Original Research Article

Ethnobotanical survey and some biological activities of *Ageratum conyzoides* collected in Southern-Benin

ABSTRACT

Aims: *Ageratum conyzoides L.* is a small annual herbaceous plan and highly odorous, and invasive of the *Asteraceae* family with widespread use in traditional medicine. The aims of this study a to evaluate *in vitro* antioxidant potential, toxicity and antimicrobial activity of aerial part extracts of *A. conyzoides* on strains responsible for vaginal infections.

Methodology: An ethnobotanical survey has been carried out on *A. conyzoides* among ethnobotanists and traditional therapists in fifteen markets shared between Abomey-Calavi, Cotonou, Zogbodomey, Bohicon and Abomey. The phytochemical screening was a qualitative analysis based on staining and precipitation reactions. Antimicrobial activity is evaluated on reference and clinical strains of *Staphylococcus aureus, Candida albicans and Escherichia coli* by the method of micro dilutions in wells from aqueous and ethanolic extracts of the leafy stem of *A. conyzoides*. The toxicity test was performed with two extracts on *Artemia salina* larvae, FRAP method was used to evaluate the antiradical activity.

Results: The survey showed that the population of Southern-Benin uses *A. conyzoides* according to different modes of preparation. Also, the administration in the treatment of a variety pathologies affects the female reproductive system. The phytochemical screening revealed the presence of flavonoids, tannins, anthocyanins, triterpenes and C-heterosides. The yield of the two extracts used is 6.18% for the aqueous extract and 4.32% for the ethanolic extract. The highest inhibition diameter (24.05 ± 0.5 mm) is obtained through the aqueous extract against the clinical strain of *S. aureus*. In contrast, the lowest inhibition diameter (10±0 mm) is obtained against the reference strain *S. aureus* ATCC29213 with the same extract. The Minimum Inhibitory Concentration varied from 2.5 to 5 mg/ml for the different extracts tested. Both extracts show a bactericidal and fungicidal effect on the different strains studied but the sensitivity of the strains to the aqueous extract is better considered compared to the ethanolic extract. So, the aqueous extracts of the plant showed higher antioxidant power compared to the ethanolic extract. No toxicity is revealed for both extracts.

Conclusion: The results obtained show that the aqueous and ethanolic extracts of the aerial part of *A. conyzoides* have antioxidant and antimicrobial properties on strains responsible for vaginal infections and do not present a toxicity.

Keywords*: Ageratum conyzoides, antioxidant potential, antimicrobial activity, toxicity, Staphylococcus aureus, Candida albicans, Escherichia coli*

1. INTRODUCTION

Infectious diseases result from the interaction between an infectious agent, its host and environmental factors [1]. They cause numerous deaths per year worldwide in general and in developing countries in particular [2]. Among these diseases are genital infections, which are not only highly endemic in the African region [3], but more importantly, have serious consequences such as infertility, ectopic pregnancy, miscarriage, and increased risk of human immunodeficiency virus transmission. Depending on the location (vulva, vagina, and cervix) of the germ involved, they include low infections and high infections localized to the tubes and ovaries [4]. Infectious diseases represent a global health problem in women of reproductive age and present in various forms (bacterial vaginosis, aerobic vaginosis, vulvovaginal candidiasis, and trichomoniasis). Almost all of these women are affected by a vaginal infection, sometimes recurrently, characterized by painful or embarrassing physical symptoms that can affect their life quality and self-esteem [5-6]. They are extremely prevalent [7], and are among the most common reasons for gynecological consultations in Benin. To address genital infections, modern antimicrobials are commonly used. Incompetent diagnosis, inappropriate treatment, resistance to antimicrobial molecules, inaccessibility to modern care, high cost of drugs, and the manifestation of severe and in some cases toxic side effects are the main causes of unsatisfactory results of conventional treatment of these infections [8-10]. To face this problem, there is an urgent need for research to discover other active ingredients that can effectively treat genital infections. Thus, medicinal plants usually used in traditional medicine could constitute an alternative source of new molecules with antimicrobial activity that are economically accessible [11-12] to populations with relatively very low-income levels. Considering that traditional practitioners hold an impressive number of plant-based recipes that can be used as a basis for screening, it makes sense to continue or even intensify research in this direction [13].

Indeed, plants are potential natural remedies that can be used in curative and preventive treatment [14], despite the advances in modern medicine. Thus, according to WHO estimates, 80% of the world's population in developing countries and 70% of Benin's population use traditional medicine in the treatment of various ailments [15]. In Benin in particular, medicinal plants remain the source of most of the extracts used in the composition of pharmacopoeia products. Various medicinal plants including are used for their biological properties in the treatment of many infectious pathologies [16]. Among these medicinal plants we can mention *Ageratum conyzoides* occupies, used in many purposes such as treatment of several infections (genital, urinary, skin, oral, viral and eye) [17]. It is also known for its anti-inflammatory, antispasmodic, hypoglycemic, analgesic, anti-diarrheal, diuretic, antitussive, antirheumatic properties [18].

Several studies reported the use and potential antimicrobial activity of medicinal plants [17, 19-21] including *A. conyzoides* [22], it should be noted however that studies related to the antimicrobial activity of *A. conyzoides* in the specific treatment of vaginal infections are almost inexistent. Thus, despite its medicinal use, the toxicity profile of the *A. conyzoides* plant remains to be explored and requires further studies. Thus, in order to strengthen the scientific knowledge on the medicinal usefulness of *A. conyzoides* and to contribute to its valorization, this study focused on the evaluation of the antioxidant potential, toxicity and

antimicrobial activity of aerial part extracts of this plant on the growth of strains, *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*.

2. MATERIAL AND METHODS

2.1. Ethnobotanical survey on the medicinal use of *A. conyzoides*

To investigate on the use of *A. conyzoides*, an ethnobotanical survey was conducted among herbalists, traditional practitioners, phytotherapists and all persons with endogenous knowledge in 5 municipalities of Southern-Benin (Bohicon, Abomey, Zogbodomey, Abomey-Calavi and Cotonou). The survey was conducted in the markets of Abomey-Calavi (Godomey, Agontikon, Ouédonou, Ouèdo, Togba, Calavi-Tokpa and Djadjo), Cotonou (Vèdoko, Gbégamey, Dantokpa and Sèdégbé); Zogbodomey (Massi and Zogbodomey), Bohicon and Abomey. During the survey, questions were asked through an individual interview using a survey form. These questions relate to information on the age, gender, level of education, professional experience and ethnicity of the respondents on the one hand; and on the vernacular name, selling price, different parts used, different pathologies treated, modes of preparation, modes of administration, contraindications and dosage of the plant on the other hand [23]. A total of 153 people were surveyed for this study.

2.2. Plant material samples collection

After harvesting the plant was certified on April 11, 2022 at the National Herbarium of Benin (University of Abomey-Calavi) under the number YH696/HNB. Once identified, aerial part was washed and then dried at laboratory temperature (25 ± 2°C) for two weeks. The dried sample was ground using a Retsch mechanical grinder type SM 2000/1430/Upm/Smf. The powder thus obtained were weighed and stored (protected from light) until their use for phytochemical screening and different extractions.

2.3. Extractions of the plant powder

Two types of extracts (aqueous and ethanolic) were performed. The choice of these types of extracts is based on the way the plant is traditionally used.

2.3.1. Aqueous extract

Maceration was used to get aqueous extract. Thus, 50 g of previously obtained powder was dissolved in 500 mL of distilled water and left under continuous stirring for 72 hours. The homogenate obtained was filtered successively twice on absorbent cotton and once on Whatman 1 paper. The filtrate was dried at 50°C and the powder obtained constituted the total aqueous extract.

2.3.2. Ethanolic extract

For the ethanolic extract, 50 g of powder was macerated under continuous stirring for 72 hours in 500 ml of 70° ethanol. The mixture was then filtered to remove solids; this filtrate was filtered again using a Whatman 1 paper to obtain a solids-free solution. In order to remove ethanol, the solution was concentrated in a rotary evaporator at 50°C and stored at 2-4°C to be used for further bioassays.

2.3.3. Yield

The extraction yield is defined as the ratio of the mass of dry extract obtained to the mass of plant material processed (Harborne, 1998). It was obtained according to the following formula: R (%) = (Me/Mv) × 100 with R (%): yield in %, Me: mass of dry extract, Mv: Mass of plant material used.

2.4. Phytochemical screening

The presence of metabolites was investigated directly on the powder of the plant. It is a qualitative analysis based on differential staining and precipitation reactions of the main groups of chemical compounds contained in the plant as described by Houghton and Raman [24] and used successfully by Chabi-Sika et al. [16] under laboratory conditions.

2.5. Evaluation of the antimicrobial activity

The antimicrobial activity was performed in three steps: susceptibility test, determination of the Minimum Inhibitory Concentration (MIC) and determination of the Minimum Bactericidal Concentration (MBC) and Fungal Concentration (FMC). Six microorganisms including three reference strains (*Staphylococcus aureus* ATCC29213, *Candida albicans* MHMR, *Escherichia coli* ATCC 25922) and three (03) strains isolated from vaginal swabs by Sina et al. [25] were used in this work for the antimicrobial activity.

2.5.1. Sensitivity test

The susceptibility of the microorganisms to the two extracts was performed using the Mueller Hinton (MH) solid media diffusion method, as previously described [16]. The sterile discs (Ø=6mm) containing 30 µl of each extract were deposited, under aseptic conditions, on previously inoculated microbial culture dishes. For each extract, the experiment is duplicated and a negative control is performed with distilled water. The plates are then left for 30 min at room temperature before being incubated at 37°C in for 24 h and then 48 h. The inhibition diameters are measured after incubation times.

2.5.2. Determination of the Minimum Inhibitory Concentration (MIC)

The lowest concentration for which no growth is visible (MIC) was determined following the microdilution method using iodonitrotetrazolium (INT) as an indicator of bacterial viability [26]. Briefly, a range of nine concentrations (10 to 0.039 mg/ml) of the extracts was tested on the microbial strains. Indeed, 150 µl of distilled water was distributed in all wells (wells 1 to 9) of the plate and 150 µl of each extract (20 mg/ml) was added into the first wells. Successive dilutions of ½ ratio were then made from well 1 to well 9 and 150 µl of the last well was discarded. To end, 150 µl of bacterial inoculum (10^6 CFU/ml) was added to all wells. The microplate was covered with the parafilm paper and incubated at 37°C for 18h. After incubation, 10 µl of para-INT violet solution (INT, 0.2 mg/ml) was added to all wells. Plates were re-incubated at 37°C for 30 min and the MIC is represented by the first well in which there is no appearance of red/pink staining.

2.5.3. Determination of the Minimum Bactericidal Concentration (MBC)

The lowest concentration at which 99.99% of germs are inhibited (MBC) was determined on the basis of the results of the determined MIC. Thus, after identifying the MIC, the content of wells with concentrations ≥ MIC were sought on petri dishes containing MH agar medium. The plates were examined after 24 h of incubation at 37°C. The antibacterial effect [27] will be considered as bactericidal (CMB/CMI ≤ 4) or bacteriostatic fungistatic (BMC/MIC≥ 4).

2.6. Larval toxicity evaluation

The test is performed according to the method described by Kawsar et al. [28] and successfully used recently by Chabi-Sika et al. [16]. Thus, larvae used are obtained by hatching 10 mg of *Artemia salina* eggs placed under continuous agitation in 1L of seawater for 72 hours. A stock solution of 20 mg/ml per extract was prepared by adding DMSO. From extracts, a ½ ratio serial dilutions were made. To 1ml of each dilution, was added 1ml of seawater containing 16 larvae. After 24h of incubation, the count of dead, moribund and live larvae was performed for the determination of LC_{50}. If deaths were recorded among the control, the data were corrected by Abbott's formula: %death = [(test-control)/control)] x 100.

2.7. Antioxidant activity evaluation

The reducing power of the extracts was determined by the Ferric Reducing Antioxidant Power (FRAP) method according to the protocol described by Dieng et al. [29]. Briefly, using a bach of 8 tubes (numbered from 1-8), 0.5ml of 25% DMSO were distributed in tubes 2 to 7 and then 0.5ml of the extract (5mg/ml) were introduced in tubes 1 and 2. A series of successive ½ dilution from tube 2 into all other tubes was then performed. In addition, 0.5 ml of sample at different concentrations was mixed with 1ml of phosphate buffer (0.2M; pH=6.6) and 1ml of 1% potassium hexacyanoferrate [K3Fe(CN)6]. After incubating the mixture at 50°C for 30 minutes, 1ml of 10% trichloroacetic acid was added to stop the reaction, then the tubes were centrifuged at 3000 rpm for 10 minutes. Then, 1ml of the supernatant from each tube was mixed with 0.2ml of 0.1% FeCl3 solution and allowed to stand in the dark for 30 minutes before measuring the optical densities (OD) at 700 nm. The antioxidant activity related to the reducing power of the extracts is expressed as Reducing Power (RP) using the following formula: $\mathrm{RP} = [\mathrm{OD}\ (extract) - DO\ (blanc)\ /\mathrm{OD}\ (extract)] \times 100$

2.8. Data Analysis

Collected data were encoded using Excel 2013 Spreadsheet. Data analyses were done using GraphPad Prism 8 software. For each extract, the lethal concentration that causes 50% larval death (LC_{50}) was calculate with a 95% confidence interval by linear regression analysis and also using the Probit analysis method following. A regression line equation, obtained from the larval mortality curve, is used to calculate the concentration (LC_{50}) corresponding to the death of half the larvae. The degree of leaf toxicity was evaluated based on the correspondence table established by Mousseux [30].

3. RESULTS AND DISCUSSION

3.1. Results Subheading

3.1.1 Ethnobotanical survey on the medicinal use of *A. conyzoides*

During this survey, it was revealed that *A. conyzoides* is well known by the population who give it different names in the different ethnics of southern-Benin (table 1).

Table 1. Different names in function the ethnics in southern-Benin

Ethnics	Names in the different ethnics
Goun	Awovitakin, Kouvito-takin and Soungnonu
Fon	Awovitakinman, Gnor-sounouman and Kouvito-takin
Xwla	Zounxosou, Azétorxontin and Togbé
Mahi	Assoukousi-xwawé

3.1.2. Socio-demographic parameters of respondents

Table 2 presents the socio-demographic results of respondents. The table shows that the extremes of age are 20 years and 80 years with an average age of 44.22 ± 7.11 years. The majority of respondents had a primary (45.10%) or secondary (31.37%) education; some of them had no schooling (22.22%) or rarely had a university education. In addition, 93.46% of the respondents are of Fon ethnicity followed by Goun (3.92%), Xwla (1.96%) and Mahi (0.65%) ethnicity. The professional experience of the respondents varies between 5 and 35 years, with 54.54% having professional experience ranging from 15 to 30 years, followed by those with experience ranging from 5 to 11 years (33.33%) and finally 12.12% with professional experience exceeding 30 years.

Table 2. Socio-demographic parameters of respondents

Sociodemographic Parameters	Workforce (N)	Proportions (%)

Sex		
Male	32	20,92
Female	121	79,08
Age (Year)		
[20;40[	45	29,41
[40;60[	99	64,71
[60;80[	9	5,88
Professional experience (Year)		
[5; 10[	19	12,42
[10; 15[	42	27,45
[15; 20[	46	30,07
[20; 25[	30	19,61
[25; 30[	8	5,23
[30; 35[	8	5,23
Education level		
Primary	69	45,10
Secondary	48	31,37
Superior	2	1,31
Unschooled	34	22,22
Ethnic group		
Fon	143	93,46
Goun	6	3,92
Xwla	3	1,96
Mahi	1	0,65

3.1.3. Uses of the different parts of *Ageratum conyzoides* in Southern-Benin

Figure 1 below presents the results of the medicinal and pharmacological use of the various parts of *A. conyzoides* in Southern-Benin. It should be noted that the population uses this plant in the treatment of various pathologies. They mainly use the leaves (87.58%) and the stem (53.59%), sometimes the whole plant (14.38%), few uses roots (1.96%) and very rarely the flower (0.65%) of *A. conyzoides* (figure 1).

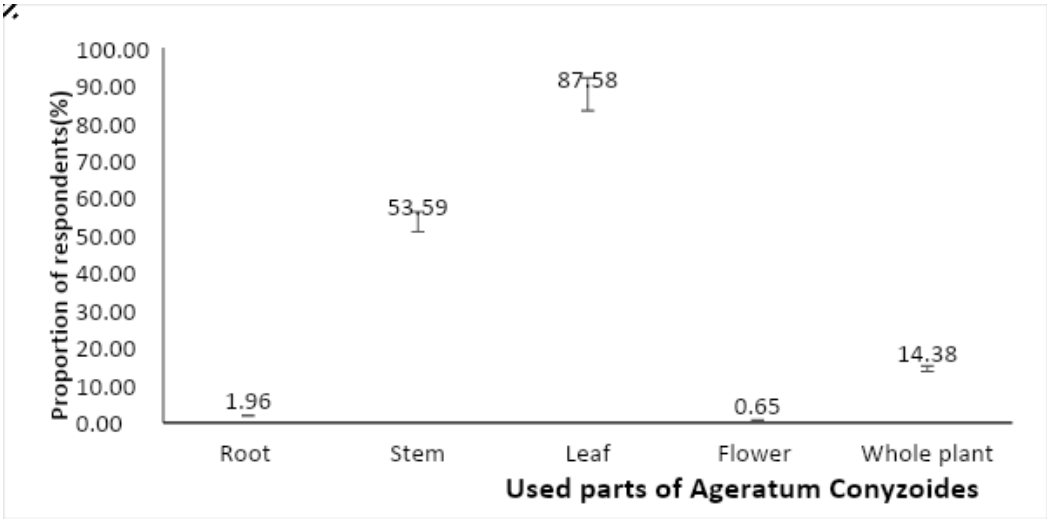

Figure 1: Proportions of respondents according to the different parts of *A. conyzoides*.

In addition, it appears that the studied population uses this species essentially in the treatment of vaginal infections (32.08%), stomach aches (18.30%), skin infections (14.38%),

female sterility (5.88%), pathologies affecting the female genital tract [painful periods (10.46%), blocked trunk (5.88%), cysts (1.96%)], wounds (7.84%), intestinal worms (7.19%), pain (5.23%), urinary tract infections (3.92%), diarrheal diseases (2.61%) and malaria (1.96%) (figure 2).

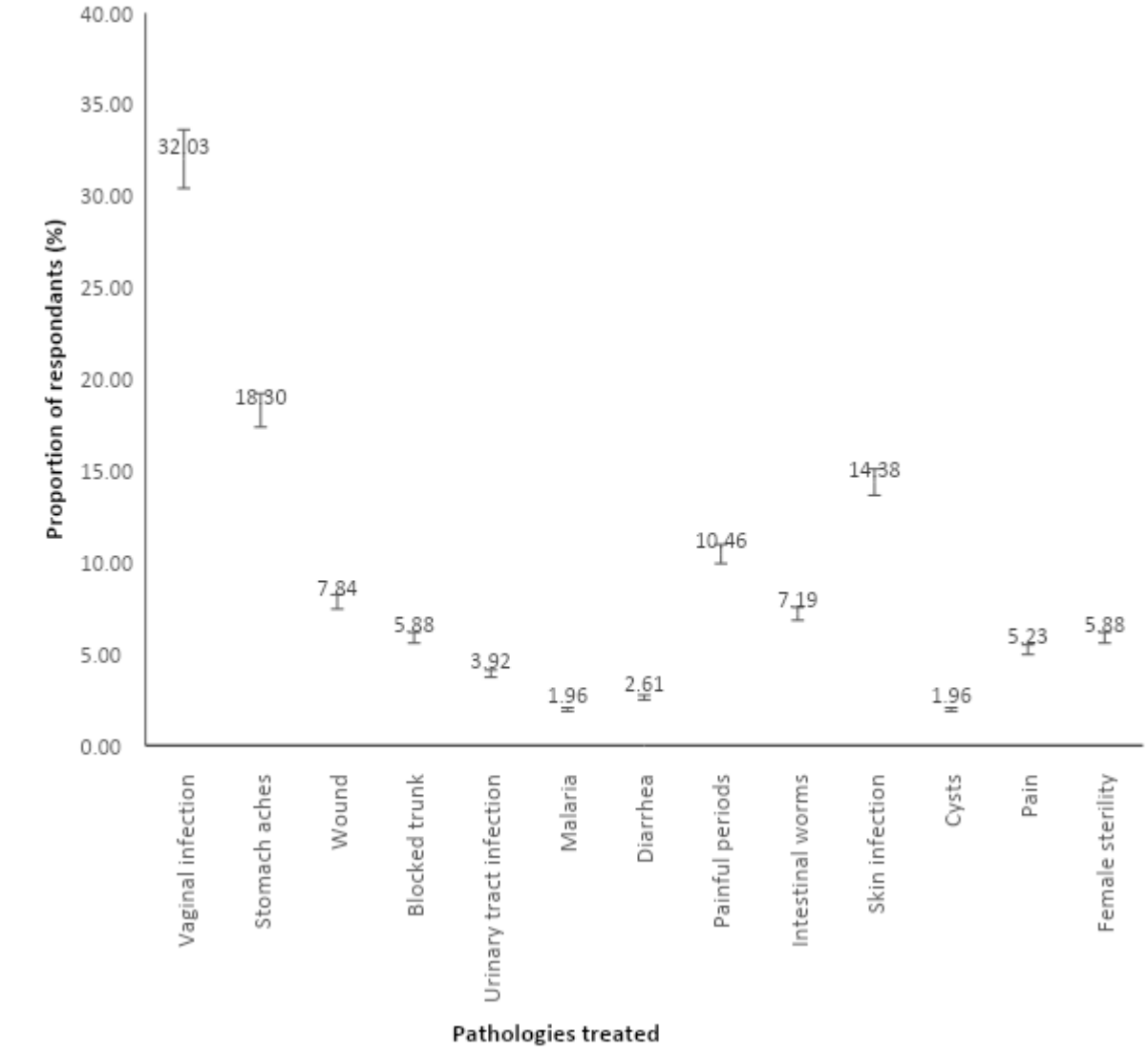

Figure 2. Distribution of the different pathologies reported to treated by *A. conyzoides*

To treat the various pathologies, the population uses this species alone or in combination in various forms, namely decoction (72.55%), trituration (16.99%), maceration (14.38%), infusion (9.8%) or carbonization (0.65%) (figure 3).

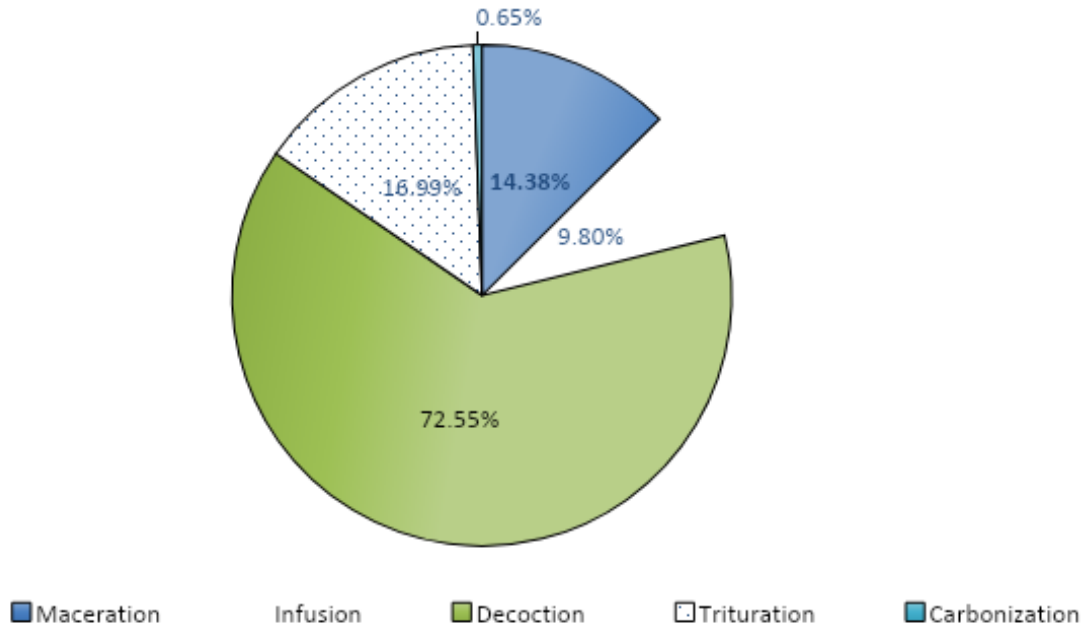

Figure 3. Method of preparation of *A. conyzoides*

Moreover, depending on the pathology to be treated and the method of preparation, the species can be administered orally (62.75%), dermally (36.60%), vaginally (14%) or rarely by the laryngeal route (0.65%) as shown in Figure 4.

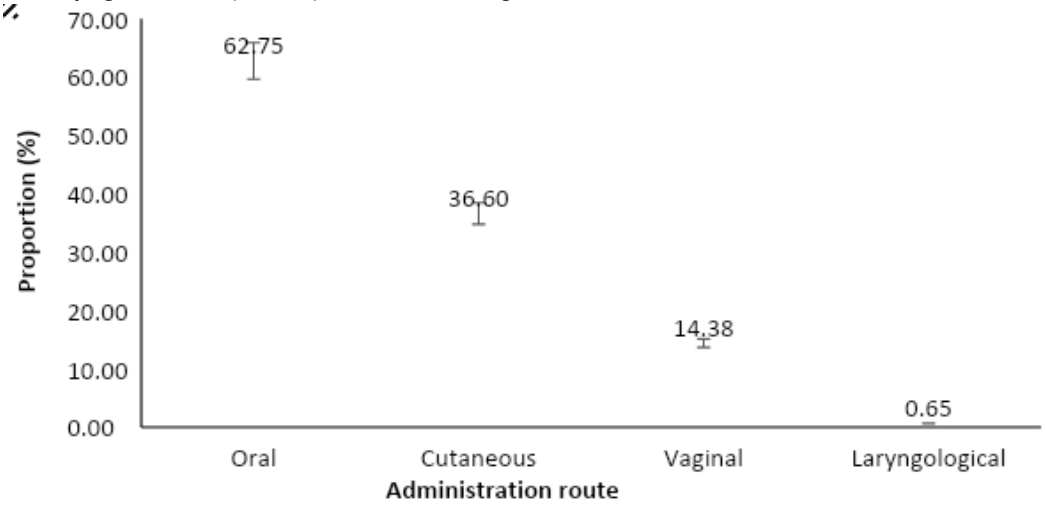

Figure 4. Proportion of the administration routes of *A. conyzoides* based preparations

The duration of treatment shown in Figure 5 is generally indefinite (use until satisfaction) but varies according to the pathology. It ranges from 3 to 5 days in the treatment of stomach aches and pain, from 7 to 15 days in the treatment of infections, from 1 to 3 months in the treatment of infertility. However, the therapeutic use of this plant is limited in pregnant women. Also, its use is accompanied by some restrictions, namely: the intake of alcohol

during the treatment, the combination with other drugs or pharmaceutical products during the treatment, the consumption of sticky sauces and the excessive consumption of red oil during its use.

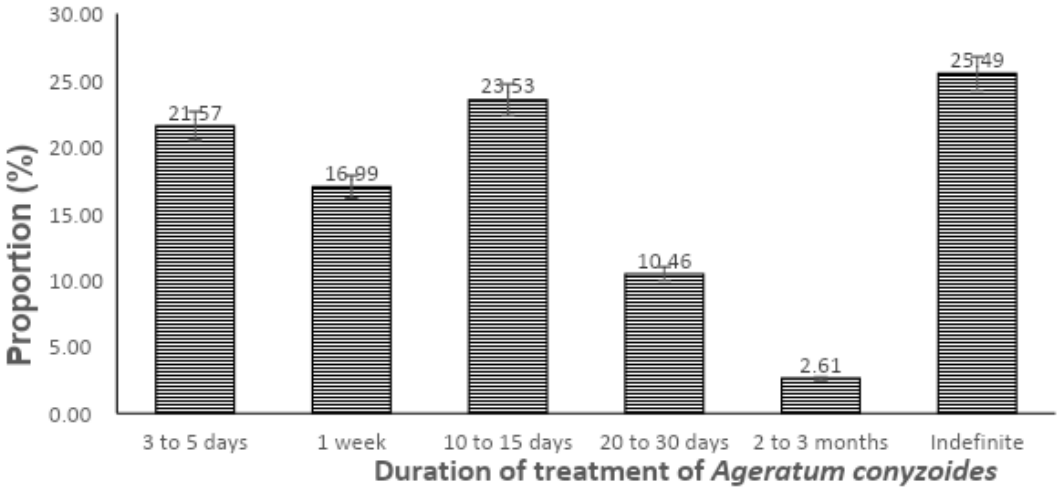

Figure 5. Compilation of the *A. conyzoides* based preparations treatment duration

3.1.4 Phytochemical screening of *Ageratum conyzoides*

The analysis of this table 3 shows that *A. conyzoides* contains a several secondary metabolites such as: flavonoids, catechic tannins, gall tannins, anthocyanins, triterpenes and C-heterosides. However, leuco-anthocyanins, alkaloids, reducing compounds, mucilages, saponosides, steroids, coumarins, quinone derivatives, free anthracenics, O-heterosides, O-heterosides with reduced genines are absent. The plant does not contain cardiotonic and cyanogenic derivatives either.

Table 3. Results of phytochemical screening of the leafy stem of *A. conyzoides*.

GROUPS OF METABOLITES	**PRESENCE**
Catechic tannins	+
Gallic tannins	+
Flavonoids	+
Leuko-anthocyanins	-
Anthocyanin	+
Alkaloids	-
Reducing compounds	-
mucilage	-
Saponosides	-
Cyanogenic derivatives	-
Triterpenes	+
Steroids	-
Coumarins	-
Quinone derivatives	-
Free anthracenes	-
C-glycosides	+

O-heterosides	-
O-heterosides with reduced genins	-
Cardiotonic derivatives	-

3.1.5 Yield of the extracts

Figure 6 shows the yield of the two extracts: aqueous and ethanolic. The analysis of this figure shows that the extraction yield of the aqueous extract (6.18%) is higher than that of the ethanolic extract (4.32%).

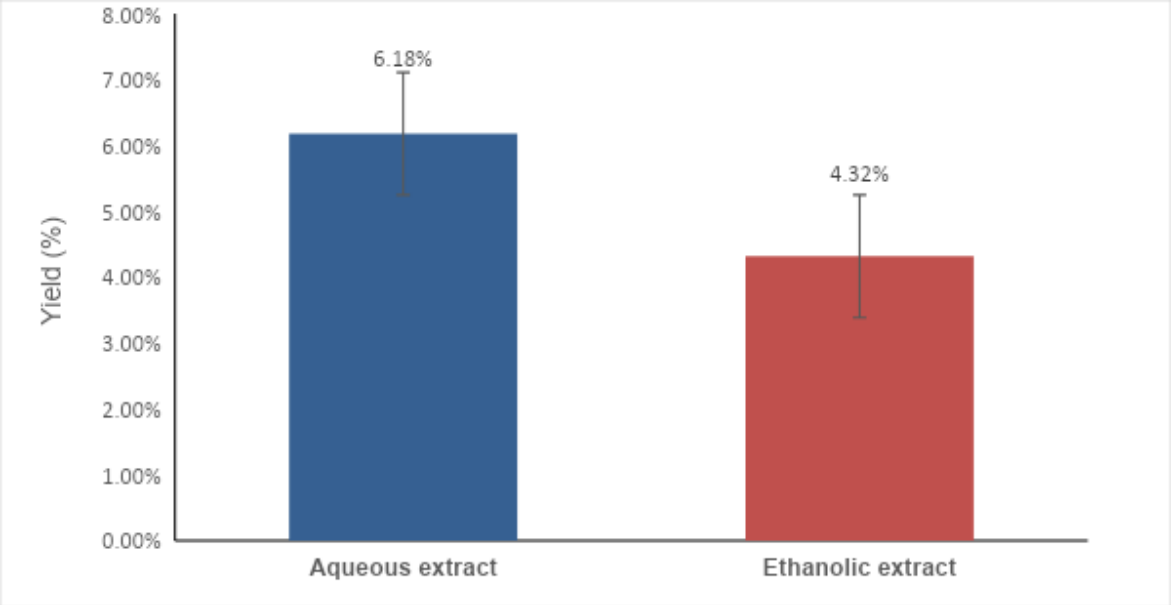

Figure 6. Yield of *A. conyzoides* extracts

3.1.6 Antimicrobial activity of *A. conyzoides* extracts

3.1.6.1. Sensitivity test

The results of the inhibition tests reveal that these strains are very sensitive to the different extracts tested (Table 4). However, *Candida albicans* was more sensitive to the ethanolic extract (with an inhibition diameter of 18±0.5 mm and 16±0 mm respectively for *C. albicans* MHMR and clinical *C. albicans*) than to the aqueous extract (against an inhibition diameter of 12±0 mm and 12±0.5 respectively for *C. albicans* MHMR and clinical *C. albicans*).

The *Staphylococcus aureus* strain was found to be the most sensitive to the aqueous extract. However, clinical *S. aureus* was more sensitive (inhibition diameter 24.05±0.5 mm) than the reference strain *S. aureus* ATCC29213 (10±0 mm) with the same extract. Similarly, the reference strain *Escherichia coli* ATCC 25922 was more sensitive than the clinical strain with the aqueous extract respectively with an inhibition diameter of 14.5±0.5 mm and 10±0.5 mm.

Table 4. Inhibitory activity of the aqueous and ethanolic extracts of the aerial part of *A. conyzoides* towards the reference and clinical strains tested.

Strains Tested	**Inhibition diameter (mm)**

	Aqueous extract		Ethanolic extract	
	24 h	**48 h**	**24 h**	**48 h**
S. aureus ATCC29213	10.5±0,5	10±0	15±0,5	10±1
C. albicans MHMR	11±1	12±0	18±0,5	17.5± 0,5
E. coli ATCC 25922	14.5±0,5	-	13±0,5	11.5±0,5
Clinical isolated S. aureus	24.5±0,5	10±1	10±0	11±1
Clinical isolated C. albicans	12±0,5	10.5±0,5	15±0,5	16±0
Clinical isolated E. coli	10.5±0,5	10±0,5	-	-

The clinical strains tested showed variable sensitivity in the presence of *A. conyzoides* extracts (Figure 7). Over a period of 24h and 48h, the strains show a higher sensitivity (75% of the tested strains) to the aqueous extract of the plant as opposed to the ethanolic extract (62.5%).

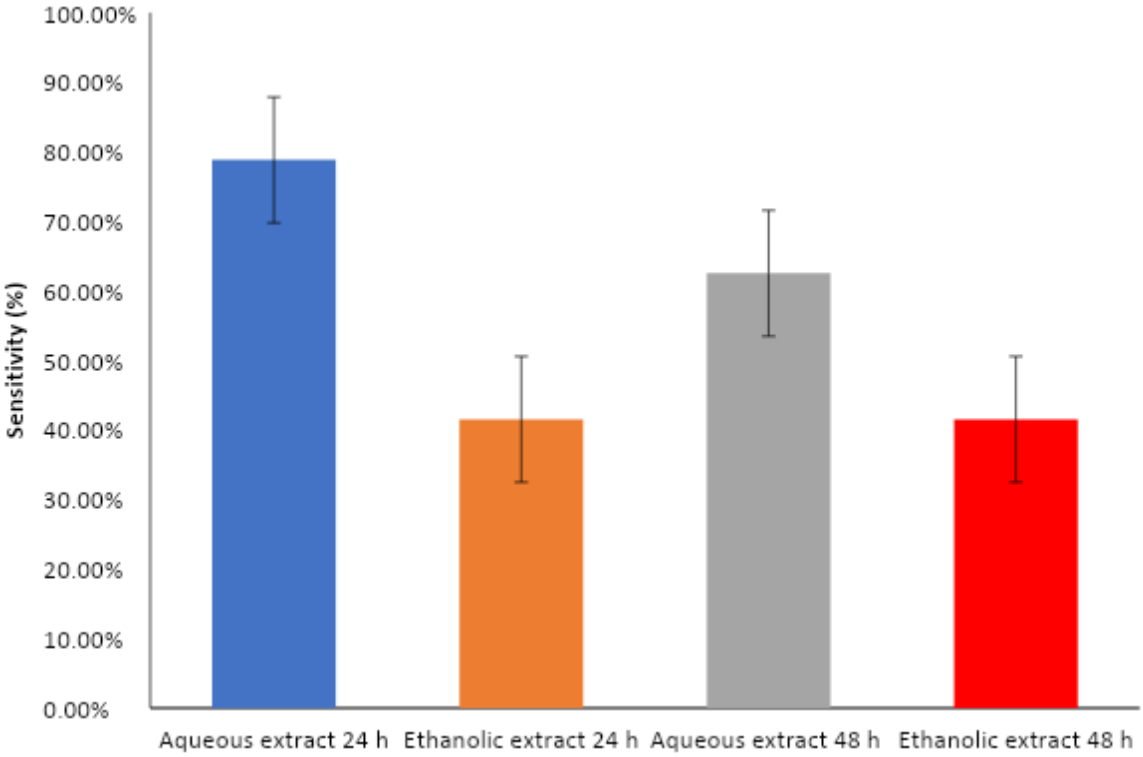

Figure 7: Sensitivity rate of clinical strains to extracts.

3.1.6.2. Minimum Inhibitory Concentration and Minimum Bactericidal or Fungal Concentration

Table 5 presents the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal or Fungal Concentration (MBC or MFC) of the *A. conyzoides* extracts on the strains studied. As a result, the minimum inhibitory concentrations of the extracts vary between 2.5 and 5 mg/ml. The MICs of the ethanolic extract of the reference strains of *S. aureus* and *E. coli* are 2.5 mg/ml and 5 mg/ml respectively. In contrast to the reference strains, the lowest MICs

were observed with the aqueous extract with MICs of 3.75±1.25 mg/ml; 2.5 mg/ml and 2.5 mg/ml for the clinical strains of *S. aureus*, *C. albicans* and *E. coli* respectively.
The BMC or FMC of both extracts on the different strains is 10 mg/ml. Both extracts are bactericidal and fungicidal respectively on the clinical strains of *S. aureus* and *C. albicans* but show no effect on the clinical strain of *E. coli*. Moreover, we notice that on the reference strain of *C. albicans*, both extracts present a quasi-stable activity with the same GPC/MIC ratio (2mg/ml). They are therefore fungicidal on the reference strain of *C. albicans*.

Table 5. Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of the two extracts of *A. conyzoides* on the strains studied.

Strains	CMI et CMB (mg/ml) of*Ageratum conyzoides* sur les souches étudiées							
	Aqueous Extract				Ethanolic extract			
	CMI	CMB CMF	CMB/CMI CMF/CMI	Effects	CMI	CMB CMF	CMB/CMI CMF/CMI	Effects
S. aureus ATCC29213	5	10	2	Bactericidal	2.5	10	4	Bactericidal
C. albicans MHMR	5	10	2	Fungicide	5	10	2	Fungicide
E. coli ATCC 25922	5	-	-	-	5	10	2	Bactericidal
Clinical S. aureus	3.75±1.25	10±0	2.67	Bactericidal	5	10	2	Bactericidal
Clinical C. albicans	2.5	10	4	Fungicide	5	10	2	Fungicide
Clinical E. coli	2.5	-	-	-	-	-	-	-

3.1.6 Antioxidant activity

The results of antioxidant activity of the aqueous and ethanolic extracts of *A. conyzoides* and Beta-Hydroxy Acid (BHA) are presented in figure 8. This figure reveals that the aqueous and ethanolic extracts of the *A. conyzoides* plant sample show antioxidant activity with respective inhibitory half-concentrations (IC50) of 0.16 mg/ml and 0.41 mg/ml while the IC50 of the control (BHA) is 0.23mg/ml. It should also be noted that the aqueous extract of the plant has a stronger antioxidant power than the ethanolic extract.

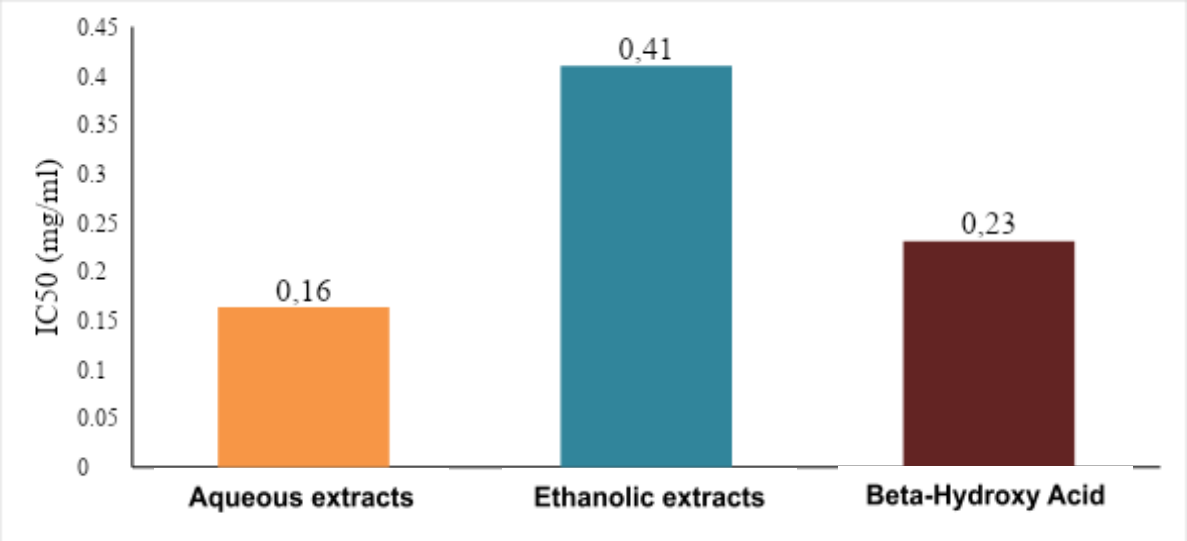

Figure 8: IC50 of aqueous and ethanolic extracts of *A. conyzoides*

3.1.7 Larval toxicity

Figure 9 shows the results of the toxicity tests of the aqueous and ethanolic extracts of the aerial part of *A. conyzoides* on the larvae of *Artemia salina*. The results showed variability in the lethality rate on *Artemia salina* larvae. The lethal LC_{50} concentrations were determined using the linear regression curve equations for each extract. The highest LC_{50} was obtained with the ethanolic extract (4.84 mg/ml) and the lowest with the aqueous extract (4.28 mg/ml). It is found that for all the obtained graphs the correlation coefficient R^2 is lower than 0.8. By referring to the scale of toxicity established by Mousseux (1995), the extracts whose LC_{50} higher than 0.1 mg/ml, are regarded as not presenting any toxicity. Indeed, the extracts tested on *Artemia salina* were found to be non-toxic at the doses tested. However, mortality of brine shrimp (*A. salina*) increases as the concentration of extracts increases. The sensitivity of the larvae to the extracts thus follows a dose-response relationship.

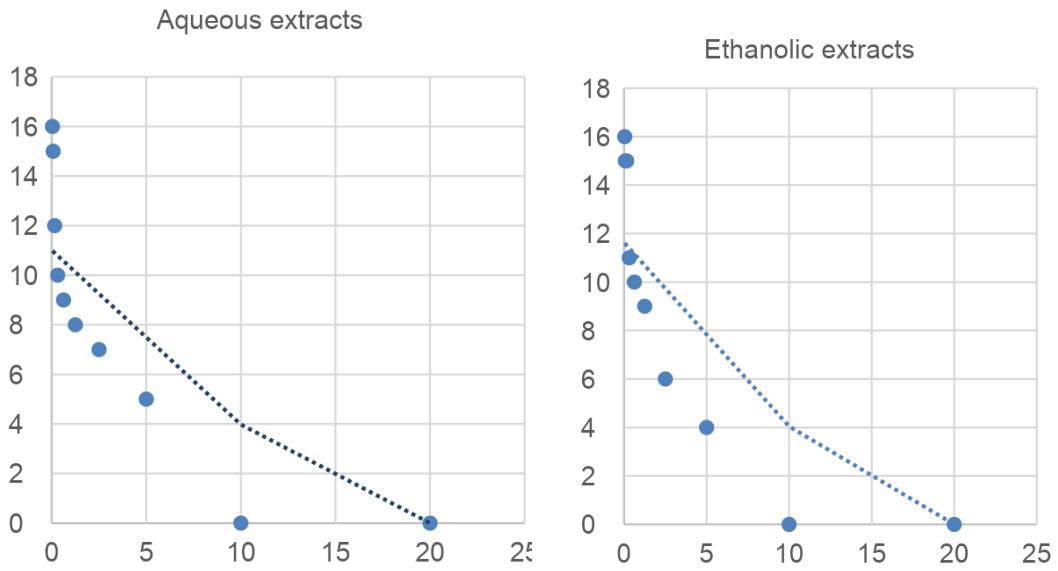

Figure 9: Toxicity curve of aqueous and ethanolic extracts of *A. conyzoides*

3.2. Discussion

Antibiotic and antifungal treatment of infections is not always effective and refers the population to the use of medicinal plants [7]. In this study, the ethnobotanical survey conducted on *A. conyzoides* in southern-Benin showed that traditional practitioners and herbalists are the professionals who hold endogenous knowledge. These professionals are mostly female (79.08%) and have a primary school education (45.10%) with at least five years of professional experience. This could be explained by the fact that in Benin, selling items at the market is usually reserved for women. Our results are in agreement with those of Yapi et al. [17] in Côte d'Ivoire who showed that 93% of herbalists are female compared to 7% male. However, their results show that 65% of herbalists are uneducated. Similarly, *A. conyzoides* is well known in the traditional treatment of various pathologies, in this case in the treatment of vaginal infections. The leaves and the stem are the parts of the plant most used essentially by maceration in water and by oral or cutaneous way. Our results are similar to those obtained by Yapi et al. [17] in Côte d'Ivoire who reveal a strong use of *A. conyzoides* for antimicrobial purposes especially in the treatment of conditions that can lead to female infertility. Also, they showed that the drink from the leaves is mostly (43.18%) used and the oral route is the most frequently used (60.93%). The high use of leaves would be explained by the fact that this part of the plant is the seat of photosynthesis and secondary metabolites responsible for biological properties [31]. Similarly, Ouattara [32] and N'Guessan [33] in Côte

d'Ivoire, have shown that drinking is the most requested mode of administration in traditional medicine for the fact that diseases can be related to bacterial, fungal and/or parasitic infections.

Furthermore, phytochemical screening of *A. conyzoides* leafy stem powder revealed the presence of secondary metabolites such as gall and catechin tannins, flavonoids, anthocyanins, triterpenes and C-heterosides. These results are little similar to those obtained recently in Cameroon [34] and previously by other researchers [35-37]. These different authors have shown that phytochemical analyses on *A. conyzoides* provide evidence for the presence of a wide variety of phytochemicals, such as alkaloids, tannins, terpenoids, chromenes, coumarin, flavonoids, saponins, glycosides, phenols, and resins. This difference could be explained probably to the difference between the organs of the plant used. Other factors that could be responsible for these variations are differences in detection methods, nature of the solvent, concentration and polarity of the solvent, collection area, nature of the soil, and stage of plant development [22, 38]. However, the absence of cyanogenic derivatives and cardiotonic heterosides shown by our results is confirmed by these different authors. The presence of these large groups of chemical compounds, would be at the origin of the pharmacological properties of this plant and could justify its empirical use in various traditional medicines and especially in the treatment of vaginal infections in South-Benin.

The majority of the constituents of plants used in the treatment of female infertility possess antimicrobial activities [18]. Thus, the antimicrobial activity of the extracts showed that the extracts had a broad spectrum of antimicrobial activities, inhibiting *Staphylococcus aureus* ATCC29213, *Candida albicans* MHMR, *Escherichia coli* ATCC 25922, clinical *S. aureus*, *C. albicans* and *E. coli*. These results are in agreement with the work of other authors [39-41]. The results of these authors reveal on the one hand that the aqueous and ethanolic extracts showed potential antibacterial activity on *Alcaligenes viscolactis*, *Klebsiella aerogenes*, *Bacillus cereus* and *Streptococcus pyogenes* as well as on methicillin-resistant *S. aureus* (MRSA). On the other hand, the literature review conducted by Chahal et al. [42], reveals that *A. conyzoides* effectively suppressed the growth of the *genera Aspergillus*, *Alternaria*, *Candida, Fusarium*, *Phytophthora* and *Pythium*.

The antimicrobial activity of *A. conyzoides* extracts would thus be linked to a synergistic effect between the different phytochemical groups present, namely tannins, flavonoids and triterpenes, all of which have antibacterial activity according to the literature. For example, polyphenolic compounds such as flavonoids exhibit various biological activities and are attributed to their ability to form complexes with the microbial extracellular wall [40]. Tannins exhibit antiparasitic, antiseptic, antibacterial, antioxidant, and anti-inflammatory activity [43]. Triterpenoids have antimicrobial, antifungal, analgesic, virostatic and immunostimulatory properties [44].

The Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations obtained are variable depending on the types of strains and the type of extract. In this study, the MICs are between 2.5mg/ml and 5 mg/ml for the reference strains tested and for the clinical strains. These values are largely lower than those obtained by Odeleye et al. [45] in Nigeria who had found MICs values between 120mg/ml and 200 mg/ml with *A. conyzoides* extracts on the strains studied. The differences may be explained by the extraction method, solvents used and the plant organ used. Therefore, depending on the extraction method, the solvent used and even the plant organ, the antimicrobial active ingredients will not have the same concentrations in the extracts. These low values obtained in our studies encourage the idea of the effectiveness of the antimicrobial activity of the extracts of *A. conyzoides* in the treatment of infectious pathologies due to the tested strains.

In this study, the BMCs are 10 mg/ml for the reference strains tested and for the clinical strains. Our results are contrary to those obtained by other authors with the ethanolic extract of *A. conyzoides* [39]. This difference could be justified by the microbial strains used. The ratio of MIC and BMC parameters the aqueous and ethanolic extracts all have a bactericidal

and fungicidal effect on the different strains tested with the exception of the ethanolic extract towards clinical *Escherichia coli*. Also, in this study, the tested strains show a low sensitivity to the ethanolic extract (62.5%) contrary to the aqueous extract (75%). This could be explained by the fact that water better concentrated the secondary metabolites present in *A. conyzoides* compared to ethanol. This is justified by the higher yield (6.18%) given by the aqueous extract compared to the ethanolic extract (4.32%) in our study. These results are similar to those obtained by Wuyep et al. [40] in a similar study in Nigeria who showed that *A. conyzoides* gave a better yield (10.796%) with the aqueous solvent than the ethanolic solvent (6.409%) as well as a higher antifungal activity compared to the ethanolic extract. According to Ouattara et al. [46], water is used as the main solvent especially in the treatment of mycoses. This would justify on the one hand the use of this plant mainly in the form of maceration or decoction in water and on the other hand, the restriction of alcohol consumption during its use for more effectiveness.
In our study, the results of the antioxidant activity of the *A. conyzoides* extracts show that with an IC50 of 0.16 mg/ml, the aqueous extract of the plant presents a good antioxidant activity contrary to the ethanolic extract which presents an IC50 of 0.41 mg/ml. Our results are similar to those obtained by Acheampong et al. [47] in Ghana; It showed that methanolic extract of *A. conyzoides* has high antioxidant power between 7.82-1000 µg/ml against gallic acid. Acheampong et al. [47] showed that aqueous extracts of *A. conyzoides* possess remarkable antioxidant effects. The results obtained provide evidence that *A. conyzoides* extracts through the studied organs (leafy stem) exhibit antioxidant activity [48] and would therefore be useful as a free radical scavenger and thus would help in the treatment of many diseases caused by reactive oxygen species. These diseases include aging, inflammation, cancer, diabetes and in this case microbial infections. The antioxidant activity is due to the presence of major chemical groups including tannins and flavonoids. This result corroborates well with the phytochemical screening results presented above.

Toxicity evaluation of *A. conyzoides* extracts on shrimp larvae shows that the two leafy stem extracts do not show toxicity. The non-toxic character of these extracts, revealed by the toxicity test, comes to justify the results of the phytochemical screening which showed the absence of cardiotonic heterosides, cyanogenic derivatives and quinonic derivatives which are generally toxic compounds [49]. Moreover, these results are contrary to those of Djeneb et al. [50] who showed in their study that when mice were treated orally with the 70% ethanolic extract of *A. conyzoides* that no death of the mice was observed in the experiment but that the presence of slightly toxic effects on proliferating human HFF cells and an increase in the activity of cells that no longer divide were still noted.

4. Conclusion

An ethnobotanical survey was conducted on the use of *A. conyzoides* in the traditional treatment of infections. This survey, carried out among herbalists and traditional therapists in Abomey-Calavi, Cotonou, Zogbodomey, Bohicon and Abomey, revealed its strong therapeutic use by the populations in the treatment of genital affections, mainly vaginal infections. The present work allowed to highlight the antimicrobial and antioxidant properties and the toxic power of the different aqueous and ethanolic aerial parts extracts of *A. conyzoides*. The evaluation of the toxicity of the said extracts on shrimp larvae shows that they do not present larval cytotoxicity. The leafy stem of *A. conyzoides* presents a chemical profile that justifies its antimicrobial and antioxidant power and the safety of its use in human health. These results allow us to suggest the use of the aqueous extract of the leafy stem of *A. conyzoides* in the traditional treatment of vaginal infections. However, further studies need to be conducted to determine the appropriate dosage for safer human use.

Consent (where ever applicable)

References

1. Hilly M, Adams ML, Nelson SC. A study of digit fusion in the mouse embryo. Clin Exp Allergy. 2002;32(4):489-98.

1. Cuzin L, Delpierre C. Epidemiologie des maladies infectieuses. EMC-Mal infect. 2005. 2(1) :157-62.

2. Holmes KK, Bertozzi S, Bloom BR, et al. Major Infectious Diseases: Key Messages from Disease Control Priorities, Third Edition. *In*: Holmes KK, Bertozzi S, Bloom BR, et al., editors. Major Infectious Diseases. 3rd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 2017 Nov 3. Chapter 1. Available from: https://www.ncbi.nlm.nih.gov/books/NBK525197/ doi: 10.1596/978-1-4648-0524-0_ch1
3. WHO. 2002. Strategy for Traditional Medicine for 2002–2005, OMS/WHO ed. Geneva, 65p. (French)
4. Pellati D, Mylonakis I, Bertoloni G, Fiore C, Andrisani A, Ambrosini G, Armanini D. Genital tract infections and infertility. Eur J Obstet Gynecol Reprod Biol. 2008. 140(1): 3-11. DOI: http://doi:10.1016/j.ejogrb.2008.03.009.

5. Bilardi JE, Walker S, Temple-Smith M, McNair R, Mooney-Somers J, Bellhouse C. The burden of bacterial vaginosis: Women's experience of the physical, emotional, sexual and social impact of living with recurrent bacterial vaginosis. PLoS One 2013; 8(9): e74378. http://dx.doi.org/10.1371/journal.pone.0074378.

6. Willems HME, Ahmed SS, Liu J, Xu Z, Peters BM. Vulvovaginal Candidiasis: A Current Understanding and Burning Questions. J Fungi. 2020; 6(1), 27. https://doi.org/10.3390/jof6010027
7. Palmeira-de-Oliveira R., Palmeira-de-Oliveira A., Martinez-de-Oliveira J., 2015. New strategies for local treatment of vaginal infections. *Adv. Drug Deliv. Rev. 92*, 105–122.

8. Zirihi GN. Botanical, pharmacological and phytochemical study of some antimalarial and/or immunogenic medicinal plants used among the Bété of the Department of

Issia, in the west of the Ivory Coast. State Doctorate Thesis, University of Cocody Abidjan, UFR Biosciences; 2006. 126 p. (French)
9. WHO (World Health Organization), Antimicrobial Resistance. Regional Office for the Western Pacific, Seventieth Session Manila, Philippines. 2019; 8p. (French)
10. Superti F, De Seta F. Warding off recurrent yeast and bacterial vaginal infections: *Lactoferrin* and *Lactobacilli*. Microorganisms. 2020; *8*(1), 130.doi:10.3390/microorganisms8010130
11. Sanogo R, Diallo D, Diarra S, Ekoumou C, Bougoudogo F. Activité antibactérienne et antalgique de deux recettes traditionnelles utilisées dans le traitement des infections urinaires et la cystite au Mali. Mali Méd. 2006 ; Tome 21(1) :18.
12. Dembélé LD, Dramé BSI, Haïdara M, Koné C, Sanogo R. Paramètres physicochimiques et activité antibactérienne de trois plantes médicinales, utilisées dans la prise en charge des infections urinaires au Mali. J Soc Ouest-Afr Chim. 2022 ; 51 : 10–16
13. Biswas B, Rogers K, Claughin F, Daniels D, Yadav A. Antimicrobial Activities of Leaf Extracts of Guava (*Psidium guajava* L.) on Two Gram-Negative and Gram-Positive Bacteria. Int J Microbiol. 2013, ID 746165, 7 p https://doi.org/10.1155/2013/746165
14. Oullai L, Chamek C. Contribution to the ethnopharmacognosic study of medicinal plants used for the treatment of digestive tract disorders in Kabylie. Dissertation Doctor of Pharmacy, Mouloud Mammeri University, Faculty of Medicine, 2018, 199pp. (French)
15. WHO/MS-Benin, WHO Country Cooperation Strategy 2009–2013 Benin. WHO Regional Office for Africa, 2009. ISBN: 978 929 031 1249 (NLM Classification: WA 540 HB35)
16. Chabi-Sika K, Sina H, Boya B, Bade F, Hounnou T, Badoussi ME, Adjatin A, Baba-Moussa L. *Richardia brasilensis* collected in Southern-Benin: Phytochemical, Antimicrobial Activity and Toxicity. Asian J Biol. 2021; 13(4):22-23.
17. Yapi AB, Kassi NJ, Fofie NBY, Zirihi GN. Etude ethnobotanique des Asteraceae médicinales vendues sur les marches du district autonome d'Abidjan (Côte d'Ivoire). Int J Biol Chem Sci. 2015; 9(6): 2633-47. DOI : http://dx.doi.org/10.4314/ijbcs.v9i6.10
18. Telefo PB, Lemfack MC, Bayala B, Lienou LL, Goka CS, Yemele MD, Mouokeu C, Tagne SR, Moundipa FP. Enquête ethnopharmacologique des plantes utilisées dans le traitement de l'infertilité féminine dans les localités de Fossong-Wentcheng et Foto, Cameroun. Phytothérapie. 2012 ; 10(1) : 25-34.
19. Akoegninou A, van der Burg WJ, van der Maesen LIO, Adjakidjè V, Essou JP, Sinsin B, Yédomonhan H. Flore Analytique du Benin. Université d'Abomey-Calavi, Cotonou, République du Bénin. Cotonou & Wageningen. Backhuys Publishers, 2006. 1034p.
20. Fanou BA, Klotoe JR, Fah L, Dougnon V, Koudokpon CH, Toko G, Loko F. Ethnobotanical survey on plants used in the treatment of candidiasis in traditional markets of southern Benin, BMC Complementary Med Ther. 2020, 20(1), 1-18.
21. Akabassi BS, Djossou JA, Tchobo PF, Tchatcha DA, Houénon GHA, Yovo M, Dédjiho CC, Bogninou-Agbidinoukoun RSG, Soumanou MM. Criblage phytochimique et évaluation des activités antiradicalaire et antimicrobienne des organes du *Detarium microcarpum* Guill. & Perr de la zone soudanienne du Benin. J Soc Ouest-Afr Chim. 2021 ; 050 ; 68- 75
22. Adjou ES, Aoumanou MM. Efficacité des extraits de plantes dans la lutte contre les moisissures toxinogènes isolées de l'arachide en post-récolte au Bénin. J Appl Biosci. 2013 ;*70*, 5555-66.
23. Roko OG, Dougnon V, Hounkpatin A, Klotoé JR, Baba-Moussa L. Anti-inflammatory, Analgesic and Antipyretic Properties of Ethanolics Extrats of Three Plants of

Beninese's Pharmacopoeia: *Euphorbia hirta*, *Citrus aurantifolia* and *Heterotis rotundifolia*. Asian J Biol. 2019, 8(4) :1-8.
24. Houghton P, Raman J, 1998. A Laboratory handbook for the fractionation of natural extracts. Chapman et Hall, London, 199 p
25. Sina H, N'tcha C, Dah-Nouvlessounon D, Gnama-Tchao G, Boya B, Socohou A, Sanni ARA, Baba-Moussa F, Adjanohoun A, Baba-Moussa L. Molecular characterization of high-risk infection vaginal bacteria isolated from pregnant women in CHU-MEL of Cotonou (Benin). Afr J Microbiol Res. (2021); 15(12), 592-604.
26. Amoussa AMO, Sanni A and Lagnika L. Antioxidant activity and the estimation of total phenolic, flavonoid and flavonol contents of the bark extracts of *Acacia ataxacantha*. J Pharmacogn Phytochem. 2015; 4(2) :172-178.
27. Kamanzi AK**.** Medicinal plants of Côte d'Ivoire: phytochemical investigations guided by biological tests. Doctoral thesis, University of Cocody, Abidjan, Ivory Coast. 2002; 176p
28. Kawsar SMA, Huq E, Nahar N. Cytotoxicity assessment of the aerial parts of Macrotyloma uniflorum linn. Intl J Pharmacol. 2008; *4*(4), 297-300.
29. Dieng SIM, Fall AD, Diatta-Badji K, Sarr A, Sene M, Sene M, Mbaye A, Diatta W, Bassene E**.** Evaluation de l'activité antioxydante des extraits hydro-ethanoliques des feuilles et écorces de *Piliostigma thonningii* Schumach. Int J Biol Chem Sci. 2017; 11(2): 768-776
30. Mousseux M. Toxicity test on *Artemia salina* larvae and maintenance of a farm and blanes, second year internship report. DEUST Aquaculture ; University Center of New Caledonia, France, 1995 ; (French)
31. Mangambu M, Mushagalusa K, Kadima N. Contribution à l'étude phytochimique de quelques plantes médicinales antidiabétiques de la ville de Bukavu et ses environs (Sud-Kivu, R.D. Congo). J Appl Biosci. 2014 ; 75: 6211-20. http://dx.doi.org/10.4314/jab.v75i1
32. Ouattara D. Contribution to the inventory of significant medicinal plants used in the Divo region (southern forest of the Ivory Coast) and to the diagnosis of the Guinea pepper plant: *Xylopia aethiopica* (Dunal) A Rich (*Annonaceae*). Doctoral thesis University of Cocody, Abidjan (Ivory Coast), UFR Biosciences, 2006. 184 p. (French)
33. N'Guessan K. Medicinal plants and traditional medical practices among the Abbey and Krobou peoples of the Department of Agboville (Ivory Coast). State Doctorate Thesis, University of Cocody-Abidjan, U.F.R. Biosciences. 2008; 235 p. (French)
34. Ndacnou MK, Pantaleon A, Tchinda JS, Mangapche ELN, Keumedjio F, Boyoguemo DB. Phytochemical study and anti-oomycete activity of *Ageratum conyzoides* Linnaeus. Industrial Crop Products. 2020, 153, 112589. https://doi.org/10.1016/j.indcrop.2020.112589
35. Kasali AA, Winterhalter P, Adio AM, Knapp H, Bonnlander B. Chromenesin *Ageratum conyzoides L*. Flavour Fragr J. 2002; 17: 247–50.
36. Usman LA, Zubair MF, Olawore NO, Muhammad NO, M'Civer FA, Ismaeel RO. Chemical constituents of flower essential oil of *Ageratum conyzoides* growing in Nigeria. Elixir Org Chem. 2013; 54:12463–5.
37. Okereke SC, Chukwudoruo CS, Nwaokezie CO. Phytochemical screening using GC-FID and sub-chronic assessment of Hydroethanolic leaf extract of *Ageratum conyzoides Linn*. On albino rats. J Med Plants Stud. 2017; 5: 282–7.
38. Folashade KO, Omoregie EH, Ochogu AP. Standardization of herbal medicines–A Review. Int J Biodiver Conserv. 2012; 4(3): 101-112
39. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC Complementary Altern Med. 2005; 5(1): 1-7

40. Wuyep PA, Musa HD, Ezemokwe GC, Nyam DD, SilaGyang MD. Phytochemicals from *Ageratum conyzoides* L. Extracts and their Antifungal Activity against Virulent *Aspergillus* spp. J Academia Industrial Res. 2017**;** 6(3): 32-39.
41. Omole OA, Oladipo JO, Orimolade BO, Ajetomobi OO, Olorumaiye KS, Dosumu OO. Anti-Oxidant and Anti-Microbial Activities of the Root and Leaf Extracts of *Ageratum conyzoides* L. Agriculture Conspectus Scientificus. 2019; 84(3):295-304.
42. Chahal R, Nanda A, Akkol EK, Sobarzo-Sánchez E, Arya A, Kaushik D, Dutt R, Bhardwaj R, Rahman MH, Mittal V. *Ageratum conyzoides* L. and Its Secondary Metabolites in the Management of Different Fungal Pathogens. Molecules. 2021; 26, 2933. https://doi.org/10.3390/.
43. Yessoufou A, Gbenou J, Grissa O, Hichami A, Simonin A-M, Tabka Z, Moudachirou M, Moutairou K, Khan NA. Anti-hyperglycemic effects of three medicinal plants in diabetic pregnancy: modulation of T cell proliferation. BMC Complementary Altern Med. 2013; 13(77) 13p.
44. Connolly JD, Hill RA. Triterpenoids. Natural Product Reports. 2007; 24: 465-486.
45. Odeleye OP, Oluyege JO, Aregbesola OA, Odeleye PO. Evaluation of preliminary phytochemical and antibacterial activity of *Ageratum conyzoides* (L.) on some clinical bacterial isolates. Int J Eng Sci. 201; 3: 1–5.
46. Ouattara KE, Doga D, Zirihi GN. Evaluation In Vitro du Pouvoir Fongicide des Extraits De *Erigeron floribundus* (Kunth.) Sch. Bip. (Asteraceae) sur *Sclerotium rolfsii* et *Colletotrichum musae* Deux Champignons Phytopathogènes. Eur Sci J. 2019; 15(9) : 370. https://doi.org/10.19044/esj.2019.v15n9p370
47. Acheampong F, Larbie C, Arthur FKN, Appiah-Opong R, Tuffour I. Antioxidant and anticancer study of *Ageratum conyzoides* aqueous extracts. J Global Biosci. 2015; 4(1):1804-15.
48. Bédié AP, N'guessan BB, Yapo AF, N'guessan JD, Djaman JA. Activités antioxydantes de dix plantes médicinales de la pharmacopée ivoirienne. Sci Nat. 2011 ; 8(1):1 -11
49. Houngbèmè AG, Gandonou C, Yehouenou B, Kpoviessi SDS, Sohounhloue D, Moudachirou M, Gbaguidi FA. Phytochemical analysis, toxicity and antibacterial activity of Benin medicinal plants extracts used in the treatment of sexually transmitted infections associated with HIV/AIDS. Int J Pharmaceutical Sci Res. 2014 ; 1739-1745.
50. Djeneb C, Basile YA, Yvette BNF, Etienne OK, Noël ZG. Etude Comparative des Toxicités Cellulaires et Aigües de *Ageratum conyzoides L*. et de *Acanthospermum hispidum* DC. Eur Sci J. 2021; 17(40), 74. https://doi.org/10.19044/esj.2021.v17n40p74