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Original Article

Phytochemical Screening by Metabolites Histolocalization in the Medicinal Plants *Artemisa Annua* and *Argania Spinosa*

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Abstract

Background and Aim: Plants have been used since antiquity to treat different diseases. *Artemisia annua* is particularly an interesting plant due to its anti-malarial and anti-cancer properties. *Argania spinosa* is also a bioactive drug. Secondary metabolite histolocalization made it possible here to show molecules presence, with antioxidant properties.

Materials and Methods: The method chosen here is metabolites chemical characterization at tissue level by histolocalization.

Results: The histolocalization study of the drug *A. annua* showed the presence of phenolic compounds, both at stem level in hypodermis and in marrow as in parenchyma and xylem level leaf. Flavonoids are more particularly localized at xylem and pericycle in stem level and at xylem, pericycle and parenchyma in leaf level. Finally, catechic tannins are found in the parenchyma of these 2 organs. The histolocalization study of *A. spinosa* showed phenolic compounds precisely located in phelloderm and phloem for vascular bundle. Terpenoids and phenols are found in the bark, parenchyma and marrow for stem and in palisade and spongy mesophyll for leaf. Alkaloids are found in small amounts in stem liber and in leaf parenchyma. Finally, a slight presence of carotenoids is found in palisade and leaf phloem.

Conclusion: Good antioxidant activity was obtained by DPPH assay for *A. annua* (*aerial part decoction*) with an EC_{50} of 0.29 mg/L and for *A.spinosa* with an EC_{50} of 0.20 mg/L (*testa decoction*), 0.0075 mg/L (*testa ethanolic extract*), 0.0762 mg/L (*ethanolic almond extract*) and 0.0206 mg/L (*ethanolic leaf extract*).The results showed that testa has twice as powerful antioxidant activity as reference control (trolox).

Keywords: Histolocalization, *Artemisia annua*, *Argania spinosa*, Antioxidant

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Introduction

Some 2600 to 3000 years before J-C, Sumerians wrote the first known text dealing with plants medicinal use (1). The first precise manuscript indicating doses, durations and administration modes, is Egyptian and

dates 1600 years before J-C. Since then, medicinal plants used for treatment of diseases, the so called herbal medicines, have come a long way. Gradually separating from its mythological aspects to become a science, botany owes its advances and discoveries to great characters, such as Hippocrates and Theophrastus in Greece, or Dioscorides and Galien during Roman Empire who wrote many collections grouping their knowledge. In the Middle Ages, the contribution of Arab-Muslims' knowledge on the subject allowed a considerable increase in medicinal plants use (2), that were reserved in France only by apothecaries and herbalists from the thirteenth century (3).

Nevertheless, the boom in pharmacy and chemical industry, as well as synthetic medicines introduced in the twentieth century, were gradually reducing medicinal plants role in pharmacopoeia. In France, the training and the diploma, issued since 1778 by the faculty of medicine, were removed in 1941 from the course of health education in France. Despite various attempts, he has not recovered to date (4, 5).

Today, complex legislation regarding their sale makes the use of medicinal plants difficult. However, their virtues are no longer to prove and their active substances, objects of much research, remain important candidates for diseases treatment.

This is particularly true for sagebrush plant from asteraceae family present, generally, in most temperate regions. *Artemisia annua* has been used since ancient times for its therapeutic properties, i.e. tonic, hypoglycemic, emmenagogue, antiseptic, expectorant, diuretic and analgesic properties, due to its secondary metabolites. For example, they can be used for rheumatism treatment, asthma, cancer and epilepsy (6).

A. annua has interesting properties. That is, it synthesizes a sesquiterpene lactone, artemisinin, and is used as an antimalarial drug. It is potentially effective against lung and breast cancer (7). In 1967, malaria ravages in Asia prompted research inspired by traditional Chinese medicine research and finally highlighted artemisinis extracted from *A. annua* (8). In 2011, WHO recommended artesunate use as an intravenous monotherapy (9).

Traditionally, *Argania spinosa* (L.) Skeel (Sapotaceae) (argan tree) could be used for several purposes (10). Leaf tea is used in the treatment of gastritis, diarrhea, fever, and headache. The testa is used against hives and dandruff but is mainly used for tanning skin. Almond paste is recommended for scaly scalp, hair loss, eczema and hives. Its in-depth study could thus be important and decisive for new cancer treatments development.

The histochemical characterization is therefore at the same time chemical, morphological and topographical (localization) and aims to highlight, rather than define substances, functions or substances groups. This technique also uses precipitation reactions among others. In this perspective, it seemed interesting to carry out a study of the antioxidant activity of this plant and to carry out a phytochemical screening by histolocalization to directly highlight the responsible metabolites. This could then be the first step in a more extensive valuation of the drug. The purpose of this study is to show histolocalization interest, especially in *A. annua* and *A. spinosa* which are used for herbal medicine elaboration.

Materials and Methods

The Tested Plant

A. annua L. (Asteraceae) (IPNI database, WFO (2020), and the plant list accessed in January 2020 were collected from Toulouse Botanical Garden Henri Gaussen (JBHG) during the seed bearing stage in 2018. Voucher specimens were identified by Doctor Fatiha El Babili and deposited at JBHG herbarium under the number JBHG2566 and JBHG2567. *A. spinosa* (L.) Skeel (Sapotaceae) (*IPNI, 2020)* seeds were purchased in Essaouira province and were grown in JBHG during 2019 spring.

Histochemistry and Histolocalization of Interesting Molecules

Plant thin transverse sections were made for every sample using a razor blade on the fresh plant material to denature the least possible cellular constituents. For each of them, five cross-sections replicas were made. Subsequently, the sections were immersed in carmino-green for a few minutes and then rinsed with distilled water before being mounted in gelatined glycerin. This control color makes it possible to visualize different tissues. Stains with different reagents are also performed as described in Table 1.The prepared slides were then observed under an optical microscope

MOTIC BA210, and observations were captured with Motic 3.0 software.

Carmino-green alune R staining: A bath of 3 minutes in carmino-green staining (Begenat) makes it possible to identify tissue organization.

Staining with reagents: Free-hand sections were submitted to various histochemical tests.

Universal developers:

- Sulfuric Vanillin (H_2SO_4 - 3% - Prolabo), universal developer
- Sudan III (Begenat) revealing terpene compounds such as essential oils
- DPPH (2,2'-diphenyl-1-picrylhydrazyl) for antioxidant metabolites.

Primary metabolites developers:

- Lugol reagent for starch,
- Sudan III for lipids or lipophilic substances (wax)
- Phloroglucin for lignin,
- Hydrochloric acid for establishing calcium oxalate crystals nature.
- Red ruthenium method as mucilage and pectin substances reagent

The phenolic compound developers:

- $FeCl_3$: revealed in violet for phenol, green for catechol and red for pyrogallol; blue-black for hydrolysable tannins and greenish-brown for condensed tannin.
- Phosphomolybdic (Fluka analytical) revealed aldehydes, thiols, flavonoids and total tannins and also steroids.
- Phloroglucinol (11) and KOH: colors lignified tissues (red-violet)
- Folin-Ciocalteu reagent (Sigma): flavonoid and tannins in blue color
- H_2SO_4 (Prolabo) revealing phenolic pigment such as curcumin in yellow,
- HCl (Prolabo) and Aluminum Nitrate (Prolabo): tannin but also lignified elements.
- Aluminium chloride: flavonoids (yellow) and a bluish for acid-phenol.

Terpenoid developers:

- HCl + H_2SO_4: yellow for colchicine, brown-red for tanins, blue for iridoids, blue-violet for carotenoids
- Lugol : green-blue for carotenoids
- Anisaldehyde: blue-violet for sesquiterpenes
- Erlich reagent: blue coloring for diterpens

Alkaloid developers:

- Dragendorff reagent (Fluka Analytical). orange for alcaloids

Heterosides developers:

- Raymond-Marthoud reagent (Prolabo): cardiotonic glycosides and anthracenes

Antioxidant developers: DPPH

For the majority of tests, fresh-hand sections were immersed for 5 minutes in reagent and subsequently they were rinsed with water prior to being mounted in a drop of water or in gelatined glycerin. Control sections were subjected to histochemical tests in accordance with the standard procedure (Table1). We photographed the untreated sections in order to examine the natural appearance of the studied plant organ. One of the references we used to conduct our study was the thin layer chromatographic knowledge summarized in Wagner's book (12).

Observation and Image Processing

Following the treatment, the images were taken with a light microscope (MOTIC BA210LED) equipped with a Digital camera (Moticam 3+ 3.0 MP). The photos were made using MOTICAM 3+, analyzed, and processed by Motic plus 3.0 image analysis software.

Methods for Assessing the Presence of Antioxidants in Plants

When free radical DPPH˙ reacts with an antioxidant, a discoloration results. This reaction can be followed by spectrophotometry between 515 and 518 nm.

DPPH Solution Preparation

A methanolic solution with the concentration of 375µM was prepared.

Extract Preparation (*Ph. Eur. 10th Edition – monograph 6.04* (13))

Working from traditional uses, we deliberately chose to work only on extracts that can be edible for medicinal use (decoction and the ethanolic extract). 1 g of dried drug was extracted hot in 100 mL of water (decoction) or ethanol (extract) for 15 min

Measure of Absorbance and Absorbance Reduction Measurements

The absorbance is measured at reaction end at 517 nm. The absorbance decay corresponded to the decrease in DPPH° concentration. The results of absorbance reduction measurements can be represented as a percentage:

$$\% \text{ DPPH consumed} = 1 - \left(\frac{\text{Absorbance in antioxidant presence}}{\text{Absorbance without antioxidant}}\right) \times 100$$

EC_{50} was defined as concentration required to cause a 50% decrease in initial DPPH° concentration. Trolox was used as a standard at 150µM.

Statistical Analysis

All the data (excel analysis utility) were expressed as means ± standard deviations of triplicate measurements. The confidence limits were set at $P < 0.05$. Standard deviations (SD) did not exceed 5% for majority of obtained values.

Results and Discussion

Histochemistry and Histolocalization of Molecules of Interest (Table 2)

***Artemisia annua* (photos 1 to 8)**

The drug *A. annua* histolocalization study showed the presence of phenolic compounds, revealed with Folin Ciocalteu reagent, both at stem level in hypodermis and in marrow as in parenchyma and xylem level leaf. The treatment with phloroglucinol and phosphomolybdic acid confirmed this presence in stem xylem and leaf parenchyma. Flavonoids are more particularly localized by KOH at xylem and pericycle in stem level and at xylem, pericycle and parenchyma in leaf level. Finally, catechic tannins are found in the parenchyma of these 2 organs. Artemisinin is present mainly in leaves, but flavonoids were found in *A.annua* stems. It is therefore preferable to collect not only leaves but also young stems to make the drugs.

***Argania spinosa* (photos 9 to 13)**

The *A. spinosa* histolocalization study has shown that cross-sections react strongly to sulfuric vanillin by highlighting a certain number of metabolites presence, in particular at the bark (hypodermis in particular) level and conductive tissue level in stem and leaf. It is the parenchyma which are the most qualitatively colored.

Table 1: Some types of reagents used to highlight metabolites of interest in medicinal plants cross-sections.

Types of compounds detected	Reagent	References	Expected colorations
ESSENTIAL OIL LIPOPHILIC COMPOUNDS (SUBERIZED WALLS CUTINIZED)	• phosphomolybdic acid • Soudan III (10-20 min)	(17) (20), (21)	• Blue task on yellow background • Red-orange
STARCH (GRANULES)	• Lugol reagent	(22)	• dark blue-colored
PECTINS / MUCILAGES	• ruthenium red	(22)	• pink color
PROTEINS	• Eosin (extremely dilute solution) for 10 min	(22)	• proteins stain red
ACETOGENINS	• Kedde's reagent	(23)	• pink/magenta-colored
PHENOLIC COMPOUNDS	• Phloroglucin – Hcl • Folin-Ciocalteu reagent	(11) , (24)	• Yellow • Green-blue
ESSENTIAL OIL	• Ferric chloride • phosphomolybdic acid • Vanillin-sulfuric acid • Soudan III	(22) (17) (12) (20)	• Red-purple task • Blue/yellow background for terpenes. • Dark and variable task • Orange/red task
TERPENOIDS	• phosphomolybdic acid	(17)	• bluish area corresponding to anethole and chemically close substance
SAPONINS	• sulphuricacid	(22)	• Immediately yellow, red within 30 min and finally violet, or blue-green in a few instances
ALKALOIDS	• Dragendorff • Lugol	(27) (20)	• reddish-brown-coloured reaction • golden/brown-coloured drops
FLAVONOIDS	• Neu reagent • Ferric chloride	(12) (22)	• Specific fluorescence • Greenish task
DITERPENES	• Erlich	(12)	• Night blue task
TANNINS	• Ferric chloride	(22)	• Blackish green-brown task
ANTIOXIDANTS	• DPPH • ABTS	(28) (29) Modified method	• Discoloration on purple background • Discoloration on a blue background

Table 2: Results of specific histolocalisation qualitative analyzes performed on transverse sections of *A. annua* L. and *A. spinosa* (L.) Skeels.

Metabolites Type Studied	**Reagent used**	**Colorimetric Reaction results**				**Tissue Reacting Positively**			
		Artemisia annua		***Argania spinosa***		***Artemisia annua***		***Argania spinosa***	
Plant tested		***Stem***	***Leaf***	***Stem***	***Leaf***	***Stem***	***Leaf***	***Stem***	***Leaf***
essential oils, steroids, saponins, etc.	Universal developer : Sulfuric Vanillin	-	-	++++	+++	-	-	Cortex (hypodermis) and vessels (phloem)	Palisade Spongy mesophyll
Phenolic compound	Folin Ciocalteu	++++	++++	+++	+	Hypoderma (-), marrow	Hypoderma, parencyma (all) and xylem (little)	Cortical parenchyma, phloem and vessels	Spongy mesophyll
Phenolic compound	Phloroglucinol	+	+++	+++	-	Xylem (little Iaire)	All parenchyma	Phelloderma, phloem, marrow	-
Terpenoids, Phenols	Phosphomolybdic acid	-	-	+++	++	Hypoderma, xylem	**Xylem**	Cortical parenchyma, marrow	Palisade, spongy mesophyll
Flavonoids	KOH (alcoholic potash)	++	++	+++	+++	Xylem, pericyclic fiber	Xylem, pericycle and parenchyma	Phelloderma, cortical parenchyma, narrow	Palisade, spongy mesophyll
Catechic tannins	HCl	++	+	-	-	Cortical Parencyma	parenchyma	-	-
Alkaloids	Dragendorff	**(+)**	**(+)**	+	+++	-	-	Phloem	palisade spongy
Essential Oil	Soudan III Chloral hydrate	+	+	+++	+++	cuticle	Striated cuticle, gland	Epidermis, hypodermis, canals in pericycle with orangey content and cambium.	Epidermis, palisade
Iridoides	HCl	-	-	-	-	-	-	-	-
Carotenoids	HCl+ H_2SO_4	-	-	-	++	-	-	-	Palisade, liber
Heterosides Cardiotonic	Raymond-Marthoud reagent + 5 drops of KOH	-	-	-	-	-	-	-	-

+**:** positive reaction (graduation of 1 to 5 crosses for a qualitative evaluation of the colorimetric reaction); -: negative reaction; nd: not determined**.**

Moreover, we know that vanillin colors pectocellulosic walls in yellowish in general, particularly at stems level, which confirms the observation already found in stem.With Folin Ciocalteu

Table 3: Extracts antioxidant activity with DPPH test.

Tested plant	Extracted type	DPPH assay IC_{50} 50(mg/L)
Artemisia annua L.	aqueous extract of aerial part- R	0.29 ± 0.24
Argania spinosa (L.) Skeel	aqueous extract of seedtesta - R	0.20 ± 0.13
Argania spinosa (L.) Skeel	Ethanolic extract of seedtesta – N	0.0075± 0.001
Argania spinosa (L.) Skeel	Ethanolic extract of seedalmond - N	0.0762 ± 0.006
Argania spinosa (L.) Skeel	Ethanolic extract of leaf - N	0.0206 ±0.05
Trolox	aqueous extract	**0.015**± 0.0002

reagent, the results obtained previously are confirmed and show that most of localized metabolites are of phenolic nature. At leaf level, it is spongy mesophyll which reveals the presence of phenolic compound. The results become clearer through the reaction of stem sections with phloroglucinol. Phenolic compounds are located more precisely in phelloderm and phloem for vascular bundles. The use of phosphomolybdic acid places terpenoids and phenols in the bark, parenchyma and marrow for stem and in palisade and spongy mesophyll for leaf. The alkaloids are found in small amounts in stem liber and in leaf parenchyma. Finally, carotenoids are slightly found in palisade and leaf phloem.

Absorbance Reduction Measurements

Good antioxidant activities were obtained by DPPH assay for *A. annua* (aerial part decoction) with an EC_{50} of 0.29 mg/L and for *A. spinosa* with an EC_{50} of 0.20 mg/L (testa decoction), of 0.0075 mg/L (testa ethanolic extract), of 0.0762 mg/L (almond ethanolic extract) and of 0.0206 mg/L (leaf ethanolic extract) (Table 3). The ethanolic extract activity of seed testa is particularly spectacular since it is twice as powerful as trolox.

Spectrophotometric assays of antioxidant activity corroborate qualitative analytical results obtained by bioactives metabolites histolocalization. That is, Table 2 indicates the presence of phenols, flavonoids and tannins essentially. A significant antioxidant activity was also demonstrated by TLC because in histolocalization, reaction is too fast to be photographed but can be easily observed. These metabolites are none other than phenolic compounds which corroborate and attest to antioxidant therapeutic properties traditionally accorded to these plants.We are now able to show screening usefulness by histolocation for the purpose of conducting research on medicinal plants (*in particular antioxidant, even anticancer properties*) more efficiently by a more complete phytochemical study, which can then end up establishing a relationship

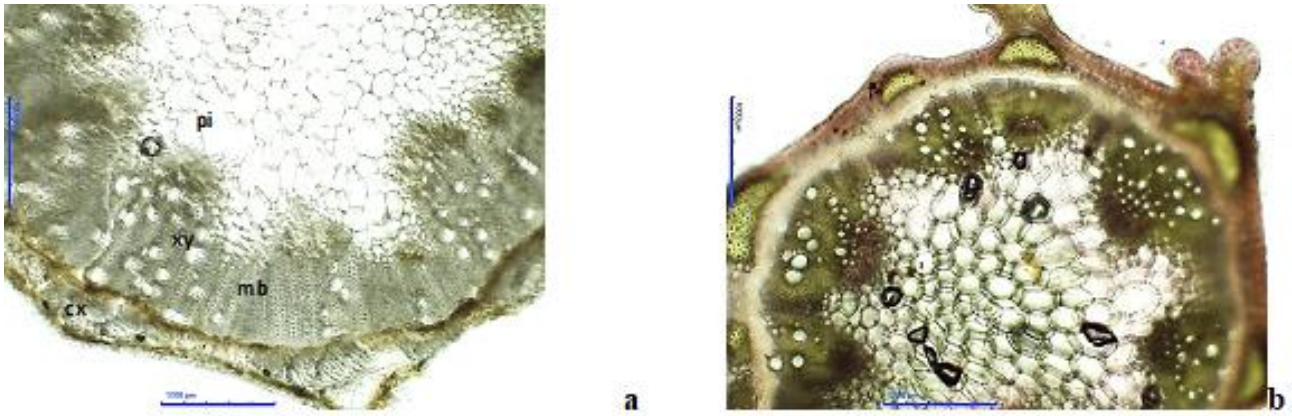

Figure 1. Hand-sectioned fresh material in *A. annua* stem without treatment (a) and stained with carmino-green reagent (b). Blade (magnification x 10); Scale bars: 1000µM; Abbreviations: cx, cortex; pi, pith; xy, xylem; mb, medullary bundle; tr, trichome.

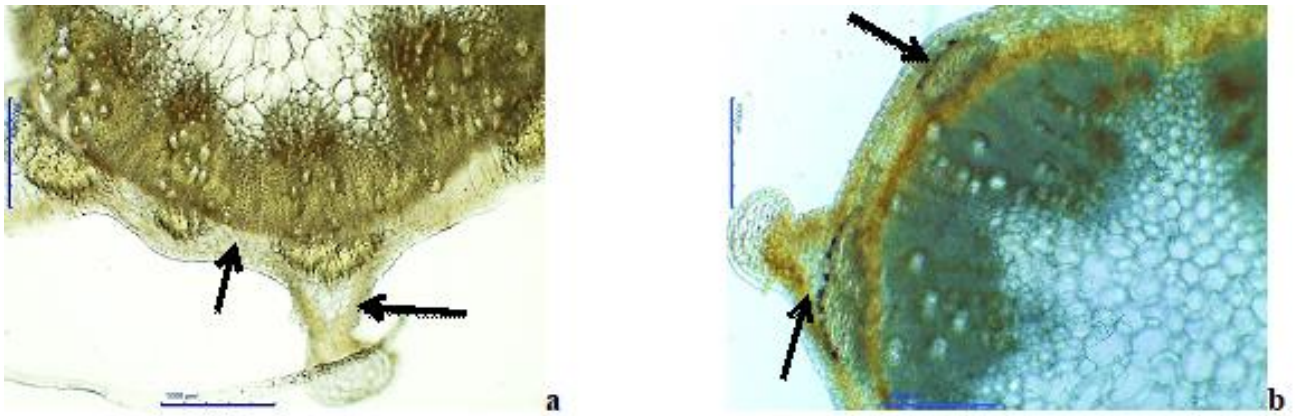

Figure 2. Hand-sectioned in *A. annua* stem submitted to histochemical tests with KOH reagent (a) and with phosphomolybdic reagent. Blade (magnification x 10); Scale bars: 1000µM; Arrows: sites of positive results.

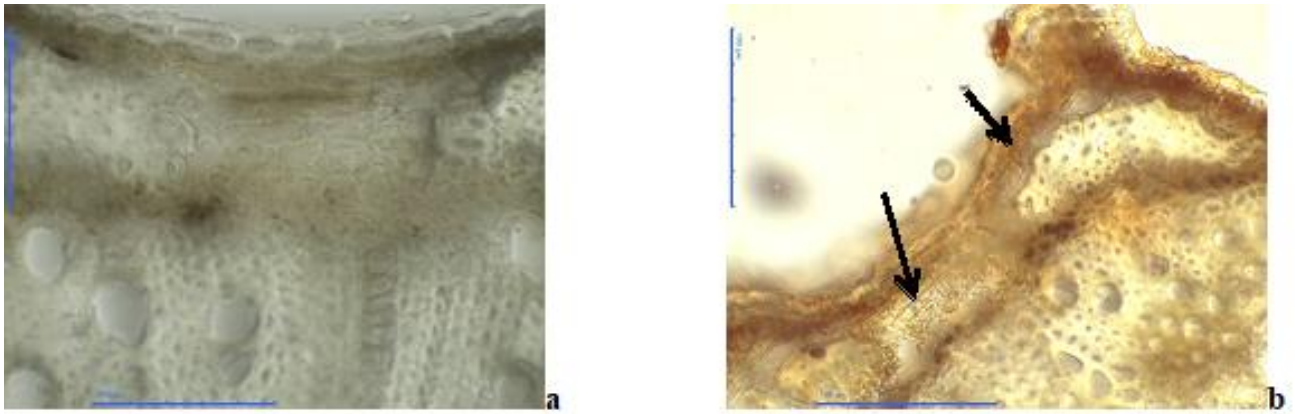

Figure 3. Hand-sectioned in *A. annua* stem without staining (a) and submitted to histochemical test with phloroglucinol reagent. Blade (magnification x 40; Scale bars: 100µM; Arrows: sites of positive results.

"structure-activity" that is faster and more economical. On the one hand, mother tincture, decoction and infusion are commonly used forms in traditional medicine because of their ease of access for patients. On the other hand, these modes of preparation are more sensitive to current ecological concerns and

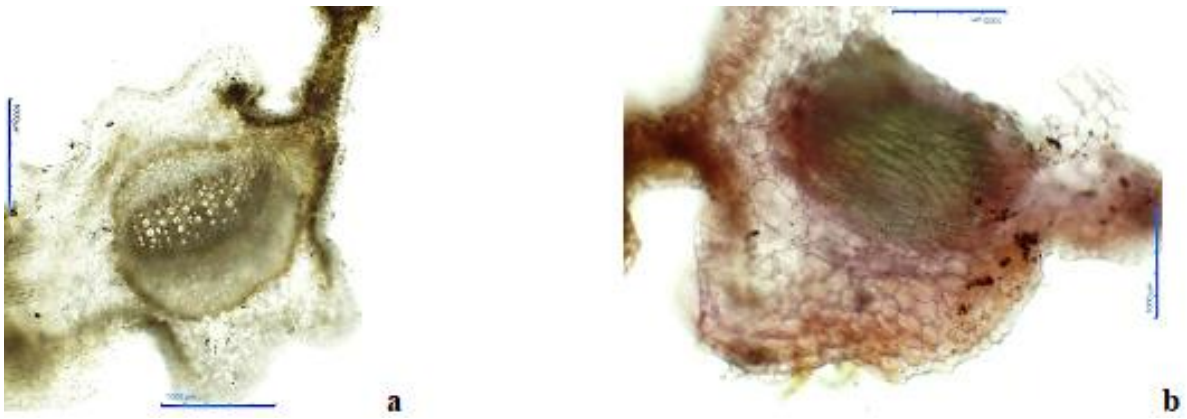

Figure 4. Hand-sectioned in *A. annua* leaf without staining (a) and stained with carmino-green alun R (b). Blade (magnification x 10); Scale bars: 1000µM.

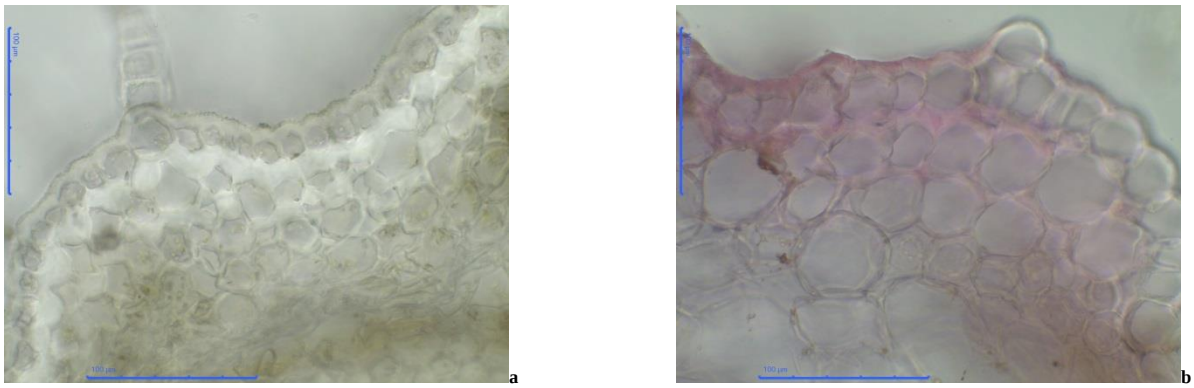

Figure 5. Hand-sectioned in *A. annua* leaf without staining (a) and stained with carmino-green alun R (b). Blade (magnification x 40); Scale bars : 100µm.

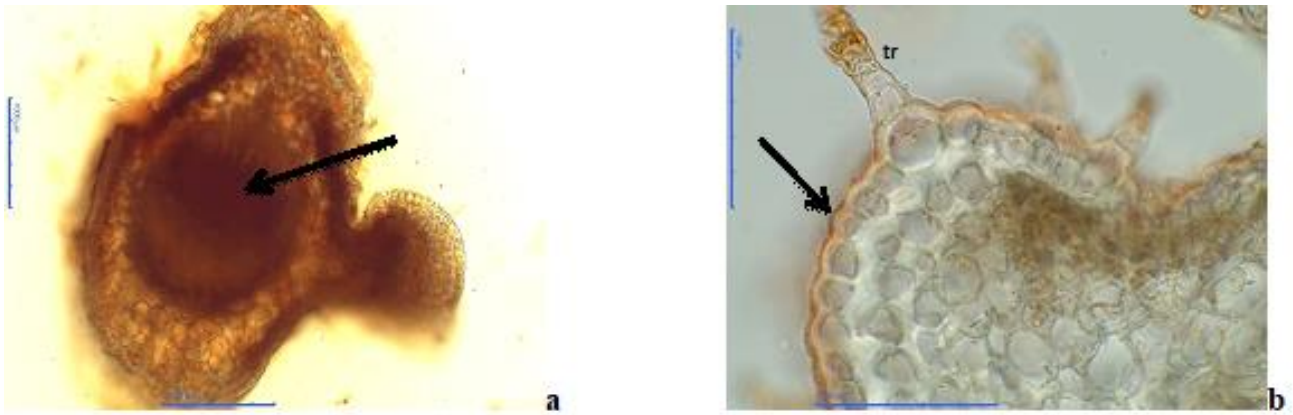

Figure 6. Hand-sectioned in *A. annua* leaf submitted to phloroglucinol reagent (a) (magnification x 10), Scale bars: 1000µM; and with soudan III reagent (b) (magnification x 40), Scale bars: 100µM; (b). Arrows: sites of positive results.

the search for universal access to care (WHO). Hence, the use of histolocalization applied to unprocessed plants (fresh or dry) in the preliminary analysis makes sense. In fact, we will then be able to better control transformation stages in herbal medicines preparation, as found very precisely in traditional Chinese medicine. Indeed, drug definition and preparation is extremely codified in China. Histolocalisation, among other sciences, allows today to valorize (14) an ancestral experience lost widely in the world. This science will

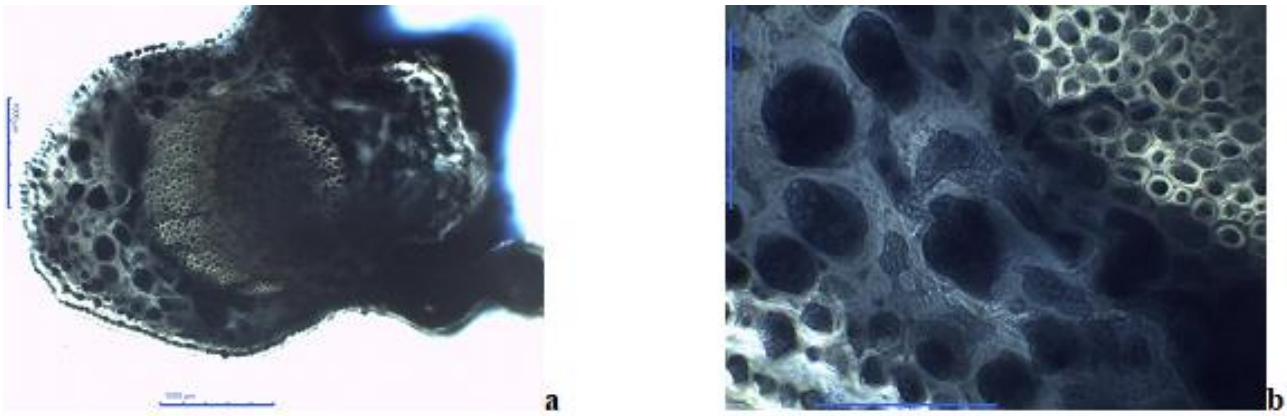

Figure 7. Hand-sectioned in *A. annua* leaf submitted to histochemical test with Folin Ciocalteu reagent: blade (magnification x 10), Scale bars: 1000 µm (a) and blade (magnification x 40), Scale bars: 100 µm (b).

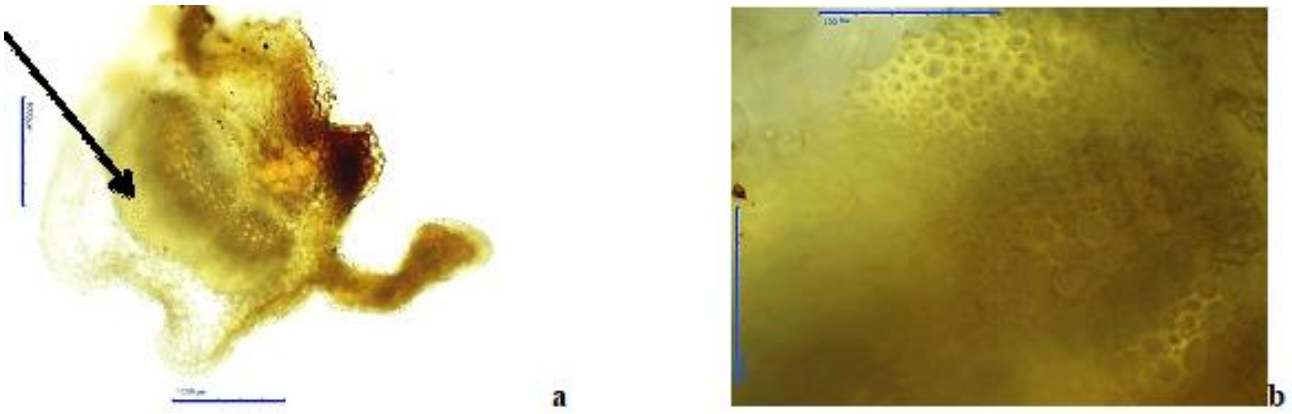

Figure 8. Hand-sectioned in *A. annua* leaf submitted to KOH reagent: blade (magnification x 10), Scale bars: 1000µM (a) and blade (magnification x 40), Scale bars: 100µM (b).

Figure 9. Transverse section of *Argania spinosa* leaf blade without staining: magnification x 10 (a) and magnification x 40 (b).

thus help to better prepare herbal medicines by allowing a more precise identification of tissues rich in bioactive metabolites. For example, stem bark or only the marrow can be selected because of its high content of bioactive compounds, localized by histolocation. Finally, there is the possibility of more

Figure 10. Transverse section of *A. spinosa* leaf blade treated with KOH reagent: magnification x 10. Abbreviations: p, palisade; sm, spongy mesophyll, epidermis.

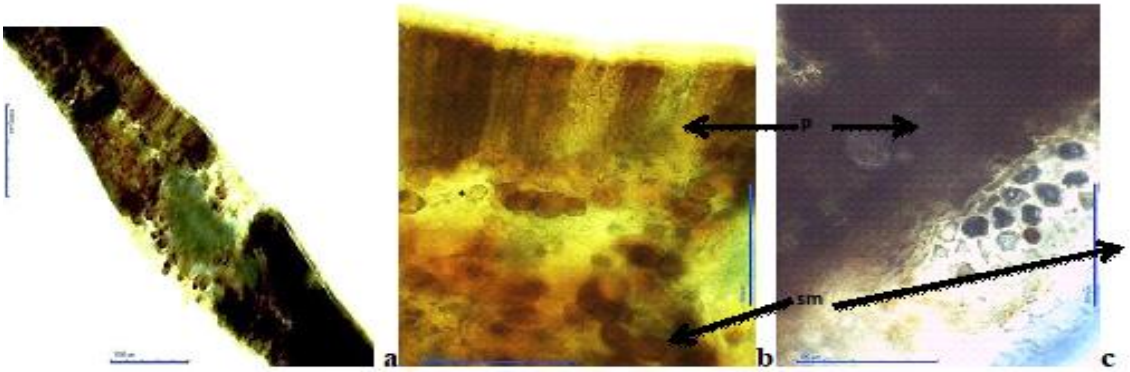

Figure 11. Transverse section of *A. spinosa* leaf blade colored with phosphomolybdic reagent: magnification x 10 (a) and magnification x 40 (b and c (heated)).

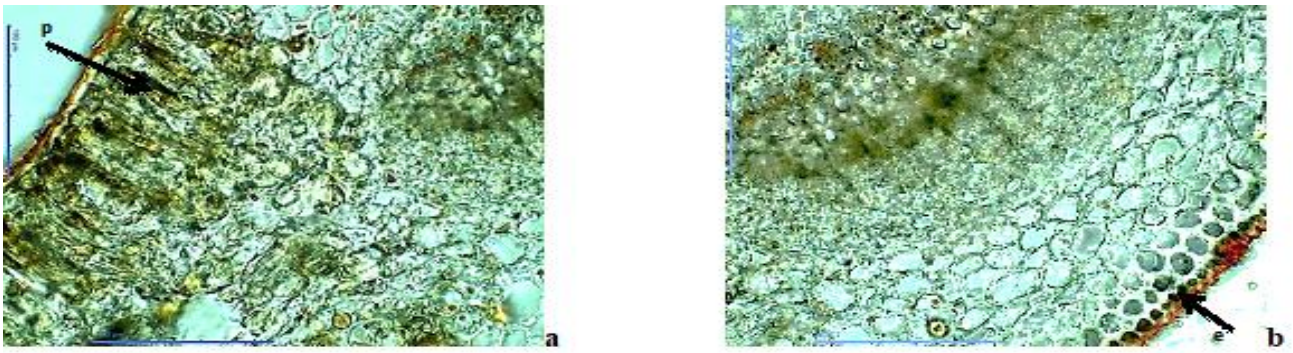

Figure 12. Transverse section of *A. spinosa midrib* leaf blade stained with soudan III reagent (magnification x 40) upper side (a) and lower side (b)**.**

targeted extractions.

Phytochemical screening has therefore revealed secondary metabolites produced by *A.annua* and *A. spinosa*. Essential oils, which cause pleasant smells, can have antiviral, anti-inflammatory and other properties. Flavonoids, revealed by KOH and phloroglucinol, are often present in plants in the form of soluble glycosides (15). They all share a basic structure: they are polyphenols with two aromatic rings bound by three carbons. This structure therefore has many phenolic functions that confer antioxidant properties to these molecules. In fact, antioxidants fight oxidative stress. The latter corresponds to an imbalance which results in an excess of oxygenated radicals produced during the metabolism of oxygenated products (16). Although these free radicals may have beneficial effects, in antimicrobial defense, for example, they nevertheless play a role in the development of cancer, degenerative diseases such as Alzheimers, and cardiovascular diseases (17).

The existence of considerably high levels of phenolic compounds and their localization in conductive tissues enables us to explicate and confirm the traditional medicinal use practiced in Thailand with A. annua. Indeed, compounds with antioxidant properties have generally proven to be very good anticancer agents. Given that recent studies and clinical trials have shown that artemisinin and its derivatives have anti-cancer activity in patients with human cancer (18), our work thus takes on its full meaning. It makes it possible to specify molecules location in order to implement more effective isolation and purification techniques.Our histolocalization results on *A. spinosa* show that seed decoction has high percentages in two main chemical families: polyphenols (99.3 + 2.9 eq gallic acid) and tannins (39.3 + 1.3 eq catechin), and allows us to understand that the organ which contains catechic tannins is essentially seed, because in stem and leaf

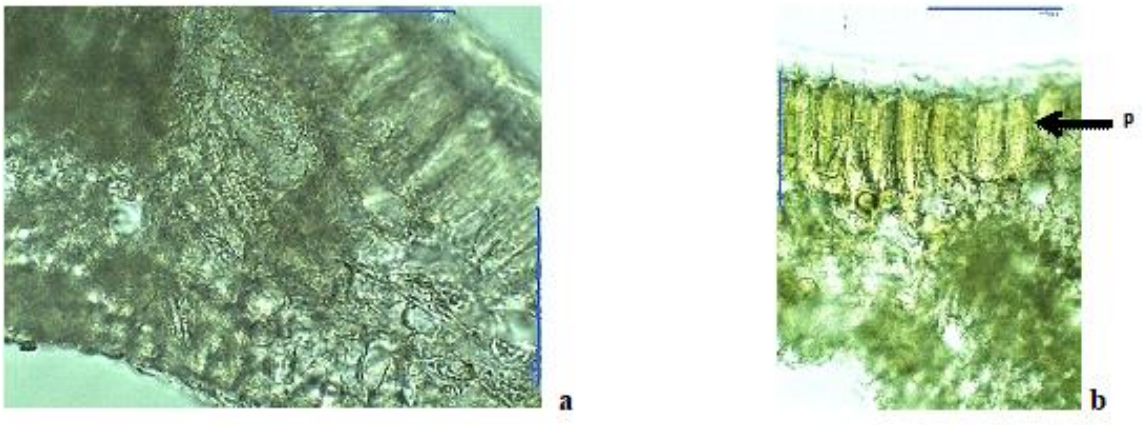

Figure 13. Transverse section of *A. spinosa midrib* leaf blade reagent (magnification x 40) without staining (a) and stained with dragendorff (b)**.**

there is none. The tradition essentially concerns seed use and we understand its meaning. But there remains a fact which can explain that, only almond is used in argan tree. Morocco is essentially a Muslim faith country, where drugs maceration practices such as liqueurs and other alcohol-based preparations are less common. This could explain why the exploitation of this miraculous seed both in food and in traditional medicine was limited until then to seeds expression. Anyway, Argan tree, endemic and emblematic of Morocco, will not cease to surprise us. Our new work on *Argania* complements the previous one by specifying better antioxidant properties depending on the seed part and especially the precise location of bioactive compounds families already studied. Today, seed testa is mainly used for external cosmetic use (hair mask according to our ethnobotany field survey in progress in Agadir and Essaouira). The results show that testa has twice as powerful antioxidant activity as reference control (trolox). It would perhaps seem relevant to improve research on this residue exploitation, generated by Argan oil expression.

However, if phytochemical screening allows a qualitative analysis of *A. annua* secondary metabolite composition, it does not allow knowing concentrations. Novertheless, this qualitative analysis remains interesting because it is important to note that plant composition varies considerably according to its origin. In China, for example, a significant difference in composition is observed between *A. annua* from the north and the south (19).

Conclusion

In *A. annua*, phenolic compounds (flavonoids, tannins) are localized both in stem and leaves in reserve areas. In *A. spinosa*, it is the conductive tissues that are rich in phenols while stem bark contains terpenoids, alkaloids in small quantities (stem and leaf) and few carotenoids in leaves. The antioxidant activity of testa seed extract is particularly spectacular since it is twice as powerful as that of trolox. Hence, antioxidants such as flavonoids found in *A. annua* and *A. spinosa* are a notable track for treatment and understanding of pathologies due to free radicals. In this way, being able to precisely locate metabolites will make it possible in the future to improve phytochemical methods for studying metabolites. We have regularly observed, in our purification work, problems of degradation, denaturation or even disappearance of studied metabolites studied and also disappearance of properties extracts when metabolites are purified. In recent times, these problems of inter-molecular synergy have prompted phytochemists to turn to metabolomic, a more comprehensive technique for studying chemical composition of plant extracts, without necessarily having to purify targeted metabolites. Thus, with histo-localization, precise mapping can be established at tissue level. It will be a powerful tool for preparation of enriched bioactive extracts, sufficiently specific as they will be characterized by majority compounds.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Founding

None.

References

1. Shoeb M. Anti-cancer agents from medicinal plants. Bangladesh J Pharmacol. 2006;1(2):35-41.
2. Bashar Saad. Greco-Arab and Islamic Herbal Medicine: A Review. 4(3):249-58.
3. Hajar R. The Air of History (Part II) Medicine in the Middle Ages. Heart Views Off J Gulf Heart Assoc. 2012;13(4):158-62.
4. Jorite S. La phytothérapie, une discipline entre passé et futur: de l'herboristerie aux pharmacies dédiées au naturel:156.
5. Cloatre E. Regulating Alternative Healing in France, And the Problem of 'Non-Medicine' | Medical Law Review | Oxford Academic. Spring 2019;Volume 27, Issue 2, ,:Pages 189-214,.
6. Abiri R, Silva ALM, de Mesquita LSS, de Mesquita JWC, Atabaki N, de Almeida EB, et al. Towards a better understanding of Artemisia vulgaris: Botany, phytochemistry, pharmacological and biotechnological potential. Food Res Int. 1 juill 2018;109:403-15.
7. Efferth T. From ancient herb to modern drug: Artemisia annua and artemisinin for cancer therapy. Semin Cancer Biol. 2017;46:65-83.
8. Tu Y. Artemisinin—A Gift from Traditional Chinese Medicine to the World (Nobel Lecture). Angew Chem Int Ed. 2016;55(35):10210-26.
9. WHO | Cost-effectiveness of parenteral artesunate for treating children with severe malaria in sub-Saharan Africa [Internet]. WHO. World Health Organization; [cité 24 nov 2020]. Disponible sur: https://www.who.int/bulletin/volumes/89/7/11-085878/en/
10. El Babili F, Bouajila J, Fouraste I, Valentin A, Mauret S, Moulis C. Chemical study, antimalarial and antioxidant activities, and cytotoxicity to human breast cancer cells (MCF7) of Argania spinosa. Phytomedicine. 2010;17(2):157-60.
11. Sass JE. Elements of botanical microtechnique. New York, N.Y.: McGraw-Hill Book Company, Inc.; 1940.
12. Schmauder H-P. H. Wagner, S. Bladt and E. M. Zgainski, Plant Drug Analysis — a Thin Layer Chromatography Atlas. XIII + 320 S., 170 farb. Abb. 23 Tab. Berlin-Heidelberg-New York-Tokyo 1984. Springer Verlag. DM 169,00. ISBN: 3-540-13195-7. J Basic Microbiol. 1986;26(2):128.
13. EDQM. La Pharmacopée Européenne (Ph. Eur.) 10e Édition | EDQM - Direction européenne de la qualité du médicament [Internet]. 2020 [cité 24 nov 2020]. Disponible sur: https://www.edqm.eu/fr/Pharmacopee_Europeenne_10e_Edition
14. Wick MR. Histochemistry as a tool in morphological analysis: a historical review. Ann Diagn Pathol. 1 janv. 2012;16(1):71-8.
15. Nsemi FM. Identification de polyphénols, évaluation de leur activité antioxydante et étude de leurs propriétés biologiques. :296.
16. Migdal C, Serres M. Espèces réactives de l'oxygène et stress oxydant. médecine/sciences. 2011;27(4):405-12.
17. Hogstad A. A Chemical and Morphological Study of Nicandra Physalodes (L) Pers. :88.
18. Tu Y. Artemisinin—A Gift from Traditional Chinese Medicine to the World (Nobel Lecture). Angew Chem Int Ed. 2016;55(35):10210-26.
19. Zhang X, Zhao Y, Guo L, Qiu Z, Huang L, Qu X. Differences in chemical constituents of Artemisia annua L from different geographical regions in China. PLOS ONE. 7 sept 2017;12(9):e0183047.
20. Kraus JE, Arduin M. Manual básico de métodos em morfologia vegetal. 1997 [cité 16 nov 2020]; Disponible sur: https://repositorio.usp.br/item/000930286
21. Foster AS. Practical plant anatomy [Internet]. New York : D. Van Nostrand company, inc.,; 1942. 172 p. Disponible sur: https://www.biodiversitylibrary.org/bibliography/5975
22. Johansen DA. Plant microtechnique. 1940.
23. Brechú-Franco A, Laguna-Hernández G, De la Cruz-Chacón I, González-Esquinca AJEjohE. In situ histochemical localisation of alkaloids and acetogenins in the endosperm and embryonic axis of Annona macroprophyllata Donn. Sm. seeds during germination. 2016;60(1).
24. Jensen WA. Botanical histochemistry. 1962.
25. 627.full.pdf [Internet]. [cité 24 nov 2020]. Disponible sur: https://www.jbc.org/content/73/2/627.full.pdf
26. Folin O, Ciocalteu VJJobc. On tyrosine and tryptophane determinations in proteins. 1927;73(2):627-50.
27. Yoder LR, Mahlberg PG. Reactions of Alkaloid and Histochemical Indicators in Laticifers and Specialized Parenchyma Cells of Catharanthus roseus (Apocynaceae). Am J Bot. 1976;63(9):1167-73.
28. Blois MS. Antioxidant Determinations by the Use of a Stable Free Radical. Nature. 1958;181(4617):1199-200.
29. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1 mai 1999;26(9):1231-7.

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