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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITIES OF TANNINS EXTRACT FROM *ALOE SAPONARIA*

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ABSTRACT

The objective of this work was to study the chemical composition and antioxidant activity of tannins extract of *Aloe saponaria* (TEAS). The *Aloe saponaria* (syn. *Aloe maculata*) is a succulent plant belonging to the Xanthorrhoeaceae family, this succulent plant native to South Africa was introduced in Morocco as an ornamental plant, rustic and easy to grow. In order to ensure this, morphological and histological study method of the species, phytochemical screening, characterization of TEAS by gas chromatography coupled to mass spectrometry (GC/MS), and the study of antioxidant activity by two methods (2,2-diphenyl-1-picrylhydrazyl (DPPH) and total antioxidant capacity (TAC) assays) were examined. The results of phytochemical screening indicated that the *Aloe saponaria* leaf was rich of alkaloids, tannins catechics, flavonoids, sterols, triterpenes, oses, holosides, mucilages and reducing sugars. In addition, the main constituents of TEAS identified by GC/MS analysis were Linolenic acid (32.22%), Palmitic acid (17.42%), and Phytol (11.30%). Regarding antioxidant activity, the TEAS showed a significant antioxidant effect.

Keywords: *Aloe saponaria* (syn. *Aloe maculata*), GC/MS, Phytochemical screening, Tannins, Antioxidant activity, Histological study method

INTRODUCTION

Oxidative stress plays a significant role in the development of numerous diseases that persist over time. Free radicals and other reactive oxygen species are recognized as factors concerned in the pathogenesis of sicknesses such as inflammatory arthropathies, asthma, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. Reactive oxygen species are also said to be responsible for the human aging (**Chiavaroli *et al*., 2011; Kanwar *et al*., 2009)**.
Generally, antioxidants prevent or delay oxidation damage to targeted molecules by definition (**Yamagishi and Matsui, 2011)**. They also have the ability to trap free radicals. Herbal plants have always been considered natural sources of antioxidants. Many contain phenolic compounds, which remove harmful free radicals like peroxide, hydroperoxide and lipid peroxyl. Thus they protect the body from degenerative illnesses caused by oxidative stress (**Nimse and Pal, 2015; Wu *et al*., 2011**).
In recent years, researchers are increasingly interested in plants with antioxidant activity. Plants are the source of many secondary metabolites, many of which are natural antioxidants (**Rojas and Buitrago, 2019).** These secondary metabolites can be found in vegetables, nuts, seeds, leaves, flowers, fruits, roots and bark. These natural antioxidants of plant-based are interesting from a human health perspective, in that these compounds could help promote health and well-being, and from a storage perspective, the fact that antioxidants help extend the shelf life of food products (**Amarowicz and Pegg, 2019**).
The plant family Xanthorrhoeaceae contains the genus *Aloe*, which has been used medicinally for many years in cultures around the world. Some of the most well-known species of *Aloe* are *Aloe vera* (syn. *Aloe barbadensis* Mill), which is used medicinally for treating burns and sunburn. *Aloe vera* is the most studied species of *Aloe*. It has been used as a clinical remedy for various illnesses; many other aloes have also been used for this purpose. These include *Aloe ferox* Mill., *Aloe arborescens* Mill., *Aloe succotrina* and *Aloe maculata* (syn *Aloe saponaria*) **(Chinchilla *et al*., 2013; Jia *et al*., 2008; Sandeep Kumar *et al*., 2017c, 2016; Yadav *et al*., 2016**).
Two known medicinal products that are made from leaves of *Aloe* species; a bitter exudate known as ' drug *aloes*' or ' bitter *aloes*', derived from pericycle cells beneath the epidermis and a gel '*Aloe* gel' obtained from parenchymal tissue of the leaf. In addition, the bitter leaf exudate has been used universally as a laxative (**Lobine *et al*., 2018**). Whereas the leaves from many other *Aloes* have been documented as traditional medicinal remedies (**Grace *et al*., 2008; Sandeep Kumar *et al*., 2017a, 2017b**) there is a comparative lack of scientific proof that documents the chemistry and biological activities of the less broadly known *Aloe* species, to validate their reputed medicinal effects.
In this context, many studies have concentrated on the employ of natural sources, especially plants that contain compounds with antioxidant activity. The *Aloe* family is composed of tropical and subtropical plants and is characterized by lanced-shaped leaves with jagged edges and, in some species, sharply tapered leaves (**Silva *et al*., 2014**). Phytopharmacological studies of different *Aloe* species, like *A. vera, A. spicata* and *A. ferox*, have shown antioxidant property as well as the capacity to enhance burn healing (**De Barros *et al*., 2007**). As with other *Aloe* species, *Aloe saponaria* treats burn injuries. Furthermore, HPLC analysis of *Aloe saponaria* showed various chromatographic peaks, indicating a wide variety of chemical compounds. These compounds include some phenolic substances, which usually exhibit antioxidant property **(Silva *et al*., 2014**).
To the best of our knowledge the tannins extract composition and antioxidant activity of *Aloe saponaria* has not been studied previously. Therefore, the objective of this study was to perform a preliminary phytochemical screening, extraction of *Aloe saponaria* tannin and determination of their chemical composition (by GC/MS) and antioxidant activity (by DPPH and TAC).

MATERIAL AND METHODS

Plant material

Freshly harvested, washed and dried *Aloe saponaria* is stored in the open air for 15 days at room temperature. After this process, the plant is processed into a dry powder that can be stored for later use. To determine the water content of this plant, a fresh portion of *Aloe saponaria* is used.

Morphological description

In 1981, the plant classification system by Cronquist included the Xanthorrhoeaceae family, which includes *Aloe saponaria* (= *Aloe zebra* or *Aloe*

maculata). This family is also part of the classification APGIII, which lists the families of plants, in 2009. This plant native to South Africa and is often grown as an ornamental plant in Morocco.
The leaves in rosettes are succulent, persistent, green and marked with oblong whitish spots (Figure 1). Their margins are provided with tiny, slightly pungent teeth. The leaves are narrow and long (10 cm/30 cm) thick and filled with gel. In late spring, the plant blooms giving a cluster sometimes panicle of orange and greenish flowers at the top in the young state; they are long stalked with a bract at its base. The floral stem measures approx 40 to 60 cm (Figure 1). The flower consists of 6 tepals welded and arranged on two whorls, 6 stamens also in two whorls and then 3 carpels welded. Its multiplication is done either by sowing or by discharges that grow around its foot.

Figure 1 The flowers and leaves of *Aloe saponaria*

Histological study method

A histological analysis determines the *Aloe saponaria* leaf, after its morphological classification. This is performed via a double-stained (carmine iodine green) process (**Prat, 2007**). Briefly, treated leaf sections were treated with alum for a few minutes before being rinsed in distilled water until all the iodine green excess was removed. Next, the sections were stained with iodine green for a few seconds. More exactly, this technique consists in staining the cell wall with iodine carmine-green: the pecto-cellulosic wall is colored pink as well as the lignified wall is colored blue-green. The anatomical sections are then mounted between the two slides in a drop of water and then they were observed under an optical microscope at multiple levels of magnification. After determining the plant tissues that composed the leaf, a series of photographs were taken with a digital camera.

Phytochemical screening of plant material

Different tests on the leaves of the *Aloe saponaria* plant determine its chemical constituents. These include saponins, tannins, flavonoids, alkaloid, holosides and anthraquinones as well as reducing sugars. Other tests performed by the methods described below include mucilages, sterols and triterpenes (**Benzidia *et al*., 2019; Yeasmin *et al*., 2016**).

Extraction and determination of the tannins contents of *Aloe saponaria* leaf

Extraction of Tannins

The Extraction of tannin

The extraction process for *Aloe saponaria* tannin was detailed in a study by **Benzidia *et al*.,** (**2019**). The dried and crushed bark powder was placed into a beaker, which 10 g was placed into a second beaker containing 100 mL of 70% acetone and 30% distilled water. This mixture was left to sit for three days before being strained. This is the first step in the extraction process. With a second step, we filtered our solution to remove the acetone and evaporated it at 40 °C. Next, we washed our solution with 60 mL of dichloromethane to remove pigments and fats. Two 60 mL portions of ethyl acetate were used to extract the aqueous phase after the separation of the two phases. The organic phases were combined, evaporated it at 77 °C, weighed, and taken up with methanol.

Tannins extract analysis

A Clarus® SQ 8 Gas Chromatograph/Mass Spectrometer (GC/MS) by PerkinElmer, equipped with GC Capillary Column Rxi-5ms 30 m, 0.25 mm ID, 0.25 µm performed the TEAS's analysis process. With a rate of 4°C/min, the oven maintained a temperature of 40°C for the first two minutes. After that, the temperature increased by 4°C/min until it reached 180°C. Then it was maintained at 180 to 300°C at a rate of 20°C/min and kept constant at 300 °C for 2 min. The injector temperature was also set at 220 °C split 1/20 of 1 mL was injected. The helium is the carrier gas with a flow rate of 1 mL/min. The ion source temperature is 200 °C, and 70 eV are used for ionization. Additionally, 40 to 450 Da masses were scanned for electron ionization mass spectra. The chemical constituents of TEAS were identified by their retention indices (RI) and mass fragmentation patterns with those on the stored NIST library (version 2014).

Antioxidant activities

DPPH free radical scavenging activity

The antioxidant activity of TEAS was assessed according to the method of **Brand-Williams *et al*.,** (**1995**). This involved adding 0.05 mL of the material to 1.95 mL of DPPH solution in a test tube. DPPH was freshly prepared (25 mg of DPPH in 100 mL ethanol). After 30 minutes in the dark, a 515 nm reading on a spectrophotometer was performed on the control sample, which contained Butylated hydroxytoluene (BHT). The percentages of inhibition (I%) were determined according to the following formula:

$$I\,(\%) = [(A_{Control} - A_{Test})/A_{Control}] \times 100$$

The IC_{50} values indicate the concentration of samples that is needed to trap 50% of DPPH free radical. The IC_{50} was estimated by nonlinear regression via Excel, version 2013.

Evaluation of total antioxidant capacity (TAC)

In the TAC assay, the phosphomolybdenum compound is used according to the **Barbouchi *et al*.,** (**2019**) protocol. A volume of 0.3 mL of TEAS extract is mixed with 3 mL of reagent solution containing 28 mM Na_3PO_4, 0.6 M H_2SO_4 and 4 mM $(NH_4)_6Mo_7O_{24}$. This mixture is then used to determine the antioxidant potentials of the extracts. The TEAS is prepared in test tube. They is incubated at 95°C for 90 minutes before being cooled. Next, a reading is taken at 695 nm against the blank (0.3 mL of methanol and 3 mL from reagent solution), which is then incubated under the same conditions as the sample. The ascorbic acid (AA) is employed as a standard and the TAC assay is presented in milligram equivalents of AA per gram of TEAS extract (mg EAA/g of TEAS extract).

RESULTS AND DISCUSSION

Histological study of *Aloe saponaria* leaf

Figure 2 presents the microscopic structure of the *Aloe saponaria* leaf, from this figure we can distinguish:

- A superior epidermis and an inferior epidermis with a thick cuticle on the two external faces of the limbus and stomata;
- Towards the inside of the epidermis, an assimilative parenchyma formed of about 8 to 10 layers of chlorophyll cells, some of which contain crystals of calcium oxalate.
- An aquiferous parenchyma with hypertrophied cells, rich in mucilage (= hydrated gel) allowing the retention of the water, thus giving a succulence to the leaf which reduces the loss of water.
- In the contact zone between the two parenchyms, the ribs (= crib-vascular bundles) are located.

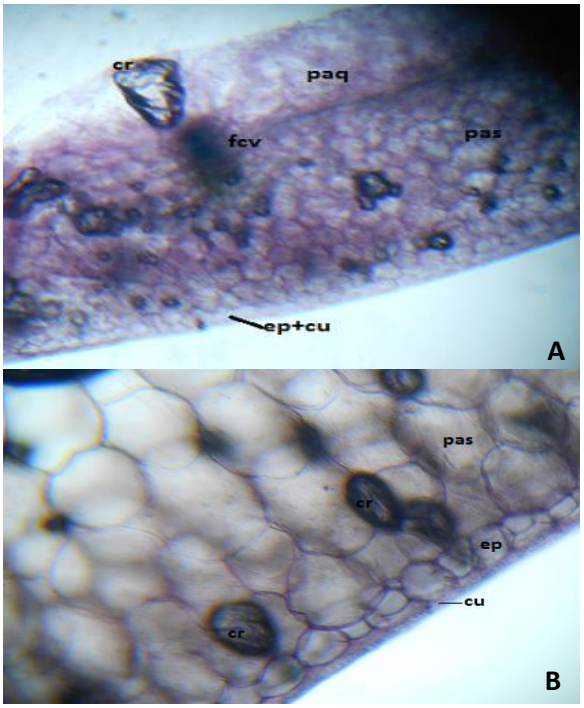

Figure 2 Cross section of the upper side of the Aloe saponaria leaf. A : Cross section of the upper side of the *Aloe saponaria* leaf (x40). B : Detail of the epidermis of the limbus of *Aloe saponaria* (x100). Legend : cu, cuticule ep, superior epidermis pas, assimilative parenchyma fcv, faisceau criblo-vasculaire paq, aquiferous parenchym cr, crystals of calcium oxalate

Phytochemical screening

The phytochemical screening of *Aloe saponaria* leaf (Table 1) showed the presence of alkaloids, tannins catechics, flavonoids, sterols, triterpenes, oses, holosides, mucilages and reducing sugars and the absence of tannins gallics, saponins and coumarines. In this respect, our results thus obtain are in global agreement with those existing in the literature suggests that the genus *Aloe* is rich in secondary metabolites such as alkaloids, tannins, flavonoids and sterols (**Cardarelli *et al.*, 2017; Dagne *et al.*, 2000; Ombito *et al.*, 2015**).

Table 1 Phytochemical analysis of *Aloe saponaria* leaf

Phytoconstituents		Test used	Presence/ absence
Alkaloides		Dragendorff's	++
		Mayer's	++
Tannins Catechics		Stiansy reaction	+++
Tannins Gallics		Lead acetate	----
anthraquinons Free		Borntrager's	----
Anthra quinons combined	O-heterosides reduced genins	Modified Borntrager's	----
	C-heterosides		----
Flavonoids		Shinoda's	++
Saponins		Foam Index	----
Sterols and Triterpenes		Liberman-burchard	+++
Oses and holosides		saturated alcohol with thymol	++
Mucilages		Alcohol 95%	+++
Reducing sugars		Fehling's	+++
Coumarines		Fluorescence	----

High concentration (+++); moderate concentration (++); low concentration (+); absence (----).

Extraction yield of tannins extract from *Aloe saponaria* leaf

$$R = \frac{m_2}{m_1} \times 100 = \frac{0.1424}{10} \times 100 = 1.424\%$$

With: m_1 = Mass of *Aloe saponaria* leaf starting (g) and m_2 = Mass of tannins extract (g).

Chemical composition of the tannins extract of *Aloe saponaria*

The chemical composition of TEAS was performed using GC-MS analysis and the following result was exposed in (Table 2). The TEAS showed that the major components were Linolenic acid (32.22%), Palmitic acid (17.42%), and Phytol (11.30%). Our results show that the TEAS is rich in fatty acid with a percentage of 58.39%. Fat-based oils and natural fats contain a significant amount of fatty acids that have antibacterial and antifungal properties. These ubiquitous molecules are essential to the healthy functioning of living organisms (**Agoramoorthy *et al.*, 2007; Desbois and Smith, 2010; Golebiowski *et al.*, 2014**).
Our research included the chemical makeup of the tannins found in *Aloe saponaria*. This was the first time this information was recorded in a scientific study, and it was compared to other research about *Aloe vera* tannins (**Benzidia *et al.*, 2019**). This last species is rich in fatty acids with a percentage of 38.52%, which the major constituents this species were Linolenic acid (16.59%), Phytol (14.40%), Palmitic acid (11.91%), and Diisooctyl phthalate (11.84%). Hence, it can be concluded that the extract of the *Aloe* species is rich in fatty acids.

Table 2 Chemical composition of the tannins extract of *Aloe saponaria* (TEAS)

N°	Compounds of TEAS	Area %
1	Butanoic acid	-
2	Pyranton	-
3	9-Oxononanoic acid	-
4	Palmitic acid	17.42
5	Phytol	11.30
6	17-Octadecynoic acid	8.75
7	Linolenic acid	32.22
8	Butyl palmitate	4.61
9	Chrysarobin	-
10	Chrysophanol	5.02
11	Diisooctyl phthalate	7.71
12	β-Sitosterol	4.88
Total identified		91.91
Fatty acid		58.39
Other		33.52

(-) : means trace state (<0.2).

Antioxidant activities of TEAS

In recent years, interest in natural antioxidants, in relation to their therapeutic properties, has increased considerably. There has been a growing interest in natural antioxidants for use in foods or medicinal substances to replace synthetic antioxidants, which in some cases have been reported as carcinogens (**Lourenço *et al.*, 2019**).
In this context, our study investigated the evaluation of antioxidant activity using two different methods, as TAC and DPPH. The results of TAC determined by phosphomolybdenum complex assay are given in Table 2. From this Table it can be seen that a significant TAC in TEAS with 265.2 ± 1.16 mg EAA/g of extract. As regards the measurement of DPPH radical scavenging activity of TEAS assessed, the results obtaining as shown in Figure 3 and Table 3. From these results, a significant anti-radical activity was found in TEAS compared to the BHT standard. BHT is a well-known conservative used as a food additive as well as in hygiene products. Furthermore, the TEAS can be very beneficial to be used as a food additive. The antioxidant activity detected in TEAS may be due to some major compounds in particular the Phytol and Palmitic acids that are known for their antioxidant activity (**Benzidia *et al.*, 2019; Costa *et al.*, 2016; Kumar *et al.*, 2010**). Also, recent studies have demonstrated antioxidant properties of water, ethanol, methanol and acetone extracts of *Aloe saponaria* (**Yoo *et al.*, 2008; Zapata *et al.*, 2013**).

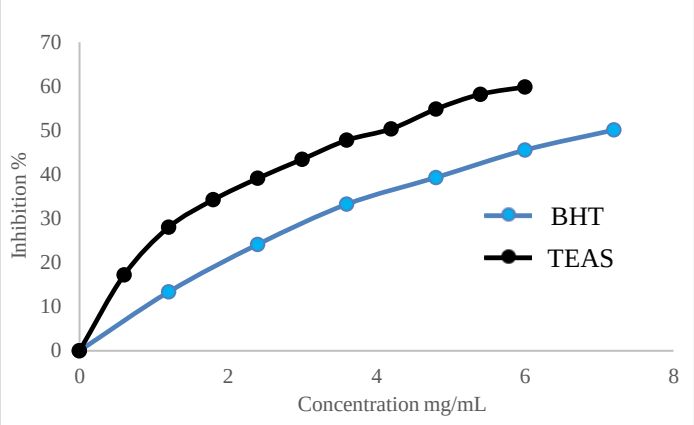

Figure 3 DPPH radical scavenging activity of BHT and TEAS

Table 3 Antioxidant activity of the tannins extract of *Aloe saponaria*

Bioactive compounds	Antioxidant activity[a]	
	TAC (mg EAA/g)	DPPH assay (IC_{50}(mg/mL))
TEAS	265.2 ± 1.16	3.90 ± 0.04
BHT	-	7.38 ± 0.00

[a] Mean values ± standard deviations of triplicate determinations are reported.

CONCLUSION

Based on the results, it can be seen that the phytochemical screening showed that the *Aloe saponaria* leaf is rich in secondary metabolites such as alkaloids, tannins, flavonoids, sterols, triterpenes and mucilages. The chemical composition showed that the TEAS are rich in fatty acids with a percentage of 58.39%. The major components were Linolenic acid (32.22%), Palmitic acid (17.42%), and Phytol (11.30%). From the results of the antioxidant activities, a significant anti-radical activity was found in TEAS, which is stronger than the BHT standard with an IC_{50} values are 3.9 and 7.38 mg/mL respectively and a significant TAC with 265.2 ± 1.16 mg EAA/g of extract. It can be concluded that TEAS can be very beneficial for use as an antioxidant natural, which will be developed in our future goals.

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