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# Contribution to the phytochemical study and evaluation of the antibacterial activity of extracts from the leaves of *Ageratum conyzoides* (Asteraceae) from Côte d'Ivoire

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## Abstract

*Ageratum conyzoïdes* is a plant known for its therapeutic virtues. Its leaves are used by traditional healers to treat several pathologies. Phytochemical screening and quantitative analysis revealed the richness of this plant thanks to its multiple secondary metabolites. The aqueous, ethanolic and hexanic extracts were used for phytochemical screening. Thus, these extracts showed overall the presence of tannins, flavonoids, coumarins, free quinones, anthraquinones, alkaloids, sterols-triterpenes, terpenoids, saponosides, reducing compounds, anthocyanins, volatile oils and cardiac glycosides in the leaves of *Ageratum conyzoïdes*. Qualitative analysis by dosage showed that the ethanolic extract is more concentrated than the aqueous extract in total polyphenols, total flavonoids and total condensed tannins. The evaluation of the antibacterial activity of the ethanolic and aqueous extracts was carried out. It appears that only the ethanolic extract exhibits a bacteriostatic effect on the bacterial strain *Staphylococcus aureus* ATCC 25923.

**Keywords:** *Ageratum conyzoïdes;* Antibacterial activity; Phytochemical screening*;* Côte d'Ivoire

## 1. Introduction

The populations of the world, and particularly those of Africa, are increasingly faced with the resurgence of certain pathologies such as cardiovascular disease, cancer, diabetes [1] and bacterial diseases [2]. Faced with this situation, a great deal of work is being done to find new biomolecular sources, precisely in medicinal plants [3, 4]. The use of plants for therapeutic purposes is reported in ancient Arabic, Chinese, Egyptian, Hindu, Greek and Roman literature [5, 6]. In Africa, the therapeutic power of plants was known empirically for centuries [7, 8].

However, the chemical composition of the traditional medicinal preparations used for health care was unknown. In order to improve this African medicine, a number of phytochemical and biological investigations have been carried out to provide scientific justification for the traditional use of medicinal plants [9]. It was with this in mind that this research work focused on *Ageratum conyzoides.* This plant is often used in the traditional treatment of various ailments such as rheumatism and sleeping sickness, as a disinfectant for toothache, cough suppressant, vermifuge and tonic [10]. With

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the aim of developing medicinal plants, the aim of this work is to prove or disprove certain pharmacological properties of *Ageratum conyzoides* leaves using chemical and biological approaches.

## 2. Material and methods

### 2.1. Material

#### *2.1.1. Plant material*

The plant material used in this study consisted of *Ageratum conyzoides* leaves. The leaves were harvested at the Jean Lorougnon Guédé University in Daloa (Côte d'Ivoire). The organ was dried for 5 days in a room at room temperature. The dried leaves were crushed in a mortar and then sieved to obtain fine powders that were used to prepare the various extracts to be tested.

#### *2.1.2. Bacterial strains*

Six (06) bacterial strains were used:

- 02 *Staphylococcus aureus* species: *S. aureus* ATCC 25922 and *S. aureus* 1568 UB/23 CNRa: both collected from infected skins.
- 02 *Pseudomonas aeruginosa* species: *P. aeruginosa* ATCC 27853 and *P. aeruginosa* 1587 UB/23 CNRa.
- 02 species of Enterobacteria: *Escherichia coli* ATCC 25922 and *Escherichia coli* UB/23 CNRa.

All these bacterial strains are from the Natural Substances Antibiotics and Anti-Infectious Microorganisms Surveillance Unit (ASSURMI) of the Bacteriology and Virology Department of the Institut Pasteur de Côte d'Ivoire (IPCI).

#### *2.1.3. Technical equipment and reagents*

The technical equipment consisted of the usual laboratory glassware, an electronic balance, a permanent stirrer, an oven and a spectrophotometer, etc...

The chemicals and reagents used were ethanol, hexane, methanol, reagent reagents (1% $FeCl_3$, 10% KOH, HCl, NaOH, 10% ammonium hydroxide, acetic anhydride, sulphuric acid, chloroform, copper sulphate, tartaric acid ($C_4H_6O_6$), Folin-Ciocalteu, gallic acid, catechin, quercetin, vanillin, sodium carbonate, magnesium, etc..)

### 2.2. Methods

#### *2.2.1. Extractions : preparation of aqueous, ethanolic and hexanolic extracts*

In three Erlenmeyer flasks each containing ten (10 g) of *Ageratum conyzoides* leaf powder, 120 mL of distilled water, 120 mL of ethanol and 120 mL of hexane were added individually. The three different aqueous, ethanolic and hexane mixtures obtained were stirred continuously for 15 min. They were then filtered and each filtrate separated into two parts. One part of each extract was used for phytochemical screening in tubes and the other part, evaporated to dryness in an oven (50 °C), was used for quantitative analysis by assay to assess antibacterial activity.

#### *2.2.2. Phytochemical screening using colour reaction and precipitation tests*

For phytochemical screening, the methodologies used are those described in the literature [11, 12, 13, 14].

Tannin detection test

A few drops of aqueous iron III chloride solution (1%) were added to tubes containing 5 mL of aqueous, ethanolic and hexanolic extract. The presence of gallic or catechic tannins results in the development of a greenish or blackish-blue coloration.

Flavonoid detection test

A few drops of concentrated hydrochloric acid and 3 magnesium chips were added to tubes containing 5 mL of each extract. The presence of flavonoids in the extracts is indicated by the change in colour to red or yellow.

Coumarin detection test

To 5 mL of each extract contained in test tubes are added 10 drops of KOH (10%). The resulting solution was neutralised with a 10% HCl solution. The presence of a cloud or precipitate in the tubes confirms the presence of coumarins in the extracts.

Test for the detection of free quinones

A few drops of sodium hydroxide (1%) were added to tubes containing 5 mL of the different extracts. The appearance of a yellowish green colour indicates the presence of free quinones.

Anthraquinone detection test

A few drops of ammonium hydroxide (10%) were added to tubes containing 5 mL of each extract tested. The presence of anthraquinones is confirmed when the solution turns yellow.

Alkaloid detection test

A few drops of HCl are added to 5 mL of each extract contained in test tubes, then each solution is divided into two equal volumes in tubes labelled A and B. A few drops of Mayer's reagent were added to tube A and a few drops of Wagner's reagent to tube B. The alkaloids are detected by a precipitation reaction in the presence of Mayer's and Wagner's reagents.

Sterol and triterpene detection test

A reagent consisting of an equal volume mixture of acetic anhydride and sulphuric acid is prepared. A few drops of this reagent are added to 5 mL of each extract. After incubation for 15 minutes, the appearance of a move (green) or violet colour indicates a positive test.

Terpenoid detection test

A reagent consisting of a mixture of 2 mL chloroform and 3 mL sulphuric acid is prepared. A few drops of this reagent were added to 5 mL of each extract. The formation of two phases and the appearance of a brown colour confirmed the presence of terpenoids.

Test for the detection of saponosides or saponins

After adding 5 mL of distilled water to 5 mL of each extract, the test tubes were stoppered and vigorously shaken for 15 seconds. The formation of a persistent foam greater than 1cm in height indicates the presence of saponins.

Test for the detection of reducing compounds

Fehling's reagent is prepared from liquor A, which is a mixture of copper sulphate and water, and liquor B, which consists of tartaric acid ($C_4H_6O_6$), sodium hydroxide (NaOH) and water. A few drops of Fehling's reagent are added to 5 mL of each extract. The appearance of a brick-red precipitate indicates a positive test.

Anthocyanin detection test

A few drops of sulphuric acid (10%) and a few drops of ammonium hydroxide (10%) were added to 5 mL of each extract. The presence of anthocyanins was confirmed by a black coloration.

Volatile oil detection test

A few drops of sodium hydroxide (10%) and a few drops of chloridric acid (10%) are added to 5 mL of each extract. The presence of volatile oils in the extracts is indicated by the appearance of a blackish colour.

Cardiac glycoside detection test

A few drops of chloroform and sulphuric acid (concentrated) are added to 5 mL of each extract. The presence of cardiac glycosides is confirmed when there are two phases and a brown colour in the test tube.

*2.2.3. Quantitative analysis by assay*

The quantitative analyses by assay were carried out on the aqueous and ethanolic extracts in order to be in the same conditions of traditional use of the plant. Extractions are traditionally made either with water or with traditionally produced alcoholic beverages.

Determination of total polyphenols

The amount of total phenols in the extracts was determined using the Folin-Ciocalteu colorimetric method. This is based on the oxidation of polyphenolic compounds by the Folin-Ciocalteu reagent in a basic medium. The blue reduction products absorb at 760 nm and the intensity is proportional to the quantity of polyphenols present in the sample. 0.01 g of each crude extract tested was dissolved in 10 mL of distilled water to give the stock solution, which was diluted 1 :10. To 1 mL of the diluted solution, 0.5 mL of Folin-Ciocalteu reagent (0.5 N) and 1.5 mL of sodium carbonate ($Na_2CO_3$) (17%, w/v) were added. The mixture was incubated in the dark for 30 min. Absorbance was determined at 760 nm using distilled water as a blank.

A calibration line was performed with gallic acid at different concentrations. The operation was repeated three times, and the concentration of polyphenols was expressed in micrograms per gram of gallic acid equivalent extract (mg EAG/g) [15, 16]. The amount of total phenols was determined by the following formula :

$$\text{Quantity (mg/g)} = (Vf \times Q \times D)/M \text{ ; with :}$$

**Vf** : final volume of extract ;
**Q** : quantity of phenols in equivalent gallic acid in mg/mL ;
**M :** mass of extract in g ;
**D** : dilution factor.

The concentration of total phenols is determined from the calibration curve with equation: y=6.7469x-0.0353, with R2=0.9942, where x = Concentration and y = Absorbance.

Determination of total flavonoids

This assay was carried out according to the method used by Arvouet-Grand et al [17].

0.01 g of each crude extract was diluted in 10 mL of distilled water to give the stock solution, which was diluted 1 :10. To 2 mL of the diluted solution, 2 mL of 2% aluminium chloride ($AlCl_3$) in methanol was added. The mixture was incubated in the dark for 15 min. Absorbance was determined at 415 nm using distilled water as a blank. A calibration line was made with quercetin at different concentrations.

The operation was repeated three times and the flavonoid concentration was expressed in micrograms per gram of quercetin equivalent extract (mg EQ/g). The amount of total flavonoids was determined by the following formula :

$$\text{Quantity (mg/g)} = (Q \times D) / C \text{ ; with :}$$

**Q** : quantity of flavonoids in quercetin equivalent, mg/mL ;
**D** : dilution factor ;
**C** : initial concentration of the stock solution.

The concentration of total flavonoids is determined from the calibration curve with the equation: y=22.779x-0.0159 with R2=0.9994, where x : Concentration and y: Absorbance.

Determination of total condensed tannins

Condensed tannins were determined using the method described by Broadhurst & Jones [18] and Heimler et al [19], with a few modifications.

To 0.2 mL of each sample (1 mg/mL), 1.5 mL of vanillin solution (4% in MeOH) and 0.75 mL of concentrated HCl were added. The mixture was incubated for 15 min and the absorbance was read at 500 nm. The concentrations of condensed tannins are deduced from the calibration ranges established with catechin (0.15 - 0.009375 mg/mL) and are expressed in microgrammes of catechin equivalent per milligramme (mgECT/g).

The concentration of total tannins is determined from the calibration curve with equation: y=1.6546x+0.0086 with R2=0.9983; where x : Concentration and y: Absorbance.

*2.2.4. Evaluation of the antibacterial activity of Ageratum conyzoides leaves*

Preparing the inoculum for the efficacy test

Bacterial colonies 18-24 h old were picked and homogenised in 2 mL of sterile physiological water until an optical density (DO) of 0.5 Mc Farland was obtained, corresponding to a bacterial population of approximately $10^6$ UFC/mL. This inoculum was used to inoculate the Muller Hinton (MH) agar plates for the test using tight streaks and a swab [20].

Efficacy test

Aqueous and ethanolic extracts of *Ageratum conyzoides* leaves at a concentration of 134 mg/mL were poured into wells on MH agar plates that had been inoculated beforehand. Antibiotics such as amoxicillin + clavulanic acid (AMC), ticarcillin + clavulanic acid (TCC) and cefoxitin (FOX) were applied to the MH agar plates and used as positive controls [21].

Determination of MIC by dilution in liquid medium

100 µL of bacterial inoculum is distributed in the wells of a microplate. To these quantities are added 100 µL of the different concentrations of plant extract to be tested, from the highest concentration to the lowest, with the exception of the growth control wells (Tc) to which are added only 100 µL of sterile distilled water (the total volume of each well being 200 µL). Only 200 µL of distilled water was added to the wells reserved for the sterility controls (Ts). The microplate was then incubated at 37 °C for 24 hours. The MIC therefore corresponds to the concentration of the first experimental well from

Determining the minimum bactericidal concentration (MBC)

First, 4 successive 10 $^{ème}$ fold dilutions from $10^{-1}$ to $10^{-4}$ are made from the starting inoculum. These dilutions and the inoculum were inoculated in 5 cm strips on the various MH agar plates, then incubated at 37°C for 24 h. These Petri dishes were called A plates. These Petri dishes formed the A plates [21]. Next, the microplate wells in which no cloudiness was visible to the naked eye were streaked 5 cm onto the MH agar plates, starting with the MIC tube. They were then incubated at 37°C for 24 h. These plates became the B plates. The MIC corresponds to the lowest concentration of extract for which there are no more than 0.01% surviving bacteria when comparing plates A and B.

*2.2.5. Statistical analysis*

The measurements obtained during the various experiments were analysed using EXCEL software. It was used to determine the calibration lines and plot the various extract diagrams.

## 3. Results

### 3.1. Extraction of *Ageratum conyzoides* leaves

Extracts of *Ageratum conyzoides* leaves were obtained by maceration with three solvents: distilled water, ethanol and hexane. The results of the various extraction yields are given in Table 1. The values range from 3.54 ± 0.01 to 6.66 ± 0.01.

**Table 1** Different extraction yields

| Extraction solvents | Distilled water | Ethanol | Hexane |
|---|---|---|---|
| Extraction yields (%) | 6.66 ± 0.01 | 4.20 ± 0.01 | 3.54 ± 0.01 |

### 3.2. Phytochemical study

*3.2.1. Phytochemical sorting of Ageratum conyzoides leaves in tubes*

The compound families tannins, flavonoids, coumarins, free quinones, anthraquinones, alkaloids, sterol-triterpenes, terpenoids, saponosides, reducing compounds, anthocyanins, volatile oils and cardiac glycosides were investigated in

the aqueous, ethanolic and hexanolic extracts of *Ageratum conyzoides* leaves. The results obtained are recorded in Table 2 and summarised in Table 3. In general, all the compounds investigated were found to be present in *A. conyzoides* leaves.

**Table 2** Results of triphytochemical analysis of aqueous, ethanolic and hexanolic extracts of *Ageratum conyzoides* leaves

| Secondary metabolite family | Aqueous extract | Ethanolic extract | Hexane extract |
|---|---|---|---|
| Tannins | + | + | - |
| Flavonoids | + | + | + |
| Coumarins | + | + | + |
| Fre quinones | + | - | + |
| Anthraquinones | - | - | + |
| Alkaloids | + | + | + |
| Sterols and triterpenes | + | + | + |
| Terpenoids | + | + | + |
| Saponins | + | - | - |
| Reducing compounds | + | + | + |
| Anthocyanins | + | + | + |
| Volatile oil | + | + | + |
| Cardiac glycoside | + | + | + |

The "+" sign indicates a positive reaction and the "-" sign indicates a negative reaction.

**Table 3** Summary table of phytochemical sorting of *Ageratum conyzoides* leaves

| Ta | Fl | Co | Qu | An | Al | S-T | Te | Sa | C-R | A-Q | H-V | G-C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + | + | + | + | + | + | + | + | + | + | + | + | + |

Ta : Tannins ; Fl : Flavonoids ; Co : Coumarins ; Q-L : Free quinones ; A-Q : Anthraquinones ; Al : Alkaloids ; S-T : Sterols and triterpenes ; Te : Terpenoids ; Sa : Saponins ; C-R : Reducing compounds ; At : Anthocyanins ; H-V : Volatile oil ; G-C : Cardic glycoside. + : presence and - : absence.

*3.2.2. Quantitative analysis by assay*

The assay was used to quantify total phenols, total flavonoids and total condensed tannins in the aqueous and ethanolic extracts of *Ageratum conyzoides* leaves. The analysis was carried out only on these two extracts, in order to use the same conditions as those used for traditional treatments, which use aqueous and alcoholic extracts.

Determination of total polyphenols

The results of the values for the quantities of total polyphenols in the ethanolic and aqueous extracts of *Ageratum conyzoides leaves* are shown in Table 4. The quantity of total polyphenols in the ethanolic extract (0.145± 0.006 mg EAG/g MS) is slightly higher than that in the aqueous extract (0.120 ± 0.002mg EAG/g MS).

**Table 4** Total polyphenols in ethanolic and aqueous extracts of A. conyzoides

| Extracts | Total polyphenol content (mg EAG/g MS) |
|---|---|
| Ethanolic | 0.145 ± 0.006 |
| Aqueous | 0.120 ± 0.002 |

Determination of total flavonoids

Table 5 shows the values for total flavonoids in the ethanolic and aqueous extracts of *Ageratum conyzoides* leaves. The total flavonoid content in the ethanolic extract (0.206 ± 0.003 mg EQ/g MS) is much higher than that observed in the aqueous extract (0.005 ± 0.001 mg EQ/g MS).

**Table 5** Total flavonoids in ethanolic and aqueous extracts of A. conyzoides

| Extracts | Total flavonoid content (mg EQ/g MS) |
|---|---|
| Ethanolic | 0.206 ± 0.003 |
| Aqueous | 0.005 ± 0.001 |

Determination of total condensed tannins

The results of the total condensed tannin values in the ethanolic and aqueous extracts of *Ageratum conyzoides* leaves are shown in Table 6. The total condensed tannin content in the ethanolic extract (0.064 ± 0.007 mg ECT/g MS) is almost three times higher than that observed in the aqueous extract (0.022 ± 0.004 mg ECT/g MS).

**Table 6** Total condensed tannins in ethanolic and aqueous extracts of *A. conyzoides*

| Extracts | Total condensed tannins ontent (mg ECT/g MS) |
|---|---|
| Ethanolic | **0,064 ± 0,007** |
| Aqueous | **0,022 ± 0,004** |

### 3.3. Antibacterial activity

*3.3.1. Efficiency of ethanolic crude extract in solid medium*

This screening made it possible to observe the sensitivities of the ethanolic and aqueous extracts at 134 mg/mL to the bacterial strains tested compared with those of the reference antibiotics. The inhibition diameter results are shown in Table 7. The inhibition diameters of the aqueous and ethanolic extracts range from 00 to 09 mm while those of the reference antibiotics range from 00 to 35 mm.

**Table 7** Diameter of the inhibition zones of ethanolic and aqueous extracts and reference antibiotics in relation to the bacterial strains used

| | | Diameter of zone of inhibition at 134 mg/mL (mm) | | | |
|---|---|---|---|---|---|
| **Strains bacterial** | | **Extract Ethanolic** | **Extract aqueous** | **FOX** | **EDS** |
| *S.aureus* | ATCC 25923 | **09±0.35** | **07±0.032** | **35±1.30** | 00±00 |
| | 1568UB/23 CNRa | 00±00 | 00±00 | **28±1.41** | 00±00 |
| | | | | AMC | EDS |
| *E.coli* | ATCC 25922 | 00±00 | 00±00 | **23±0.58** | 00±00 |
| | 1562UB/23 CNRa | 00±00 | 00±00 | **18±0.35** | 00±00 |
| | | | | TCC | EDS |
| *P. aeruginosa* | ATCC 27853 | 00±00 | 00±00 | **17±0.35** | 00±00 |
| | 1587UB/23 CNRa | 00±00 | 00±00 | 00±00 | 00±00 |

EDS : Distilled sterile water

*3.3.2. Action of the ethanolic crude extract in liquid medium on the sensitive strain*

Only the ethanolic extract was effective, and only against *S. aureus* ATCC 25923. Its MIC and MBC were therefore determined for this bacterial strain. The results are shown in Table 8. The MBC/MIC ratio was used to determine the bacteriostatic nature of the ethanolic extract with respect to the strain tested.

**Table 8** Antibacterial parameters of crude extracts effective against S. aureus

| Bacterial strain | MIC (mg/mL) | MBC (mg/mL) | MBC/MIC | Interpretation |
|---|---|---|---|---|
| *S. aureus* ATCC 25923 | 33,5 | 134 | 4 | Bacteriostatic |

## 4. Discussion

The yield of extraction by maceration of *Ageratum conyzoide* leaves with distilled water (6.66%) is higher than that of ethanol (4.20%), which in turn is higher than that of hexane (3.54%). The higher yields observed with distilled water and ethanol would justify the common use of these two extraction solvents in traditional medicine. These solvents are reputed to extract a large number of phytocompounds [4, 23]. In addition, water allows the permeability of plants tissues and favours the phenomenon of mass diffusion in the extraction stage [24].

Phytochemical screening of aqueous, ethanolic and hexanolic extracts of *Ageratum conyzoides* leaves revealed the presence of tannins, flavonoids, coumarins, free quinones, anthraquinones, alkaloids, sterol-triterpenes, terpenoids, saponosides, reducing compounds, anthocyanins, volatile oils and cardiac glycosides. These results are in agreement with those of certain authors. Indeed, Vera [25] showed the presence of essential oils, phenolic compounds and coumarins in the plant's leaves. Similarly, Ekundayo et *al* [26] also extracted essential oils from the African species. However, these results contradict those of Bouquet & Debray [27], who noted the absence of saponosides, flavonoids, sterols and polyterpenes. This difference in results could be justified by differences in the environment, harvesting sites and extraction and analysis techniques. The presence of these secondary metabolites could explain the pharmacological properties of the leaves, which have already been proven. The alcoholic extract of the leaves has been shown to have analgesic, anti-inflammatory, antioxidant [28, 29] and spasmolytic [29] properties. Aqueous leaf extracts have also been shown to have a highly effective analgesic action in rats [30]. Flavonoids have anti-allergic, anti-inflammatory, antiviral, antibacterial, anti-tumour and anti-cancer properties [31, 32, 33] and tannins are healing, antibacterial and antiseptic [9]. The alkaloids are antimalarial and the sterols, polyterpenes, polyphenols and saponosides have various therapeutic effects [34]. Saponins are surface-active, haemolytic, antioxidant and antimicrobial [35, 36, 37]. Coumarins have a wide range of biological properties. They are antifungal, antimicrobial, anti-tumour, anti-platelet aggregation, inhibitors of several anti-viral enzymes, anti-inflammatory, anti-coagulant, diuretic and analgesic [38].

In addition to their contraceptive properties, quinones have analgesic, anti-inflammatory, antioxidant, anti-tumour and anti-bacterial properties [39]. The free anthracene derivatives belonging to the quinones have a purgative and laxative action on the large intestine [40]. Terpenoids are widely used for their aromatic qualities. They also play a role in traditional herbal remedies and are the subject of research into their antibacterial and antineoplastic effects [41]. However, the terpenes and sterols derived from terpenoids are analgesic and anti-inflammatory [9]. The presence of these phytocompounds in their entirety could therefore justify the use of *Ageratum conyzoides* leaves in the traditional treatment of numerous pathologies. Indeed, *Ageratum conyzoides* is known for its therapeutic properties. In Central Africa, the plant is particularly used to treat wounds caused by burns [42]. In Cameroon and Congo, the plant is used to treat fevers, rheumatism, headaches, diabetes and colic [30, 43, 44]. In India, it is used to treat leprosy and as an oil lotion for purulent eye infections [45]. In Vietnam, the plant is used to treat gynaecological diseases in particular [46]. In Brazil, medicinal tea made from *A. conyzoides* is used as an anti-inflammatory, analgesic and anti-diarrhoeal [47]. In Côte d'Ivoire, the plant is traditionally used in several regions. In the south, among the Abbey and Krobou people, it is used to treat migraine and malaria and to facilitate childbirth [48]. According to Ouattara [49], the Dida people of Divo, in the south of the forest, use it to treat epilepsy. In the Centre-West, it is used to treat headaches [50]. The Akan ethnic groups in the coastal region use it to treat sore eyes and measles [51].

Although the yield of water extraction is higher than that of ethanol, quantitative analysis by assay showed that the ethanolic extract of *Ageratum conyzoides* leaves is richer in total polyphenols, total flavonoids and total condensed tannins than its aqueous extract. We can therefore conclude that ethanol is more favourable for extracting phenolic compounds from *A. conyzoides* leaves than distilled water.

Evaluation of the antibacterial activity of *Ageratum conyzoides* leaves showed that only the ethanolic extract was active, and only against the bacterial strain *Staphylococcus aureus* ATCC 25923. According to Ponce et al [52], a bacterium is said to be resistant to an extract when its inhibition diameter around this extract is less than 8 mm, sensitive if this diameter is between 9 and 14 mm, very sensitive when it is between 15 and 19 mm and extremely sensitive for a diameter greater than 20 mm. The positive controls, which are the reference antibiotics, had highly sensitive activity on all the strains tested except ticarcillin + clavulanic acid (TCC), which was inactive on *Pseudomonas aeruginosa* 1587 UB/23 CNRa. This inactivity of TCC against *Pseudomonas aeruginosa* 1587 UB/23 CNRa confirms the frequently observed resistance of certain pathological bacterial strains to conventional antibiotics [53]. This result could justify the orientation of much research into plant-based antibiotics [53, 54, 55, 56]. However, in this study, the extracts studied were not very effective on all the bacterial strains tested. However, the ethanolic extract that acted on *Pseudomonas aeruginosa* 1587 UB/23 CNRa proved to be bacteriostatic with regard to the minimum bactericidal and inhibitory concentrations (MBC/MIC ratio=4). This bacteriostatic activity of the ethanolic extract observed on the bacterial strain *Staphylococcus aureus* ATCC 25923 is thought to be due to the synergistic action of the secondary metabolites detected in the leaves of *Ageratum conyzoides*, but more specifically to the high presence of phenolic compounds compared with the aqueous extract.

## 5. Conclusion

This study is part of a project to develop medicinal plants and, in particular, to find new molecules from plant extracts. The general aim of this work is to confirm or refute the traditional use of *Ageratum conyzoides* leaves using chemical and biological approaches.

Phytochemical sorting showed the presence of several secondary metabolites in *Ageratum conyzoides* leaves, namely tannins, flavonoids, coumarins, free quinones, anthraquinones, alkaloids, sterol-triterpenes, terpenoids, saponosides, reducing compounds, anthocyanins, volatile oils and cardiac glycosides. The quantitative study by assay revealed that the ethanolic extract is more concentrated in total polyphenols, total flavonoids and total condensed tannins than the aqueous extract. As for the study of antibacterial activity, the ethanolic extract only had a bacteriostatic effect on the bacterial strain *Staphylococcus aureus* ATCC 25923. The presence of all these secondary metabolites and the bacteriostatic effect observed on the *Staphylococcus aureus* ATCC 25923 strain could therefore point to the use of *Ageratum conyzoides* leaves in pathologies linked to bacterial infections of the *Staphylococcus aureus* family. In the future, however, it will be useful to study the toxicity of *Ageratum conyzoides* leaves and to assess other pharmacological properties such as antioxidant, antimalarial and analgesic activities.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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