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Phytochemical screening and thin layer chromatography of two medicinal plants: *Adansonia digitata* (Bombacaceae) and *Acacia raddiana* (Fabaceae)

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Abstract

Adansonia digitata and *Acacia raddiana* are two herbs used extensively in Saharan pharmacopoeia. To characterize these two plants in terms of secondary metabolites, qualitative reactions of staining/precipitation were performed for the different edible organs. The results obtained showed the presence of several types of phenolic compounds; namely tannins, flavonoids, anthocyanins, coumarins, and other secondary metabolites such as alkaloids, sterols and terpenes, essential oils, saponins and quinones. The thin layer chromatography (TLC) was performed first to confirm the qualitative characterization of phytochemical screening, second to separate different molecules of each secondary metabolite and show the diversity in metabolite extracts. The TLC results are presented in the form of spots and frontal reports R_fs reflecting the plurality of component molecules in both of studied plants. This diversity of secondary metabolites can be at the origin of the widespread medicinal properties and therapeutic uses of the tested plants.

Keywords: Phytochemical screening, TLC, secondary metabolites, phenolic compounds, *Acacia raddiana*, *Adansonia Digitata*

Introduction

Although modern medicine is well developed, a significant proportion of the world population, particularly in developing countries, still relies on traditional healers, medicinal plants and herbal medicines for their basic care.

In addition, in recent decades, public interest in alternative therapies has increased dramatically along with the use of medicinal plants in developed countries.

Each herb has many therapeutic properties that traditional healers employ to cure many diseases caused by different active ingredients resulting from secondary plant metabolism.

According to the World Health Organization (WHO), evaluating these products and guaranteeing their safety and effectiveness through phytochemical and biological studies in order to know their clinical and pharmaceutical value has become a public health necessity that presents significant challenges and interesting opportunities.

An ethnobotanical study in the province of Laayoune allowed inventorying some specific medicinal plants in the region, used in traditional medicine. Statistical analysis of the results showed the use of a significant number of marketed medicinal plants, and more frequently *Acacia raddiana* and *Adansonia Digitata*; mostly recommended against digestive diseases [1].

In order to research the various secondary metabolites in *Acacia raddiana* and *Adansonia Digitata* extracts, namely flavonoids, saponins, tannins, alkaloids, anthracene derivatives, sterols and terpenes... A phytochemical screening was performed based on a set of staining / precipitation reactions. This characterization was confirmed by TLC.

The baobab (*Adansonia Digitata* L.) is one of the major food tree species in the Sahelian countries [2]. Imported via Mauritania, leaves and fruits are widely consumed at the Laayoune region. The leaves and fruit pulp are known for their richness in nutrients, especially vitamins A and C [2, 3].

Acacia tortilis subsp. Raddiana is the most widespread and most common tree in the Moroccan Sahara. It symbolizes the desert in North Africa [4].

In terms of traditional medicine, *Acacia tortilis subsp. Raddiana* is especially healing of wounds deemed effective⁵. The leaves in the form of ground powder are widely used against Stomach aches at the Laayoune region [1].

These plants represent an African heritage highly used by traditional practitioners in Laayoune region in particular and in the Sahara in general. The medicinal value of these practices is mainly archived through the identification of active biomolecules constituting its plants in the first place, and later on by isolation and quantification of these biomolecules.

Materials and Methods

Vegetal Material

The plants studied; *Acacia raddiana* and *Adansonia Digitata* were purchased from traditional practitioners at Laayoune the form of milled powder.

Harvested in the Sahara of Laayoune, the leaves of *Acacia raddiana* are carefully washed, sun dried, then ground in a mortar and sieved in order to obtain a fine green powder stored and sold in labelled bags or glass jars.

Acacia is also known for the gum extraction. It holds an important place in the Moorish traditional medicine. It is a viscous substance exuded from tree trunks and used in textile, pharmaceutical, mining, food, cosmetics [6]. Inhabitants of Laayoune region buy gum from the market and use it in small quantities with tea. For the phytochemical characterization, it is milled and sieved to obtain a white fine powder.

Concerning *Adansonia Digitata*, commercialized and edible parts; leaves, fruit pulp and seeds are imported from Mauritania and sold by traditional healers in the form of a powder used against stomach ache and diarrhoea.

Phytochemical Screening

Tannins Characterization

A brew 5% was prepared from vegetable powders mentioned above in 100 ml of boiling distilled water. After 15 minutes of infusion, the solution was filtered and rinsed with hot distilled water until a volume of 100 ml is obtained. The same brew was used for the characterization of different types of flavonoids [7].

Gallic tannins have been identified by the addition of 1.5 ml of the reagent Stiasny to 3 ml of the infused. After 15 minutes of heating in a water bath at 90 °C, the mixture was filtered and saturated with 0.5 g of sodium acetate. 0.1 ml of a solution of ferric chloride (FeCl₃) (1%) was then added. The appearance of a blue-black colour indicates the presence of gallic tannins [7].

1 ml of concentrated hydrochloric acid (HCl) was added to 5 ml of the infused. The mixture is boiled for 15 minutes. The formation of a red precipitate insoluble in iso-amyl alcohol indicates the presence of catechins tannins [7].

Flavonoids Characterization

5 ml of 5% infused; previously prepared, was added to 5 ml of hydrochloric alcohol (50% HCl in ethanol) and 1 ml of iso-amyl alcohol with some magnesium chips. The appearance of a pink-orange colour (flavones) or purplish pink (flavanones) reveals the presence of free flavonoids [7].

The same reaction cyanidin cited above but without addition of magnesium chips is used to indicate the presence of anthocyanins. After 15 minutes of heating in a water bath, the appearance of a cherry-red colour is characteristic of the presence of leucoanthocyanins (flavonols and flavanonols) while a brown-red colour indicates the presence of catechol [7].

Anthocyanins were revealed by adding 5 ml of 5% infused with 5 ml of sulfuric acid (10%) (H₂SO₄) and 5 ml of

ammonia (50%) (NH₄OH). The colour of the infused is accentuated by acidification then turns blue in basic medium in the presence of anthocyanins [7].

Sterols and Polyterpenes Characterization

A 1g maceration of plant material powder in 20 ml of ether for 24 hours (in a closed glass jar) is used to search sterols and polyterpenes, carotenoids and coumarins [7].

1 ml of CHCl₃ is added to residue of 10ml evaporated maceration. The resulting solution is divided between two test tubes. 1 to 2 ml of H₂SO₄ is added to the first tube, the second will serve as a control. The presence of sterols and polyterpenes is revealed by the formation of a brownish red or a purple ring at the contact area of the two solutions [7].

Carotenoids Characterization

2 to 3 drops of saturated antimony trichloride in chloroform are added of the residue to 5ml evaporated to dryness maceration. A blue colour becoming red reveals the presence of carotenoids [7].

Coumarins Characterization

Method 1

4 ml of ethanol's solution 5% is divided between two test tubes. In one of the tubes, 0.5 ml of NaOH at 10% is added, and then the test tubes are heated in a water bath until boiling. After cooling, 4 ml of distilled water are added to each test tube. If the liquid from the test tube in which we added the alkaline solution is clearer compared to the control test tube liquid (unbuffered solution) the reaction is positive. By acidifying the clear solution with a few drops of concentrated HCl, it loses its yellow colour, is cloudy or precipitates [8].

Method 2

5ml of ether extract is evaporated to dryness. The residues are taken back in 2 ml of hot water and 1ml of NH₄OH at 25%. The observation under UV at 366 nm with an intense blue fluorescence indicates the qualitative presence of coumarins [7].

Saponins Characterization: Foam Test

0.5 g of extract was shaken with 2 ml of warm water. If foam produced persists for ten minutes it indicates the presence of saponins [9].

Characterization of Reducing Sugars

2 g of different herbal drugs from each plant are macerated in 15ml of methanol for 48 h in a closed glass bottle to prevent evaporation of the solvent. 5ml of the filtered suspension was added to 5 ml of Fehling's solution and placed in a test tube (2.5 ml of solution A is obtained by adding 2 g of SO₄Cu to 50ml of distilled water and 2.5 ml of solution B is obtained by dissolving 7.5 g of NaOH in 50 ml of distilled water and 10g of Tartrate Na / K are then added). After heating the tube in a water bath for 2 to 3 min at 70 °C, the observation of a brick red precipitate indicates the presence of reducing compounds [8].

Alkaloids Characterization

2 g of herbal drugs of each plant were macerated in 15 ml of distilled water for 24 hours under ambient climatic conditions. After filtration, the maceration of each sample is placed in a test tube to be tested by the Dragendroff reagent; a second test tube; containing distilled water and a few drops of reagent; is

considered as control. The reaction is positive with the presence of a precipitate of a reddish-orange colour [10].

Free quinones Characterization

2g of plant material are macerated in 20 ml of petroleum ether. After a few minutes of stirring, the mixture is allowed to stand for 12 hours. A few drops of NaOH 1/10 are added to the evaporated to dryness petroleum extract. The presence of free quinones is confirmed by a colour change of the aqueous phases to yellow, red or purple [11].

Mucilage test

A 1 ml decoction plant sample at 10% is added to 5ml of absolute ethanol. The formation of a flocculent precipitate indicates the presence of mucilage [12].

Drug Research

Put in a test tube 0.5 g of the plant powder and add 5 ml of petroleum ether. After stirring for 15 min filter the mixture and evaporate the filtrate to dryness in a water bath. 4 to 5 drops of KOH at 5% in alcohol are added to the residue. The appearance of a purple coloration indicates the presence of tetrahydrocannabinol type drugs (reaction BEAM) [13].

Proteins Characterization

The proteins are identified by the Biuret reaction. 1 g of plant powder was added to 2 ml of aqueous NaOH at 20% in a test tube, to which are added 2 to 3 drops of an aqueous solution of CuSO₄ at 2%. The appearance of a purple colour, sometimes tinted of red, indicates a positive reaction [8].

Lipoids Characterization

2g of plant material was macerated in 15 ml of petroleum ether for 30 minutes. The resulting filtrate was evaporated. 3 drops of H₂SO₄ were added to the residue. The appearance of a strong purple or green colour indicates the presence of lipoids [10].

Essential oils Characterization

10ml of dichloromethane extract at 1% was evaporated to dryness. The residue was then dissolved in 3 ml of ethanol. The solution thus obtained is evaporated again to dryness. A perfume smell indicates the presence of essential oils [14].

Irridoides Characterization

A decoction is prepared by adding 1 g of plant material in 20ml of distilled water. The whole is boiled for 1 hour. After cooling the filtrate and evaporation, 1 ml of concentrated HCl is added to the residue. The test is positive if there is a blackish precipitate formation after heating the solution [11].

Thin layer chromatography (TLC)

The realization of a separating analysis; namely the thin layer chromatography (TLC) completing the characterization by phytochemical screening. TLC plates were used (silica gel on an aluminium support DC-Fertigfolien ALUGRAM® SIL G / UV254) using as an eluent and an developer of different solvents and reagents. The following table summarizes the solvents and controls used for the characterization of phytochemical constituents of edible parts of *Acacia raddiana* and *Adansonia Digitata* by Thin Layer Chromatography. If necessary, a UV lamp is used to identify the components at 366 nm. The spots were spotted and the migration distances of these spots were measured.

Table 1: Extraction solvents and Chromatography Thin Layer system

Secondary metabolites	extraction solvents	migration Solvents	revelation reagents
Flavonoids [15]	Methanol	15% acetic acid in water	5% aluminium chloride in mixture methanol / water (1: 1)
Tannins [16]	Acetone	Ethyl acetate / Methanol / Water (40 : 8 : 5)	Ferric chloride / acetic acid / water (2: 2: 96)
Anthraquinones [17]	Methanol	Ethyl acetate / Methanol / Water (81 : 11 : 8)	Potassium hydroxide
Coumarins [15, 18]	Chloroform	Ethyl acetate / Toluene (10 : 93)	ammonia NH ₃
Alkaloids [16]	Acetone	Chloroform / Diethylamine (45: 5)	Reagent Dragendroff
Carotenoids	Dichloromethane	Diethyl ether / petroleum ether (60: 40)	-
Saponins [19]	Methanol	Chloroform/ Methanol/ water (60: 30: 4)	Antimony trichloride
Terpenoids [20]	Hexane	Benzene	Antimony trichloride

Results and Discussion

Phytochemical Screening

The following table shows the results of phytochemical screening obtained for the various edible parts of both plants

tested, *Acacia raddiana* and *Adansonia Digitata*.

The results are arranged in the following table according to the degree of reactivity.

Table 2: Results of the characterization of the various bodies of the two plants studied *Adansonia Digitata* and *Acacia raddiana* secondary metabolites

Phytochemicals Tests	Plants	<i>Adansonia Digitata</i>			<i>Acacia raddiana</i>	
		fruit pulp	leaves	seeds	leaves	gum
Tannins	Simples	+	+	-	+++	-
	Catechics	+	++	-	++	-
	Gallic	+	-	-	+	-
Flavonoids	Flavones	-	-	-	+	-
	Flavanones	-	-	-	-	-
	Anthocyanins	-	-	-	-	-
	Leucoanthocyanins	+	+	+	++	-
	Catechol	-	-	-	-	-

Anthracene Derivatives	Free	-	-	-	-	-
	o-glycosides	-	-	-	-	-
	o-glycosides genins	-	-	-	-	-
	c-glycosides	+++	+++	+++	+	-
Test of Mucilage		+++	++	+++	+++	-
Sterols et Polyterpenes		+	+++	++	-	-
Reducing Sugars		+++	+++	+++	-	-
Alkaloids		++	+++	-	+	-
Saponins		+	-	-	-	+++
Free Quinones		++(red)	+(yellow)	-	-	-
Coumarins						
1st method		-	+	+	+	-
After addition of HCl		-	+	+	+	-
2nd method		-	+	+	+	-
Carotenoids		-	+++ greenish blue	++ Red- orange	+++ greenish blue	-
Drugs		-	-	-	-	-
Essential Oils		-	+	+	+	+
Lipoids		-	+++	-	+++	-
Proteins		+	-	-	-	-
Irridoïdes		+++	+++	+++	+++	-

- Positive reaction: +++
- Moderately Positive reaction: ++
- Trace: +
- Negative test: -

Adansonia Digitata

Phytochemical characterization of *Adansonia Digitata* revealed the presence of traces of different types of simple tannins, Gallic and catechin at the fruit pulp as well as traces of simple tannins and a moderately positive reaction catechin tannins in the leaves. Regarding the seeds, the results indicate an absence of all tannins.

The leucoanthocyanins are the only type of flavonoid which presence is revealed in the form of traces among the three organs tested.

The anthracene derivatives are abundantly present in the form of C-glycosides in fruits, leaves and seeds.

The mucilage test was highly positive in fruit pulp and seeds and moderately positive for the leaves.

Sterols and polyterpenes are significantly present in the leaves of *Adansonia Digitata*, moderately present in the seeds and are released in the form of traces in the case of fruit pulp.

Unlike a total absence of drugs, the reducing sugars and irridoides are very abundant in the three edible parts of *Adansonia Digitata*.

Lipoids are present only but abundantly in the leaves of *Adansonia Digitata*, however saponins and proteins are present in the form of traces only in the pulp.

In terms of alkaloids, a precipitate of reddish-orange colour indicates a strong presence in the leaves, a medium presence in the pulp and a total absence in the seeds.

While the reaction of the free quinones remained colourless for the seeds, an intense red and yellow coloration indicated their presence in the pulp of the fruit and leaves.

The qualitative results also showed the presence of coumarin and essential oils in a form of traces in the leaves and seeds.

Finally, in terms of carotenoids, a green blue colour indicates an abundant presence in leaves and an orange-red colour indicates an average presence in seeds.

Acacia raddiana

The results of the phytochemical screening gum Acacia show a significant presence of saponins and a reaction in traces for essential oils as well as a total absence of other secondary metabolites.

Regarding the *Acacia raddiana* leaves; they showed a very intense reaction for simple tannins, mucilage test, carotenoids (green-blue), lipoids and irridoides. The reactions of catechin tannins and flavonoids in form of leucoanthocyanins proved to be moderately positive. Gallic tannins, flavones, anthracene derivatives (C-glycosides), alkaloids, coumarins, and essential oils are present in the form of traces.

TLC

The phytochemical screening on extracts of different organs by TLC yielded the results shown in the following table.

Table 3: TLC results

		<i>Adansonia Digitata</i>			<i>Acacia Raddiana</i>	
		Pulp	leaves	seeds	leaves	gum
secondary metabolites with Rf and color spots	Tannins	0.103 (red)	0.819 (red) 0.987 (red)	-	0.771 (red) 0.819 (red) 0.987 (red)	0.927 (bleu) 0.963 (bleu)
	Flavonoids	0.062 (bleu) 0.837 (red) 0.987 (red)	0.062 (orange) 0.137 (orange) 0.775 (red) 0.987 (red)	-	0.062 (orange) 0.137 (orange) 0.775 (red) 0.837 (red) 0.9 (red) 0.987 (red)	-

	Coumarins	-	0.114 (bleu) 0.178 (bleu) 0.658 (bleu) 0.734 (bleu) 0.886 (bleu) 0.975 (bleu)	0.114 (bleu) 0.178 (bleu) 0.658 (bleu) 0.734 (bleu)	0.114 (bleu) 0.78 (bleu) 0.886 (bleu) 0.975 (bleu)	-
	Anthraquinones	-	0.937 (red) 0.962 (red) 0.975 (red)	-	0.937 (red) 0.962 (red) 0.975 (red)	-
	Carotenoids	yellow brown	yellow green- yellow green-bleu yellow-orange	yellow brown	yellow green-yellow green-bleu brown yellow-orange	-
	Alkaloids	0.951(red)	0.048 (red) 0.951 (red) 0.975 (red)	-	0.048 (red) 0.975 (red)	-
	Saponines	-	0.4 (red) 0.72 (red) 0.867 (red)	-	0.507 (red) 0.587 (red) 0.773 (red) 0867 (red)	-
	Terpenoids	-	-	-	-	-

Adansonia Digitata

The TLC visualized with UV (366 nm) has spots of red, orange, blue, yellow, green and brown which may correspond to multiple classes of secondary metabolites.

According to the spots observed in the pulp of the fruit extracts, it contains tannins, various types of flavonoids and alkaloids with diverse colours and retention factors (R_f). The carotenoids observed are of the xanthophyll kind (yellow spot). Brown spots correspond to the pheophytin pigment. These results confirm those observed in the phytochemical screening.

The leaves also show similar results to the screening; namely the presence of tannins, flavonoids, coumarins and carotenoids (xanthophylls: yellow; carotene: yellow-orange). Chlorophylls a and b are represented by green-yellow and green-blue spots. Anthraquinones are marked by red spots with diverse R_f .

The TLC obtained for seeds indicates the presence of four types of coumarins with R_f s similar to those observed in the leaves. The spots are seen only under UV. Carotenoids of the Xanthophylls types of are also present. Pheophytin is as well observed at the TLC of carotenoids.

Acacia raddiana

The *Acacia raddiana* leaves characterization by TLC showed the presence of several types of tannins, flavonoids, coumarins, anthraquinones, alkaloids and saponins.

Xanthophyll and carotene are also present in addition to chlorophyll a and b and pheophytin.

With regard to the gum, only tannins were observed under UV.

Roughly speaking, the results of CCM were similar to those of the phytochemical screening.

Discussion

Considering the results obtained via the phytochemical screening by staining and TLC, it seems that the bodies of *Adansonia Digitata* are full of very important secondary metabolites that could justify growing interest of traditional medicine in this plant.

In particular, leaves of *Adansonia Digitata* are rich in phenolic compounds and other metabolites. This is consistent notably with other scientific studies showing that the leaves contain many phenolic compounds namely flavonoids and tannins and alkaloids, reducing sugars and quinones [21].

However the pulp is rich in flavonoids and tannins and shows no positive results for coumarin and anthraquinones. These results were confirmed by the studies of I.K.E.D. Koko and *al* [22]. It also showed that the seeds contain important secondary metabolites namely, coumarins, carotenoids, reducing sugars, irridoides, sterols and terpenes, and leucoanthocyanins.

Similarly to the studies of El-Mousallamy and *al* [23], the results found show that the leaves of *Acacia raddiana* also contain phenolic compounds such as flavonoids, tannins, coumarins and anthraquinones. They also contain carotenoids, alkaloids and saponins. The gum is particularly rich in saponins and tannins.

The conducted Thin layer Chromatography has confirmed the results of phytochemical screening by staining. Indeed, the TLC is an analytical technique used in this case first to identify specific secondary metabolites extracts, and second to separate the constituents of these metabolites.

Calculating the front ratio (R_f) or retention factor of a compound; ratio of the migration distance of substance on the migration distance of solvent-front, showed significant diversity of compounds separated from the different researched secondary metabolites. Chromatograms have validated the presence of several types of tannins, flavonoids, coumarins, quinones, carotenoids, saponins, alkaloids and terpenes relative to phytochemicals colouring reactions in various organs of the two plants tested.

The little dissimilarity observed for the absence or presence of certain secondary metabolites compared with other studies or from the TLC performed can be explained by the choice and method of extraction. Indeed, the solvent plays a crucial role in the extraction process, since the solubility is the most important parameter. The solubility of the phenolic compounds is affected by the polarity of the solvent used [24, 9].

Conclusion

Acacia raddiana and *Adansonia Digitata* are among the most medicinal plants used in traditional medicine in the province of Laayoune. For this reason, a qualitative phytochemical study by staining followed by thin layer chromatography has been developed for the different edible organs of these two plants in order to reach a characterization of the chemical substances which may be responsible of the curative characteristics. Indeed, this study showed the presence of several secondary metabolites such as phenolic compounds, sterols and triterpenes, alkaloids, reducing sugars, quinones, irridoides, and saponins. These can effectively endow the two plants studied with anti-inflammatory, antiseptic and antibacterial properties which are very important and widely sought.

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