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Original Article

INVESTIGATING THE ANTIBACTERIAL, ANTIOXIDANT, AND ANTI-INFLAMMATORY ACTIVITIES OF AQUEOUS AND HYDROETHANOLIC EXTRACTS OF *OCIMUM BASILICUM* AND *OCIMUM GRATISSIMUM* ON SOME GERMS RESPONSIBLE FOR AEROBIC VAGINITIS

DJOVA STEVE VALDI[1], NDOYE FOE FLORENTINE[2*], DONGMO ZANGUE ARLETTE[3], KENGNE GOUNMADJE LANDRY[2], NDJIB ROSETTE[4], NGONGANG TCHAMI DIMITRI[3], NYEGUE MAXIMILIENNE ASCENSION[3]

[1]Department of Biochemistry, University of Bamenda, P. O. Box 39, Bambili, NW-Region Cameroon, [2]Department of Biochemistry, University of Yaoundé I, P. O. Box 812 Yaounde, Cameroon, [3]Department of Microbiology, University of Yaounde I, P. O. Box 812, Yaounde, Cameroon, [4]Laboratory of Pharmacology, Institute of Medical Research and Study of Medicinal Plants, P. O. Box 13033 Yaounde, Cameroon

[*]Email: ndoyef@yahoo.fr

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ABSTRACT

Objective: The present work evaluates the antibacterial, antioxidant, and anti-inflammatory activities of aqueous and hydroethanolic extracts of *Ocimum basilicum* and *Ocimum gratissimum* on germs responsible for aerobic vaginitis.

Methods: University Teaching Hospital of Yaoundé and Central Hospital of Yaoundé provided the germ (*Staphylococcus aureus*) and isolates (*Enterobacter cloacae* and *Providencia stuartii*). The extracts were obtained by maceration of the plants in water and hydroethanolic system. Phytochemical screening was assessed using the standard method; sensitivity and inhibition tests were carried out on agar medium and microplates in liquid medium. The antioxidant activity of the extracts was evaluated using the 2,2-diphenyl-1-picrilhidrazil (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ferric reducing antioxidant power assay (FRAP), and β-carotene assays; total polyphenols content was obtained by the Folin Ciocalteu assay; anti-inflammatory activity by denaturation of ovalbumin.

Results: Flavonoids, catechin, and tannins were abundant in the hydroethanolic extracts (56.65±3.89 and 228.94±8.42 mg EAA/g, respectively). The extracts were bactericidal for *S. aureus* and bacteriostatic for *P. stuartii* and *E. cloacae*. The hydroethanolic extract of the leaves of *O. gratissimum* has the best anti-radical activity 23.08±3.12, while that of the leaves of *O. basilicum* had the best-reducing power (388.36±9.96). The anti-inflammatory activity was found to be significant in the leaf extracts studied with IC_{50} values of 0.358±0.013 mg/ml for *O. basilicum* to 0.269±0.008 mg/ml for *O. gratissimum*, thus justifying their anti-inflammatory properties. The equivalent contents of primary and secondary antioxidants of the extracts were also obtained.

Conclusion: The hydroethanolic extracts of *Ocimum basilicum* and *Ocimum gratissimum* have antibacterial, antioxidant, and anti-inflammatory properties.

Keywords: *Ocimum basilicum*, *Ocimum gratissimum*, Antibacterial activity, Antioxidant activity, Polyphenols, Anti-inflammatory activity

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INTRODUCTION

Aerobic vaginitis is characterized by a decrease or disappearance of lactobacillus and the proliferation of aerobic bacteria, in this case, *E. coli*, *Staphylococcus aureus*, *Streptococcus* group B, and *Enterococci* with a marked inflammatory reaction [1]. An imbalance in the vaginal flora leads to a reduction in lactobacillus activity, an increase in vaginal pH to > 4.5 and the proliferation of a polymicrobial flora dominated by *Gardnerella vaginalis*, but also Gram-positive anaerobic species (*Streptococcus sp. Staphylococcus sp*.) or Gram-negative [2]. When changing the balance, a large amount of reactive oxidative species (ROS) is generated and accumulated in the genital tract, thus leading to oxidative stress, which is responsible for cellular damage (enzymes, DNA, membrane lipids). The production of reactive oxygen species is useful but can be harmful to the body when overproduced and in the absence of defense mechanisms [3]. This pathology being part of cervicovaginal infections, constitutes one of the most common reasons for consultation in gynecology [4].

In developed countries, these infections and their complications are among the top five reasons for consultation among adults [5]. They occur in 85 to 90% of cases following the *Candida* genus; the other causes are represented by bacteria (*Staphylococcus aureus* and many others) and parasites [6]. In Cameroon, studies have been conducted on these infections in the city of Douala, showing a prevalence rate of 28% [7]. In addition, in recent years, resistance to antibiotics for other pathogens responsible for vaginal infections has also been described. This situation makes the prevention and adequate treatment of STIs (sexually transmittable infections) essential [8]. Research activities aimed at the development of effective antibiotics are particularly long and costly; antibiotics are toxic and can cause many side effects [9]. Therefore, medicinal plants are very promising and are used as medicine or a source of antimicrobial molecules. *Ocimum basilicum* and *Ocimum gratissimum* are plants of Cameroonian pharmacopeia and are also used in cooking; these plants are used in traditional medicine to treat many diseases: painful menstruation, gonorrhea, white discharge, and many others [10]. In Cameroon also, previous work on these plants showed their therapeutic effects. The activity of the essential oils of these plants in northern Benin was carried out on *Salmonella Sp*. [10]; the potential in chemical composition and antioxidant, antimicrobial, and anticancer activities of ethanolic extracts (EE) and essential oils has been demonstrated as an asset in pharmaceutical technology by Rezzoug *et al.* [11]; various articles have shown the potential of *Ocimum basilicum* on respiratory disorders [12]. However, very few studies show the potential of these plants in the treatment of genital infections and more specifically, aerobic vaginitis. Thus, this work contributes to the reduction of the incidence rate of aerobic vaginitis by showing properties likely to overcome not only the germs involved in this pathology but also the oxidative stress and the inflammation linked to that through the use of plant extracts of *Ocimum basilicum* and *Ocimum gratissimum*.

MATERIALS AND METHODS

Collection and identification of plant material

The samples of *Ocimum gratissimum* (Leaves and Stems) were collected in early October 2020 in the city of Bafoussam and those of *Ocimum basilicum* (Leaves and Stems) in the city of Yaoundé in mid-

October 2020 in the West and Central regions of Cameroon, respectively. After harvest, the samples were sent to the National Herbarium of Cameroon for identification by comparison with specimen number 42747/HNC for *Ocimum basilicum* and 40332/HNC for *Ocimum gratissimum*.

Preparation of hydroethanolic and aqueous extracts

These samples (Leaves and Stems) were dried and ground, then 100g of the dry powders of each sample of plant material were macerated for 72 h in 100 ml of water and in 20/80 ml of H_2O/Ethanol. After maceration, the various mixtures were filtered on Whatman N °1 filter paper, and the filtrates obtained were concentrated on a rotary evaporator at 80 °C and by freeze-drying to eliminate the ethanol and water. The crude extracts collected were weighed and stored in the refrigerator at 4 °C, in sterile Eppendorf tubes with labeled screw-tops [13].

Strains and isolates used

Bacteria consisting of two clinical isolates and a reference strain were used for this study. Isolates of *Enterobacter cloacae*, *Providencia stuartii* (clinical), and *Staphylococcus aureus* CIP 7625 (reference strain) were provided by the University Teaching Hospital of Yaounde and the Central Hospital of Yaounde respectively. This biological material was stored at 4 °C in a liquid nutrient medium contained in the cryotubes.

Phytochemical screening of extracts

Photochemical screening allows the identification of the different families of bioactive compounds present in the extracts using colorimetric tests. It was carried out according to the method described by Harbone [14].

Preparation of culture medium, a stock solution of bacterial extract, ATB, and inoculum

Culture media (MHB and MHA) were prepared as indicated on the container boxes; then boiled, followed by sterilization at 121 °C for 15 min in the autoclave and finally poured into 99 mm Petri dishes for MHA medium.

The bacterial inoculum was adjusted to the Mc Farland 0.5 standard of such an enclosure that after shaking in a spectrophotometer and reading at an absorbance of 625 nm, the suspensions obtained contained around 108 CFU/ml [15].

Agar susceptibility test: well diffusion method

Petri dishes containing Mueller Hinton Agar medium were inoculated aseptically by swab with a suspension of the inoculum described above so as to have a semi-confluent colony sheet. After the dishes had dried, wells of 6 mm in diameter were dug with the wide end of the yellow tips. The cavities thus formed were filled with 100 µl of plant extracts at a concentration of 100 mg/ml; likewise, a solution of 1 mg/ml of volume 10 µL of ciprofloxacin was used as reference antibiotics introduced into the central wells. The plates were incubated in a 37 °C incubator for 24 h. After incubation, the inhibition diameters are measured in millimeters using a caliper. The experiments were carried out in triplicate.

Determination of the minimum inhibitory concentration (MIC) of the extracts by the method of dilution in a liquid medium

The minimum inhibitory concentrations (MIC) of the extracts were determined as described by CLSI [16]. The MIC was determined to be the lowest concentration that inhibited any visible growth [16].

Determination of the minimum bacterial concentration (MBC) of the extracts of interest by subculture in a solid medium

The minimum bacterial concentration (MBC) is the lowest concentration of antibiotic that left at most 0.01% of survivors of the initial inoculum or the minimum concentration showing no bacterial growth, it was determined by subculture [17].

The calculation of the CMB/CMI ratio made it possible to determine the bactericidal, bacteriostatic, and tolerance effects of the microbial strains.

Evaluation of antioxidant and anti-inflammatory activities

Evaluation of antioxidant activity

Anti-free radical test: DPPH

Preparation of the 0.04 g/l DPPH solution

The method described by Fitrotunnisa *et al.* [18] was used. The DPPH reagent (10 mg) was dissolved in 25 ml of methanol. From this solution, 5 ml is taken and dissolved in 45 ml of methanol. All of this happened in the dark.

After the various solutions were prepared, 1950 µL of DPPH solution was pipetted and introduced into the tubes and 50 µL of extract for each concentration was added to obtain a final volume of 2 ml per tube. The actual concentrations of the extract reacted with DPPH in 2 ml were determined: 0.1 mg/ml; 0.05 mg/ml; 0.025 mg/ml; 0.0125 mg/ml; 0.00625 mg/ml; 0.0031 mg/ml. The tests were carried out in triplicate and in the dark. The optical density was measured after 30 min of incubation for 120 min at a wavelength of 515 nm.

A control tube (white) was also made and contained DPPH+methanol.

The SC_{50} was determined from the graph representing the entrapment percentages as a function of the concentration tested in mg/ml. This value allowed us to calculate the effective concentration 50 (EC_{50}) expressed in g of extract per mole of DPPH at the end of all this we calculated the PA.

Anti-free radical test: ABTS (2,2-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid)

The study of the antioxidant capacities was made by a discoloration test of the ABTS radical. For this, we first prepared 7 mmol of stock solution of the cationic radical ABTS by dissolving 8 mg of ABTS powder in 1 ml of distilled water (solution A). Then solution B was prepared from 13.2 mg of potassium persulfate dissolved in 10 ml of distilled water. We mixed 0.5 ml of solution A with 0.5 ml of solution B and stored it at room temperature in the dark for 16 h. Under these conditions, the concentrations of ABTS and potassium persulfate are 7 mmol and 2.45 mmol, respectively and the stability is at least two days. On the day of analysis, we diluted the stock solution with distilled water to an absorbance of 0.7 (±0.02) at 734 nm using a Jenway 6305 spectrophotometer.

Reducing power test: FRAP

The determination of the ferric antioxidant-reducing power of the extracts was made by the method described by Benzie and Strain [19].

Reducing power test: β-carotene bleaching

The β-carotene bleaching test used in this study is that described by Moure and *al.* (2001) [20].

Assay of total polyphenols by the folin-ciocalteu test

The total polyphenols were evaluated according to the spectrophotometric method using the Folin-Ciocalteu reagent described by Chew and *al.* [21].

Evaluation of anti-inflammatory activity

Method of inhibiting ovalbumin denaturation

The *in vitro* inhibitory effect of the extracts was determined using the protein denaturation method described by Chandra and *al.* [22] with some modifications.

Data analysis

Tables were obtained using the Microsoft Excel 2016 Spreadsheet. Graphs and Statistical analyzes were obtained and performed in GraphPad/Prism 7. The analysis of the variance (ANOVA) was used to compare the averages between more than two groups. The materiality threshold was set at $p<0.05$.

RESULTS AND DISCUSSION

Extraction yield

We used the leaves and stems of *Ocimum basilicum* and *Ocimum gratissimum* as samples for aqueous and hydroethanolic solvent

extraction using the maceration technic. The leaf and stem extracts of *Ocimum gratissimum* and *Ocimum basilicum* were respectively obtained and presented different physicochemical characteristics summarized in table I below. This table shows that the extracts obtained from the hydroethanolic solvent extraction all had a pasty texture with dark green, brown, and brown colors; while those obtained with the aqueous solvent had pasty, powdery, and crystalline textures, with the same colors as the hydroethanolic extracts. Similarly, the yields obtained from the different extractions vary between 12% and 34.36%; we note that the hydroethanolic extracts have a high extraction yield (21.63% to 34.36%) compared to the aqueous extracts (12.00% to 30.86%).

Phytochemical screening of plant extracts

This test allowed us to highlight ten families of secondary metabolites, namely: alkaloids, phenols, polyphenols, tannins, saponins, flavonoids, triterpenes, steroids, anthocyanins, and anthraquinones. Table 1 shows that the hydroethanolic extracts contain all families of compounds except for anthraquinones, while the aqueous extracts only present phenols, triterpenes, and steroids out of the 10 families of compounds tested.

This table shows that the hydroethanolic extracts have all the families of compounds with the exception of anthraquinones whereas the aqueous extracts have only the phenols, triterpenes, and steroids in the 10 families of compounds; this low presence of the compounds in the aqueous extracts leads us to continue our study only with the extracts hydroethanolic compounds because they are richer in bioactive compounds.

Antimicrobial activities of plant extracts

Bacterial growth inhibition parameters: (MIC, CMB, and CMB/CMI)

The values of the MICs and CMBs obtained by the microdilution method were determined and are given in table 2 below.

Table 1: **Phytochemical screening of aqueous and hydroethanolic extracts of *O. basilicum* and *O. gratissimum***

	Hydroethanolic				Aqueous			
	Leaves of *O. g*	**Stems of *O. g***	**Leaves of *O. b***	**Stems of *O. b***	**Leaves of *O. g***	**Stems of *O. g***	**Leaves of *O. b***	**Stems of *O. b***
Alkaloids	+	++	-	+	-	-	-	-
Phenols	+++	++	+++	+++	+	+	+	+
Polyphenols	++	+	++	++	+	-	-	-
Tannins	++	+	++	++	-	-	-	-
Saponins	+	-	+	+	-	-	-	-
Flavonoids	++	+	++	++	+	-	-	-
Triterpenes	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+
Anthocyanins	+	+	+	-	+	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-

Legend: (+) Presence of compound; (+++) Abundance of compound; (-) Absence of compound, *O. g: O. gratissimum and O. b: O. basilicum*

Table 2: **Parameters of inhibition of the growth of bacterial species by extracts and ciprofloxacin**

Strains	**Inhibition parameters (mg/ml)**	**Hydroethanolic extracts**				**ATB**
		O. g. F	***O. g. T***	***O. b. F***	***O. b. T***	
S. a	CMI	3.125	6.250	0.390	0.781	0.0243
	CMB	6.25	12.5	6. 25	6.25	0.195
	EFFECT	Bactericidal (2)	Bactericidal (2)	Bacteriostatic (16.02)	Bacteriostatic (8)	Bacteriostatic (8.02)
E. c	CMI	1.56	12.5	3. 12	6. 25	0. 78
	CMB	6. 25	25	12.5	25	6. 25
	EFFECT	Bacteriostatic (4)	Bactericidal (2)	Bacteriostatic (4)	Bacteriostatic (4)	Bacteriostatic (8)
P. s	CMI	3. 12	12. 5	6. 25	6. 25	0.78
	CMB	12. 5	25	12. 5	25	6. 25
	EFFECT	Bacteriostatic (4)	Bactericidal (2)	Bactericidal (2)	Bacteriostatic (4)	Bacteriostatic (8)

Legend: S. a; *Staphylococcus aureus*; E. c: *Enterococcus cloacae* P. s: *Providencia stuartii*; O. g. F: *Ocimum gratissimum* Leaves; O. g. T: *Ocimum gratissimum* Stems; O. b. F: *Ocimum* b*asilicum* Leaves; O. b. T: *Ocimum basilicum* Stems; ATB antibiotic

The analysis of table 2 shows that the MICs of the plant extracts are between the values of 0.390 mg/ml and 12.5 mg/ml and the CMBs are between 6.25 mg/ml and 25 mg/ml. Subsequently, table 4 shows that the extracts exhibit bacteriostatic activity on two species among the three strains tested, while *O. Gratissimum* leaves and stems had bactericidal activity against *Staphylococcus aureus*. The extracts of *O. basilicum* leaves and stems, although less active on *S. aureus*, showed better inhibitory activities with MIC values between 0.390 mg/ml and 0.78 mg/ml. It is also apparent that for ATB (Cipro), although MICs were 0.024 mg/ml and 0.78 mg/ml; this molecule did not show good activity on all the strains tested and all had a bacteriostatic power on them.

Antioxidant and anti-inflammatory activities

Antioxidant activities

This part of our study allowed us to evaluate two anti-radical assays, two tests of the reducing power, and a test for the dosage of total polyphenols; All these tests allowed us to determine the amount of primary and secondary antioxidants contained in the extracts of *O. basilicum* and *O. gratissimum*.

The anti-radical activity of *O. basilicum* and *O. gratissimum*

2,2-diphenyl-1-picrylhydrazile (DPPH) test

The concentrations of the extracts that inhibit 50% of the DPPH radicals (SC_{50}) were determined by graphic projection and using the equation of the regression line y= ax+b; from the SC_{50}, the effective concentration which makes it possible to trap 1/2 of the quantity of DPPH (EC_{50} expressed in mg Ex/g of DPPH), as well as the anti-free radical power (AP), was determined and summarized in table 3 below.

Analysis of this histogram shows that the hydroethanolic extracts of *O. basilicum* and *O. gratissimum* have statistically different anti-radical activities ranging from 23.08±3.123 to 0.786±0.019 for the extract of leaves and stems of *O. gratissimum* and from 7.366±0.818 to 3.033±0.330 for the extract from the leaves and stems of *O. basilicum*. Similarly, we note that the anti-radical activity of the

extracts tested is lower than that of the reference molecules in the occurrence of Ascorbic acid whose AP is 40.000±0.000 and Quercetin whose AP is 25.816±2.660 however, the AP of leaf, extracts of *O. gratissimum* is closest to that of the reference molecules and in particular that of quercetin.

2,2-Azino-bis(3-ethylbenzo-thiazoline)-6-sulfonic (ABTS+) radical test

From these curves the SC_{50}s were obtained, the EC_{50} and the AP were determined and collated in table 4 below.

Table 3: Summary of the results of the anti-radical activity by DPPH

Substances tested	SC_{50} (mg/ml)	EC_{50} (mgEx/g of DPPH)	AP
Hydroethanolic leave extracts of *O. basilicum*	0.006±0.001	140.000±15.943	7.366±0.820
Hydroethanolic stem extracts of *O. basilicum*	0.013±0.001	331.666±39.651	3.033±0.330
Hydroethanolic leave extracts of *O. gratissimum*	0.002±0.001	44.166±6.236	23.080±3.123
Hydroethanolic stem extracts of *O. gratissimum*	0.052±0.001	12.900±27.003	0.786±0.020
Ascorbic acid	0.001±0.000	25.000±0.000	40.000±0.000
Quercetin	0.002±0.001	39.166±4.249	25.816±2.660

Legend: AP: Antioxidant power; SC_{50}: Scarvenging concentration; EC_{50}: Effective concentration; number of experiments (ne):3, mean±SD. To compare the AP of the extracts of *O. basilicum* and the PAs of the *O. gratissimum* extracts between them and between the APs of the reference molecules, the histogram below was used (fig. 1).

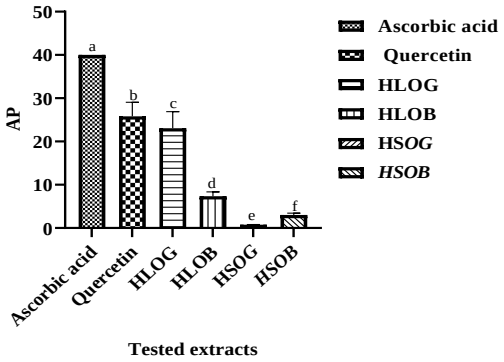

Fig. 1: **Anti-radical activity of DPPH, Legend: The bands with different letters are statistically different with a significance level *P<0.05*; n: 3, mean±SD. HLOG: hydroethanolic leaves extract of *O. gratissimum*; HLOB: Hydroethanolic leaves extract of *O. basilicum*, HSOG: Hydroethanolic stem extract of *O. gratissimum*; HSOB: Hydroethanolic stem extract of *O. basilicum***

Table 4: **Summary of the results of the anti-radical activity by ABTS**

Substances tested	SC_{50} (mg/ml)	EC_{50} (mgEx/g of ABTS)	AP
Hydroethanolic leave extracts of *O. basilicum*	0.048±0.001	3692.307±111.648	0.271±0.008
Hydroethanolic stem extracts of *O. basilicum*	0.035±0.001	2701.795±40.433	0.370±0.005
Hydroethanolic leave extracts of *O. gratissimum*	0.002±0.001	131.282±11.672	7.681±0.715
Hydroethanolic stem extracts of *O. gratissimum*	0.013±0.002	1002.820±132.216	1.014±0.127
Ascorbic acid	0.001±0.000	96.154±0.000	10.400±0.000

Legend: AP: Antioxidant power; SC_{50}: Scarvenging concentration; EC_{50}: Effective concentration; ne: 3, mean±SD. In order to compare the APs of *O. basilicum* extracts and the APs of *O. gratissimum* extracts with each other and between the APs of the reference molecules, the histogram below was used.

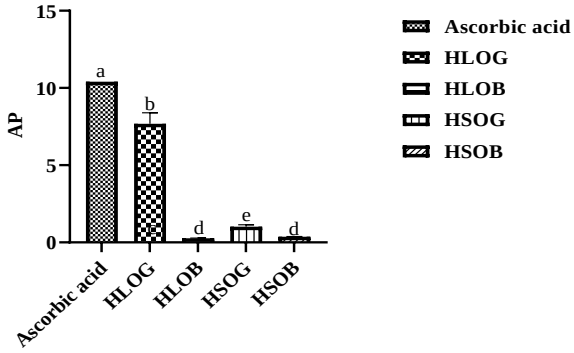

Fig. 2: **Anti-radical activity of ABTS, Legend: The bands with different letters are statistically different with a significance level *P<0.05;* n: 3, mean±SD. HLOG: hydroethanolic leaves extract of *O. gratissimum*; HLOB: Hydroethanolic leaves extract of *O. basilicum*, HSOG: Hydroethanolic stem extract of *O. gratissimum*; HSOB: Hydroethanolic stem extract of *O. basilicum***

It emerges from the analysis of this histogram that the hydroethanolic extracts of *O. basilicum* and *O. gratissimum* have statistically different anti-radical activities ranging from 7.681±0.716 to 1.014±0.127 for the extract of leaves and stems of *O. gratissimum* and 0.271±0.008 to 0.370±0.006 for *O. basilicum* leaf and stem extract; the extract of *O. gratissimum* presented a good anti radical activity statistically superior to that of the extract of *O. basilicum*. Similarly, the anti-free radical activity of the extracts tested is lower than that of the reference molecule in the occurrence of Ascorbic acid, the AP of which is 10.400±0.000; however, the AP of the leaf extract *O. gratissimum* shows an activity close to that of Ascorbic acid. Table 5 below summarizes the potency of the scavenging activity of the DPPH and ABTS tests.

Table 5: **Summary of anti-radical tests**

Samples	PA/DPPH	AP/ABTS
Hydroethanolic leave extracts of *O. basilicum*	7.366±0.817	0.271±0.008
Hydroethanolic stem extracts of *O. basilicum*	3.033±0.330	0.370±0.006
Hydroethanolic leave extracts of *O. gratissimum*	23.080±3.123	7.681±0.715
Hydroethanolic stem extracts of *O. gratissimum*	0.786±0.020	1.014±0.126
Ascorbic acid	40.000±0.000	10.400±0.000
Quercetin	25.820±2.660	/

Legend: AP: Antioxidant power; ne: 3, mean±SD.

Statistically, Ascorbic acid and Quercetin, which are reference molecules, have the highest anti-radical activities in each of the tests compared to hydroethanolic extracts of *O. basilicum* and *O. gratissimum* tested and an activity higher than that of the four extracts evaluated by each test. On the other hand, it is noted that the hydroethanolic extracts of the leaves *O. gratissimum* presented better antiradical activities in the two tests (DPPH and ABTS). In addition, it emerges from this statistical analysis that the test having the best anti-radical activity on the extracts tested is that of the DPPH test, with APs ranging from 23.08±3.123 to 0.786±0.020.

Reducing the capacity of *O. basilicum* and *O. gratissimum*

The summary reducing power results are summarized in the following table 6.

Table 6: Summary of the reduction capacity by the FRAP test

Substances tested	PR in mg EAA/g extract
Hydroethanolic leave extracts of *O. basilicum*	154.556±18.803
Hydroethanolic stem extracts of *O. basilicum*	147.66±15.322
Hydroethanolic leave extracts of *O. gratissimum*	304.156±17.579
Hydroethanolic stem extracts of *O. gratissimum*	280.783±56.463

Legend: PR: Reducing power, ne: 3, mean±SD. In order to compare the reducing powers (RP) between the extracts of *O. basilicum* and PRs of extracts of *O. gratissimum*, the histogram below was designed.

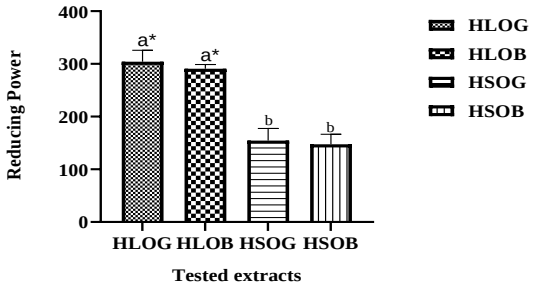

Fig. 3: **FRAP test reducing power, Legend: The bands with different letters are statistically different with a significance level (*P<0.05, n: 3, mean±SD). HLOG: hydroethanolic leaves extract of *O. gratissimum*; HLOB: Hydroethanolic leaves extract of *O. basilicum,* HSOG: Hydroethanolic stem extract of *O. gratissimum*; HSOB: Hydroethanolic stem extract of *O. basilicum***

In this test, we also notice that the hydroethanolic extracts of the leaves and stems of *O. gratissimum* have a higher and stronger metal-chelating activity than the hydroethanolic extracts of the leaves and stems of *O. basilicum*. Furthermore, the hydroethanolic extracts of the leaves and stems of *O. gratissimum* have a statistically identical PR; likewise, those of the extracts of *O. basilicum* are statistically identical to each other. *O. gratissimum* leaves have a reducing power of 304.156±17.579 mg EAA/g and have a statistically higher heavy metal chelating activity (p<0.05) than that of the leaves extract *O. basilicum* whose reducing power is 154.556±18.803 mg EAA/g and the hydroethanolic extract of the stems of *O. gratissimum* with a reducing power of 280.783±56.463 mg EAA/g extract, has a reducing activity statistically superior to that of the hydroethanolic extracts of the stems of *O. basilicum* with a reducing power of 147.660±15.322 mg EAA/g extract.

β-carotene bleaching test

The reducing powers were determined and summarized in the following table 7.

With the β-carotene bleaching test, the activated oxygen deactivating capacity is measured. It appears from the histogram that the hydroethanolic extracts of the leaves of *O. basilicum* are the most active. The hydroethanolic extract of the leaves of *O. basilicum* has a reducing power of 388.36±9.9618171 mg EAA/g extract, statistically higher than the reducing power of the hydroethanolic extract of the leaves of *O. gratissimum* whose reducing power is 351.557±23.6328175 mg EAA/g extract; similarly, the PR of the stems of *O. basilicum* 354.9833±5.3800888 mg EAA/g extract is statistically higher than that of *O. gratissimum* 323.7366±1.0492007 mg EAA/g extract which presented the lowest PR with a significant threshold P<0.05.

After evaluation of the reducing activity of the hydroethanolic extracts of *O. basilicum* and *O. gratissimum* through the FRAP and β-carotene test, by comparison with the results obtained, we can conclude that the test with the best-reducing power on the extracts tested is the β-carotene test with PR ranging from 388.36±9.9618171 mg EAA/g extract to 323.7366±1.0492007 mg EAA/g extract.

Table 7: Summary of reducing activity by β-carotene test

Substances tested	PR in mg EAA/g extract
Hydroethanolic extracts leave of *O. basilicum*	388.360±9.962
Hydroethanolic extracts stem of *O. basilicum*	354.983±5.380
Hydroethanolic extracts leave of *O. gratissimum*	351.557±23.633
Hydroethanolic extracts stem of *O. gratissimum*	323.736±1.049

Legend: PR: Reducing power, ne: 3, mean±SD. As for the FRAP test, the following histogram was used for comparative purposes of the reducing power of the plant extracts tested in this work

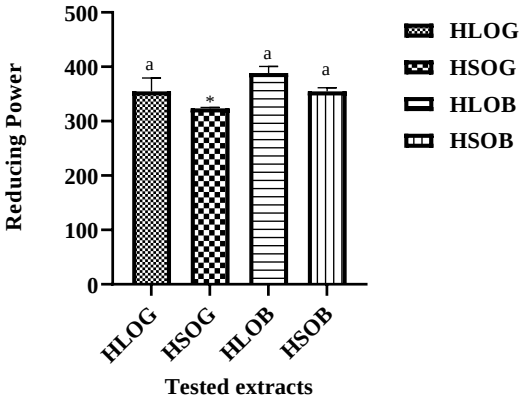

Fig. 4: β-carotene test reducing power, Legend: The bands with different letters are statistically different with a significance level *$P<0.05$, n: 3, mean±SD. HLOG: hydroethanolic leaves extract of *O. gratissimum*; HLOB: Hydroethanolic leaves extract of *O. basilicum,* HSOG: Hydroethanolic stem extract of *O. gratissimum*; HSOB: Hydroethanolic stem extract of *O. basilicum*

Table 8: Summary of the reducing power of extracts of *O. basilicum* and *O. gratissimum*

Substances tested	PR in mg EAA/g extract (FRAP)	PR in mg EAA/g extract (β-carotene)
Hydroethanolic leave extracts of *O. basilicum*	154.556±18.804	388.36±9.962
Hydroethanolic stem extracts of *O. basilicum*	147.660±15.322	354.983±5.380
Hydroethanolic leave extracts of *O. gratissimum*	304.156±17.579	351.557±23.633
Hydroethanolic stem extracts of *O. gratissimum*	280.783±56.463	323.737±1.049

Legend: PR: Reducing power, ne: 3, mean±SD.

Dosage of polyphenols and content of primary and secondary antioxidants

The evaluation of the antioxidant activity of extracts of *O. basilicum* and *O. gratissimum* ended with the determination of the polyphenol content with the spectrophotometric method using the Folin-Ciocalteu reagent. At the end of this assay, the contents of primary antioxidants or curative antioxidants and of secondary antioxidants or preventive antioxidants were determined and summarized in table 9 below.

Table 9: Summary of the polyphenol content and the primary and secondary antioxidant content in the extracts studied

Samples	Qphenols antioxidant mg EAA/g extract	Primary antioxidants mg EAA/g extract	Secondary antioxidants mg EAA/g extract
O. basilicum leaf extract	126.943±6.750	45.000±0.204	48.500±1.225
O. basilicum stem extract	56.646±3.894	38.666±0.117	44.416±0.624
O. gratissimum leaf extract	228.940±8.418	45.416±0.112	44.000±2.965
O. gratissimum stem extract	137.200±21.356	30.000±0.204	40.500±0.204

Legend: ne: 3, mean±SD.

It appears from this table that the extracts of *O. basilicum* and *O. gratissimum* have almost all similar contents of total polyphenols with the exception of stems of *O. basilicum* whose content is low (56.646±3.894 mg EAA/g extract). Similarly, these extracts have almost identical amounts of primary and secondary antioxidants with contents ranging from 48.500±1.225 mg EAA/g extract to 30.000±0.204 mg EAA/g extract. These extracts are rich in both primary and secondary antioxidants.

Anti-inflammatory activity

The IC_{50} was determined and summarized in table 10 below.

Table 10: Summary of ovalbumin denaturation inhibition

Substances tested	IC_{50} (mg/ml)
Hydroethanolic extracts leave of *O. basilicum*	0.358±0.013
Hydroethanolic extracts stem of *O. basilicum*	0.5967±0.030
Hydroethanolic extracts leave of *O. gratissimum*	0.269±0.008
Hydroethanolic extracts stem of *O. gratissimum*	0.539±0.047
Diclofenac sodium	0.071±0.004

Legend: IC_{50}: Inhibition concentration, n: 3, mean±SD. In order to compare the IC50 (mg/ml) of extracts of *O. basilicum* and the IC_{50} (mg/ml) of the extracts of *O. gratissimum* between them and between the IC_{50} (mg/ml) of the reference molecules, the histogram below has been drawn up.

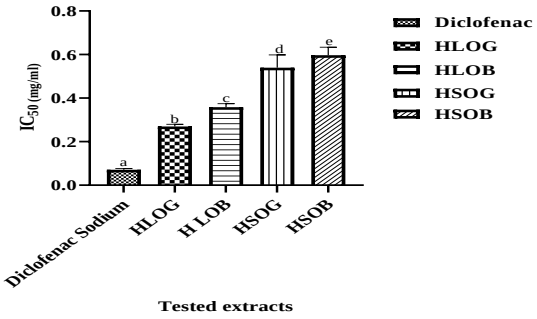

Fig. 5: Comparison of inhibitory concentrations of hydroethanolic extracts of *O. basilicum* and *O. gratissimum* and diclofenac sodium, Legend: The bands with different letters are statistically different with a significance level *P<0.05*, n: 3, mean±SD. HLOG: hydroethanolic leaves extract of *O. gratissimum*; HLOB: Hydroethanolic leaves extract of *O. basilicum,* HSOG: Hydroethanolic stem extract of *O. gratissimum*; HSOB: Hydroethanolic stem extract of *O. basilicum*

It appears from these results that the hydroethanolic extracts of the leaves of *O. basilicum* and *O. gratissimum* with an IC_{50} of 0.358±0.012 mg/ml to 0.269±0.008 mg/ml, respectively, possess a strong inhibitory activity of the denaturation of Ovalbumin, statistically superior to the activity of the stem extracts of the same plants whose IC_{50} values are 0.596±0.030 mg/ml to 0.539±0.0478 mg/ml. Note that the extracts have an activity lower than that of the reference molecule Diclofenac sodium with an IC_{50} of 0.071±0.004 mg/ml.

DISCUSSION

The yields obtained from the different extractions vary between 12% and 30.86% for the aqueous extracts and 21.63% and 34.36% for the hydroethanolic extracts. We note that those of the hydroethanolic extracts are higher than those of the aqueous extracts; this is because the alcohol-water system facilitates the extraction of the compounds.

The results obtained show us that the compounds extracted from the leaves and stems of our plants are much more polar; [23] showed that the alcohol-water mixture offers the advantage of modulating the polarity of alcoholic solvents and that solvent mixtures are ideal, selective for the extraction of a large number of bioactive compounds. The presence of highly polar compounds like tannins and steroids; and low polar ones such as flavonoids, phenols, and steroids confirm the polarity of the ethanol/water system on the extracts [24].

Kpètèhoto *et al.* [25]; and BAM *et al.* [26] have shown that these plants owe their therapeutic medicinal properties (anti-inflammatory, antibacterial, antiviral, antifungal, antiseptic, antimoral) to phenolic compounds, which are more concentrated in the leaves.

According to the determination of the bacterial growth inhibition parameters, namely the CMI, CMB and the CMI/CMB effects, the hydroethanolic extract of the leaves of *Ocimum basilicum* showed better activity on *Staphylococcus aureus* tested (CMI≤0, 39 mg/ml) and did not have too much effect on the other strains tested (3.12 mg/ml to 6.25 mg/ml) likewise the hydroethanolic extract of the leaves of *Ocimum gratissimum* was less effective on the germs tested and in particular on Gram-negative isolates; Olga *et al.* [27] observed that Gram-negative bacteria were more resistant to the extracts studied compared to Gram-positive strains; this could be explained by the chemical composition, the place of the harvest of the plants, the nature of the solvent and also that the species tested in this study are mainly clinical isolates.

The antioxidant potential of hydroethanolic extracts of *O. basilicum* and *O. gratissimum* reveal an amount of total antioxidants in each extract; the hydroethanolic extract of *O. gratissimum* as a whole presents a more significant amount (228.94±8.418 mg EAA/g extract) compared to the *O. basilicum* extract (126.94±6.75 mg EAA/g extract). The preponderance of primary and secondary antioxidants in the two extracts studied in almost equivalent quantity refers to a curative and preventive role in the treatment of diseases caused by oxidative stress and would justify the use of these plants in the curative and preventive treatment of a vaginal pathology that can degenerate into cancer. The antiradical (AP) power of Ascorbic acid proved to be more effective than in the DPPH and ABTS tests compared to those of the extracts tested, moreover, the DPPH test proved to have the strongest activity. Antiradical on the two extracts with PA ranging from 0.786±0.018 to 23.08±3.123. This antioxidant activity could be justified by the abundant presence of biomolecules contained in the extracts like those of the flavonoid family [28].

Furthermore, the greatest reducing power was obtained statistically with the data of the β-carotene test with PRs ranging from 323.736±1.049 mg EAA/g extract to 388.36±9.96 mg EAA/g extract. Thus, according to [29], biflavonoids have many aromatic groups rich in electrons, as well as unsaturated carboxyl groups of β and α nature which increase their antioxidant capacity because they can easily chelate heavy metals or deactivate active oxygen by transfer. of an electron and rapid isomerization in order to stabilize the structure of the radical formed.

In the ovalbumin denaturation inhibition test, the hydroethanolic extracts of the leaves of *O. basilicum* and *O. gratissimum* with an IC_{50} of 0.358±0.0129 mg/ml to 0.269±0.008 mg/ml respectively, have a moderate activity, significantly lower than that of the reference molecule Diclofenac sodium whose IC_{50} of 0.071±0.004 mg/ml. Thus, we can affirm that the extracts tested in this study, and in particular the leaves are able to control the production of auto-antigens at the origin of inflammations that accentuate the pain in aerobic vaginitis and these results agree with those from Neamati *et al.* [30]. Harish *et*

al. [31] showed that the anti-inflammatory activity of the hydro-ethanoic extract would be due to the flavonoids and tannins contained in the plant extracts. Indeed, the hydroethanolic extracts of *O. basilicum* and *O. gratissimum* are found to be rich in phenolic and flavonoid compounds capable of preventing the formation of prostaglandins that cause inflammation.

CONCLUSION

This study shows evidence of the antibacterial, antioxidant, and anti-inflammatory activities of aqueous and hydroethanolic extracts of *Ocimum basilicum* and *Ocimum gratissimum* on some germs responsible for aerobic vaginitis and corroborates claims of the use of these traditional medicinal plants in the management of vaginitis diseases in Cameroon. However, studies including the mechanism of action of these plant extracts are necessary to better understand the result and approve their efficacity. Also, the toxicity of those medicinal plants must be a plan for further research activities to be sure of their safety.

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AUTHORS CONTRIBUTIONS

Djova S. Valdi, Dongmo Z. Arlette, and Kengne G. Landry performed the experiments; Ndjib rosette and Ngongang T. Dimitri designed and edited the document; Nyegue M. Ascension and Ndoye F. Florentine supervised and contributed to the study. All authors have read and approved the final manuscript of this article.

CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest regarding this article.

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