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

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## ABSTRACT

**Aims:** *Ageratum conyzoides L.* is a small annual herbaceous highly odorous plant use in traditional medicine. The aim of this study is to evaluate *in vitro* antioxidant potential, toxicity and antimicrobial activity of aerial part extracts of *A. conyzoides* on strains potentially involved in vaginal infections.

**Methodology:** An ethnobotanical survey has been carried out on *A. conyzoides* among ethnobotanists and traditional therapists in fifteen markets in the communes of Abomey-Calavi, Cotonou, Zogbodomey, Bohicon and Abomey. The phytochemical screening was a qualitative analysis based on staining and precipitation reactions. Antimicrobial activity of *A. conyzoides* aqueous and ethanolic extracts was evaluated on reference and clinical strains of *Staphylococcus aureus, Candida albicans and Escherichia coli* using micro dilutions method in wells from . The toxicity of *A. conyzoides* extracts was determine using *Artemia salina* larvae, whereas the antiradical activity was evaluated using the Ferric Reducing Antioxidant Power (FRAP) method.

**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 of pathologies affecting the female reproductive system. The phytochemical screening revealed the presence of flavonoids, tannins, anthocyanins, triterpenes and C-heterosides. The yield of 6.18% for the aqueous extract and 4.32% for the ethanolic extract as recorded. The highest inhibition diameter (24.05 ± 0.5 mm) was obtained using aqueous extract against the clinical *S. aureus* strain. In contrast, the lowest inhibition diameter (10±0 mm) was obtained against the *S. aureus* ATCC29213 with the same extract. The Minimum Inhibitory Concentration varied from 2.5 to 5 mg/ml. 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 compared to the ethanolic extract. In addition, the aqueous extracts 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 involved in 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]. Those diseases 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 in the infection, we can have low and high infections [4]. Thus, 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 major 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]. Vaginal infections are extremely prevalent [7], and are among the most common reasons for gynecological consultations in Benin [8]. 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 [9-10]. To face this problem, there is an urgent need for research to discover other active compound that can effectively treat genital infections. Thus, medicinal plants commonly 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 use traditional medicine in the treatment of various ailments [15]. In Benin in particular, medicinal plants are used in the composition of pharmacopoeia products. Various medicinal plants are used for their biological properties in the treatment of many infectious pathologies [16]. Among these medicinal plants we can mention *Ageratum conyzoides*, 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]. However, it should be noted a lake of studies related to the antimicrobial activity of *A. conyzoides* in the specific treatment of vaginal infections. Thus, despite its medicinal use, the toxicity profile of the *A. conyzoides* plant remains to be explored and requires further studies. In order to strengthen the scientific knowledge on the medicinal usefulness of *A. conyzoides* and to contribute to its valorization this study is conducted. It aimed at evaluating the antioxidant potential, toxicity and antimicrobial activity of aerial part extracts of *A. conyzoides* plant on the growth of *C. albicans*, *E. coli* and *S. aureus* strains.

## 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 in the 5 municipalities between march and April 2022.

## 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 twice successively 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 debris; this filtrate was optimized thought additional filtration using a Whatman 1 paper. 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 the ratio of the mass of dry extract and the mass of plant material processed (Harborne, 1998). It was determined according to the 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 plant powder. 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 by Chabi-Sika et al. [16].

## 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) / 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. <u>Determination of the Minimum Inhibitory Concentration (MIC)</u>

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 18 h. 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. <u>Determination of the Minimum Bactericidal/Fungal Concentration (MBC or MFC)</u>

The lowest concentration at which 99.99% of germs are inhibited (MBC or 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 or fungicidal (MBC or MFC/CMI ≤ 4) or bacteriostatic fungistatic (MBC or MFC/MIC≥ 4).

### 2.6. Larval toxicity evaluation

The test is performed according to the method described by Kawsar et al. [28] and recently used by Chabi-Sika et al. [16]. Thus, larvae used are obtained by hatching 10 mg of *Artemia salina* eggs placed under continuous agitation in 1 L 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 batch 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 (5 mg/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 1 ml of phosphate buffer (0.2M; pH=6.6) and 1 ml of 1% potassium hexacyanoferrate [$K_3Fe(CN)_6$]. After incubating the mixture at 50°C for 30 minutes, 1 ml of 10% trichloroacetic acid was added to stop the reaction, then the tubes were centrifuged at 3000 rpm for 10 minutes. Then, 1 ml of the supernatant from each tube was mixed with 0.2 ml of 0.1% $FeCl_3$ solution and allowed to stand in the dark for 30 min 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: RP = [OD ($extract$) - $DO$ ($blanc$) /OD ($extract$)] × 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

#### 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 according the ethnics of southern-Benin (Table 1).

Table 1**.** Different names of *A. conyzoides* in the ethnics of 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 age are 20 years old and 80 years old with an average age of 44.22 ± 7.11 years old. 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 (1.31%). 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 | 131 |
| 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).

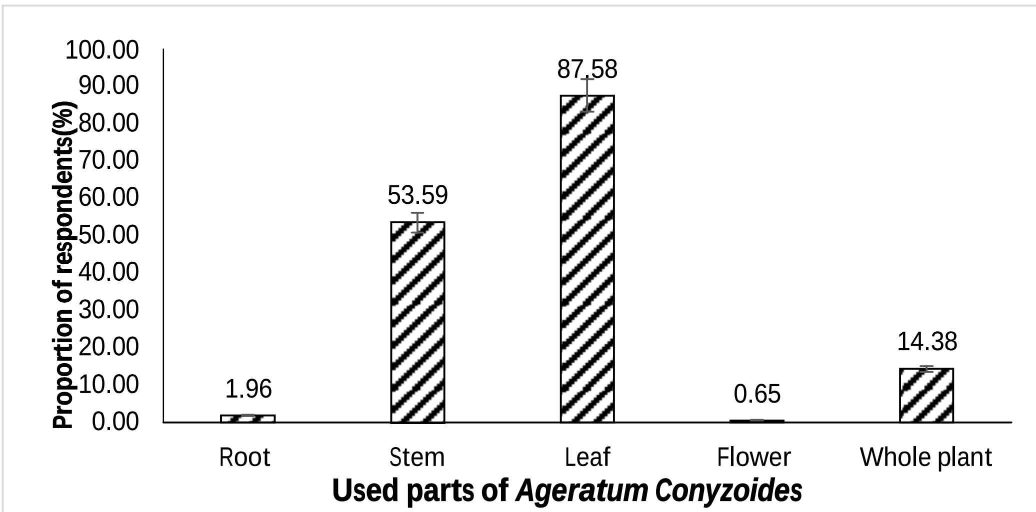


Figure 1: Proportions of respondents according to the different parts of *A. conyzoides*.

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).

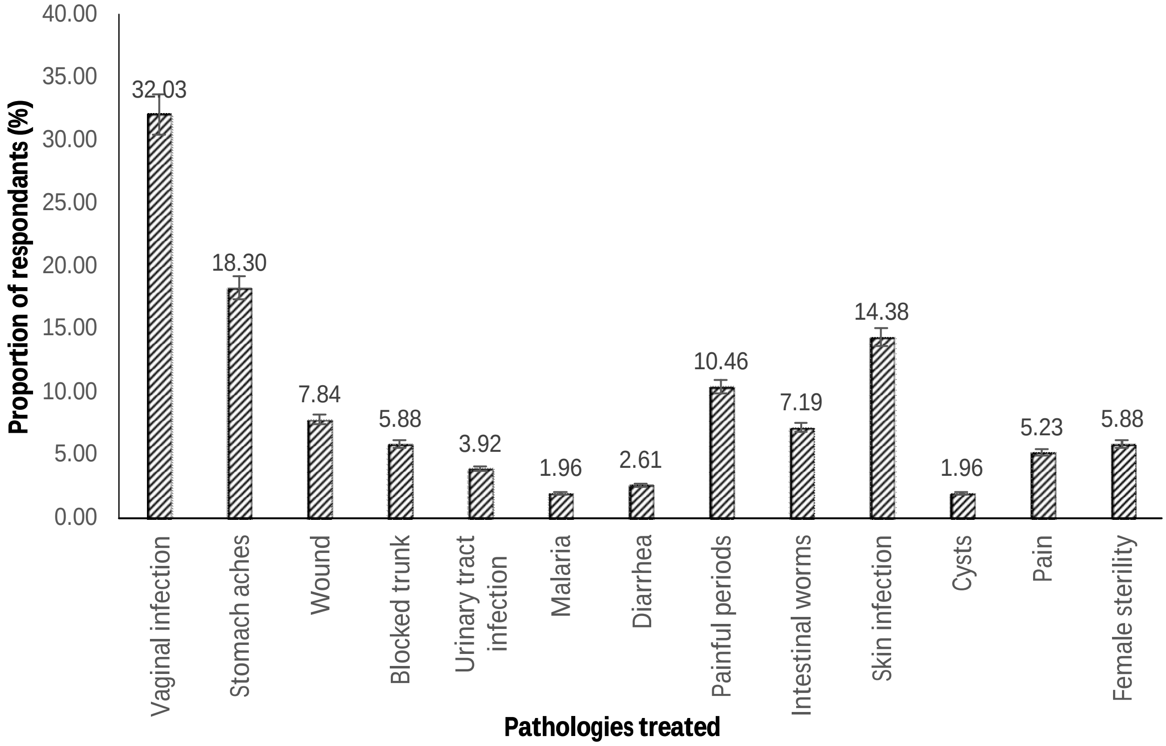


Figure 2. Distribution of the different pathologies reported to treated by *A. conyzoides*

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).

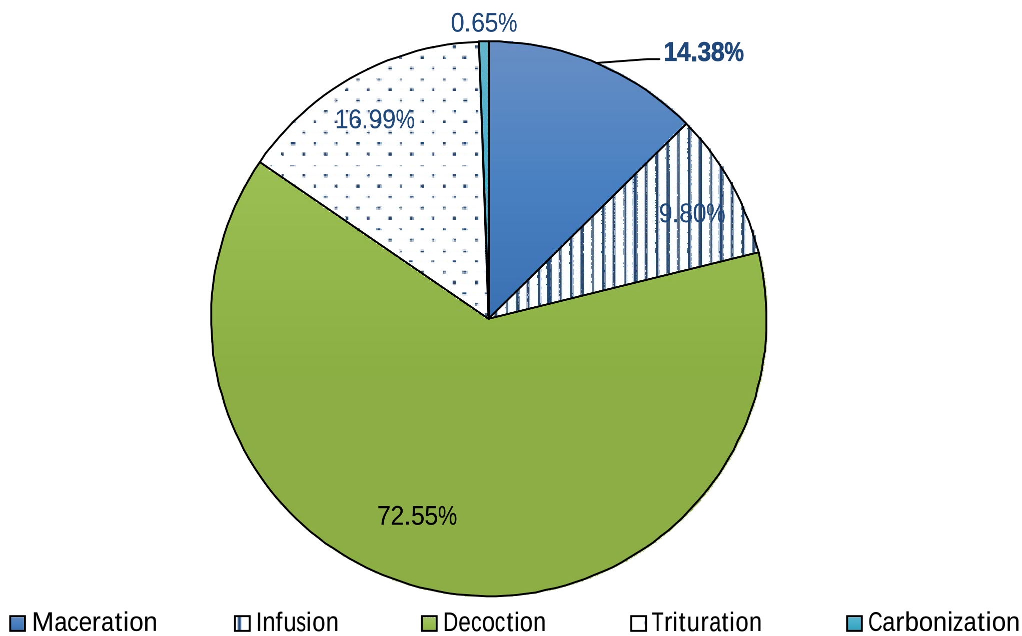


Figure 3. Preparation method of *A. conyzoides* in the study area

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

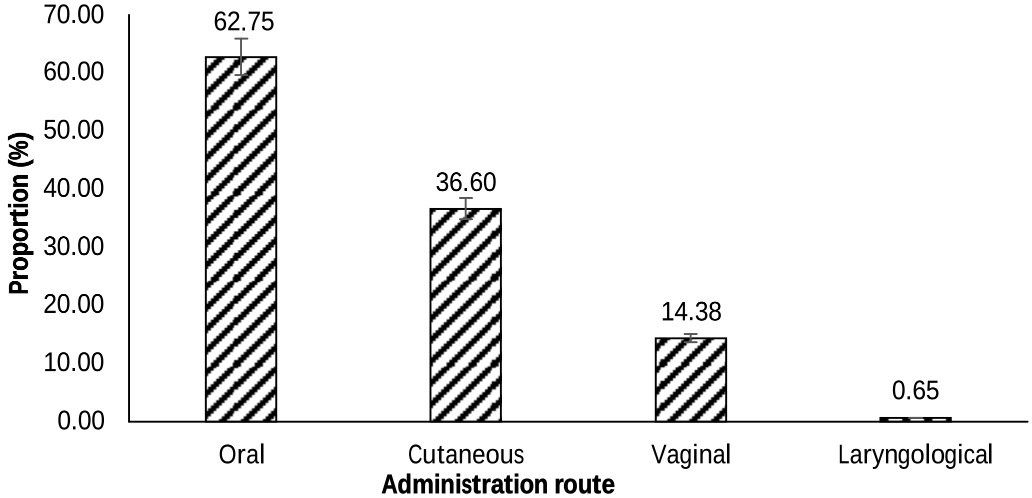


Figure 4. Proportion of the administration routes of *A. conyzoides* based preparations in southern Benin

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.

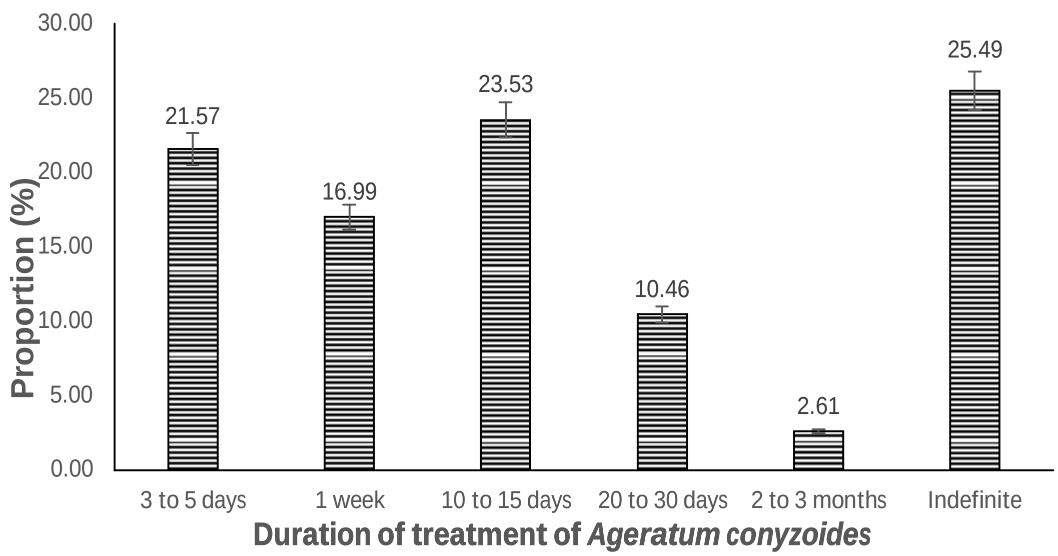


Figure 5. Compilation of the *A. conyzoides* based preparations treatment duration in southern Benin

### 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%).

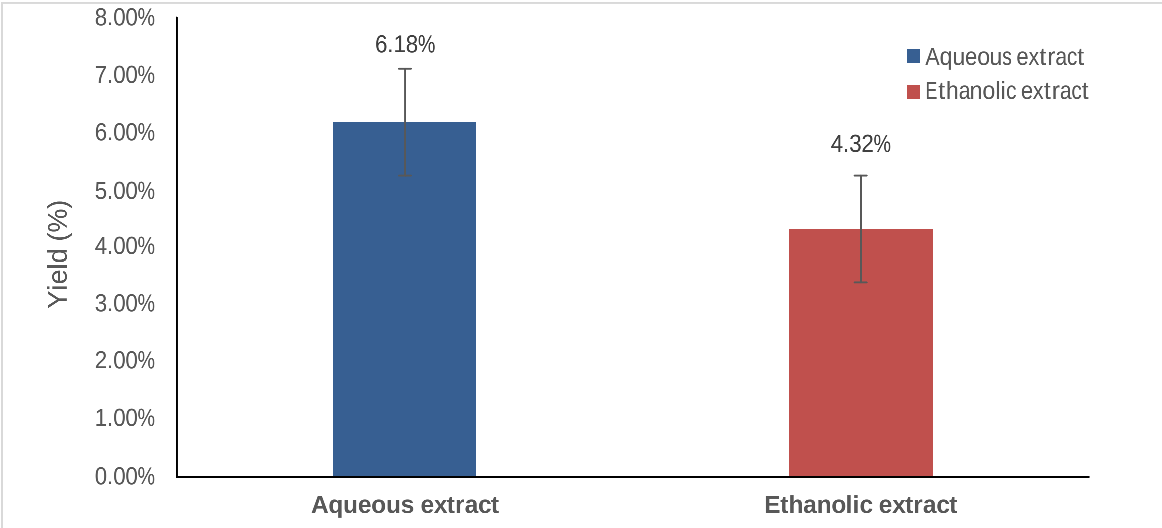


**Figure 6.** Yield of *A. conyzoides* extracts

### <u>3.1.6 Antimicrobial activity of *A. conyzoides* extracts</u>

*3.1.6.1. Sensitivity test*

The results of the inhibition tests reveal that 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 (inhibition diameter of 12±0 mm and 12±0.5 mm respectively for *C. albicans* MHMR and clinical *C. albicans*).

*Staphylococcus aureus* strains were most sensitive to the aqueous extract. However, clinical *S. aureus* was more sensitive (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 24 h and 48 h, the strains show a higher sensitivity (75%) to the aqueous extract of the plant as opposed to the ethanolic extract (62.5%).

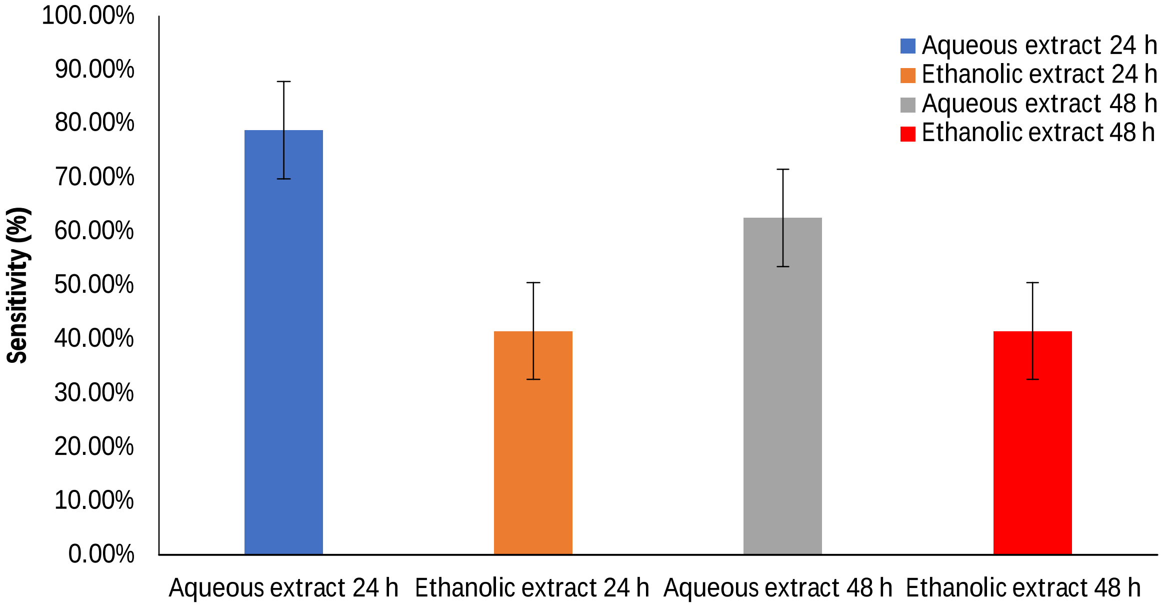


Figure 7: Sensitivity rate of clinical strains to extracts.

*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 (clinical *S. aureus*); 2.5 mg/ml (clinical *C. albicans*) and 2.5 mg/ml (clinical *E. coli*).

The MBC or MFC 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 MFC/MIC ratio (2 mg/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 | MIC and MBC (mg/ml) of *Ageratum conyzoides* on tested strains | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Aqueous Extract** | | | | **Ethanolic extract** | | | |
| | **MIC** | **MBC MBC** | **MBC/MIC MFC/MIC** | **Effects** | **MIC** | **MBC MFC** | **MBC/MIC MFC/MIC** | **Effects** |
| ***S. aureus ATCC29213*** | 5 | 10 | 2 | Bactericidal | 2.5 | 10 | 4 | Bactericidal |
| ***C. albicans MHMR*** | 5 | 10 | 2 | Fungicidal | 5 | 10 | 2 | Fungicidal |
| ***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 | Fungicidal | 5 | 10 | 2 | Fungicidal |
| ***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. The Beta-Hydroxy Acid (BHA) is used as a control in this study. This figure reveals that the aqueous and ethanolic extracts of the *A. conyzoides* plant sample show antioxidant activity with respective inhibitory half-concentrations ($IC_{50}$) of 0.16 mg/ml and 0.41 mg/ml while the $IC_{50}$ of the control (BHA) is 0.23 mg/ml. It should also be noted that the aqueous extract of the plant has a stronger antioxidant power than the ethanolic extract.

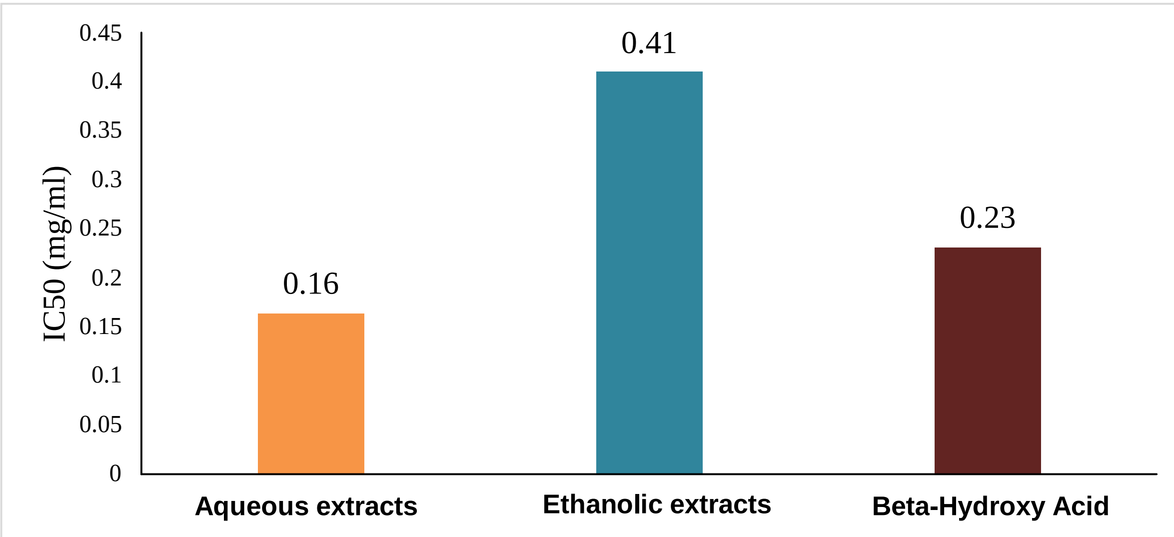


Figure 8: IC50 of aqueous and ethanolic extracts of *A. conyzoides*

### 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 *A. salina* larvae. The lethal concentrations ($LC_{50}$) 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 *A. salina* were found to be non-toxic at the doses tested. However, mortality of *A. salina* increases as the concentration of extracts increases. The sensitivity of the larvae to the extracts thus follows a dose-response relationship.

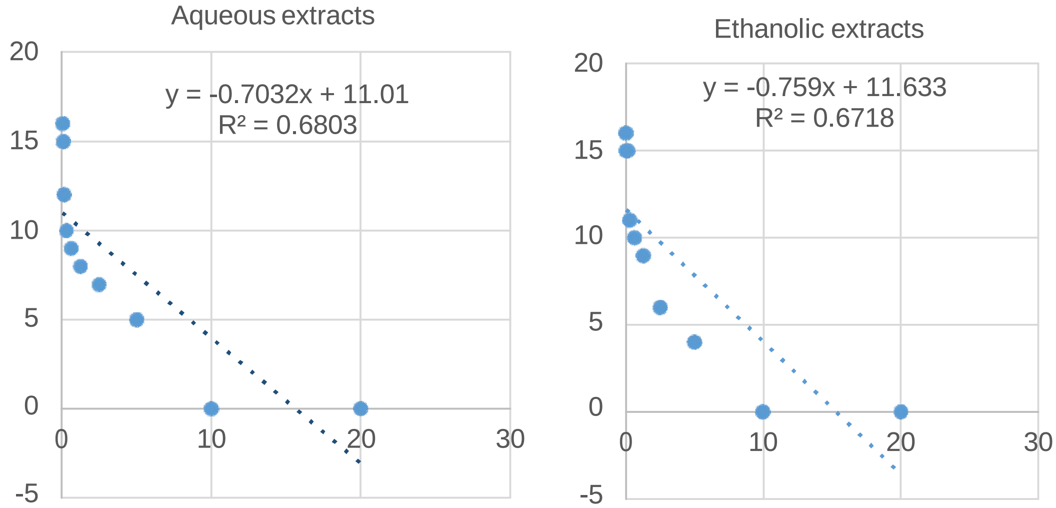


Figure 9: Toxicity curve of aqueous and ethanolic extracts of *A. conyzoides*

### 3.2. Discussion

Antibiotic and antifungal treatment of infections is not always effective and refers 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. 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 in Côte d'Ivoire [17] revealing 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 *S. aureus* ATCC29213, *C. albicans* MHMR, *E. 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 *S. 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, viro-static 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 2.5 mg/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 120 mg/ml and 200 mg/ml with *A. conyzoides* extracts on the studied strains. 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.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## AUTHORS' CONTRIBUTIONS

'KC-S, HS, GD, BB and GAD' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'KC-S, IM-S, HAS, AS, NM, DD-N and LB-M'' managed the analyses of the study. 'HS, GAD, HL, GD and BB' managed the literature searches. All authors read and approved the final manuscript.

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