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Phytochemical study of *Thymus fontanesii* and *Laurus nobilis*

Farah Haddouchi, Tarik Chaouche, Abdelhafid Benmansour and Hamadi Abderrahmane Lazouni

Laboratory of Natural Products, Department of Molecular and Cell Biology, Tlemcen, ALGERIA

ABSTRACT

Our work aims at studying tow medicinal and aromatic plants very well by the local population. They are called Laurus nobilis L (Laurel) and Thymus fontanesii (Thyme). The phytochemical tests, which had been done while studying these plants permitted to us to detect different families of the chemical compounds existing in both of these plant's leaves.

Keywords: *Thymus fontanesii*, *Laurus nobilis*, Phytochemical tests, Essential Oils.

INTRODUCTION

This study focuses on the phytochemical study of two species. These *Thymus fontanesii* of the family *Labiateae* and *Laurus nobilis* of the family *Lauraceae*. Secondary metabolites are widely used by humans at least since antiquity for their medicinal, culinary and fragrant. Their use however has always been practiced empirically [1, 2]. We chose to study these two plants for the following reasons:

- These are wild plants, very abundant in western Algeria;
- They are widely used in herbal medicine especially as antiseptic, antispasmodic and expectorant;
- Today, they are used as a condiment;
- They contain flavonoid glycosides and what is not without drawbacks from the toxicological
- They are rich in essential oils that have antioxidant, antibacterial, antifungal, anti-inflammatory and insecticide.

MATERIALS AND METHODS

The plants studied were collected in March 2010 in the region *Nedroma* located at an altitude of 450 m and 65 km north of *Tlemcen* (Algeria).

Phytochemical tests are performed on different extracts prepared from the dried leaves and ground, using five solvents of different polarities: Water-Ethanol-Chloroform-diethyl ether-

petroleum ether. They are generally simple, quick to implement, performed mostly in test tubes. The detection method of the different families of chemical compounds co-existing is a precipitation reaction or staining reagents. These reactions result in the appearance of turbidity, flocculation or a color change which may, depending on the intensity of the result, the concentration of certain constituents [3,4,5].

1. Plant products sold with ethanol

Monocle in a balloon, fitted with a condenser, put 50 g of plant material with 300 ml of ethanol. Wear under reflux for 1h. Strain the mixture, then subjecting the ethanol extract to the following tests:

1.1. A1kaloids

Evaporate 20 ml of ethanol solution to dryness. Add 5 ml of 2N HCl to the residue and heat in a water bath. Strain the mixture and divide the filtrate into two equal parts. Treat the first with a few drops of Mayer's reagent and the second with Wagner's reagent. Observation: turbidity or precipitation.

1.2. Flavonoids

Treat 5 ml of alcoholic extract with a few drops of concentrated HCl and 0.5 g of magnesium turnings.

1.3. Tannins

1 ml of alcoholic solution, add 2 ml of water and 2-3 drops of diluted solution of FeCl3. A positive test is revealed by the appearance of a blue color - black, green or blue - green and a precipitate. According to the tannins are catechism, gallic or ellagic.

1.4. Reducing compounds

Treat 1 ml of the ethanol extract with 2 ml of distilled water and 20 drops of Fehling's solution and then heated. A positive test is revealed by the formation of a precipitate - red brick.

1.5. Anthracénosides

Treat 8 ml of the ethereal solution by extractive reagent Bornträger. A positive test is revealed by the appearance of a color ranging from bright orange - red to purple - purple.

1.6. Anthocyanosides

Metering the acidic aqueous solution with NaOH. If there is a color change depending on the pH, the presence of anthocyanins was confirmed. 3 the water turns red.<- PH,
pH 6, the water turns< <- 4 blue.

1.7. Coumarins

Evaporate 5 ml of the ethereal extract solution. Dissolve the residue in 1 to 2 ml of warm water. Divide the volume into two parts. Take half as a control and add volume to another volume of 0.5 ml of NH4OH (10%). Putting two spots on filter paper and examined under UV light fluorescence intensity indicates the presence of coumarins.

1.8. Sterols and steroids

Evaporate the alcoholic extract equivalent to 10 ml and then dissolving the resulting residue in 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Treat the filtrate with Liebermann's

reagent Burchardt. If a solution is blue - green appears, it indicates the presence of glycosides.

2. Plant products exhausted with hot water

In a flask fitted with a condenser, put 50 g of plant material with 300 ml of water. Wear under reflux for 1h. Strain the mixture and subjecting the aqueous extract to the following tests:

2.1. Starch

Treating 5 ml of solution prepared with the reagent for starch. The appearance of a purplish blue color indicates the presence of starch.

2.2. Reducing compounds

Add 2 ml of aqueous solution 5-8 drops of Fehling's solution, heat the solution. A brick red precipitate marks the presence of carbohydrates.

2.3. Saponins

Add 2 ml of the aqueous solution with a little water and then stir in a strong way. A persistent foam confirms the presence of saponins. Abandon the mixture for 20 minutes and classify content saponosides:

- *No foam = Negative test*
- *Foam less than 1 cm = weakly positive test*
- *Moss 1-2 cm = positive test*
- *Foam over 2 cm = very positive test*

Index of foam:

In a flask fitted with a condenser, put 2 g of dried and ground plant material in the presence of 100 ml of water. Wear under reflux for 30 min. After cooling and filtration, the volume is adjusted to 100ml. From this stock solution, prepare 10 tubes (1.3 cm internal diameter) with 1.2, ..., 10ml, the final volume being adjusted to 10ml with distilled water. Each tube is shaken vigorously in a horizontal position for 15 seconds. After standing for 15 minutes in an upright position, there is the persistent foam height in cm. If it is close to 1 cm in the tenth tube, then the foam index is calculated by the following formula:

I = foam height (in cm) in the tenth tube x 5 / 0.0x

2.4. Tannins

Treat 1 ml of the aqueous solution with 1 ml of water and 1-2 drops of dilute solution of FeCl3. The appearance of a dark green color or blue-green indicates the presence of tannins.

2.5. Alkaloids

Place 15 ml of aqueous extract in a flask Bicol. Add 10% NH4OH to pH = 9. Extract with 3x10 ml of chloroform. Wash the chloroform solution with 3x2 ml of HCl 10%. The aqueous wash is divided into three equal parts. Test samples with reagents Mayer and Wagner. The third tube is considered a witness.

3. Plant products sold with diethyl ether

In a flask, topped with a reflux condenser, put 50 g with 300 ml of diethyl ether. Wear under reflux for 1h. Strain the mixture and subjecting it to various tests:

3.1. Volatile oils
Evaporate 20 ml of ethereal. The residue was dissolved in ethanol. The ethanolic solution obtained was then concentrated to dryness. A positive test is revealed by obtaining a residual aroma.

On the greasy residue, it is saponified at the end of the reaction add a little water and extract the solution with diethyl ether.

3.2. Fatty acids
Acidifying the alkaline aqueous solution, then extracted with diethyl ether. The ethereal solution is then concentrated to dryness. A positive test is revealed by obtaining a greasy residue.

3.3. Alkaloids
Evaporate 10 ml of the ethereal solution. Dissolve the resulting residue in 1.5 mL of HCl 2%. Add to alkaline aqueous solution from 1 to 2 drops of reagent Mayer. The formation of a yellowish white precipitate indicates the presence of alkaloid bases.

4. Plant products exhausted with petroleum ether
4.1. Quinones free
One gram of dried and ground plant material is placed in a tube with 15 to 30 ml of petroleum ether. After stirring and standing for 24 h, extracts were filtered and concentrated on a rotary. The presence of free quinones is confirmed by adding a few drops of NaOH 1 / 10, when the aqueous phase turns yellow, red or purple. In a flask, topped with a reflux condenser, add 5 g in the presence of 30 ml of petroleum ether. Wear under reflux for 1h. Strain the mixture and subjecting it to various tests:

4.2. Sterols and triterpenes
Evaporate 10 ml of ether extract. Dissolve the residue in 0.5ml of acetic anhydride and 0.5ml of chloroform. Add 2 ml of concentrated sulfuric acid. A red-brown or purple ring in the contact zone with the supernatant or purple color indicates the presence of sterols and triterpenes.

4.3. The polyuronides
10ml of ethanol are placed in a test tube; 2 ml of the ether extract are added dropwise. The appearance of a purple or blue precipitate indicates the presence of mucilage.

5. Plant products exhausted with chloroform
In a flask fitted with a condenser, add 5 g of root in the presence of 30 ml of chloroform. Wear under reflux for 1h. Strain the mixture and the chloroform extract submit the following test:

5.1. Anthraquinones
Was added aqueous 10 % KOH. After stirring, the presence of anthraquinones is confirmed by a bend in the aqueous phase to red.

RESULTS AND DISCUSSION

The results obtained are shown in Tables 01 and 02.

Tab. 01: Tests carried out on different phytochemical extracts prepared from the leaves of Laurel.

The leaves of laurel						
	Eau	**Ethanol**	**Chloroform**	**Ether diéthylique**	**Ether de pétrole**	**Résultats finals**
Tannins	+++	-				**Presence**
Alkaloids	+	-		+		
Saponins	++					
Anthracénosides		+				
Flavonoids		+++				
Sterols and steroids		-			+	
Volatile oil				++		
Quinones free					++	
Coumarins		-				**None**
Anthracyanosides		-				
Anthraquinones			-			

Tab 02: Tests carried out on different phytochemical extracts prepared from the leaves of thyme

Les feuilles du Thym						
	Water	**Ethanol**	**Chloroform**	**Ether diethyl**	**Ether petroleum**	**Final Results**
Tannins	++	++				**Presence**
Anthraquinones			+			
Saponins	++					
Anthracénosides		++				
Flavonoids		+++				
Sterols and steroids		-			++	
Volatile oil				+++		
Coumarins		-				**None**
Alkaloids	-	-		-		
Anthracyanosides		-				
Quinones free					-	

The presence of saponins in the plant is confirmed by an index above 100 [**6**].

1) Laurel:

N° tubes	01	02	03	04	05	06	07	08	09	10
Foam height (cm)	0.5	0.9	1	1.1	1.3	1.4	1.4	1.5	1.6	1.6

I = 0.9*5/0.02 = **225**

2) Thyme:

N° tubes	01	02	03	04	05	06	07	08	09	10
Foam height (cm)	0.4	1	1.5	1.9	1.9	2	2.6	2.6	2.6	3

I = 1*5/0.02 = **250**

Final result:

Tab.03 indices of foam *Thymus fontanesii* and *Laurus nobilis*

Espèces	***Saponaria officinalis*** **(**Witness cockle **)**	***Laurus nobilis***	***Thymus fontanesii***
Index of foam	**395**	**225**	**250**

We also examined four phytochemical extracts prepared from the leaves of laurel and thyme, dried and crushed, using water, ethanol, petroleum ether and diethyl ether and which has aim to highlight the presence or absence of reducing compounds, starch, fatty acids and polyuronides.

The results obtained are shown in the following tables:

Tab. 4: Searching for reducing compounds, polyuronides, starch and fatty acids in the leaves of *Laurus nobilis.*

The leaves of laurel					
	Water	**Ethanol**	**Ether diethyl**	**Ether petroleum**	**Final Results**
Reducing compounds	+++	+++			**Presence**
Polyuronides				+	
Fatty acids			+		
Starch	-				**None**

Tab. 5: Searching for reducing compounds, polyuronides, starch and fatty acids in leaves of *Thymus fontanesii*.

The leaves of thyme					
	Water	**Ethanol**	**Ether diethyl**	**Ether petroleum**	**Final Results**
Reducing compounds	+++	+++			**Presence**
Fatty acids			+		
Polyuronides				-	**None**
Starch	-				

The experimental results listed in Tables 01 and 02, show that tannins, flavonoids, anthracénosides, the saponins, volatile oil and sterols and steroids are present in the leaves of laurel and thyme, in varying quantities.

The intensity of the results shows that the laurel leaves are richer in tannins compared to those of thyme. Cons by the volatile oil, sterols and steroids and anthracénosides are present in significant quantities in the leaves of thyme over those of the laurel.

The presence of tannins in leaves of two plants was confirmed by a positive reaction with ferric chloride solution giving a dark green color, so this tannins catechists.

Indices foam presented in Table 03 confirm the presence of soponosides in leaves of both plants. However, the witness has a cockle index almost one and half times greater.

There is evidence that the volatile oils are present in significant quantities in both plants [1,7]. The presence of flavonoids in the bay is confirmed by data from Roulier G. (2005) [1], while data Valnet J. (2001) [8] and V. Fintelmann and Weiss RF (2004) [9] confirm the presence of tannins and saponins in thyme (*Thymus vulgaris*).

Thymus atlanticus and *Thymus satureioides* are two species of thyme, from the High Atlas and have been with other medicinal plants, a phytochemical study conducted by Lamnaouer D. (2002) [10]. This study showed that leafy stems of both species contain flavonoids and *Thymus atlanticus* contains gallic tannins.

Finally, we observed the presence of free quinones and alkaloids in the leaves of laurel, and anthraquinones in leaves of thyme, and the total absence of coumarins and anthracyanosides in leaves of two plants studied.

The results of the reactions and reagents Dragendorf Mayer, showed the absence of alkaloids in leafy stems of *Thymus atlanticus* and *Thymus satureioides* [10].

The results reported in Tables 4 and 5 has revealed the presence of reducing sugars and fatty acids in leaves of two plants studied, the presence of polyuronides in the leaves of laurel and their absence in leaves and thyme total absence of starch in leaves of both plants.

CONCLUSION

Laurus nobilis and *Thymus fontanesii* thus appear to be rich plant secondary metabolites, widely used in traditional medicine to combat and cure various ailments.

The antimicrobial potency of the thyme and bay leaves can be explained by the presence of essential oils, tannins and flavonoids. The anti-inflammatory, antispasmodic, antitussive and diuretic these two plants can be attributed to their high flavonoid and saponins. While the presence of alkaloids in the leaves of the laurel may explain their stimulating effects.

Exploitation of these pharmacological properties involves further investigation of these active ingredients, for the implementation techniques of extraction, purification, separation, crystallization and identification.

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