

## Phytochemical screening: Determination of total polyphenol and flavonoid contents, antioxidant and antimicrobial activity of leaves and seeds of *Abelmoschus esculentus* L.

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### Abstract

Reputed to be a plant rich in antioxidants, *Abelmoschus esculentus* or okra is a very effective vegetable against diabetes. In this work, we made a qualitative study of the bioactive compounds contained in the leaves and seeds of okra, through a phytochemical screening. Subsequently, the antioxidant and antimicrobial activities of these extracts were studied. The Folin-Ciocalteu reagent was used to evaluate the content of the phenolic compound. The DPPH• radical made it possible to measure the antioxidant power of the extracts. The phytochemical analysis made it possible to high the presence of substances with great therapeutic values (polyphenols, flavonoids, tannins, alkaloids, etc.). The antibacterial activity was measured on bacterial strains. The antioxidant capacities reveal that the extracts of the leaves and seeds of okra possess an interesting anti-free radical activity with IC<sub>50</sub> values of 06 ± 0.21 mg / mL and 8.85 ± 0.28 mg / mL respectively for the leaves and the seeds. . Biological analysis shows that the *E. faecalis* strain is resistant to different extracts. *E. coli*, *S. aureus*, and *P. aeruginosa* strains show some sensitivity to ethanolic extracts. However, *E. colis* and *S. aureus* strains show the best antimicrobial activities with a MIC of 3.75 mg/mL for crude ethanol extract and ethanol fraction of leaves and seeds.

**Keywords:** *Abelmoschus esculentus* L; Phytochemical screening; Polyphenols; Flavonoids; Antioxidant; Antimicrobial.

### 1. Introduction

In recent years, an increasing number of reports confirm that many fruits and vegetables may offer protection against certain chronic diseases caused by oxidative stress [1], [2]. Indeed, plants generally use secondary metabolites as means of defense against predators and parasites. These metabolites include many classes of compounds ranging from simple phenolic acids to complex tannins [3]. According to the World Health Organization (WHO), approximately 65-80% of the world's population in developing countries, due to poverty and lack of access to modern medicine, depend mainly on traditional medicinal plants for their primary health care [4], [5]. Medicinal plants are important for pharmacological research and drug development. Indeed, they are used directly as therapeutic agents, but also as raw materials for drug synthesis or as models for pharmacologically active compounds [6]. Plants produce 70% of our drugs, already about 170,000 bioactive molecules have been identified from plants [7]. Among these bioactive molecules, phenolic compounds arouse considerable interest in the field of food, chemistry and medicine because of their proven antioxidant potential [8]. Okra, widely used to treat and predict certain diseases such as diabetes and/or constipation, is a vegetable that contains many antioxidants. Since these biological activities are generally attributed to phenolic compounds, it becomes interesting to evaluate the antioxidant and antimicrobial activities of the different extracts of this plant. These results will provide important chemical resources for better use of okra leaves and seeds.

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## 2. Material and methods

### 2.1. Plant material

The selection of *Abelmoschus esculentus* leaves and seeds (**figure 1**) was made on the basis of their very wide use in the world. The harvest of this plant was carried out at the level of the city of Keur Massar (Dakar-Senegal) in January 2022. After having been thoroughly cleaned and freed from particles and dust, the organs of the plant studied were dried at room temperature and protected from sunlight for a week. This step made it possible to remove the water they contain and prevent contamination by insects or the development of fungi. Indeed, drying is of major importance for the extraction of compounds, because the vegetative cells contain enzymes likely to cause modifications in the phenolic composition of the plant [9].

The dried samples were pulverized using an electric grinder to obtain very fine powders. It should be noted that the smaller the diameter of the powder, the greater the exchange surface between it and the extraction solvent; therefore, the extraction yield is better [8]. After grinding, the powders obtained were sieved and then stored in well-sealed glass bottles.



**Figure 1** Image of okra leaves and seeds

### 2.2. Extraction

Several organic solvents were used for the extraction of the compounds. The general extraction process was carried out according to the method [10]: in fact, 30 g of powder from each of the two samples were macerated with 250 mL of ethanol for the crude extracts. For the large-quantity extraction, we used 100 g of plant material for 400 mL of solvent and 38 g in 100 mL of solvent, respectively for leaves and seeds. The marcs are renewed according to the increasing polarity of the solvents. The mixtures thus obtained are subjected to stirring, using a magnetic stirrer for 48 hours at room temperature and away from light. After maceration, the solutions of each extract of the two parts of the plant were filtered using a Büchner then by Whatman paper. The filtrates obtained were evaporated using a rotary evaporator and then dried until the solvent was completely exhausted. The dry extracts thus obtained are kept in the refrigerator until they are used. The yield of extracts is the ratio between the mass of the dry extract and the mass of the powdered plant material used [11]. It is expressed as a percentage according to the following formula:

$$\text{Extraction yield (\%)} = \frac{m_{de}}{m_{vp}} \times 100$$

$M_{de}$ = dry extract mass

$m_{vp}$ = mass of vegetable powder

### 2.3. Phytochemical screening of okra leaves and seeds extracts

The extracts of the different parts were subjected to various phytochemical tests in order to highlight the major chemical groups contained in these extracts. For this purpose, the standard method based on staining and precipitation reactions was used [12]. The presence of these different chemical groups was examined by referring to the techniques described in the work of Ronchetti and al. [13] and Karim and al. [14]. In this work, phytochemical screening focuses on the search for alkaloids, polyphenols, tannins, flavonoids, saponins, sterols and polyterpenes, coumarins, leucoanthocyanins, catechols and mucilages. Polyphenols and tannins were identified by the  $FeCl_3$  test and Stiasny's reagent, flavonoids, leucoanthocyanins and catechols by reaction with cyanidin; saponins by the foam test; sterols and polyterpenes by the

Liebermann-Burchard test; coumarins by the ammonium hydroxide test; mucilages by the absolute ethanol test and alkaloids by the Mayer test [15].

## 2.4. Antioxidant activity

Antioxidants are molecules naturally present in many foods and whose function is to capture free radicals. These damage our cells, including DNA, and are responsible in particular for the aging of cells. The determination of the antiradical activity by the DPPH• test was carried out using the method described by Molyneux [16] slightly modified. To perform the analysis, 3.8 mL of the methanolic solution of DPPH• are added to 0.2 mL of the solution of each extract at different concentrations (40, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.078125, 0.0390625 mg/mL) [17], [18]. These concentrations come from the dilution of a stock solution of 40 mg/mL. The latter is obtained by dissolving 80 g of dry extract in 2 mL of methanol. The mixture is vigorously shaken, then the tubes are incubated at room temperature and in the dark for 30 min. All readings are taken at 517 nm. The samples, the reference antioxidant (ascorbic acid) and the control are prepared under the same operating conditions. The antiradical activity is estimated according to the equation:

$$\text{Scavenging activity (\%)} = \frac{A_B - A_S}{A_B} \times 100$$

$A_B$ : blank absorbance after 30 min of incubation

$A_S$ : absorbance of the sample after 30 min of incubation

## 2.5. Determination of the 50% inhibitory concentration (IC<sub>50</sub>)

The IC<sub>50</sub> is defined as the quantity or concentration of antioxidants (extract or any other antioxidant substance) necessary to inhibit or eliminate 50% of the radicals. The IC<sub>50</sub> are determined from a graph of percentage inhibitors versus concentration. For each extract, a linear regression curve ( $Y = aX + b$ ) is established in order to determine the IC<sub>50</sub> which allows the characterization of the antioxidant power of the extracts. A low IC<sub>50</sub> value indicates high antioxidant activity [16].

## 2.6. Antimicrobial activities

### 2.6.1. Bacteria sensitivity test

The disk diffusion method was used to test antibacterial activities of different extracts [19]. The bacteria are seeded on Muller-Hinton (MH) agar.

From a 24-hour culture, inocula equivalent to the Mac-Ferland standard 0.5 (10<sup>6</sup> bacteria per mL) are prepared. On each Petri dish, a drop of the culture dilution (inoculum) prepared with a few micrograms of bacteria in a biological liquid (sodium chloride) was deposited and spread in tight streaks with a sterile swab, over the entire M-H agar.

Cupules (saboureaud) 0.7 cm in diameter were then placed on the upper layer of the agar medium, seeded with bacteria. The wells are impregnated with 100 µL of solution at a concentration of 30 mg/mL and the dishes are incubated in the oven at 37° C for 24 hours [20]. Throughout the incubation period (24 hours at 37°C), the strain to be studied competes with the inhibitory effect of the plant extract. A circular zone of inhibition forms around the well when the strain is sensitive to the extracts and an absence of zone of inhibition if the strain is resistant.

### 2.6.2. Determination of minimum inhibitory concentrations (MIC).

The MIC is the lowest concentration of antibiotic capable of causing complete inhibition of the growth of a given bacterium, appreciable to the naked eye, after a given incubation period [21]. The determination of the MIC was carried out for the extracts which showed zones of inhibition against the microorganism by the disk diffusion method. Square plates containing rows of 12 wells were used, in which concentration ranges were prepared from a solution of dimethylsulfoxide (DMSO) with a stock concentration of 30 mg/mL. To each well, 20 µL of bacterial culture suspension are added. For each bacterial strain, a solution of dimethylsulfoxide (DMSO) was used as a positive control, and as a negative control we used ceftriaxone (C-tri 10). Each microplate is covered and incubated for 24 h at 37°C [10]. A clear coloration of the well indicates the absence of growth (negative) and a cloudy well indicates the growth of bacteria (positive). Each experiment was repeated twice.

### 3. Results and Discussions

#### 3.1. . Extraction yield

**Table 1** shows the results of the extraction yields of secondary metabolites from the leaves and seeds of okra. The results obtained show that the yields vary from one part to another. The percentages recorded for the leaves vary from 2.46% to 10.80%. The aqueous extract gave the best yield. For the leaves, the percentages vary from 1.053% to 6.34% with a better yield for the hexane extract. It should also be noted that the leaves give a better yield compared to the seeds in the different extracts, except in the case of the hexane extract where we have a percentage of 2.46% for the leaves against 6.34% for the seeds. However, the results in Table 1 show that the yields of the fractions increase with the increasing polarity of the solvents, both for the leaves and for the seeds, with the exception of the hexane extract of the seeds. This phenomenon may be due to the existence of fatty substances in the seeds.

**Table 1** Extract of secondary metabolites of okra

Parts	Solvents	Plant matter (in g)	Solvent volume (in mL)	Mass obtained (in g)	Yield (in %)
leaves	Ethanol**	30	250	2.74	9.13
	Hexane*	100	400	2.46	2.46
	Ethyl acetate*			5.11	5.11
	Ethanol*			7.64	7.64
	Water*			10.8	10.8
Ethanol**	30			250	1.02
Seeds	Hexane*	38	250	2.41	6.34
	Ethyl acetate *			0.4	1.053
	Ethanol *			1.03	2.71
	Water *			1.27	3.34
	Ethanol**			30	250

\*fraction, \*\*crude extract

**Table 2** Results of phytochemical tests of different extracts of okra leaves and seeds

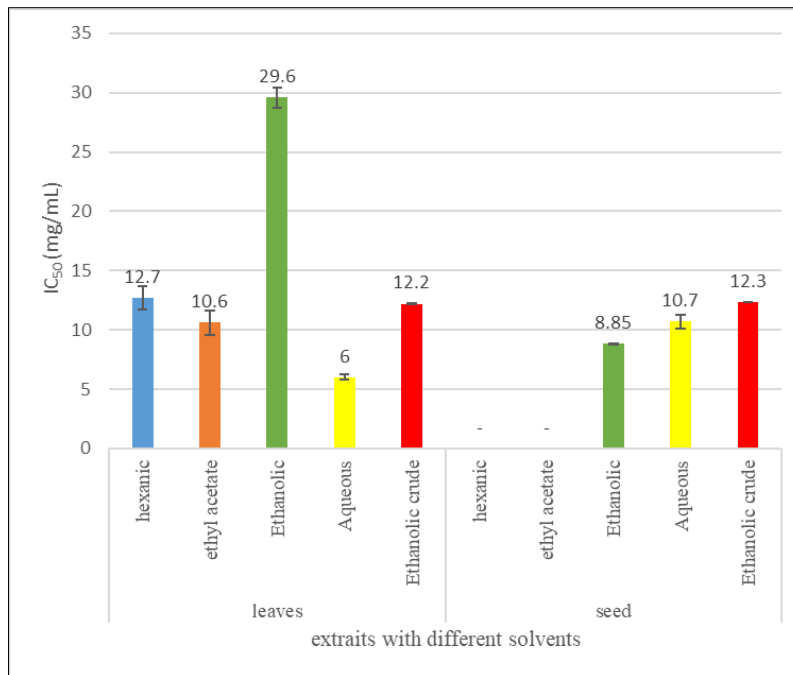
Compounds	Ethanol**		Ethanol*		Hexane*		Ethyl acetate *		Water*	
	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	leaves	Seeds	leaves	Seeds
Polyphenols	-	+++	+++	+++	-	+	+++	+	+	+
Flavonoids	++	+	++	-	+	-	+++	-	-	-
Alcaloids	+++	+	-	-	-	-	+	++	-	-
Sterols et polyterpenes	+	+++	-	-	+++	+	++	-	-	-
Saponosides	-	-	-	-	-	-	-	-	-	-
Leucoanthocyanins	-	-	+++	-	-	-	++	-	++	-
Catechols	+	-	-	-	-	-	++	-	++	-
Coumarins	-	-	+++	++	+++	++	-	+	+	+
Mucilages	+	+	+	+	+	+	+	+	+	+
Catechic tannins	++	+	-	-	++	-	+	-	-	-
Gallic tannins	++	+	-	-	-	-	+	-	+	-

+++ : Presence in large quantities, + : presence in low quantity; ++ : Presence in average quantity, - : absence, \*fraction, \*\* crude extract

**Table 3** IC<sub>50</sub> values of the different extracts studied

Part of the plant	Extract	CI <sub>50</sub> (mg/mL)
leaves	Hexanic	12.7 ± 0
	AcOEt	10.6 ± 0
	Ethanollic	29.6 ± 0.841
	Aqueous	6 ± 0.21
	Raw ethanol	12.2 ± 0.04
Seeds	Hexanic	-
	AcOEt	-
	Ethanollic	8.85 ± 0.28
	Aqueous	10.7 ± 0.56
	Raw ethanol	12.3 ± 4.45
Reference	Ascorbic acid	0.08

--: not active



**Figure 2** IC<sub>50</sub> diagram of the different extracts of okra leaves and seeds

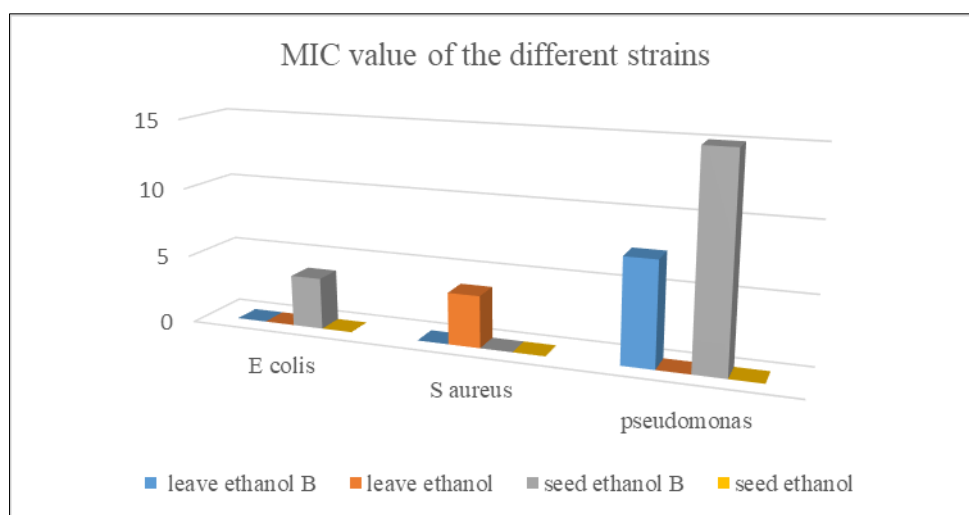
**Table 4** Inhibition diameter of the different extracts

parts				
strains	Leaves ethanol B*	Leaves ethanol*	seed ethanol b**	Seed ethanol*
<i>E. coli</i> 25922	R	R	8mm	R
<i>E. Faecalis</i>	R	R	R	R
<i>S. Aureus</i>	R	11 mm	R	R
<i>Pseudomonas</i>	8 mm	R	8 mm	R

R= resistant

**Table 5** Determination of minimum inhibitory concentrations (mg/mL)

Stains	Leaves extract		Seeds extract	
	Ethanol B	Ethanol	Ethanol B	Ethanol
<i>E.Colis</i>	-	-	3.75	-
<i>S.Aureus</i>	-	3.75	-	-
<i>Pseudomonas</i>	7.5	-	15	-

**Figure 3** Value of the minimum inhibitory concentration of the different strains

### 3.2. Phytochemical analysis

The results of the phytochemical screening are recorded in **table 2**:

The screening results show:

- The presence of flavonoids, alkaloids, sterols and polyterpenes, mucilages, gallic and catechic tannins in the leaves and seeds.
- The absence of saponosides, leucoanthocyanins and coumarins in both parts of the plant.
- The presence of polyphenols in the seeds and their absence in the leaves.
- The presence of catechols in the leaves and their absence in the seeds.
- A strong presence of polyphenols and sterols in the seeds and a strong presence of alkaloids in the leaves.

Phytochemical screening revealed the presence of all types of compounds that were tested except for saponosides. However, they are differently distributed in the parts of the plant. The results also revealed a higher concentration of secondary metabolites in the ethanolic and aqueous extracts. This shows the importance of the polarity of the solvent for the extraction of polar compounds. In summary, the results of the phytochemical screening showed that the okra plant is rich in secondary metabolites.

### 3.3. Determination of antioxidant activity

The  $IC_{50}$  is inversely related to the antioxidant capacity of a compound, because it expresses the quantity of antioxidant necessary to decrease the concentration of the free radical by 50%.

The lower the  $IC_{50}$  value, the higher the antioxidant activity of a compound [21]. The concentration of the sample essential to inhibit 50% of the radical DPPH was calculated by the linear regression of the percentages of inhibition according to the different concentrations of extracts prepared. The  $IC_{50}$  values found for the extracts studied are represented in the

following **table 3**. The results presented in **table 3** above show that the extracts of the sheets and seeds of okra tested possess an anti-radical activity with  $IC_{50}$  values of the order of  $6 \text{ mg/mL} \pm 0.21$  to  $29.6 \text{ mg/mL} \pm 0.841$  and  $8.85 \text{ mg/mL} \pm 0.28$  to  $12.3 \text{ mg/mL} \pm 4.45$  for leaves and seeds respectively.

In comparison with the reference antioxidant (ascorbic acid) which demonstrated an  $IC_{50}$  of  $0.082 \text{ mg/mL}$ , we find that these extracts are less active than the reference which is a pure product (**figure 2**). The aqueous extract of the leaves exhibits the best activity with an  $IC_{50}$  of  $6 \text{ mg/mL}$ . The latter is consistent with the results of the extraction yield where we had obtained the best yield with the aqueous extract. For the seeds, we notice that the ethanolic extract is more active with an  $IC_{50}$  of  $8.85 \text{ mg/mL}$  compared to the other extracts. There is also a lack of activity in the hexane extract and ethyl acetate. This result is not consistent with that of the extraction yield where the hexane extract presented a better yield. This result could be justified by the presence of secondary metabolites other than lipids in the seeds, which would be responsible for the biological activity of this plant.

### 3.4. Determination of antimicrobial activity

#### 3.4.1. Antibacterial power of extracts

Much work has been done on the antimicrobial power of natural products extracted from plants. During this study, we tested the activity of the different extracts of the two parts (leaves and seeds) of okra on a few bacterial strains. The scale for estimating antimicrobial activity is given by Moreira et al. 2005. They classified the diameter of the zones of inhibition (D) of microbial growth for a concentration of  $30 \text{ mg/mL}$  as follows <sup>[10]</sup>:

- Not sensitive (-):  $D < 4.8 \text{ mm}$
- Sensitive (+):  $5.4 \leq D \leq 8.4 \text{ mm}$
- Very sensitive (+ +):  $9 \leq D \leq 11.4 \text{ mm}$
- Extremely sensitive (+ + +):  $D \geq 12 \text{ mm}$

After 24 hours of incubation at  $37^\circ\text{C}$ , the zones of inhibition observed around the discs impregnated with the different extracts studied were measured. The results obtained are shown in **Table 4**:

Antimicrobial tests were performed on a few bacterial strains including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*.

Biological analysis shows that the *E. faecalis* strain is resistant to the different extracts so it does not show any activity. The *E. coli*, *S. aureus* and *P. aeruginosa* strains show a certain sensitivity to the different extracts. The sensitivity diameters of *Pseudomonas* and *E. coli* are  $8 \text{ mm}$  respectively for the ethanolic extract of leaves and seeds, so they are more or less sensitive. The *S.aureus* strain has the best sensitivity for the ethanolic extract of the leaves with an inhibition diameter of  $11 \text{ mm}$ .

#### 3.4.2. Determination of the minimum inhibitory concentration

According to **table 5**, we have all the minimum concentrations on the different strains.

For the determination of the minimum inhibitory concentration (MIC) (**figure 3**), the ethanolic part of the seed shows no activity compared to the different strains. This result is not consistent with antioxidant tests where the ethanolic extract is the most active part.

On the other hand, the strain *E. coli* and *S. aureus* presents the best activities with an MIC of  $3.75 \text{ mg/mL}$  respectively for the crude ethanolic extracts of the seeds and ethanolic of the leaves. The *Pseudomonas* strain shows the lowest sensitivity of  $15 \text{ mg/mL}$  with the crude ethanolic extract of the seeds. By comparing the results from the extraction and those from the antioxidant and antimicrobial activities, it can be said that the compounds responsible for these activities are found in the vast majority in the extracts of the most polar solvents, which are of an alkaloidal, tannic or flavonoidal nature.

Additional research on the chemical composition of each extract will allow us to know the nature of the active substances present in these different extracts.

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#### 4. Conclusion

Our present study focused on the species of okra which belongs to the Malvaceae family. It has made it possible to highlight through phytochemical screening the presence of tannins, saponosides, alkaloids, polyphenols, etc. The leaves and seeds of the studied plant have a fairly high water content, hence the interest of drying before extraction in order to obtain significant yields. Phytochemical test results on separate okra leaf and seed extracts were much better with aqueous and hexane extracts respectively. The study of the antimicrobial activity of our extracts proved to be very interesting, since we obtained positive results on the strains tested except for the *Enterococcus faecalis* strain. The results of the microbiological tests have enabled us to show that the different extracts of the two parts of the plant (leaves and seeds) have an interesting antioxidant and antibacterial activity. Regarding the antioxidant activity, the results showed that the aqueous extract of the leaves is more active compared to the other extracts.

Regarding the antibacterial power evaluated by the disk diffusion method of the different extracts (crude ethanol and fraction) of the two organs of the plant studied, our results show that the bacterium *Staphylococcus aureus* has the best sensitivity towards the ethanolic extract of okra leaves compared to other bacteria.

In perspective, a subsequent study with the aim of isolating and identifying molecules with antioxidant and antimicrobial activity by chromatographic and spectroscopic methods could make it possible to better understand the

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#### Compliance with ethical standards

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##### *Disclosure of conflict of interest*

No conflict of interest

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