Phytochemical Screening and Antioxidant Activity of *Laurus nobilis* L.

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ABSTRACT

Reactive oxygen species (ROS) are high reactive molecules involved in many physiological processes and have been associated with many diseases, such as cancer, diabetes, cardiovascular, inflammatory and neurodegenerative diseases. Nowadays, there is an increasing interest in discovering natural antioxidants for use in food and medicinal materials to replace synthetic antioxidants since such antioxidants are being restricted due to their side effects like carcinogenicity. Many studies suggested on medicinal plants have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems. *Laurus nobilis* L. (bay leaves) which commonly known since ancient times as daphne tree, belongs to Lauraceae family, being a native plant from the Mediterranean region. It's a plant of industrial importance, used extensively in the food industry as well as in drugs and cosmetics. There are many investigations on antibacterial and antioxidant activities of the essential oil obtained from *Laurus nobilis* L. However, in Morocco still almost little work has been done about phytochemical screening, polyphenolic compounds and antioxidants activity of this important plant. In this study, we have determined phytochemical compounds include tannins, flavonoids, alkaloids and saponins with different standard phytochemical methods. Total phenolic was estimated by Foline-ciocalteu method, total flavonoid content was determined by colorimetric method. Hydroacetonic (70% acetone) and hydroalcoolic extracts (70% ethanol) with maceration for one hour at room temperature with stirring were also monitored by their antioxidant ability by using different in vitro methods (DPPH and ABTS). These preliminary results suggest that *Laurus nobilis* L., is a promising source of natural products including phenols, flavonoids and antioxidants that could offer protection against oxidative stress, and can reduce free radicals and prevent chronic diseases.

Keywords: Antioxidant activity, *Laurus nobilis* L., phenols, phytochemical screening, total flavonoid.

INTRODUCTION

Reactive oxygen species (ROS) such as hydrogen peroxide (H2O2), superoxide radical (O2-), hydroxyl radical (OH ˚) and singlet oxygen (O2) have a high capacity to damage various types of cellular components in the body, causing many degenerative diseases. Supplementation of exogenous antioxidants in the body is very useful against these harmful species. Plants naturally are a rich source of secondary metabolites and novel therapeutic compounds. These compounds are well known for their various beneficial effects on human health . *Laurus nobilis* is an evergreen shrub belongs to the Lauraceae family and is native to the Mediterranean region it's used in tradional Moroccan medicine and as condiments. It is reported that this plant is a rich source of bioactive molecules, such as phenolic compounds and essential oil addition to their use as conservatives. In recent years, the antioxidant compounds are the subject of many researches because, in in the foodstuffs by replacing synthesis antioxidants, they intervene in the treatment of many diseases.

OBJECTIVE:

The study of phenolic compounds and the evaluation of the antioxidant properties of the plant *Laurus nobilis* L.

Figure 1: *Laurus nobilis* L. plant (a: Shurbs, B: Flowering C: Fruit) present in northern of Morocco.

MATERIAL AND METHODS

Plant Material

Laurus nobilis L. bay leaves and shoots were collected from female tree in November 2016 from the region of Tangier-Tetouan in the northeast of Morocco (Fig.1). The samples were cleaned manly to remove all foreign materials. Origins, locality, latitudes, longitudes and altitudes are presented in Table 1. The plant material authenticated and confirmed by Dr. Ahmed Lamarti, a specialist in plant physiology, Faculty of sciences, Abdelmalek Essaadi University Tetouan, Morocco.

Figure 2 : Garmin GPSMap Handheld GPS

Table 1. Geographic coordinates (latitude and longitude) and altitudes of laurel trees in Tazia regions from Moulay Abdeslam commune of Tangier-Tetouan.

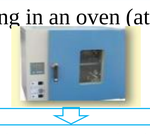
Locality	Latitude	Longitude	Altitude (m)
Tazia, Tetouan, Morocco	N 35°21.652'	W005°3 .739'	731 m

Figure 3 : Geographic localisation of the studied site in Moulay Abdeslam commune of Tetouan region, Morocco

Preparation of extracts

Healthy leaves (harvested in November 2016)

Drying in an oven (at 50 °C)



Grinding and sieving

Sample preparation (Powder is composed of particles whose size is 0.2 mm in diameter)

Figure 5: Equipment used for the preparation of powder

Figure 4 : The leaves of *Laurus nobilis* L.

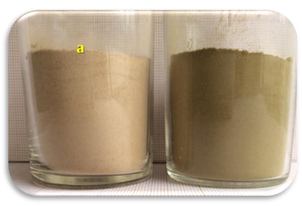


Figure 6: Powder of leaves (a) of *laurus nobilis* L.

Maceration extraction

Homogeneous powder

Maceration of dry plant material in extraction solvent.

Extraction for 1h at room temperature with stirring

Centrifugation for 15 min at 5000 rpm.

Recovery of the crude extract

Phytochemical Screening of extract

Phytochemical analysis *of Laurus nobilis* L., were carried out by using various standard methods references with slight modifications. The qualitative results are expressed as (+) for the presence and (−) for the absence of phytoconstituents.

Colorimetric determination of polyphenolic constituents in hydroethanolic and hydracetonic extracts.

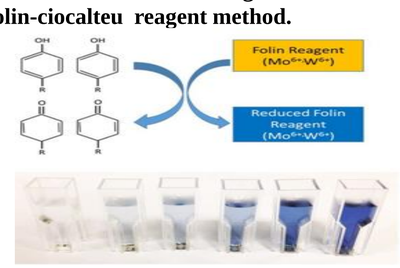
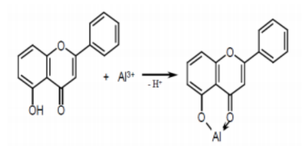
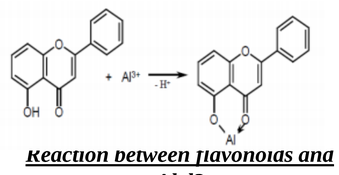
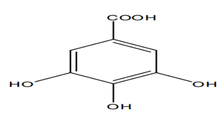
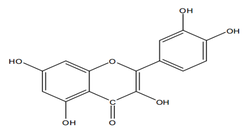
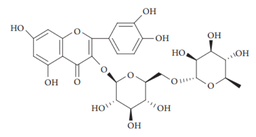
Bioactive compounds and methods of assay		
Total polyphenols	**Total flavonoids**	**Total flavonols**
❖ **Determination according to the Folin-ciocalteu reagent method.**	❖ **Determination according to the aluminum trichloride method.**	❖ **Determination according to the aluminum trichloride method.**
		
Folin-ciocalteu reagent method	***Reaction between flavonoids and Alcl3***	***Reaction between flavonoids and Alcl3***
❖ Gallic acid (AG) as standard	❖ Quercetin (Q) as standard	❖ Rutine (R) as standard
		
Chemical Structure of Gallic Acid (3,4,5-Trihydroxybenzoic acid)	**Chemical structure of Rutine**	**Chemical structure of Quercetin**

Figure 6: UV-visible spectrophotometry

In order to control and analyze these compounds, we opted for molecular absorption spectrophotometry.

Expression of results

❖Quantification was done according to a calibration curve by the linear regression equation (y = ax + b).

❖ The results are expressed in milligram equivalents of the standard used (AG, Q and R) per gram of dry matter (ms) studied (mg EE / gms).

In vitro Antioxidant Activity

DPPH Free Radical Scavenging Activity

❖DPPH radical scavenging activity is a very simple, reproducible and rapid used method to measure antioxidant capacities of natural product.

❖This test is based on the capacity to scavenge synthetic free radical 2.2-diphenyl-1-picryhydrazyl to react with hydrogen donors (figure). The color changes from purple to yellow after reduction with an antioxidant (AH) or radical (R.).

❖ The percentage

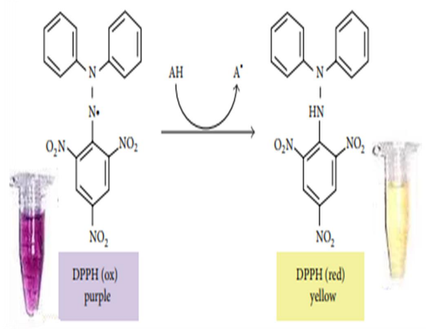


Figure: Reaction of a hydrogen donor (antioxidant) with the radical DPPH ●

ABTS free radical scavenging activity

❖This assay is based on the neutralization of ABTS·+ radical, a green chromophore by antioxidant of plant extract in a dose response curve. This reactions involves the electron-donating ability by the synthetic chromophore 2, 2 – azino-bis (3 ethylbenzothiazoline-6- sulfonic acid) and results in the decolorization of the radical (Figure).

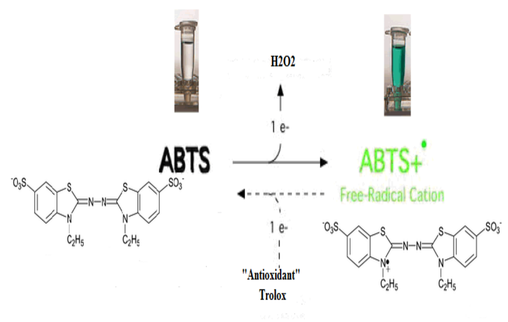


Figure : Chemical reaction between the ABTS radical and the antioxidant compound

Statistical Analysis

All measurements were run in triplicates (n =3) and the values were averaged and given along with standard error (±SE). Analyses were performed with IBM SPSS Statistics 20, averages were compared by Duncan test and values beyond $p \leq 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Phytochemical Screening of Extract

Table 2: Phytochemical constituents of different parts of *laurus nobilis* L.

Phytochemical tests	Extraction solvents	Female tree	
		Leaves	shoots
Terpenoids	70% Ethanol	+++	+++
	70% Acetone	+++	+++
Saponins	70% Ethanol	+++	+++
	70% Acetone	+++	+++
Phlobatannins	70% Ethanol	+++	+++
	70% Acetone	+++	+++
Resins	70% Ethanol	+++	+++
	70% Acetone	+++	++
Emodins	70% Ethanol	-	+
	70% Acetone	+++	+
Starch	70% Ethanol	-	-
	70% Acetone	+	+
Proteins	70% Ethanol	+	+
	70% Acetone	+	+++
Tannins	70% Ethanol	+++	+++
	70% Acetone	++	++
Quinones	70% Ethanol	+	+
	70% Acetone	+++	+++
Anthraquinones	70% Ethanol	+	++
	70% Acetone	++	+++
Alkaloids	70% Ethanol	++[a]	+++
		+++[b]	++
	70% Acetone	++[a]	+++
		+++[b]	++
Flavonoids	70% Ethanol	+++	+++
	70% Ethanol	+++	+++
Glycosides	70% Ethanol	+++	++
	70% Acetone	+++	++

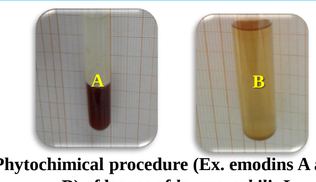
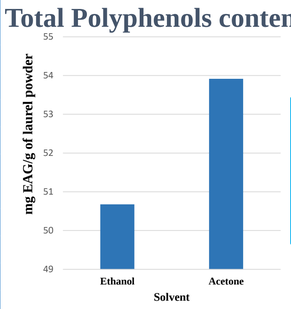


Figure: Phytochimical procedure (Ex. emodins A and glycosides B) of leaves of *laurus nobilis* L.

❖The results suggest that this plant is a rich source of secondary metabolites, such as. The present investigation has shown that starch was present in all samples, but the previous research studies showed that starch was absent in it [1].

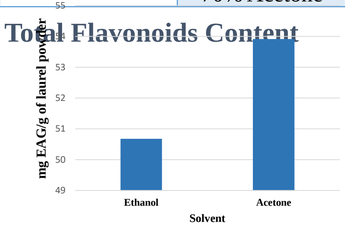
Total Polyphenols content



❖ For acetone extracts, TPC was 53.917mg GAE/g. Powder from female tree (shoots) was the richest in polyphenol **Figure**.

❖For ethanol extracts, TPC was 50.677 mg GAE/g. Powder from shoots female tree was also the richest in polyphenol followed by acetonic extracts **Figure**. These results were according with other studies [4, 5, 2, 15, 4,5] but with using others solvents and methods of extractions.

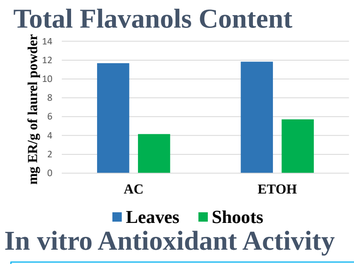
Total Flavonoids Content



❖For acetone extracts, TFC was 18.60 mg QE/g. Powder from leaves of female showed the highest TFC

❖For ethanol extracts, TFC was 19.267 mg QE/g. Powder from leaves of female tree showed the richest in TFC, **Figure**. These results were according with other studies [4,5] but using others solvent and methods of extraction.

Total Flavanols Content



❖For acetone extracts, TFC was 11.670 mg QE/g. Powder from leaves of female showed the highest TFC

❖For ethanol extracts, TFC was 11.840 mg QE/g. Powder from leaves of female tree showed the richest in TFC. These results were according with other studies [4,5] but using others solvent and methods of extraction.

In vitro Antioxidant Activity

❖The antioxidant activity of laurus nobilis L. was evaluated by two in-vitro antioxidant methods: DPHH free radical scavenging and ABTS free radical scavenging activity. The results were shown in Table 3 and Table 4.

DPPH Free Radical Scavenging Activity

Table 3: % inhibition measured by the DPPH method for 0-5 min and 5-10 min.

Sample	% inhibition (0-5min)		% inhibition (5-10min)	
	Acetone extract	Ethanol extract	Acetone extract	Ethanol extract
Leaves	21.905±0.952	20±1.429	24.761±0.952	22.381±1.259

❖ Antioxidant, or free radical scavenging, activities of the extracts of *laurus nobilis* L. were determined using DPPH radical scavenging assay. The results are displayed in table 1. In the assay, the both extracts showed a slightly higher activity. The others studies suggest the antioxidant activity by DPPH but with others solvents and method of extraction [2, 3, 4, 5].

ABTS free radical scavenging activity

The results are shown in table 2. The hydroacetonic extract displayed the highest antioxidant capacity in this assay, followed by the hydroethanolic in leaves of *laurus nobilis* L. These results were according with other studies but by using others solvents and methods of extractions [2,3].

Table 4: % inhibition measured by the ABTS method for 0-3 min and 3-6 min

Sample	% inhibition (0-3min)		% inhibition 3-6min)	
	Acetone extract	Ethanol extract	Acetone extract	Ethanol extract
Leaves	24.468±0.614	22.859±1.343	26.950±0.354	24.822±0.938

CONCLUSION

❖The present study confirms the various class of phytochemical of *laurus nobilis* L. and their total phenolic and flavonoid content. These *in vitro* assays indicate that all extracts of *laurus nobilis* are a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. It should be also noted that polyphenols contents were positively and statistically significantly correlated with the antioxidant activity of the studied extracts. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the *in vivo* antioxidant activity of theses extracts needs to be assessed prior to clinical use.

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