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Determination of Polyphenolic Compounds of *Washingtonia robusta* H. Wendl Extracts

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The aim of this study is to screen and to evaluate total amount of flavonoids and polyphenols methanolic extracts from *Washingtonia robusta* H. Wendl leaflets, rachis and roots. *Washingtonia robusta* leaflets and roots were powdered and subjected to extraction with 80% methanol solution and screened by various chemical tests and TLC studies. The total amounts of polyphenols and flavonoids were determined by the modified colorimetric method using the Folin-Ciocalteu reagent. Phytochemical analysis revealed the presence of flavonoids, tannins, coumarins and quinons. Alkaloids were not detected. In leaflets, the total amount of flavonoids is higher than in rachis and roots. However, the polyphenolic content is nearly the same in all parts of plant.

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1. Introduction

The therapeutic use of plants is practiced since old times and it is experiencing a resurgence of interest among the population. It is possible to use the whole plant or the extract of products that they provide. In the socio-economic context of developing countries, the study of plants can result in obtaining adequate therapeutic responses and low prices, joining scientifically proven efficacy and optimal cultural acceptability. The scientific development of traditional medicine in particular should lead to the development of herbal medicines.

Flavonoids and other phenolic compounds of plants have been known for their property of free radical scavengers. Hence, currently search for natural antioxidant source gains much importance.

Washingtonia robusta H. Wendl belongs to the Arecaceae family, represented by 200 genera [1] and about 3000 species, divided into six sub-families; the subfamily Coryphoideae is in turn divided into three tribes. Washingtonia genus belongs to the tribe Corypheae and includes two species: *Washingtonia filifera* and *Washingtonia robusta*. This later is native to northwestern Mexico. It is reportedly naturalized in Spain, the Canary Islands and has been cultivated in Algeria for its adaptation to climate and soil.

The chemical composition of the Arecaceae plants includes: diterpenes, triterpenes, steroids, proantocianidines, flavonoids (kaempferol, quercetin, tricin and luteolin), saponins and rarely alkaloids [2]

In this paper extracts of *W. robusta* grown in Algeria were screened for their chemical composition and polyphenols contents.

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2. Materials and methods

This study is carried on *Washingtonia robusta* H Wendl, grown in natural conditions in the littoral of the West Region of Algeria.

2.1. Extraction

Dried and crushed leaflets, rachis and roots (5 g) were subjected to extraction for 1 h in 100 ml of aqueous methanol (80%) by reflux method. The extraction was repeated twice and the extract was filtered through nylon tissue. Each filtrate was concentrated to dryness under reduced pressure using a rotary flash evaporator. Finally the dry extracts were lyophilized and then stored in refrigerat for further analysis.

2.2. Analyses

The total phenolic content of the extracts was measured using the modified Folin-Ciocalteu method. 100 μl of the sample were added to 150 μl of Folin Ciocalteu and aqueous sodium carbonate Na_2CO_3 (2%). The mixture was incubated for 1 h and the total amount of phenols was determined using a spectrophotometer (UVIKONUV/VIS) at 765 nm. All determinations were carried out in triplicate. The total phenolic concentration was expressed as gallic acid equivalents (GAE).

The flavonoid content was measured using the colorimetric method adapted by Kim (2003). The methanolic extract (500 μl) was mixed with 1500 ml of distilled H_2O and 150 μl of a 5% $NaNO_2$ solution. 150 μl of a 10% $AlCl_3$ solution was added. About 500 μl of 1 M NaOH were added to the mixture, the absorbance was measured at 510 nm using a UVIKON UV/VIS spectrophotometer. All determinations were carried out in triplicate. The total flavonoid concentration was expressed as catechins equivalents (CE).

Tannins and quinons were screened by specific reagents.

Test for tannins: few drop of ferric chloride test reagent were added. An intense green, purple, blue or black colour developed was taken as an evidence for the presence of tannins.

Test for quinons: the presence of free quinons is confirmed by the changing of the color to red after adding a few drops of 1/10 NaOH.

Qualitative analysis by fluorescence TLC was performed to separate alkaloids, coumarins and flavonoids. Revelation is made by spraying the Neu's reagent (2-aminoethyl-diphenyl borate) for identifying coumarins and flavonoids. The presence of alkaloids is proved by spraying the Draggendorff's reagent (Potassium bismuth iodide solution).

3. Results and discussion

3.1 Screening

The phytochemical composition of methanolic extracts of leaflets, rachis and roots of *W. robusta* showed the presence of tannins, quinons, coumarins and flavonoids.

The plant tested negative for the presence of alkaloids in all organs (Table I).

TABLE I

Phytochemical screening of *W. robusta* leaflets, rachis and roots.

Constituents analyzed	Leaflets	Rachis	Roots
Polyphenols	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Quinons	+	+	+
Coumarins	+	+	+
Alkaloids	−	−	−

+: Present −: Absent

The presence of these bioactive substances in the extracts, which may act synergistically, could be responsible for the pharmacological properties of the leaflets, rachis and root extracts. Tannins and flavonoids are actually reported to show antimicrobial activities [3].

3.2 Total phenolic and flavonoid contents

The content of total phenols is expressed as gallic acid equivalents. The results showed that the total phenolic content in the leaflets was 24.5 mg of GAE per gram of extract, which is lower than in rachis and roots, where it was 27 mg of GAE per gram of extract.

Results from Table II show that the maximum flavonoid content was found in leaflets 32.2 mg CE g^{-1} of extract, which was followed by rachis 27.4 mg CE g^{-1} of extract, but it was lower in roots, as compared to the other parts.

The chromatographic TLC profiles of methanolic extract of the *W. robusta* leaflets suggested the occurrence of a high content of flavonoid in this species.

TABLE II

Total polyphenolic and flavonoids content in leaflets, rachis and roots of *W. robusta*.

Content	Leaflets	Rachis	Roots
Total polyphenols (mg GAE g^{-1})	24.5	27.1	27.8
Total flavonoids (mg CE g^{-1})	32.2	27.4	18.6

The flavonoids were identified by comparison of their *Rf* and data from the literature. Flavons, rutin, kaempferol, quercetin and luteolin were found out in *W. robusta* leaflets. Flavone C-glycoside and kaempferol were identified in *W. robusta* and *W. filifera* [5]. Luteolin 7-*O*-glucoside was identified in *W. filifera* [6]. Polyphenols have been reported to be responsible for the antioxidant activities of botanical extracts [7]. It was reported that *W. filifera* has an antiviral activity [8].

The analysis of TLC chromatogram of coumarins shows the presence of several spots corresponding to coumarins after spraying with Neu reagent. These are furanocoumarins and pyranocoumarines, which were detected only in roots.

The further studies have to be carried out for isolation and purification of molecules in the extract and assessing the efficacy.

4. Conclusion

Phytochemical analyzes of extracts of *W. robusta* show that this specie is rich in secondary metabolites. The content of flavonoids and polyphenols varies from one plant organ to another. The detected phenolic compounds are known for their industrial and medicinal importance. We suggest that *W. robusta* can be used in the medicinal field.

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