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Phenolic profile and biological activities of *Aloe barbadensis* (Miller) from western Algeria

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ABSTRACT: *Aloe vera* is widely used in conventional medicine in Algeria to treat various diseases. This study aims to evaluate the chemical composition and biological activities of *Aloe vera* collected from western Algeria. Two extracts of ethanolic (EEA) and aqueous (AEA) were used to determine the total phenolic and flavonoid content. HPLC was applied to determine the amount of 15 compounds they contain, while the antioxidant activity was determined by the DPPH method. The antimicrobial activity experiment was conducted against five selected bacterial strains. Finally, an *in vivo* study on Swiss albino mice was conducted to discover the toxicity using Lorke's method and anti-inflammatory activity using the Carrageenan method. The EEA extract shows the highest total phenol content of 37.00±0.37mg GAE/g and total flavonoid content of 9.14±0.19 mg CE/g. The AEA contains hydroxybenzoic and benzoic acid with other ingredients (0.84 and 0.82 mg/g, respectively). The EEA contains 0.93 mg/g of benzoic acid. *Aloe vera* has antioxidant activity with IC_{50} values equal to 0.821 mg/ml for EEA and 1.993 mg/ml for AEA. The AEA inhibits *E. coli and S. aureus* with a bacteriostatic effect; EEA is the best inhibitor of *S. aureus and S. mutans* with the bactericidal effect. *Aloe vera* is practically nontoxic (LD_{50} is 3800 mg/kg of the AEA and superior to 5000 mg/kg of EEA). The AEA gives the best inhibition of edema, 85.96% at (100 mg/kg). *Aloe vera* leaves are an important resource of polyphenols, which have interesting antioxidant power, and antimicrobial and anti-inflammatory activities.

Keywords: *Aloe vera*; Antimicrobial; Antioxidant; Anti-inflammatory; HPLC.

1. INTRODUCTION

Finding natural and biologically active compounds has always been the goal of the food, pharmaceutical and cosmetic industries because of their therapeutic and commercial properties. Many studies have proven the existence of various herbals with pharmaceutical effects, but *Aloe* plants, specifically *Aloe vera* (*Aloe barbadensis* Miller), are the most commonly applied medicinal plant worldwide. *Aloe vera* has long been utilized in traditional medicine for its curative and therapeutic properties [1]. *Aloe vera* gel contains over 75

different bioactive compounds [2], sugar, vitamins and amino acid [3]. It has been an important medicine for centuries and is still a common household remedy [4].

Moreover, it is an important medicinal plant in the Indian system of medicine [5], the Yemeni culture [1] and the Egyptian civilization since ancient times [4]. Thanks to its anti-inflammatory, antimicrobial, and cicatrizing properties, *A. vera* has been traditionally used to cure skin injuries (burns, insect bites, cuts and eczemas) and digestive disorders [6]. It is an antioxidant [2] with hypoglycemic agents, hypolipidemic effects [7], photoprotective activities and cytotoxic effect [8]. *A. vera* extract improves insulin secretion and pancreatic β-cell function by restoring pancreatic islets [9].

A. vera also exhibits anticoccidial activity [10]. It has shown other therapeutic properties, including anticancer [6]. Most of the diseases treated with *Aloe vera* are symptoms of inflammation, microbial infection, or the accumulation of oxidants in the organism.

Thus, it is important to test the antimicrobial activity against five selected microbial strains that cause an infection disease and *in vivo* study of plant toxicity and anti-inflammatory activity. To our knowledge, there are no prior reports about an in-depth and sequential study of *Aloe vera* original in western Algeria. Therefore, this study aims to estimate the biological activities, total phenolic contents, and total flavonoid contents into ethanolic extracts (EEA) and aqueous (AEA) of *A. vera*. To consider if a natural composite has an antioxidant substance, antibacterial and anti-inflammatory properties, it is important to investigate these activities *in vitro* and *in vivo*. Our study adopts this approach and it aims at showing the value of TPC, TFC, IC_{50}, MIC, MBC, LD_{50} and inhibition capacity of edema inflammation. Despite the value of this medicinal plant, its high biological activity, and its availability, it is not utilized in Algeria as it should be.

2. MATERIALS AND METHODS

2.1. Collection of samples

The *Aloe vera* plant was harvested in July 2019 in Sfisef, Sidi Bel Abbes, located in western Algeria (35.22683683211121, - 0.3025574118455309) and (latitude 35°13'36.61" North, longitude 0°18'9.21" West). Botany experts at the University of Mascara confirmed the plant. The plant's aerial part (leaves) was washed with distillate water and then shade-dried at 25–35°C for 21 days. Afterward, it was ground in mills (SM-450TR), then sieved (size 125 µm < ø < 1 mm) to become a fine powder.

2.2. Extraction

Ethanolic extract (EEA): Ten grams of powder of dried leaves were extracted with 80% ethanol (1:10 w/v) (Sigma-Aldrich) and macerated for 24 h in obscurity. The output was then filtered and evaporated with rotavapor [11].

Aqueous extract (AEA): The aqueous extract was in the form of decoction. A quantity of 50 g of powdered leaves was boiled in 500 ml distilled water for 20 min at 80°C [12] (at a 1:10 w/v sample to solvent ratio) and followed by filtration and lyophilization to produce a dark brown powder. The yield was calculated and stored at 4°C [11].

2.3. Determination of total polyphenol concentration (TPC)

The concentration of total phenolics in the extracts was determined according to the modified method of Singleton [13]. Briefly, 100 µl of various concentrations of extracts solutions was added to 500 µl of Folin-Ciolcalteu (10% v/v) (Sigma-Aldrich), supplemented with 400 ml of 7.5% (p/v) Na_2CO_3 after 4 min. The mixture was agitated for 2 h at room temperature, and the absorbance was recorded at 765 nm. Gallic acid was

also used as a standard. The concentration of total phenolic compounds in these extracts was determined as mg of gallic acid equivalent per 1 g of extract (mg GAE/g extract). All experiments were conducted in triplicate.

2.4. Determination of flavonoid concentration (TFC)

The total flavonoid concentration in these extracts was determined according to Zhishen et al. [14]. Briefly, 1 ml of 2% $AlCl_3$ in ethanol was added to 1 ml of the extracts (2 mg/ml). After 10 min of incubation at room temperature, the absorbance was measured at 430 nm. The catechin (Sigma-Aldrich) was used as a standard. In this study, the total flavonoid content is indicated as mg of catechin equivalent per 1 g of extract (mg CE/g extract).

2.5. Determination of antioxidant activity using the DPPH method

The antioxidant activity was measured through DPPH free radical scavenging assay. The method was carried out as described [15]. First, the DPPH solution was prepared by the solubilization of DPPH (Sigma-Aldrich) (2.4 mg) in methanol (100 ml). Next, 0.05 ml of concentration of each extract (0.01 to 2 mg/ml) (w:v) was removed and mixed with the DPPH solution (1.95 ml) in a test tube. After 30 min in a dark room, the absorbance of these solutions was read at 517 nm. A duplicate reading was performed for each concentration using ascorbic acid as a positive control. The radical scavenging activity was calculated using the following formula: the radical scavenging activity (%) = Ac–As/Ac × 100, where Ac is the absorbance of the control (DPPH without the addition of test solution), and as the absorbance of the sample. The IC_{50} value was determined graphically from the sigmoidal-shaped curve of antioxidant concentration (µg/ml) versus % inhibition. For comparison purposes, the reciprocal $1/IC_{50}$ values were used [16].

2.6. High-pressure liquid chromatography- diode-array detection (HPLC-DAD)

The phenolic composition analysis of different extracts was made [17] with slight modifications and performed using an HP-Agilent 1290 Infinity HPLC equipped with a C18 column and diode array detector DAD. As a mobile phase, 3% acetic acid in (A) water and methanol (B) was used. The injection volumes were 1 µl, and the extract concentrations were 20 mg/ml. The eluates were detected at 278 nm. The tested samples were prepared in methanol, and 20 µl was the injecting volume. The elution gradient was applied at a flow rate of 0.8 ml/min is: 93% A-7% B (0.1 min), 72% A-28% B (20 min), 75% A-25% B (8 min), 70% A-30% B (7 min); and the same gradient for 15 min was 67% A-33% B (10 min), 58% A-42% B (2 min), 50% A-50% B (8 min), 30% A-70% B (3 min), 20% A-80% B (2 min), and 100% B in 5 min until it reached the end of the run. In addition, the standards used were gallic acid, catechin, chlorogenic acid, caffeic acid, hydroxybenzoic acid, epicatechin, syringic acid, coumaric acid, trans-ferulic, sinapic acid, benzoic acid, acid hesperidin, rosmarinic acid, cinnamic acid and quercetin. In this study, the identification and quantitative analysis are presented by comparison to these standards. The quantity of each phenolic compound is expressed as mg per gram of extract by external calibration curves, which were evaluated for each phenolic standard [18].

2.7. Antimicrobial activity assay and serial microdilution assay

Minimal inhibitory and minimal bactericidal concentrations (MIC and MBC) were determined by a broth microdilution assay [19] using a 96-well polypropylene plate and Mueller-Hinton broth. After 24 h at 37°C, the MIC was the lowest substance concentration that inhibited visible bacterial development in the well. Similarly, the MBC was defined as the lowest concentration yielding negative subcultures for 24 h of a 10 µl aliquot withdrawn from each well that expresses no bacterial growth. They were subcultured in MH agar. The experiment was carried out in triplicate. The MBC/MIC report of extract provides information on the

antimicrobial power. Indeed, when this ratio is equal to or less than 4, the extract is bactericidal while when it is greater than 4, the extract is bacteriostatic.

2.8. Bacteria and growth conditions

Five microorganism species were employed as test organisms: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, *Bacillus cereus* ATCC 6633 and *Candida albicans* ATCC 10231 (Laboratory for Research on Local Animal Products, Ibn Khaldoun Tiaret). The strains were in Eppendorf tubes, each containing a bacterial culture preserved in nutrient broth supplemented with 30% glycerol and maintained at -20°C. A volume of 100 µl of each tube was transferred into the BHI broth (Brain heart infusion) and then incubated at 37°C. After 24 to 48 h, the referenced strains developed, indicating their reactivation and that they were ready for use.

2.9. In vivo study

2.9.1. Experimental animals

Swiss Albino mice were left at room conditions in polypropylene cages for acclimatization within 7 days [20] (T 25 ± 2°C) and a 12 h light/dark cycle, with liberated access to standard pellet diet and water throughout breeding. The weight of the mice used was between 25–30 g. The animals fasted for 16 h before each experiment with free access to water. All the experimental protocols were prepared and performed based on the ethical guidelines of the Institutional Animal Ethical Committee of Mascara University, Mustapha Stambouli (ARECM), according to the Adelaide University Animal Ethics Committee (Ethics number M/76/98).

2.9.2. Extract toxicity

Lorke's method was used to determine the extract toxicity in two phases. During the first phase, nine animals were divided into three groups, i.e., three animals per group (n=3). Afterward, each group was administered a different dose intra-peritoneal (10, 100 and 1000 mg/kg) of extract mixed with a saline solution of 0.9%. Subsequently, they were put under observation for 24 h to scrutinize their comportment and mortality. The second phase involved using six animals, distributed into three groups of two animals each [21]. Then, we applied to each group different doses of intra-peritoneal (1600, 2900 and 5000 mg/kg) extract mixed with a saline solution of 0.9%. After that, the animals were inspected every 10 mins, followed by 24, 48, and 72 h observations of any changes in motor activity, respiration, writhing, and piloerection. The LD_{50} was calculated using the basic formula of Lorke:

$LD_{50}=\sqrt{axb}$, where a=highest non-lethal dose, b= least lethal dose.

2.10. Anti-inflammatory activity

Carrageenan (Sigma-Aldrich Co. (St. Louis, MO, USA) induced hind paw edema exemplar was used for the anti-inflammatory activity [22]. It is a useful model to detect the action of anti-inflammatory agents. The animals, *Swiss albino* mice (25–30 g), were divided into 4 groups (n = 6) and treated by intraperitoneal injection with saline 0.9% (control group), aqueous extract with (100 mg/kg), ethanolic extract (100 mg/kg) and (experimentation groups) 60 min after the administration of a test sample. Each mouse was injected with the freshly prepared suspension of carrageenan (1%) in physiological saline into the subplantar tissue of the right hind paw. After the control was injected (group control), the measure of paw edema was achieved every 60 min for 6 h after the induction of inflammation. Diclofenac (10 mg/kg) (Saidal, Algeria) was used as the reference drug [23]. The difference in foot-pad thickness was scaled by a gauge caliper (Fischer Darex). The mean values

of treated groups (six animals in each group) were compared with those of a control group and analyzed using statistical methods.

2.11. Statistical analyses

The data are presented as the mean ± SEM (standard error of the mean) or mean ± SD (standard deviation). The differences between the means were evaluated through the analysis of variance (ANOVA), followed by Dunnet's test. The statistical differences are considered significant, with $P < 0.05$ [24].

3. RESULTS AND DISCUSSION

Table 1 lists the results of extraction yield water extracts and ethanolic extract of *A. vera* leaves. Accordingly, the extraction procedure using the ethanol solvent shows that the crude extract of the *A. vera* leaves (11.11 ± 0.16%) is superior to the one obtained by aqueous extract (9.11±1.19 %) AEA<EEA. In the study [8], the yield of the aqueous extract is 13.56 ± 0.78%, and solvent extraction is 20.56% ± 0.38. Ethanol is still considered the best solvent for the extraction of *A. vera* [25]. The reasons why ethanol extraction gives superior yields may be attributed to the followings: (i) the use of an organic solvent that can easily be evaporated than water, (ii) the high concentration used (80% ethanol) and (iii) most components of this plant are organic compounds that easily dissolves in an organic solvent.

The total phenolic contents of plant extracts are observed to be a maximum of 37.00±0.37 mg GAE/g of EEA. However, the TPC of AEA is 16.23±0.47 mg GAE/g (Table 1), AEA<EEA. The ethanolic extract of Tunisia Aloe showed the highest total phenolic (40.500 ± 0.041 µg GAE/g of extract) [26]. The value of total flavonoids is observed to be higher, that is, 9.35 mg CE/g obtained from ethanolic *A. vera* extract. The aqueous extract gives 4.06±0.07mg CE/g. (Table 1) AEA<EEA. The results obtained in Nepal [12] show that TPC is equal to 54.95±2.46 (mg GAE/g), and TFC equals 1.13±0.19 (mg QE/g) in ethanolic extract. The Yemeni AVG contains a total phenolics of 97.2±12.0 mg/g; total flavonoids of 9.27±1.07 mg/g [1].

Table 1. The yields, total phenolic, total flavonoid contents, total antioxidant activities, IC_{50} and the equations of the graphics.

***Aloe vera* (*A. barbadensis* Miller)**		
Type of extract	**AEA**	**EEA**
Yield %	9.11±1.19	11.11±0.16
TPC (mg GAE/g extract)	16.23±0.47	37.00±0.37
TFC (mg CE/g extract)	4.06±0.07	9.14±0.19
Total antioxidant activity%	29.33±0.57	58.38±1.99
IC50 µg/ml	199.305	82.107
$1/IC_{50}$ µg/ml^{-1}	0,00501744	0,0121792
Y=ax+b	0.19x+12.238	0.3018x+25.22
R^2	0.9827	0.987

The results are the average of three determinations± standard deviation. AEA: Aqueous Extract of Aloe vera. EEA: Ethanolic Extract of Aloe vera. TPC: Total phenolic content (mg GAE/g extract). TFC: Total flavonoid content (mg CE/g extract) Total antioxidant activity % at 100 µg of extract. IC_{50}: The concentration of drug required for 50% inhibition. GAE/g: Gallic acid equivalents/g of extract. CE/g: Catechin equivalent/g.

3.1. DPPH radical scavenging activity assay

The results show a considerable diversity of the capacity to scavenge free radicals between extracts and ascorbic acid when its capacities show the strongest DPPH scavenging capacity is 98.39% at 2 mg/ml, with IC_{50} of 0.42 mg/ml and (R^2=0,9973). The extract exhibited DPPH scavenging capacity assay demonstrates that the maximum inhibition is the EEA (58.38±1.99%) at 2 mg/ml, with IC_{50} of 0.821g/ml and (R^2=0.987). On the other hand, the minimum inhibition showed by AEA (29.33±0.57%) at 2 mg/ml with IC_{50} values equal to 1.993 mg/ml. The rapport ($1/IC_{50}$) is present in Table 1.

The ethanolic extract of Tunisia *Aloe* showed an antioxidant capacity (471.3± 0.013µg/ml) [26], which means that it has a higher antioxidant capacity than Algeria *Aloe*. Therefore, it explains that the high amount (37.00±0.37mg GAE/g) of polyphenols positively affected the antioxidant capacity. The antioxidant activity of plant materials is closely related to the content of their phenolic compounds [27]. If a bad inhibition of radicals happens, the phenomenon of transient cavitation initiates chemical reactivity through thermolysis, supercritical water oxidation and free radical oxidation. The impact of thermolysis but also the generation of hydrogen ions (H^+), free radicals (O^-, OH^- and HO^{2-}) and hydrogen peroxide (H_2O_2) [28]. The radical scavenging activity of the extracts could be related to the nature of phenolics and their hydrogen-donating ability.

3.2. High-pressure liquid chromatography-diode-array detection (HPLC-DAD)

The analysis of HPLC of the extracts of 15 compounds (gallic acid, catechin, chlorogenic acid, caffeic acid, hydroxybenzoic acid, epicatechin, syringic acid, coumaric acid, trans ferulic acid, sinapic acid, benzoic acid, hesperidin, rosmarinic acid, cinnamic acid and quercetin) was investigated for each extract. Table 2 lists the amount of each phenolic compound expressed as mg per gram. The results show that 14 of these 15 compounds are identified in the aqueous extract of *Aloe vera* (Table 2, Figure 1). Hydroxybenzoic acid and benzoic acids are the most abundant components accounting for 0.84 mg/g and 0.82 mg/g extract, respectively.

Table 2. Chemical composition of aqueous extract of *Aloe vera* (AEA) from Algeria.

Peak	Ret Time (min)	Area [mAU.s]	Quantity (mg/g of extract)	Identification
1	4.359	802.10480	0.84	Hydroxybenzoic Acid
2	3.436	1578.86145	0.82	Benzoic Acid
3	13.197	1114.65344	0.46	Catechin
4	80.963	107.76672	0.27	Gallic Acid
5	3.973	304.65063	0.20	Chlorogenic Acid
6	7.762	1143.38696	0.17	Coumaric acid
7	12.743	313.83231	0.15	Epicatechin
8	79.919	672.40771	0.15	Rosmarinic acid
9	4.494	499.51447	0.14	Syringic Acid
10	24.267	199.43004	0.12	Trans-Ferrulic Acid
11	15.935	246.26178	0.11	Quercitin
12	35.461	1223.61169	0.10	Cinamic Acid
13	16.961	29.27682	0.08	hesperidin
14	77.218	274.47412	0.02	Sinapic Acid
Total identified			14 compounds	
Total of phenolic acids			3.63 mg/g of extract	
Total of flavonoids			0.80 mg/g of extract	
Total of phenolic compound			2.83 mg/g of extract	

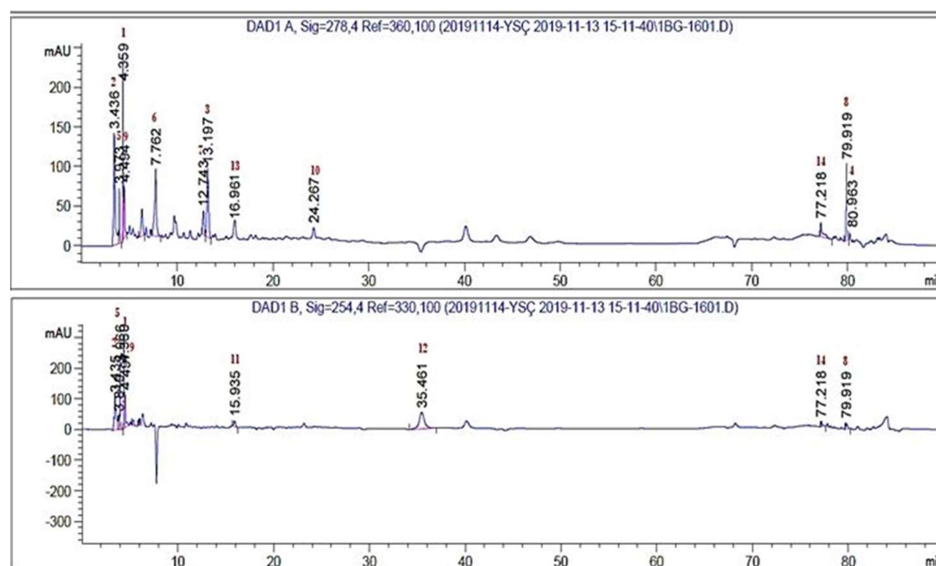

Figure 1. HPLC chromatogram of aqueous extract of *A. vera* (AEA).

In the ethanolic extract (Table 3, Figure 2), 14 components are determined, and benzoic acid is the most abundant compound in this extract at 0.93 mg/g. Compared to previous studies, the GC MS analysis of *Aloe vera* results in some important constituents, such as benzoic acid [29]. The HPLC-UV and LC-MS of the Yemeni *Aloe vera* show anthrones and chromones as the main components [1]. With reversed-phase high-performance liquid chromatography (RP-HPLC), *A. vera* extracts are characterized by the abundance of catechin, sinapic acid and quercitrin. Gentisic acid, epicatechin and quercitrin are the most prominent phenolic compounds of the flowers [30].

Table 3. Chemical composition of ethanol extract of Aloe vera from Algeria.

Peak	Retention Time (min)	Area [mAU.s]	Quantity (mg/g of extract)	Identification
1	3.826	750.39935	0.93	Benzoic Acid
2	15.454	85.55801	0.29	Quercitin
3	4.285	323.12646	0.27	Hydroxybenzoic Acid
4	85.891	1066.77112	0.25	Trans-Ferrulic Acid
5	79.784	257.47772	0.20	Rosmarinic acid
6	16.962	43.45979	0.15	Hesperidin
7	24.331	881.78558	0.14	Chlorogenic Acid
8	12.764	18.22491	0.09	Catechin
9	4.368	374.07138	0.08	Syringic Acid
10	77.222	251.01648	0.07	Sinapic Acid
11	80.250	127.87583	0.07	Coumaric acid
12	83.895	121.82565	0.03	Gallic Acid
13	82.455	108.61681	0.03	Cinamic Acid
14	74.372	13.50143	0.02	Caffeic acid
Total identified			14 compounds	
Total of phenolic acids			2.62 mg/g of extract	
Total of flavonoids			0.53 mg/g of extract	
Total of phenolic compound			2.09 mg/g of extract	

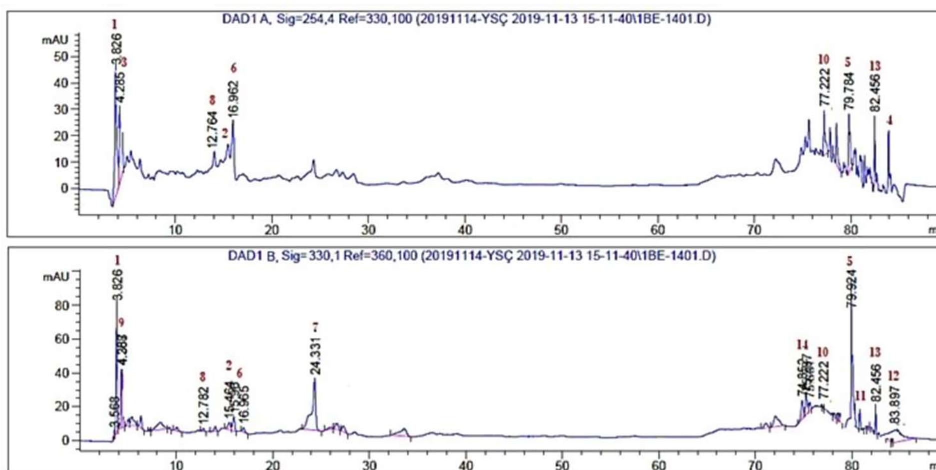

Figure 2. HPLC chromatogram of ethanolic extract of *A. vera* (EEA).

3.3. Antibacterial activity assay

Antibacterial activity assay and serial microdilution assay. The antimicrobial activity of all plant extracts is evaluated against four pathogenic bacterial strains and one fungal strain. The antimicrobial effect of herbal plant extracts is evaluated in terms of MIC and MBC of microbe's development, as presented in Table 4. Results revealed that aqueous extracts are more effective against microbes than ethanolic extracts. All the extracts of *Aloe* show better antibacterial and antifungal activity. The AEA inhibits *E. coli* ATCC 25922, *S. mutans* ATCC 25175 and *C. albicans* ATCC 10231, with MIC ranging from 12.5 to 100 µl/ml with bacteriostatic effect against *E. coli* and bactericidal effect against other strains (Table 4). EEA is the best inhibitor of *S. aureus* ATCC 25923. The same result was reported [5]. The aqueous extract effectively inhibits the growth *of B. subtilis*, *S. aureus*, and *C. albicans*.

Table 4. The minimal inhibitory concentrations (MICs) and (MBCs) of extracts against bacteria and yeast.

	MIC µl/ml		MBC µl/ ml		MBC/MIC ratio	
	AEA	EEA	AEA	EEA	AEA	EEA
Escherichia coli ATCC 25922	25	12.5	100	50	4	4
Staphylococcus aureus ATCC 25923	12.5	50	50	100	4	2
Streptococcus mutans ATCC 25175	50	25	100	25	2	1
Bacillus cereus ATCC 6633	100	6.25	200	50	1	8
Candida albicans ATCC 10231	100	100	200	100	2	1

MIC: Minimal inhibitory concentrations MBC: Minimal bactericidal concentrations. MBC/MIC ratio ≤2: bactericidal effect. MBC/MIC ratio ≥ 4: bacteriostatic effect. AEA: Aqueous Extract of Aloe vera. EEA: Ethanolic Extract of Aloe vera.

The EEA is the best inhibitor of *S. aureus* ATCC 25923 with a MIC of 50 µl/ml and a bactericidal effect (Table 4). In the study [31], *Aloe* has an effect on the three bacterial organisms, *S. pyogenes*, *S. aureus* and *E. coli*. *A. vera* leaf gel can relates the development of the Gram-positive bacteria. *A. vera* gel inhibited (79%) in vitro growth of *E. faecalis* [4].

The fresh 'juice' from cut leaves down *S. aureus*, but the activity is unstable unless the extract is refrigerated, heated, and then freeze-dried. The freeze-dried extract inhibited some species of *Staphylococcus* and *Streptococcus* [4]. *Aloe vera* contains six antiseptic agents (sulfur, lupeol, salicylic acid, cinnamic acid, urea nitrogen and phenol), which act as a team to provide antimicrobial effects [5]. Specific plant compounds such as anthraquinones, dihydroxy anthraquinones, and saponins have been suggested to have direct antimicrobial

activity [6, 23]. This effect may be due to the existence of benzoic acid and hydroxybenzoic acid in these extracts because the benzoic acid and hydroxybenzoic acid showed an inhibition for all the microorganisms tested: *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* [32].

3.4. Toxicity of extracts

In the acute extract toxicity study, no mortality was observed in all mice 24 h after testing extracts at 600 mg/kg, and 1000 mg/kg and no mortality was recorded in mice tested at 5000 mg/kg EEA. One mouse died at doses from 5000 mg/kg of the AEA. The lethal dose is 5000 mg/kg. The LD50 is equal to 3800 mg/kg of the AEA, and the lethal dose of the EEA is superior to 5000 mg/kg. *Aloe vera* caused moderately adverse effects [4]. These extracts are practically nontoxic according to Hodge and Sterner toxicity scale.

3.5. Anti-inflammatory activity

The anti-inflammatory activity of AEA and EEA against carrageenan-induced paw thickness is represented in Table 5, and the inhibition of edema is presented in Figure 3. In the control group of this study, the edema volume increased progressively depending on the number of hours (Figure 4), reaching its peak at 4 h after the carrageenan injection. Besides, the AEA at 100 mg/kg gives the best inhibition of 85.96% of edema after 6 h of induced carrageenan. The EEA inhibits 83.51% of edema. All inhibitions are less than the standard of 10 mg/kg, inhibiting 90.87% of edema paw.

Table 5. Anti-inflammatory of extracts on paw edema induced by carrageenan in mice.

	Paw Thickness (mm)						
	h_0	h_1	h_2	h_3	h_4	h_5	h_6
Control	1.91±0.04	2.07±0.09	3.24±0.24	3.37±0.25	3.57±0.06	3.89±0.11	3.88±0.07
Standard	1.92±0.02	2.05±0.08	2.85±0.10*	2.78±0.15***	2.73±0.17***	2.49±0.08***	2.10±0.06***
AEA	1.89±0.05	2.02±0.10	2.92±0.10*	2.67±0.24***	2.52±0.29***	239±0.26***	2.17±0.16***
EEA	1.93±0.05	2.04±0.05	2.84±0.17*	2.74±0.29***	2.51±0.37***	2.34±0.43***	2.25±0.42***

Mean ± SEM (n = 6); Control: vehicle (NaCl, 0.9%); Standard: Diclofenac 10 mg. h: hour. *p<0.05;***p< 0.001 significance (comparison with control group). AEA: Aqueous Extract of Aloe vera. EEA: Ethanolic Extract of Aloe vera.

The AEA and EEA (100 g/kg) give a good inhibition of edema better than diclofenac (10 mg/kg) from the 3rd hour (2.67±0.24 mm, 2.74±0.29 mm and 2.78±0.15 mm, respectively) to the 5th hour (2.39±0.26 mm, 2.34±0.43 mm and 2.49±0.08 mm, respectively) after inducing the carrageenan (Figure 4). The treatment of mice with the two extracts at a dose of 100 mg/ml show a highly significant decrease in edema (p< 0.001) from 3 h compared with the control. Meanwhile, the same could be observed for diclofenac compared with the control. This indicates that it has a faster effect, whereas diclofenac has a good effect when it reaches the 6th hour.

This significant activity of all the extracts and standard drugs highlighted in this study may be due to the inhibition of mediators of inflammation such as histamine, serotonin, and prostaglandin. The extracts' anti-inflammatory activity results can be attributed to their main compounds: hydroxybenzoic and benzoic acids. Plant benzoic acids [11] and their derivatives are common and widespread mediators of plant responses to biotic and abiotic stress [33]. The biosynthesis of all plant benzoic acids and their products ultimately starts from the shikimate pathway [34]. Benzoic acid converts to salicylic acid in plants [35]. Many natural products obtained from plant benzoic acids or have benzoyl/benzyl moieties are also of medicinal or dietary value to humans [34].

Carrageenan-induced paw edema is a usually utilized experimental model of acute inflammation represented by a biphasic development of edema. The first phase (1–2 h) depends on mediator release, e.g., histamine, serotonin, and bradykinin [36]. At the same time, the second phase (3–6 h) is sustained by the release of prostaglandins, leukotrienes, lysozymes, proteases, nitric oxide and by local infiltration by neutrophils producing free oxygen radicals, e.g., O_2^- and OH [37].

In a carrageenan-inflamed synovial pouch model of inflammation, a 10% *A. vera* treatment of synovial pouches reduces vascularity by 50% and the number of mast cells in synovial fluid by 48%. However, the aloin and aloe-emodin, two anthraquinones present in the exudate, are pronounced to cause anti-inflammatory effects by inhibiting inducible nitric oxide synthase (iNOS) and COX-2 expression [4].

4. CONCLUSION

The study demonstrates that the yield, total phenolic content, total flavonoid continent and antioxidant activity are higher in the ethanolic extract of *Aloe vera* compared to the aqueous extract of *Aloe vera*. Nevertheless, the aqueous extract is the best antimicrobial, so four of five bacteria are sensitive to the aqueous extract (diameter greater than 15 mm) and anti-inflammatory based on the percentage of edema inhibition. Our results confirm the correlation between phenolic content and antioxidant activity. This plant could treat various infections caused by microbes and reduce inflammation.

Authors' Contributions: FFF practiced laboratory experiments and wrote the article. MB, ML and AT elaborate on a research plan and correct the article. MY performed the antimicrobial activity. YSK analyzed and interpreted the HPLC of extracts. All authors read and approved the final version of the manuscript.

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