++	+	-	+
	Lead acetate	-	+	++	-	+
	shinoda	-	-	+++	-	+
Proteins	Millon	-	-	-	-	-
	Biuret	-	-	-	-	-
	Xanthoprotien	-	-	-	-	-
	Ninhydrine	-	-	-	-	-
Terpenes		++	+	+	++	-
Diterpenes		+++	-	+++	+++	+++
Quinones		++	-	-	+++	+++
Anthraquinones		+	-	-	-	-

(-): absence of compound; (+): low compound content (++) : medium compound content; (+++): High compound content

The important nutrient, carbohydrates were present clearly in all extracts, Glycosides were found in all extracts clearly, except for methanolic extract using Borntrager test, glycosides are used in cardiac muscles activation and strengthen blood vessels, in

addition to treatment of arthritis (15). Phytosterols were found in all extracts clearly, phytosterols may act as an adjuvant in the prevention and treatment of cardiovascular diseases (16).

Positive forth test indicates presence of saponines in all extracts, saponines are very important components of tooth paste and shampoo manufacturing (17). Tannins were observed in all extracts applying both Lead acetate and gelatin tests, tannins are working as anticancer, virucides, antioxidant, antimicrobial and anti-inflammatory agents (18).

Conversely to the tannins, phlopatannins were absent from all extracts except methanolic extract. Phenolic compounds, flavonoids were observed clearly in ethyl acetate extract, in addition to ether and chloroform extracts, many flavonoids are exhibited with antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities (19). In addition to the alkaloids, the other nitrogen containing molecules, the proteins, were absent from all extracts. The non-polar class, terpenes was found in all extracts except ether extract, while diterpenes were absent only from chloroform extract. Recently, modern medication uses large scales of terpene for various treatment drugs (20).

Anthraquinones were only observed in aqueous extract, while quinones were present in aqueous, methanolic and ether extracts, anthraquinone derivatives have been used as laxatives and antimicrobial and anti-inflammatory agents, in addition to treatment of constipation, arthritis and cancer (21).

### **Antioxidation activity Evaluation**

Roots ethanolic extract of *R. partita* plant showed better antioxidant potential when compared with the standard, ascorbic acid by DPPH scavenging assay method. The better antioxidant agents should have lower IC<sub>50</sub> value, especially comparing with well-known super scavenging agent, ascorbic acid (22). IC value of studied plant was 10.78 µg/ml, lower than ascorbic acid with 13.49 µg/ml and indicates that, *R. Tripartita* roots is a strong and promising natural anti oxidation agent. The obtained result for roots extract directs to the fact that, pure isolated compounds from this extract would show higher scavenging effect. The IC value mostly attributed to the high content of phenols and flavonoids as shown in phytochemical screening results.

### **Essential Metals Levels**

As shown in Table 2, Potassium element have the highest percentage (A3 sample, 0.789 %), K activating many enzymatic processes, such as protein synthesis, sugar transport, nitrogen and carbon metabolism, and photosynthesis, in addition, Potassium is crucial for cell growth (23), while Li was the less element in the roots powder (0.003%).

Table 2: Basic elements levels in roots of *R.Tripartita*

Sample No.	Na%	K%	Li%	Ca%	Ba%
A1	0.125	0.714	0.005	0.696	0.241
A2	0.116	0.785	0.003	0.739	0.152
A3	0.120	0.789	0.005	0.725	0.548
A4	0.119	0.719	0.005	0.739	0.221
A5	0.121	0.766	0.005	0.725	0.257

Na concentration of all samples was similar and low comparing to Potassium, A1 sample was the highest (0.125 %). Calcium was found with reasonable percentage (0.696% - 0.739%), Barium level was poor but still higher than sodium, A3 was the richest sample with barium (0.548 %).

### Conclusion

*Rhus Tripartita* roots could be used as a natural pool for biologically active compounds as revealed from phytochemical screening, free radical scavenging capacity was done in excellent manner by ethanolic extract, the results motivate researchers to work on isolation of pure compounds and examine them as potential strong anti-oxidants agent.

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