Histolocalization of polyphenols in leaves, stems and thorns of *A. spinosa* and involving of these components in the adaptation of argan tree to its area

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Abstract

The leaves of *Argania spinosa*, an endemic tree of Morocco, were investigated for flavonoids and condensed tannins. Four flavonol glycosides seem to be good markers for this species. Chromatographic and histochemical techniques were used to analyse flavonoids and condensed tannins and to localise these molecules in the leaves, the stems and the thorns of *A. spinosa*. 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. Condensed tannins detected histochemically by using DMCA reagent give a blue coloration under white light. The histochemical studies of the different parts of *A. spinosa* 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 levels of phenolic compounds and their localization allow us to explain this tree adaptation to the difficult conditions of its arid area.
Key words : *Argania spinosa* - Flavonol glycosides – Condensed tannins – Histochemistry.

1. Introduction

Argania spinosa (L.) Skeels (*Sapotaceae*) is an endemic tree located mainly in south-western Morocco. It grows over about 320 000 square miles.

The argan trees play a special role for a population that could not survive in a region otherwise disadvantaged. This tree has an essential ecological and socioeconomic fonction in this area [1]. Argan tree protects the soil against erosion; the leaves are used as hanging forage for goats and camels. Additionally, this species have an ability to survive to arid conditions, so it helps to stop the progression of the desert.

The chemical composition of the leaves has been studied by Tahrouch [2]. Four flavonoids have been isolated; these are quercetin and myricetin derivatives. These flavonoids are known to have antioxidant, fungicide and bactericide activities.

The aim of this study is to localize the phenolic compounds of Argan tree *in situ*. So, leaves, stems and thorns were investigated for phenolic compounds. The analysis of these phenolic components, flavonoids and condensed tannins, were carried out using thin layer chromatography combined to histochemical techniques.

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2. Material and methods

The leaves, stems and thorns of *Argania spinosa* were collected in the Ademine reserve, located in the south western of Agadir, (Morocco). Voucher specimens were deposited in the Herbarium of laboratoire de Biotechnologies Végétales, Agadir.

2.1. TLC extraction

Dry leaves were extracted three times with MeOH/H2O (4/1) at room temperature for use on thin layer chromatography (TLC).

2.2. Histochemistry

Sections of leaves, stems and thorns (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 [3]. Sections were immersed into the reagent for one minute and then observed by epi-fluorescence [4]. DMCA (4- dimethylaminocinnamaldehyde) reagent was used to locate condensed tannins [5]. Stained sections were observed with a light microscope.

3. Results and discussion

A. spinosa was investigated for flavonoids and condensed tannins, molecules well known for their broad spectrum of biological activities, [6]. The main flavonol glycoside [7] was myricitrin. The content of myricetin derivatives was higher than the quercetin derivative (table 1). With regard to chemotaxonomy, these four molecules seem to be good markers for this species [8].

It might be advisable to combine analytical and histological methods. With histochemistry we are able to localize *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 (doc. 3), stems (doc. 4a and 4b) and thorns (doc. 5) 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 (doc. 6). The high content of total flavonoids and tannins in specimens of *A. spinosa* could play a protective role in the expression of tolerance to UV-radiations as showed by Lois (1994) [9] in *Arabidopsis thaliana* (*Brassicaceae*). Olsson *et al*. (1998) [10] 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 [7].

The increase of phenolic compounds biosynthesis in plant under UV-radiation and during periods of water stress and nutrient deficiency [11] might be adaptation phenomena [12]. Indeed, the biosynthesis of flavonoids in plants is enhanced in response to changes in the

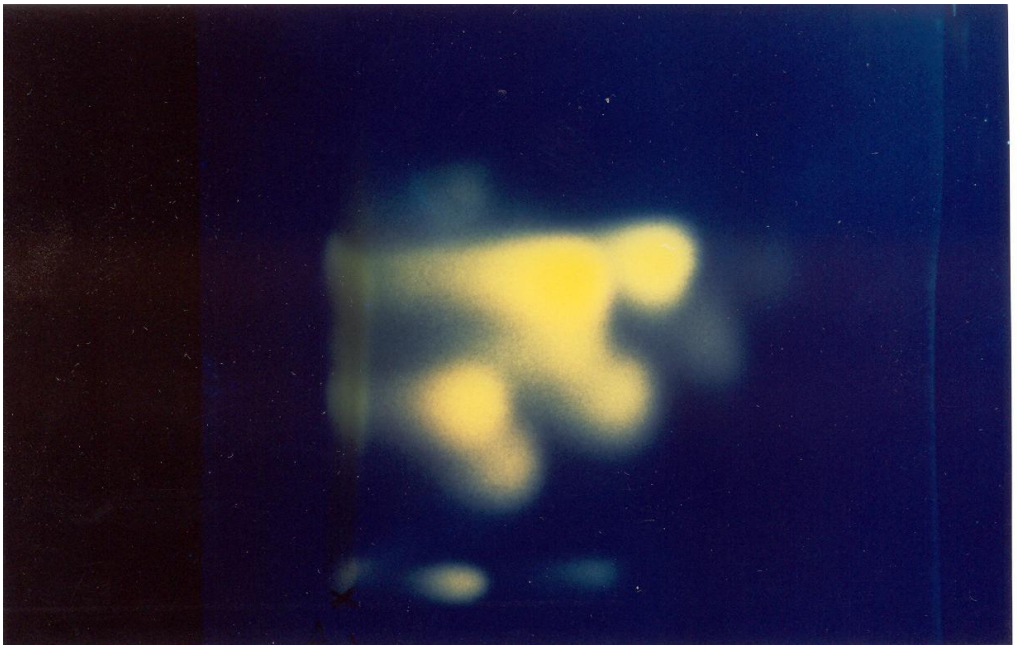
external environment [13]. According to this hypothesis, plants that normally occupy arid area and infertile habitats, such as Argan trees, could have continuously high levels of phenolic constituents.

Conclusions

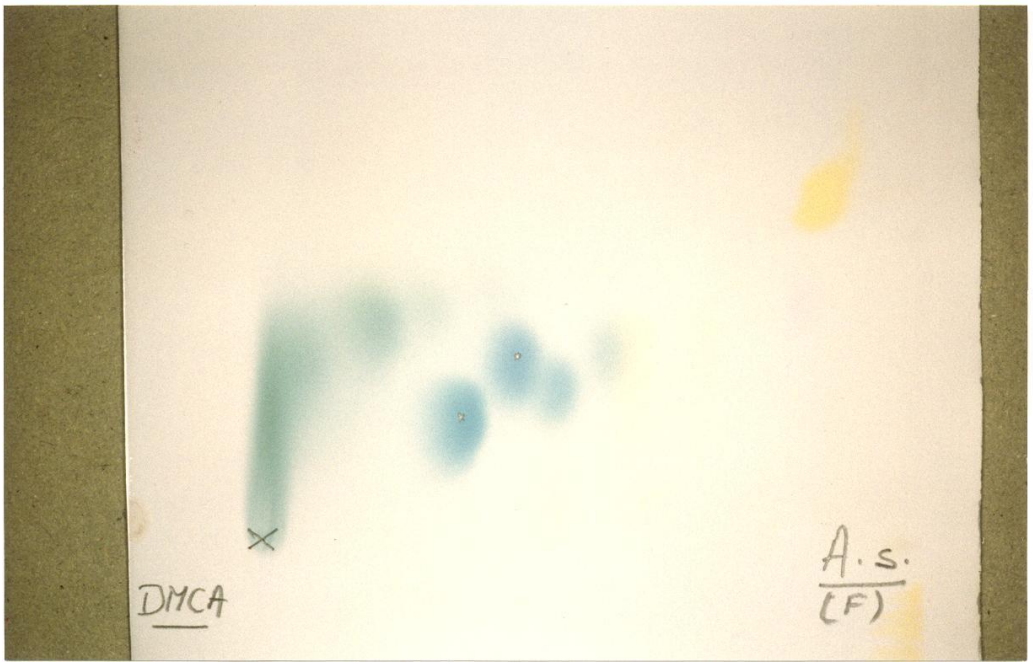
Given its socio- economic interest and its ecological and chemical characteristics, *Argania spinosa* , an endemic species in southwestern of Morocco , is a tree with many uses.
Looking for substances such as phenolic compounds and other secondary metabolites would justify the valorization of derivatives of the argan tree.
The high amount of phenolic components and their localization in the peripheral tissues might contribute to understand the relationship between accumulation of polyphenols and adaptation of *Argania spinosa* to its area.

Table I: quantitative data of flavonol glycosides from leaves of *A. spinosa* (DW : dry weight).

Components	Myricetin 3-O-galactoside	Myricitrin	Hyperosid	Quercitrin
Content $mg.g^{-1}$ DW	3.3±0.3	16.8±1.4	2.5±0.2	5.3±0.4

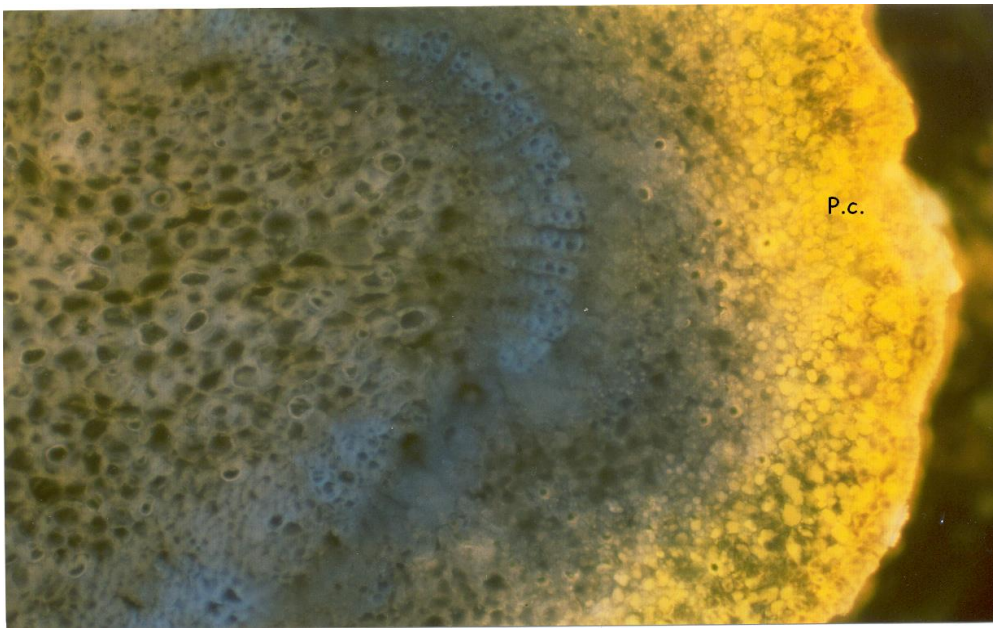


Document 1 : TLC of flavonoids. They were detected using Neu's reagent and UV.

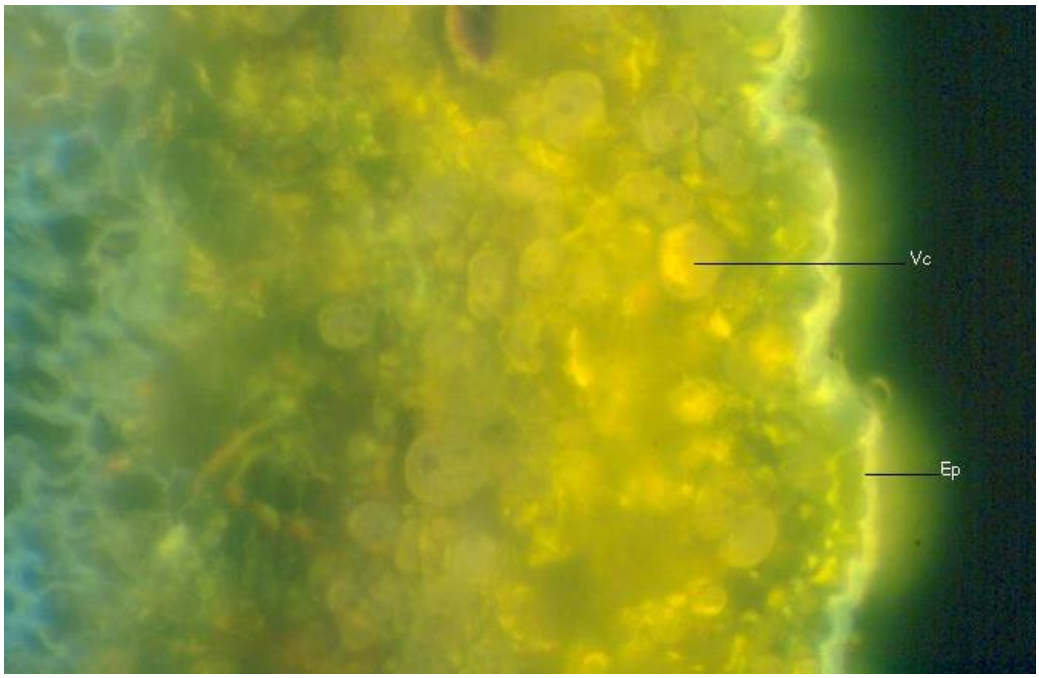


Document 2 : TLC of condensed tannins. They were detected using DMCA reagent.

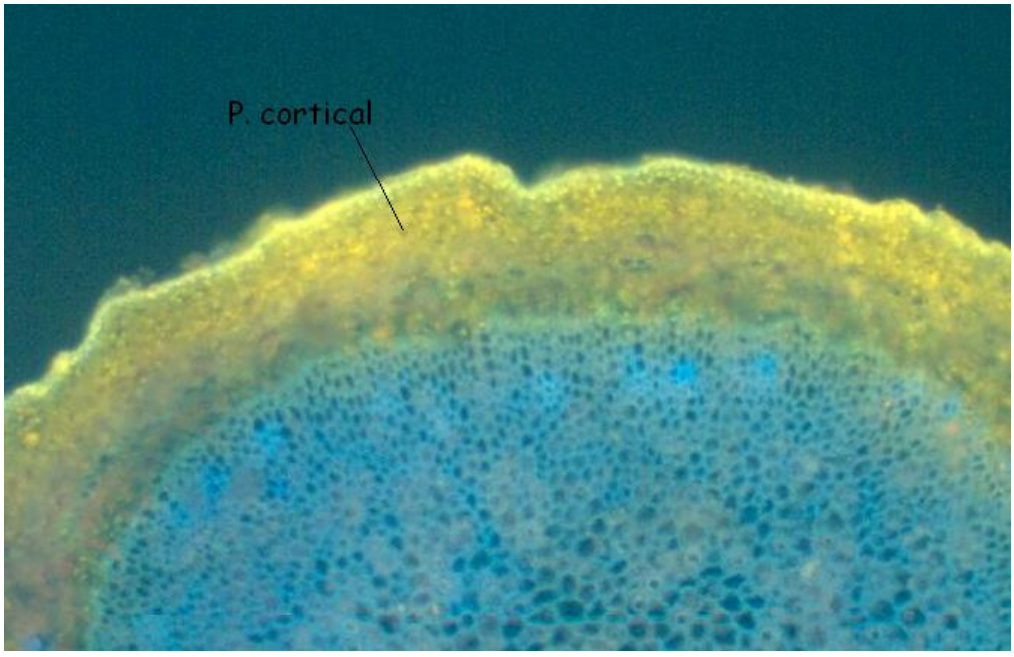
Document 3 : The histochemical studies from the leaves of *A. spinosa* showed a high content of flavonoids in the peripheral tissues particularly in epidermis cells (Ep), palisade mesophyl and xylem (Xy).



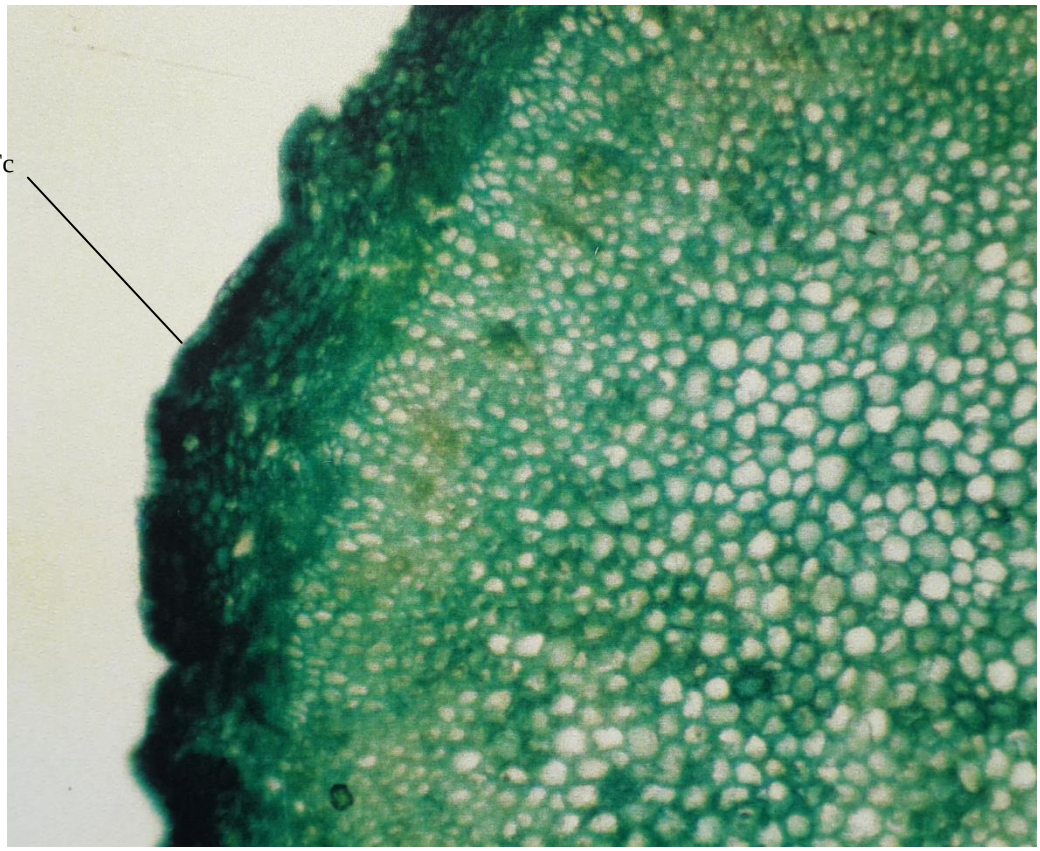
Document 4a: The histochemical studie from the stem of *A. spinosa* showed a high content of flavonoids in the peripheral tissues (P.c.).



Document 4b: The histochemical studies from the stem of *A. spinosa* showed a high content of myricetin and quercetin derivatives in the vacuole (Vc) and lipophylic components in epidermis cells (Ep).



Document 5 : The histochemical studie from the thorn of *A. spinosa* showed a high content of flavonoids in the cortical parenchyma (P. Cortical).



Document 6 : The histochemical studie from the stem of *A. spinosa* showed a high concentration of condensed tannins (Tc) mainly deposited in the cortex.

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