

Valorization of *Aloe vera* (L) as medicinal plant: antibacterial and hemostatic activities of *Aloe vera* sap and gel

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After having identified the plant study material (*Aloe vera* L.) by the macroscopic appearance and under the photonic microscope observations of the stem, leaves, flowers and rhizomes we carried out extractions of the juice and the gel. Studies of antimicrobial activities have been completed.

The results obtained show that the extracts of the gel and sap of *Aloe vera* (L) revealed that the sap of *Aloe vera* has an antibacterial power versus *Escherichia coli* and *Staphylococcus epidermidis*.

Hemostatic study revealed that the sap and gel of *Aloe vera* have a coagulation power.

Key words: medicinal plant, *Aloe barbadensis* Miller, *Aloe vera* (L), gel, sap.

1. Introduction

For a long time, medicinal plants have played a decisive role in the preservation of human health and the survival of humanity. They are a sacred and precious heritage and constitute a response of choice to provide the body, in a natural way, the substances necessary to maintain its vital balance.

Medicinal plants are used for their particular beneficial properties for human health (Dutertre, 2011). Indeed, they are used in different ways, decoction, maceration and infusion. One or more of their parts can be used, root, leaf, flower (Dutertre, 2011).

According to Demirtas et al., (2017), medicinal plants have been used by humans for nearly 7000 years and some animals also consume them for therapeutic purposes. About 35,000 plant species are used globally for medicinal purposes, which constitutes the widest range of biodiversity used by humans. The objectives expected during this experiment are study of antibacterial, hemostatic and toxic effects of *Aloe vera* (L).

2. Methods and material

2.1 Plant study material

Our study focused on the extraction, drying (at 50 ° C in a ventilated oven) and the characterization of the juice and gel of *Aloe vera* (L) its fresh leaves were harvested at the EL Botanical Garden. HAMMA (Algiers). Extraction of *Aloe vera* (L) gel and juice is made from leaves of *Aloe vera* L. aged 3 to 4 years.

2.2 Microbiological study material:

Our study focused on five bacterial strains (Table 1), of which four are from the microbiology laboratory at the Blida University Hospital Center (Frantz Fanon), and one was provided by the Dutch firm SKALAR (supplier of laboratory equipment and consumables).

Table 1: Results of extraction of *Aloe vera* L. pulp, gel and sap.

	Weight (g)	Percentage%
Whole leaf	288,79	/
Fresh pulp	216,59	75,45
Gel	144,39	50,60
Juice	1,33	0,46

2.3. Methods of analysis

2.3.1. Study of the biological activities of *Aloe vera* (L)

It includes all the laboratory tests carried out "in vitro" in reference organisms (Vandenberghe and Vlietinck, 1991, Murengezi, 1993).

2.3.1.1. Determination of the antimicrobial activity of the juice and gel of *Aloe vera* (L)

It makes it possible to prove the existence of an antimicrobial activity of the juice and the gel which results in the formation of an inhibition zone around the disk containing the extracts prepared. The technique used is the diffusion of agar medium by the method of the disks (Bendjilali et al., 1986 ; Malan et al., 1986, Menghini et al., 1987).

a) Principle

It simply involves impregnating Whatman paper disks with the substances to be tested. These discs will be deposited on previously prepared petri dishes containing 10 ml of agar medium and seeded with the chosen seed.

Operating mode

1. Preparation of extracts from juice and gel:

Extraction from the juice of *Aloe vera* (L)

The purpose of the preparation of the extracts from the juice is to release the anthraquinones present in the juice, which are endowed with an antimicrobial power.

Excerpt 1:

- Filter the pure sap by passing it through a filter paper;
- Sterilize the extract by passing it through a microbial filtration membrane of 0.45 µm millipore.

Extract 2:

- Dissolve 100 mg of sap powder in 100 ml of ethyl acetate;
- Shake for 5 minutes;
- Centrifuge at 3500 rpm **for 5 min**;
- Take the supernatant;

Sterilize the extract by passing it through a microbial filtration membrane of 0.45 µm millipore.

Excerpt 3:

- Dissolve 100 mg of the powder in 100 ml of methanol;
- Shake for 5 min;
- Centrifuge at 3500 rpm for 5 min;
- Take the supernatant;

Sterilize the extract by passing it through a microbial filtration membrane of 0.45 µm millipore.

Excerpt 4:

- Dissolve 100 mg of sap powder in 100 ml of sterile ultra- pure water;
- Shake for 5 min;
- Centrifuge at 3500 rpm for 5min;
- Take the supernatant;

Sterilize the extract by passing it through a microbial filtration membrane of 0.45 µm millipore.

Extraction from the gel of *Aloe vera* (L)

The gel of *Aloe vera* L. is very rich in saponin, the latter are soluble in water and endowed with an antimicrobial power.

Excerpt 1:

- Filter the gel by filter paper

Sterilize the extract by passing it through a microbial filtration membrane of 0.45 µm millipore.

Extract 2:

- Dissolve 100 mg of the gel powder in 100 ml of sterile ultra- pure water;
- Shake for 30 minutes then filter;

Sterilize the extract by passing it through a microbial filtration membrane of 0.45 µm millipore.

2. Preparation of the bacterial inoculum

The inoculum of each bacterial type is prepared from broth cultures 18 to 24 hours before handling for bacterial propagation. The dilution is carried out with 10 cc of sterile physiological water and a few drops of culture broth until a visible opacity is obtained.

Inoculum 1: *Escherichia coli*

Inoculum 2: *Pseudomonas aeruginosa*

Inoculum 3: *Staphylococcus aureus*

Inoculum 4: *Staphylococcus epidermidis*

3. Preparation of culture media

The development medium for this type of strain is Muller Hinton. Twenty-four petri dishes containing the previously liquefied agar medium are prepared.

4. Seeding of bacteria

Using a sterile swab soaked in the culture broth, the plates are spread on the agar at the level of eight dishes per germ.

5. Depositing discs

Using a Pasteur pipette, soak the previously sterilized Wattman paper discs with two discs per extract, a total of 12 discs per germ and 4 per box for sap and two extracts for gel. Add 50 μ l of DMSO to each extract.

The soaked discs are placed on the nutrient medium agar at equal distances.

6. Incubation

The boxes in question are first placed in the refrigerator at + 4 ° C. for 15 to 20 minutes, in order to allow the extract to diffuse into the nutrient agar.

The boxes thus seeded are placed in the oven for 24 hours at 37 ° C.

Four "control" boxes are started at the same time as the experiment and under the same conditions containing only the culture medium, the seeds in question and the Wathman paper disks soaked with the distilled water and DMSO solution, methanol, and ethyl acetate

b) Expression of results:

The reading is done with the naked eye after the incubation by the observation of the existence of a free zone of inhibition (Figure 1).

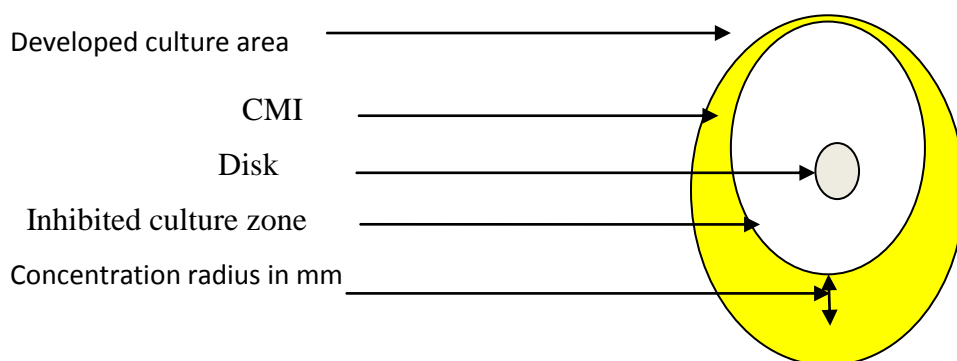


Figure 1: Diffusion technique of the agar extracts (Menghini and *al.*, 1987)

3. Results and discussion

3.1. Botanical study

3.1.1. Macroscopic appearance

The observation with the naked eye of the plant *Aloe vera* (L), allowed us to deduce that it is a tree plant about 80 cm high. It has an aerial part, consisting of a very short stem, fleshy leaves and yellow flowers, and a terrestrial part (Rhizome), which includes the roots (Figure 2).



Figure 2 : Plant of the *Aloe vera* (L)

Judd et al., (2001) reports that *Aloe vera* (L) is a 60 to 80 cm tall, shallow- rooted trees whose very ligneous stems bear a bundle of smooth fleshy leaves. The author's findings corroborate our observations.

3.1.1.1. The stem

Following observation with the naked eye, we found that the stem of *Aloe vera* (L) is very short 15 cm, robust, thick and woody (Figure 3).



FIGURE 3: stem of *Aloe vera* (L)

3.1.1.2. Leaves

The leaves of *Aloe vera* (L) are fleshy, lanceolate in shape with a triangular section and pointed ends (Figure 4.A), are arranged in a rosette (the young leaves growing in the middle and the older ones being outside) (Figure 4.B). Indeed Goetz and Paris (2007), describe the leaves of *Aloe vera* (L) as being smooth with thick cuticles, a very beautiful green color, up to 80cm long and 10cm wide binocular examination of the histological section of the *Aloe vera* L. leaf showed the existence of peripheral cells that secrete the sap and an aquifer parenchyma representing the pulp of *Aloe vera* (L) (Figure 5 A and B).



Figure 4.A : Leaf of the *Aloe vera* (L) **Figure 4.B:** Insertion of the lanceolate leaves of *Aloe vera*

(L)

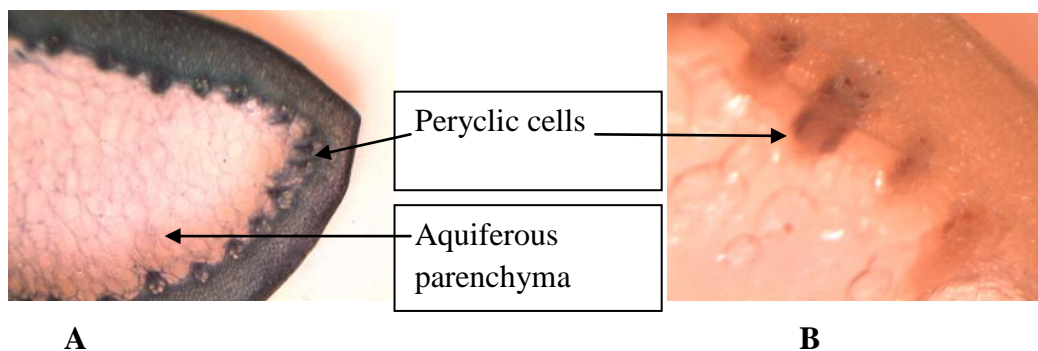


Figure 5 : Transverse section of the leaf under a binocular loupe; A : (Gr x 40) et, B (Gr x 20)

3.1.1.3. Flowers

The flowers are spread over two or three 90 cm stems (each of them carrying several tens). They are tubular, pendulous, in the shape of small trumpets of yellowish color, and hatch successively. The one at the base of the shaft hatch and open first. The fruit is a capsule (Figure 6).



Figure 6: Inflorescence of the *Aloe vera* (L)

The flowers of *Aloe vera* (L) are borne by pedicels about 0.5cm long. They are characterized by a perianth of bright yellow color measuring about 2.6 cm, composed of six tepals and organized in two whorls welded at the base.

These flowers are equipped with androecia which are dialystem, it is longer than the perianth and composed of six free stamens of the type didynome (three large and three small) surrounding a pistil located at the center of the flower (Figure 7).

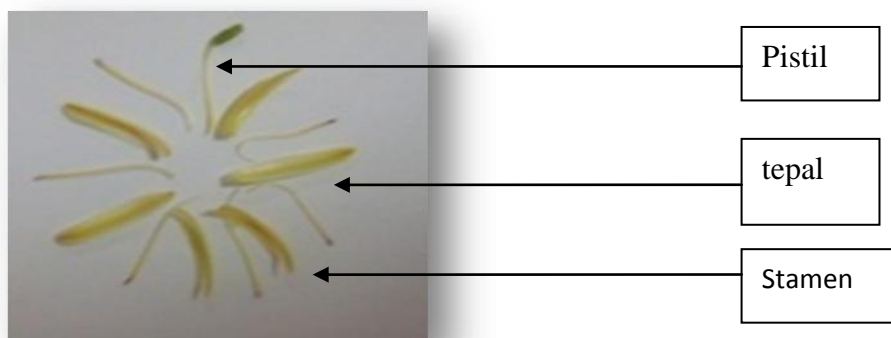


Figure 7 : *Aloe vera* shelled flower

Our observations are consistent with those of Reynolds (2004), who found that flowers of *Aloe vera* (L) are characterized by a bright yellow color, in which there is a developed perianth. The latter has three comprising three alternating internal parts and all similar, free and frequently petaloid, with six stamens in two whorls of three.

3.1.1.4. The rhizome

The study revealed that the rhizome of *Aloe vera* (L) is thick, and shallow 19 cm, on which are inserted more or less long roots which ramify by their turn into secondary roots (Figure 8).



Figure 8: Rhizome and roots of *Aloe vera* (L)

Binocular observation of the rhizome revealed the existence of several zones (Figure 9). The first being the epidermis, consisting of a layer of juxtaposed cells, then comes the cortical parenchyma which is composed of large polyhedral cells. Finally, the conductive tissues gathered in clusters are superposed with xylem and phloem. The xylem is located towards the center of the rhizome and capped, outward by the phloem. The observation of the *Aloe vera* (L) root under a

binocular loop showed the presence of a sub-steroid, a cortical parenchyma and a medullary parenchyma (Figure 10).



Figure 9: Cross section of the rhizome under a binocular loupe (Gr \times 40)

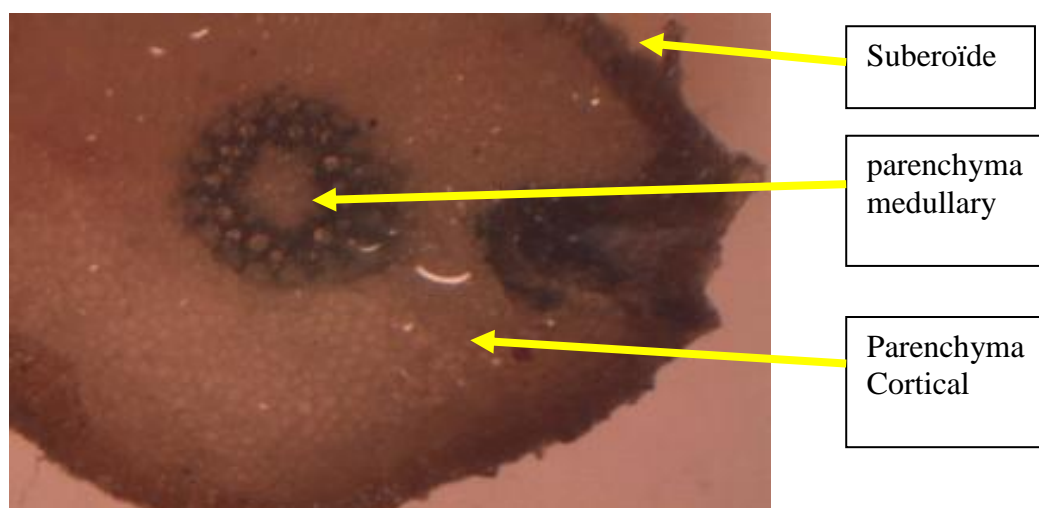


Figure 10: Cross section of the root under a binocular loupe (Gr \times 40)

3.2. Microscopic appearance

3.2.1 The Leaf

The light microscopic observation at high magnifications (Gr \times 40) and (Gr \times 100), transverse histological sections of the leaves of *Aloe vera* (L) (Figure 11) reveal the presence of the periphery towards the center of the following fabrics:

- A thick striated cuticle surrounding an epidermis formed of a base of small epidermal cells. They are closely related to each other (Figure 11 A, B);
- A chlorophyllian parenchyma palisades formed by five seats of elongated cells of rectangular shape. The latter envelops the entire sheet and lines the upper and lower faces (Figure 11 A);
- A pericycle formed of two seats of closely linked small cells. The latter surrounds all the conducting beams (xylem and phloem) (Figure 11 B, C);

- A parenchyma aquifer reserve with meatus formed of large rounded cells occupying the central part and which represents the major part of the sheet.

According to Pitton (1995), the cross-section of the leaf of *Aloe vera* (L) makes it possible to distinguish successively, going from outside to inside, the cuticle a chlorophyllian epidermal layer, a cellulosic dermis and finally, a parenchyma mucilaginous. While Bruneton (2015) shows that the cross-section of the leaf of *Aloe vera* (L) is provided with a chlorophyllian and amyloiferous parenchyma, mucilage cells and between the two isolated pericycles and endoderms.

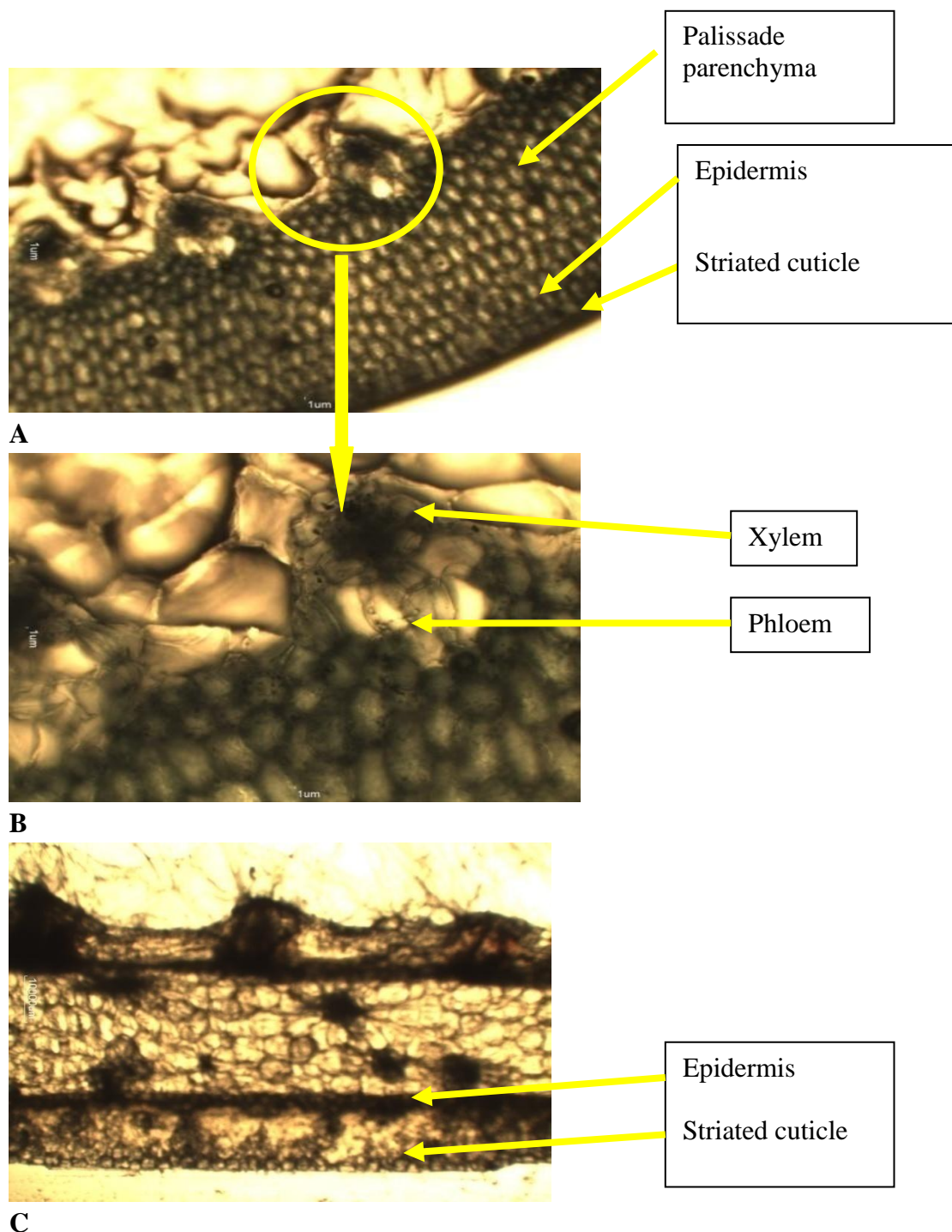


Figure 11: Cross section of the leaf under optical microscope;
A : (Gr x 40), B : (Gr x100), C : peripheral area (Gr x 40)

3.2.2. The root

The light microscopic observation at magnifications (Gr x40) and (Gr x100) of the cross sections made at the level of the *Aloe vera* L. root (Figure 12) reveals the presence of a monocotyledonous anatomical structure, composed of from the outside to the inside 2 protective layers: This is the piliferous base formed of small cells and absorbent hairs (Figure 12 B), and the suberoid formed of three layers of strongly centered walled cells. to each other (Figure 12 A, B). In fact, the cortical parenchyma occupies most of the root. It is formed of large rounded cells presenting meatus between them (Figure 12A). On the other hand, the pericycle consists of 2 seats of small rectangular epicyclic cells closely linked to each other surrounding the whole and the marrow.

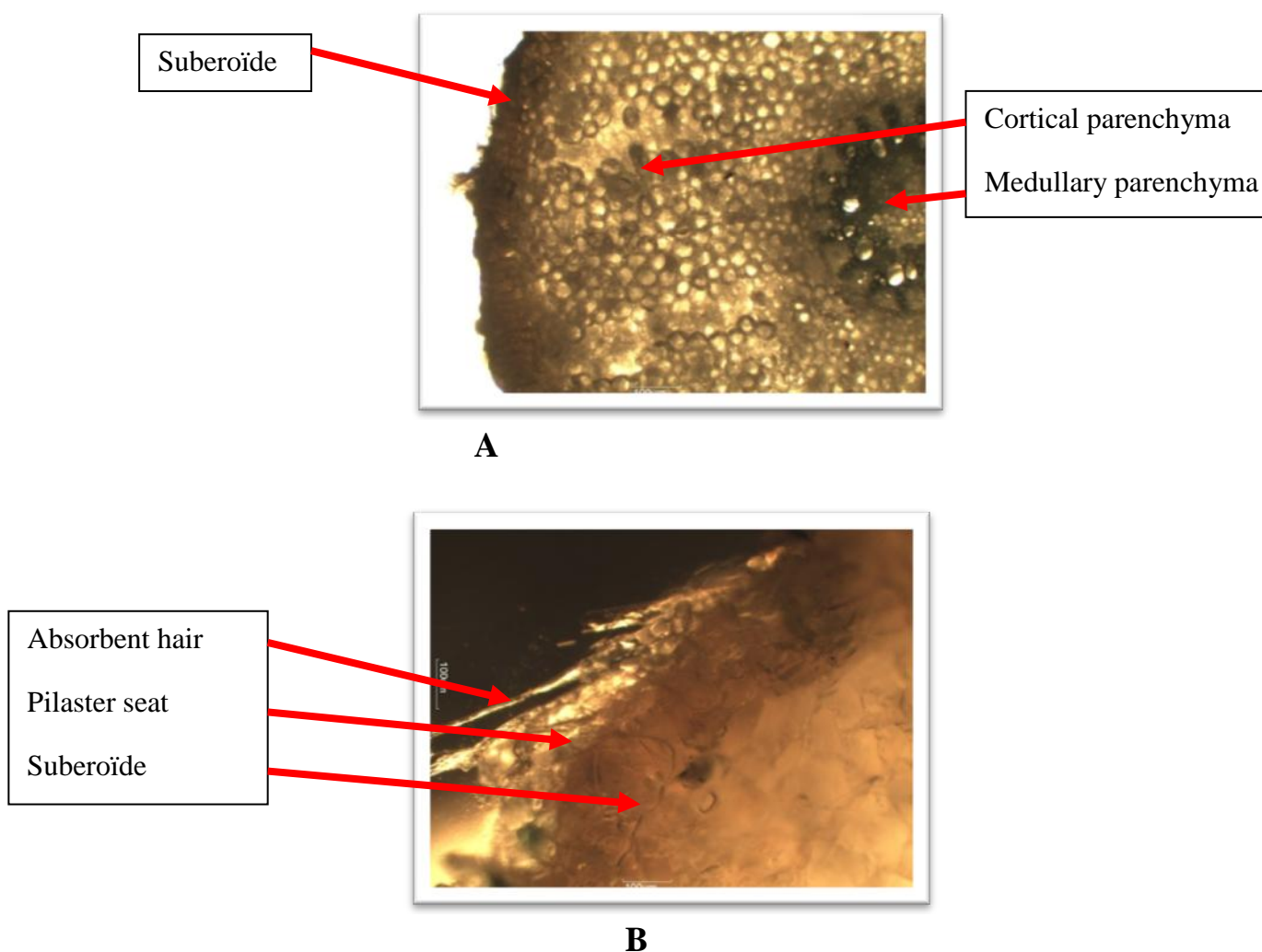


Figure 12: Cross section of the root under optical microscope, after double staining;

A: overall view Gr X40; B: peripheral area

The other tissues that form the root are in the form of conductive tissues.

The latter consist of xylem and phloem arranged alternately; the xylem is formed of two types of vessels: the metaxylem vessel with large caliber located towards the center and protoxylème vessel with a small caliber located towards the periphery arranged in V the differentiation of these vessels is centripetal (Figure 13 A, B).

The medullar parenchyma or the marrow of the root formed of small rounded cells which have meatus between them.

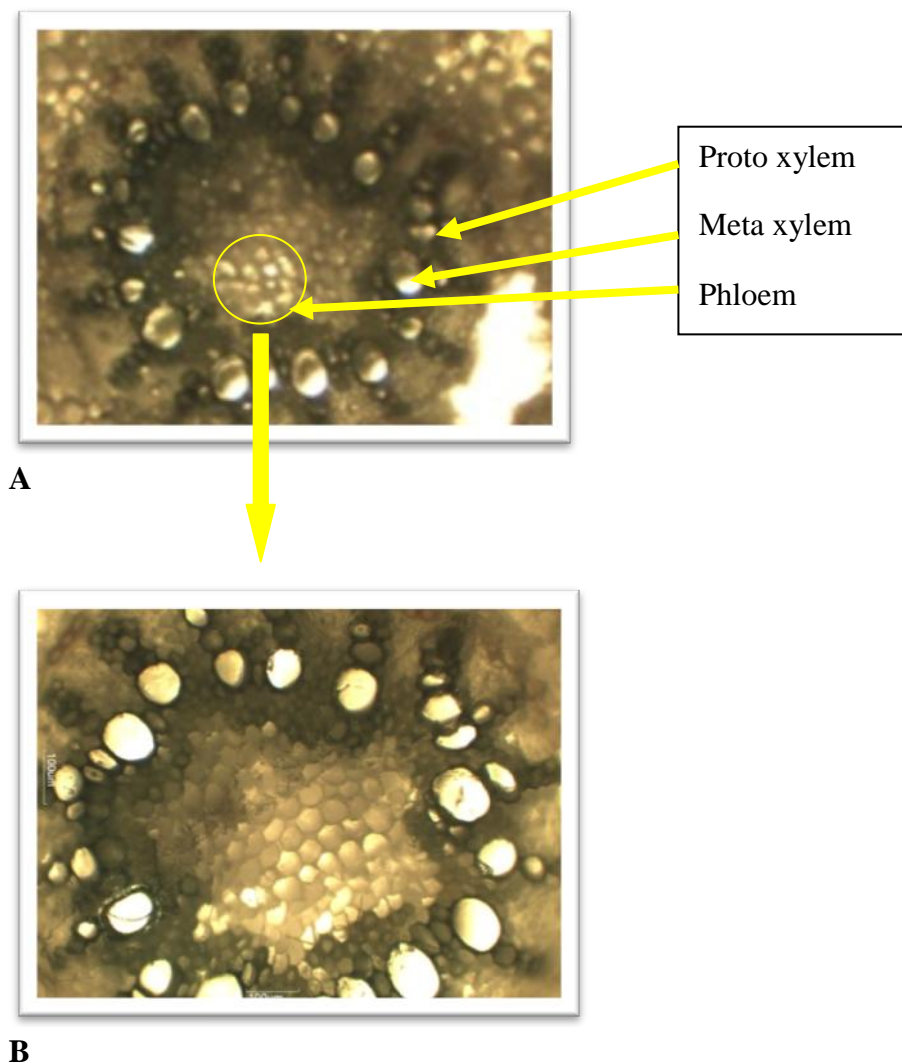


Figure 13: Cross section of the root under optical microscope;

A: central zone (G x100) B: central zone (Gr x100).

3.3. Extraction and yield of juice and gel from the leaf of *Aloe vera* (L)

After letting the sap out and extracting the pulp and recovering the gel from the leaves of *Aloe vera* (L), we obtained the results presented in Table 1.

The latter show that the *Aloe vera* (L) leaf, 54 cm long, weighs on average 288.79 g, it contains approximately 216.59 g of pulp, is 75% of the weight of the leaf, 144.39 g of gel or 50% of the weight of the leaf and 1.33g of sap is 0.46% of the weight of the leaf, which is not the same for Zapata et al., (2013), who worked on a variety of *Aloe vera* (L) from Spain, which found in their experimental condition, that a leaf of *Aloe vera* (L) of length equal to 68.29 cm weighs 559.9 g and contains about 66.47%.

3.3.1. Properties of the gel and the sap

The fresh pulp of *Aloe vera* (L) is transparent and very gelatinous (Figure 14), as well as the fresh gel which is translucent and very gelatinous, whereas the powdered gel is wrinkled and beige (Figure 14). While the fresh sap is viscous yellow in color that turns brownish in contact with the air. Finally, the sap powder is dark brown opaque resinous (Figure 15).



Figure 14: Pulp of *Aloe vera* L.



Figure 15 : *Aloe vera* L. gel

3.4. Study of the biological activities of juice and gel

3.4.1. Antimicrobial activity

The objective of this work is to highlight a possible antibacterial effect of the prepared extracts.

The principle as already described above, consists of inoculating a plate of agar medium by a test microorganism and bringing it into contact with the substance to be tested at a small determined zone. After standing in an oven for 24 hours, the action of the extract is determined by the diameter of the inhibition halo which appears clear around the contact zone. (Paris and Moyse, 1965). According to these same authors, the diameter calculation of the zone of inhibition of microorganisms is as follows:

- No inhibition diameter (-)
- Inhibition diameter less than 7 mm (m)
- Inhibition diameter between 7 and 10 mm (+)
- Inhibition diameter between 10 and 16 mm (+ +)
- Inhibition diameter greater than 16 (+ + +)

It should be recalled that the reading is always performed in comparison with a control box containing only the culture medium and the test microorganism. This box is always sown at the same time as the other boxes of the experiment and under the same conditions.

It is evident that at the control dishes, bacterial growth is complete and complete after incubation. Which allowed obtaining the following results.

After the incubation time, we removed the boxes for the interpretation of the results.

During their stay in the incubator, the extracts contained in the discs are diffused in the medium, and according to the sensitivity of the 4 germs tested, there will or will not occur zones of inhibition. The results of the microbial activity are summarized in Table 2.

Table 2: Antimicrobial Activity of Juice and Gel Extracts

Strain	E 1 s	E 2 s	E 3 s	E 4 s	E1 g	E2 g	DA +DMSO	DE+DMSO	T
<i>Escherichia coli</i>	-	+	-	-	-	-	-	-	-
<i>Pseudomonas. aeruginosa</i>	-	-	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	-	-
<i>Staphylococcus. epidermidis</i>	-	m	-	-	-	-	-	-	-

E : Extract ; DA et DE : Acetate disc and ethanol ; T : Witness; - : Absence of inhibition zone

3.4.1. Action of the juice

It is clear from this reading that the ethyl acetate extract of *Aloe vera* (L) (Extract 2) has an antibacterial activity with respect to *E. coli* and *S. epidermidis*. This results in the appearance of growth inhibition zones in the boxes containing this extract. Furthermore we noted the absence of inhibition zones for *Pseudomonas aeruginosa* and *Staphylococcus aureus*. On the other hand, the extracts 1,3 and 4 have no antimicrobial action in view of the total absence of the zones of inhibition.

The sap of *Aloe vera* L. contains hydroxyanthracene derivatives (free anthraquinones, glucosides and tannins) (Bruneton, 2008; Bezanger *et al.*, 1986).

Ethyl acetate, a medium-polar solvent, made it possible to extract the hydroxyanthracene derivatives in particular. In the literature consulted, these derivatives are known only for properties emmenagogues and purgatives. Our results show an inhibitory action on the growth of *Escherichia coli* and *Staphylococcus epidermidis*.

Results do not agree with those obtained by Bensegueni (2001), who reports that the juice extracted from *Aloe vera* (L) has no antibacterial action with respect to the same species tested.

3.4.2. Action of the gel

After reading the dishes containing extracts 1 and 2 (gel), it always appears, in comparison with the control, that the two extracts remain without any inhibitory action on the growth of the seeds in question. This results in the absence of the zone of inhibition, it is deduced that the gel fresh or powdered *Aloe vera* L. has no antibacterial power vis-à-vis bacterial strains tested.

Similar results were obtained by (Dakiche 2011), which reports that the gel extracted from *Aloe vera* L. has no antibacterial action with respect to the same species tested.

3.5. The hemostatic activity of juice and gel

The results of hemostatic activity are summarized in Table 3.

Table 3: Results of the hemostatic activity of the gel of *Aloe vera* (L)

	Coagulation time (min)					
	100µl		200µl		300µl	
Control	-	-	-	-	-	-
Distilled water	-	-	-	-	-	-
Suc Extract	4.05	4.07	3.56	3.57	2.47	2.47
Gel Extract	3.47	3.48	3.00	3.00	2.36	2.37

According to the results obtained, we found the appearance of coagulation in the tubes containing the extracts of the juice and the gel indeed the coagulation by the extracts from the gel is faster than those from the sap. Moreover, the amount of the extract is not proportional to the coagulation time, the more the amount of the extract increases, the more the clotting time decreases.

Although it is not, the powdered gel has not stimulated the growth of the bacteria and this is due to the heat treatment which generally decreases the nutritional quality of the plant.

3.5.3. Fresh gel

The results obtained are summarized in Table 4. After reading the results we, we found that the behavior of the bacterium with respect to the gel is very positive, which leaves us to think that it has been a beneficial contribution for In addition, with inhibition rates below zero, we noted a complete absence of inhibition of the bacterium. Indeed the *Aloe vera* (L) gel stimulated the growth of this luminescent microorganism, this confirms again our hypothesis concerning the richness of the *Aloe vera* (L) gel essential active ingredients, which are substrates highly valued by the bacteria.

Table 4: fresh frost action on *Vibrio Fesheri*

Sample	Dilution	(%) inhibition	(%) inhibition of color	(%) inhibition after color correction
Fresh gel	0,010	-11,32	-	-
	0,025	-19,36	-	-
	0,050	-21,31	-	-
	0,100	-19,12	-	-
	0,200	-9,13	-	-

3.5.4. Powder gel

The results obtained are summarized in Table 5. For the powdered gel prepared extract, the percentages of inhibition calculated for each dilution are very low with negligible values of 5.23%, 6.01%, 6.66%, 9.7% and 10%, these values all remain in effect below 50% and therefore can

conclude that the gel powder is not toxic. Although it is not, the powdered gel has not stimulated the growth of the bacterium and this is due to the heat treatment which generally decreases the nutritional quality of the plant.

Table 5: Powdered gel action on *Vibrio fischeri*

Sample	Dilution	(%) inhibition	(%) inhibition of color	(%) inhibition after color correction
Powder gel	0,010	69,10	63,87	5,23
	0,025	88,13	82,12	6,01
	0,050	95,31	88,65	6,66
	0,100	98,20	88,45	10.00
	0,200	99,38	89,68	9,70

3.5.5. Fresh sap

The results obtained are summarized in Table 6.

From these results, we found that fresh sap is toxic with a maximum inhibition percentage of 97.78%. Indeed at high concentrations Aloin contained in the sap of *Aloe vera* (L) can be toxic and irritating. The abuse of *Aloe vera* (L) can cause the consumer to experience acute diarrhea, as its maximum authorized concentration in Switzerland is 0.1 mg / kg (Bieri, 2013).

This explains its use which is limited to external use in certain regions.

Table 6 : Fresh sap action on *Vibrio fischeri*:

Sample	Dilution	(%) inhibition	(%) inhibition of color	(%) inhibition after color correction
Fresh sap	0,010	11,24	-	-
	0,025	61,15	-	-
	0,050	81,26	-	-
	0,100	93,86	-	-
	0,200	97,78	-	-

3.5.6. Sap powder

The results obtained are summarized in Table 7. From the results obtained we found that the percentage of maximum inhibition of sap powder is equal to 56.4%, this suggests that the reduction

of the toxicity of the juice is likely to be due to the heat treatment (50 ° C). So it is better to use the powdered juice in traditional use or in galenic preparations than fresh juice.

Table 7: Action of fresh powdered juice on *Vibrio feschleri* :

Sample	Dilution	(%) inhibition	(%) inhibition of color	(%) inhibition after color correction
fresh powdered juice	0,010	95,52	46,54	48,98
	0,025	99,17	47,04	50,13
	0,050	99,63	47,13	52,50
	0,100	99,72	47,22	54,50
	0,200	99,90	68,40	56,40

General conclusion

Aloe vera (L) is a very rich plant that has not yet delivered all its secrets. It has become one of the first medicinal plants grown in the world.

In the study of biological activities, we carried out in vitro tests, which made it possible to study the antibacterial, hemostatic and toxic activity of *Aloe vera* (L) gel and juice.

According to the results obtained from the biological study of the gel and juice of *Aloe vera* (L), we can conclude that the juice has an anti-microbial action on *Escherichia coli* and *Staphylococcus aureus*.

On the other hand, the gel does not have an antimicrobial action on the species tested. In addition, the gel and juice of *Aloe vera* (L) have an interesting coagulation power. Finally unlike the juice, the gel of *Aloe vera* (L) is not toxic.

The study of the antibacterial activity tested on the four strains most encountered in cutaneous infections has shown that the juice of *Aloe vera* (L) has a power of reduction of growth of *E. coli* and *S. epidermidis* in a solid medium. While the *Aloe vera* (L) gel remains without bactericidal power.

The hemostatic study revealed that the gel and juice of *Aloe vera* (L) have a coagulating power of the blood.

The study of the toxic activity, tested on the luminescent bacteria, *Vibrio feschleri* showed that the gel of *Aloe vera* (L). is not toxic. Unlike gel, the fresh juice of *Aloe vera* (L). is toxic.

Throughout the results obtained in our experimental conditions, it is clear that the gel and juice of *Aloe vera* (L) are very rich in water, sugars and minerals. These results justify the use of the gel as a dietary supplement.

Although the gel of *Aloe vera* (L) is rich and widely used, but it oxidizes quickly by releasing the peroxides that make it toxic. In order to preserve the *Aloe vera* (L) gel, it would be interesting to test the stabilization.

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