PHENOLIC COMPOUNDS OF ARGAN TREE, *ARGANIA SPINOSA* (ENDEMIC SPECIES OF SOUTH WESTERN MOROCCO)

Saïda Tahrouch[1], Abdelhakim Hatimi[1], Sylvie Rapior[2], laurence Mondolot[2], Annick Gargadennec[2] and Claude Andary[2]

[1]Laboratoire de Biotechnologies Végétales, Département de Biologie, Faculté des Sciences, Université Ibnou Zohr, B.P. 8106-Dahkla Agadir, Maroc.
[2] Laboratoire de Botanique, Phytochimie et Mycologie, UMI-CNRS (UPR 9056, CEFE), Faculté de Pharmacie, Université Montpellier I, F-34060 Montpellier cedex 2.

e-mail: tahrouch@hotmail.com

Abstract: *Argania spinosa* (L.) Skeels (Sapotaceae) is an endemic tree located mainly in south-western of Morocco. The argan tree plays medicinal, ecological and socioeconomic roles in this area. The fruit of A. spinosa has oil-producing kernels with a high unsaturated fatty acid content. The argan oil is greatly used in food and cosmetic products. Kernel, pulp of fruit and trunk have also been studied for sterols, triterpenes and saponins. Our goal in this study is to investigate the leaves for phenolic compounds by HPLC in 90 specimens of argan tree from three localities in Souss Massa area (south-western Morocco). Quantification and histolocalisation of phenolic components, i. e. flavonoids and condensed tannins (molecules well known for their broad spectrum of biological activities) in the three localities were carried out using chromatographic and spectroscopic methods combined to histochemical technics. Flavonol glycosides were quantified by HPLC from argan leaves. The main flavonol glycoside was myricitrin. The content of myricetin derivatives was higher than the quercetin derivative content. With regard to chemotaxonomy, four flavonol glycosides seem to be good markers for this species as they were detected by HPLC in 90 specimens of argan tree from the 3 localities. The histochemical studies of the different parts of A. spinosa (leaves, stems and thorns) have shown a high concentration of myricetin derivatives in the peripheral tissues, this cell localisation of the flavonoids could explain the Argan tree adaptation to aridity.

Key words**: *Argania spinosa* - Flavonol glycosides – Condensed tannins – Histochemistry.**

INTRODUCTION

Argania spinosa (L.) Skeels (*Sapotaceae*) is an endemic and medicinal tree (Bellakhdar J., 1997) located mainly in south-western Morocco. The argan tree plays ecological and socioeconomic roles in this area (Boukhobza and Pichon-Prum, 1988).

The fruit of *A. spinosa* has oil-producing kernels with a high unsaturated fatty acid content (Maurin *et al*., 1992). The argan oil is greatly used in food (Huyghebaert and Hendrickx, 1974) and cosmetic products (Pierre Fabre Patent). Whereas kernel, pulp of fruit and trunk have been studied extensively for sterols, triterpenes and saponins (Farines *et al*., 1984; Charrouf *et al*., 1991, 1992; Maurin, 1992; Nerd *et al*., 1994; Oulad-Ali *et al*., 1996), relatively little is known about chemistry and histochemistry of the leaves, the stems and the thorns (Tahrouch *et al*., 1998 ; Tahrouch *et al*., 2000).

In order to understand both vigour and resistance of this endemic plant to an arid habitat, the leaves were investigated for phenolic compounds by HPLC in 90 specimens of argan tree from three localities. Quantification of phenolic components, i. e. flavonoids and condensed tannins in three localities were carried out using chromatographic and spectroscopic methods combined to histochemical techniques.

MATERIAL AND METHODS

The leaves of *Argania spinosa* were collected in the Ademine reserve (**A**) 60 m, Ait Baha (**AB**) 500 m and Immouzzer (**I**) 900 m, Agadir (Morocco). Voucher specimens were deposited in the Herbarium of Laboratoire des Symbiotes Racinaires et de Biochimie Végétale in Agadir. Dry leaves were extracted three times with MeOH/H_2O (4/1) at room temperature.

Histochemistry

Sections of leaves (45-60 µm thickness) were cut with a cryostat microtome (Frigocut 2800 E) operating at -20°C and examined using either a light microscope or an epi-fluorescence microscope (Nikon Optiphot) with two filter sets: UV filter set with 365 nm excitation and a 400 nm barrier filter. Flavonoid compounds were detected using Neu's reagent (Neu, 1956). Sections were immersed into the reagent for 1 min and then observed by epi-fluorescence (Dai *et al*., 1995). DMCA (4-dimethylaminocinnamaldehyde) reagent was used to locate condensed tannins (Feucht *et al*., 1986). Stained sections were observed with a light microscope.

Quantification of flavonoids and condensed tannins

HPLC was carried out using an isocratic mobile phase (Acetonitrile/MeOH/H_2O, 2/8/15) running through Nucleosil C18 (250 x 4 mm, 5 µm particle size). UV-Visible data were recorded using a Photodiode Array Detector coupled to HPLC system. Flavonoids of argan leaves were quantified using myricitrin as internal standard at 350 nm.

Quantitative determination of condensed tannins were carried out by UV and visible spectrophotometry according to Mc Murrough and Mc Dowell (1978).

RESULTS

A. spinosa was investigated for condensed tannins and flavonoids, molecules well known for their broad spectrum of biological activities, (Di Carlo *et al*., 1999). Flavonol glycosides were quantified by HPLC from argan leaves (figure 1). The main flavonol glycoside (Tahrouch *et al*., 2000) was myricitrin [**2**]. The content of myricetin derivatives [**2**, **4**] was higher (≅ 20 $mg.g^{-1}$ D. W.) than the quercetin derivative [**1**, **3**] content (≅ 8 $mg.g^{-1}$ D. W.).

Compounds	Quercitrin [**1**]	Myricitrin [**2**]	Hyperoside [**3**]	Myricetin-3-O-galactoside [**4**]
Rt[a]	15.2	8.8	10.1	6.8
Q[b]	5.3±0.4	16.8±1.4	2.5±0.2	3.3±0.3

[a] retention time (minutes) ; [b] quantity ($mg.g^{-1}$ D. W. ± s.e.)

With regard to chemotaxonomy, these four molecules seem to be good markers for this species as they were detected by HPLC in 90 specimens of argan tree from the 3 localities : A, AB and I (tables 1, 2 and 3).

These results showed that there is a close relationship between A and AB. The amount of flavonoids in these 2 localities (A and AB) is higher than in I. A and AB are located in arid areas.

It might be advisable to combine analytical and histological methods. With histochemistry we are able to localise *in situ* the flavonoids by Neu's reagent, which give a bright orange-yellow fluorescence

under UV. Neu's reagent is a borate salt that forms complex with certain groups of phenolic compounds giving them specific fluorescence (doc. 1). Condensed tannins detected histochemically by using DMCA reagent give a blue coloration under white light (doc. 2).

The histochemical studies of the different parts of *A. spinosa* (leaves, stems and thorns) have shown a high concentration of myricetin derivatives in the peripheral tissues particularly in epidermis while condensed tannins were mainly deposited in the cortex and palisade mesophyl.

The high content of total flavonoids in specimens of *A. spinosa* that located in localities A and AB, could play a protective role in the expression of tolerance to UV-radiations as showed by Lois (1994) in *Arabidopsis thaliana* (*Brassicaceae*). Olsson *et al.* (1998) explained that flavonoids afforded a protective role not only through the absorption of UV-radiations, especially in the epidermal layers, but also through a selective increase after UV-B irradiation that happens for flavonoids which possess an additional hydroxyl group in the B-ring of the flavonoid skeleton such as quercetin and myricetin derivatives that we identified from *A. spinosa* leaves.

The increase of phenolic compound biosynthesis in plant under UV-radiation and during periods of water stress and nutrient deficiency (Keller and Hrazdina, 1998) might be an adaptation phenomena (Gershenzon, 1984). Indeed, the biosynthesis of flavonoids in plants is enhanced in response to changes in the external environment (Cooper-Driver and Bhattacharya, 1998). According to this hypothesis, plants that normally occupy arid area and infertile habitats, such as argan trees, could have continuously high levels of phenolic constituents. The high amount of phenolic components and their localisation in the peripheral tissues might contribute to understand the relationship between accumulation of polyphenols and adaptation of argan tree to his area.

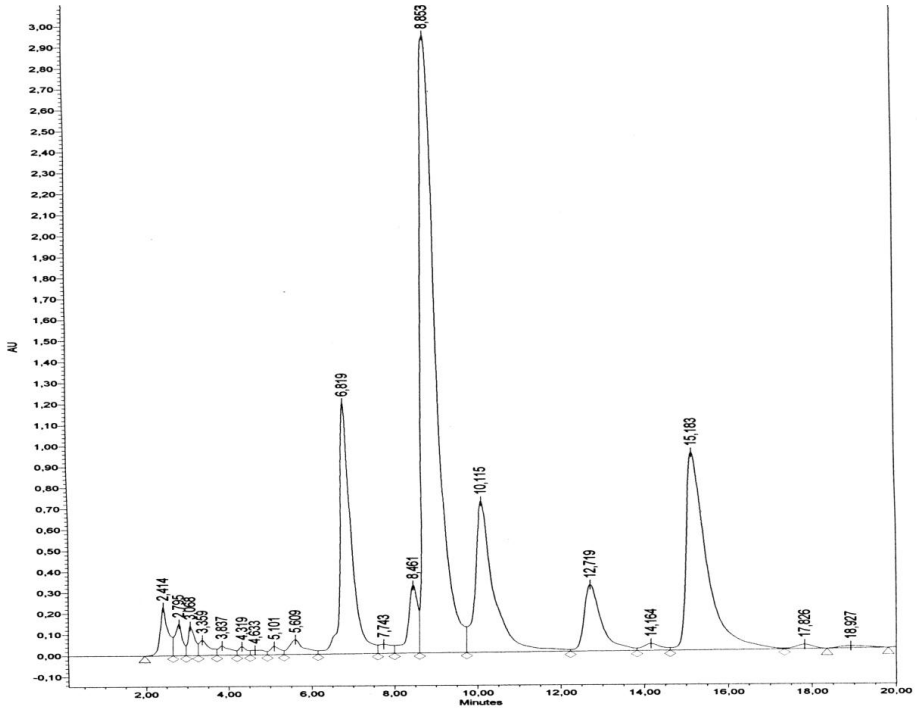

Figure 1 : HPLC of methanolic extract of Argan leaves.

components	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
retention time	2,41	2,80	5,60	6,81	8,46	8,85	10,11	12,71	14,16	15,18

M4 : Myricetin 3-O-galactoside (**D**)
M6 : Myricitrin (**B**)
M7 : Hypéroside (**C**)
M10 : Quercitrin (**A**)
M5 : Myricetin derivatives
M8 : Quercetin derivatives
The other flavonoids (M1, M2, M3, and M9) were not identified.

Table 1 : Quantification of flavonoids (M1-M10) and condensed tannins (M11) in Admine reserve ($mg.g^{-1}$ D.W.).

	M1	M2	M3	M4 (D)	M5	M6 (B)	M7 (C)	M8	M9	M10 (A)	M11
1	1,460	1,426	0	2,740	0	10,037	2,318	1,517	1,134	2,214	59,90
2	1,623	1,800	1,055	2,081	1,143	15,484	1,054	1,118	1,729	5,892	44,00
3	1,327	1,545	1,093	1,875	0	8,670	2,368	1,438	1,503	4,674	139,35
4	1,536	1,330	0	0	0	7,190	0	0	0	1,698	47,35
5	1,504	1,368	1,089	2,646	1,062	9,635	1,945	1,404	1,182	2,454	78,75
6	1,248	1,219	1,169	3,488	0	15,129	2,330	1,187	1,566	4,734	69,55
7	1,288	1,281	0	1,608	0	10,392	0	1,042	1,244	3,162	81,30
8	1,334	1,341	1,096	4,330	0	12,842	3,184	1,804	1,252	3,115	72,90
9	1,228	1,266	0	1,643	0	6,312	0	1,040	1,066	1,955	60,35
10	1,447	1,724	0	2,231	0	12,562	2,499	1,305	1,354	3,924	60,35
11	1,338	1,547	1,085	2,607	1,147	18,753	1,039	1,205	1,596	5,219	90,55
12	1,356	1,526	1,163	2,763	1,340	14,220	3,522	1,539	1,608	5,494	68,30
13	1,486	1,592	1,224	3,098	1,305	19,165	2,678	1,375	1,901	7,860	144,30
14	1,256	1,345	1,142	2,443	1,063	9,150	2,742	1,649	1,467	4,131	32,70
15	1,768	1,833	0	4,107	0	28,365	4,159	1,429	2,819	14,381	91,55
16	1,285	1,349	0	2,891	1,097	11,639	2,739	1,559	1,664	5,669	52,40
17	1,594	1,666	1,468	5,402	0	21,789	3,439	1,632	1,318	3,686	103,50
18	1,267	1,378	1,038	2,858	0	14,132	2,421	1,451	1,323	3,318	65,80
19	1,547	1,662	1,414	4,776	1,327	26,907	1,067	1,587	1,522	4,724	63,70
20	1,546	1,513	1,135	4,204	1,202	21,866	2,999	1,300	1,734	5,550	69,60
21	1,115	1,196	0	1,579	0	8,061	0	1,080	1,156	2,398	45,40
22	1,580	1,698	1,090	2,947	1,079	16,483	3,196	1,464	1,956	7,040	78,10
23	1,534	1,564	1,196	4,648	1,246	27,859	3,920	1,695	2,894	11,972	87,85
24	1,158	1,269	1,050	2,627	0	11,538	2,051	1,218	1,309	3,347	111,10
25	1,196	1,383	1,317	2,096	0	12,266	2,410	1,201	1,646	5,044	102,00
26	1,311	1,396	1,270	3,391	0	20,390	0	1,235	1,574	4,705	39,90
27	1,617	1,570	1,465	2,337	1,208	15,267	2,446	1,438	1,851	6,541	61,15
28	1,325	1,450	1,354	2,286	1,040	10,558	2,804	1,636	1,446	4,291	61,55
29	1,498	1,466	1,434	3,618	1,113	18,156	2,744	1,502	1,522	4,914	72,60
30	1,557	1,534	1,302	2,676	1,091	17,152	2,577	1,430	1,608	5,267	70,05

Table 2 : Quantification of flavonoids (M1-M10) and condensed tannins (M11) in Aït Baha reserve ($mg.g^{-1}$ D.W.).

	M1	M2	M3	M4 (D)	M5	M6 (B)	M7 (C)	M8	M9	M10 (A)	M11
1	1,459	1,4264	0	2,740	0	10,036	2,317	1,516	1,133	2,2136	60,70
2	1,622	1,7996	1,054	2,080	1,143	15,483	1,054	1,117	1,729	5,8924	62,00
3	1,326	1,5452	1,092	1,874	0	8,669	2,367	1,437	1,502	4,674	50,95
4	1,536	1,33	0	0	0	7,190	0	0	0	1,6976	58,15
5	1,504	1,3684	1,088	2,646	1,062	9,635	1,945	1,404	1,182	2,4536	99,00
6	1,248	1,2192	1,169	3,487	0	15,129	2,330	1,187	1,565	4,7344	70,90
7	1,287	1,2812	0	1,608	0	10,392	0	1,042	1,244	3,162	50,10
8	1,334	1,3412	1,095	4,330	0	12,842	3,184	1,804	1,251	3,1148	53,50
9	1,227	1,2656	0	1,642	0	6,311	0	1,039	1,064	1,9548	51,80
10	1,447	1,7244	0	2,230	0	12,562	2,499	1,304	1,353	3,924	112,2
11	1,338	1,5472	1,085	2,607	1,146	18,753	1,038	1,205	1,595	5,2192	49,65
12	1,356	1,5256	1,163	2,763	1,340	14,219	3,522	1,539	1,608	5,494	72,15
13	1,485	1,592	1,223	3,098	1,305	19,165	2,678	1,374	1,901	7,8596	72,15
14	1,256	1,3452	1,141	2,442	1,063	9,1504	2,741	1,649	1,466	4,1308	60,30
15	1,768	1,8328	0	4,107	0	28,364	4,158	1,429	2,819	14,3808	130,30
16	1,284	1,3492	0	2,890	1,096	11,639	2,738	1,559	1,663	5,6692	43,30
17	1,594	1,666	1,467	5,401	0	21,789	3,438	1,632	1,318	3,6856	81,50
18	1,267	1,378	1,038	2,858	0	14,132	2,421	1,450	1,322	3,318	47,10
19	1,546	1,6624	1,414	4,775	1,327	26,906	1,067	1,586	1,522	4,724	59,45
20	1,546	1,5128	1,134	4,204	1,202	21,865	2,999	1,300	1,733	5,55	64,10
21	1,115	1,196	0	1,578	0	8,061	0	1,080	1,156	2,3976	31,40
22	1,580	1,6984	1,090	2,947	1,078	16,482	3,195	1,464	1,955	7,04	70,05
23	1,534	1,5644	1,196	4,647	1,246	27,858	3,920	1,695	2,894	11,9716	44,55
24	1,158	1,2688	1,049	2,626	0	11,538	2,050	1,218	1,309	3,3468	30,55
25	1,196	1,3828	1,316	2,096	0	12,266	2,409	1,201	1,646	5,0444	37,80
26	1,310	1,3964	1,269	3,391	0	20,390	0	1,234	1,574	4,7048	58,15
27	1,616	1,57	1,464	2,337	1,208	15,267	2,446	1,438	1,850	6,5408	64,95
28	1,325	1,45	1,354	2,285	1,040	10,558	2,803	1,636	1,446	4,2912	57,30
29	1,498	1,4656	1,434	3,617	1,112	18,156	2,744	1,501	1,522	4,9144	61,95
30	1,557	1,534	1,302	2,676	1,090	17,152	2,576	1,429	1,607	5,2672	103,00

Table 3 : Quantification of flavonoids (M1-M10) and condensed tannins (M11) in Immouzer reserve (mg.g^{-1} D.W.).

	M1	M2	M3	M4 (D)	M5	M6 (B)	M7 (C)	M8	M9	M10 (A)	M11
1	0	0	0	1,270	0	4,234	1,834	1,205	1,248	2,682	9,80
2	0	0	0	0	0	5,142	1,340	0	1,153	2,208	17,95
3	0	0	0	1,326	0	6,211	1,960	1,334	1,227	2,862	20,40
4	0	0	0	0	0	1,865	0	1,247	0	0	8,63
5	0	0	0	1,600	0	3,102	1,812	1,145	0	1,588	14,70
6	0	0	1,225	2,238	0	6,707	2,258	1,401	1,186	2,478	26,30
7	0	0	0	2,107	0	7,148	3,265	1,446	1,531	4,302	26,50
8	1,073	0	0	2,019	0	8,776	2,946	1,662	1,493	4,170	28,55
9	0	0	1,200	1,078	0	2,554	1,399	1,120	1,114	2,080	19,60
10	1,064	1,130	0	2,096	0	8,979	2,496	1,505	1,333	3,231	33,85
11	1,130	1,150	1,836	3,552	0	15,080	5,596	2,871	2,285	7,092	55,25
12	0	1,096	0	1,716	0	7,125	1,966	1,273	1,259	2,859	29,60
13	0	0	0	3,013	0	9,233	2,688	1,714	1,311	2,642	47,75
14	0	1,138	0	1,930	0	6,905	2,670	1,352	1,442	3,643	26,50
15	1,458	1,470	1,434	3,086	0	17,116	4,060	2,266	2,401	6,754	64,10
16	1,097	1,042	1,836	2,861	0	6,348	2,474	1,867	1,289	2,756	30,20
17	1,150	1,096	0	1,609	0	6,210	1,888	1,284	1,502	4,257	39,80
18	0	1,050	0	2,746	0	10,995	3,082	1,556	1,994	7,053	31,60
19	1,440	1,377	0	1,466	0	7,841	1,881	1,291	1,442	3,740	97,55
20	1,260	1,362	0	3,098	0	11,747	4,166	1,962	1,875	5,566	49,45
21	--	--	--	--	--	--	--	--	--	--	12,65
22	1,232	1,476	0	5,232	0	13,406	5,368	2,608	1,992	5,956	55,25
23	1,207	1,255	0	1,328	0	3,524	1,517	1,150	1,086	1,962	54,45
24	1,059	0	1,045	3,211	0	10,314	3,852	1,904	1,740	5,054	36,85
25	1,170	1,246	0	2,663	0	7,971	3,128	1,739	1,529	4,084	51,10
26	1,247	1,344	1,495	2,847	0	10,469	3,654	1,967	1,856	5,230	45,25
27	0	0	0	1,900	0	6,533	2,755	1,489	1,520	3,834	19,60
28	1,503	1,462	0	2,370	0	17,876	3,230	1,742	2,158	6,254	59,10
29	1,041	1,141	0	0	0	3,987	0	0	1,302	2,928	38,10
30	0	1,127	0	2,153	0	6,617	2,544	1,458	1,242	2,586	27,75

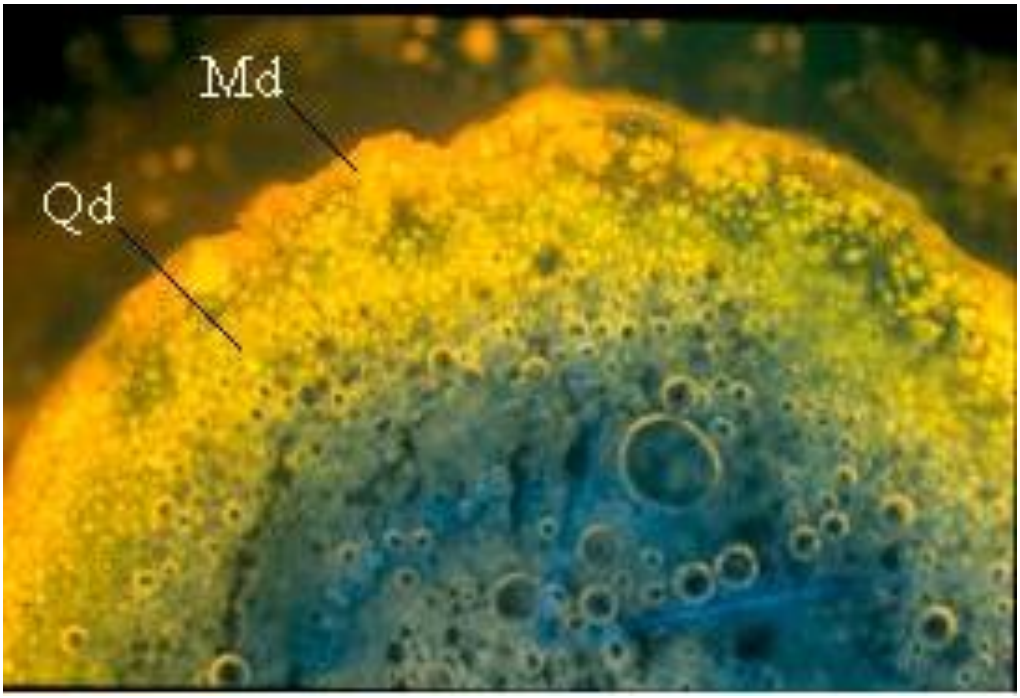

Figure 2 : The histochemical study from the stem of *A. spinosa* showed a high content of myricetin derivatives (Md) in the peripheral tissues. Qd : Quercetin derivatives.

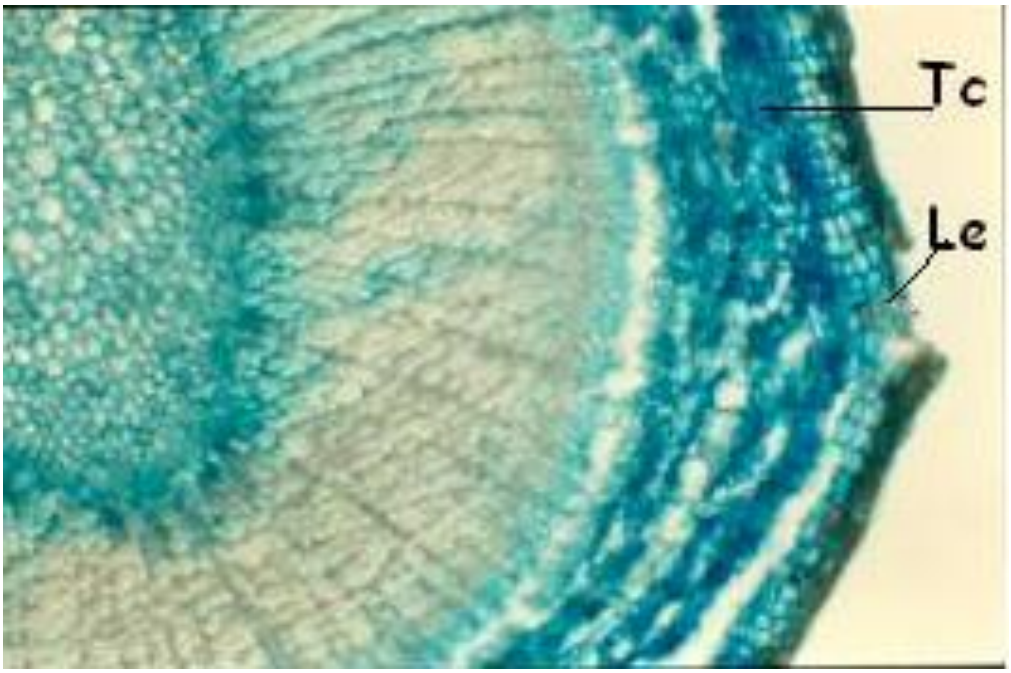

Figure 3 : The histochemical studie from the stem of *A. spinosa* showed a high concentration of condensed tannins (Tc) mainly deposited in the cortex. Le : lenticel.

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